Morphine effects on monetary reward

An fMRI (pilot) study

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Abstract

The μ-opioid receptor system is central to reward and pain relief across species. In rodents, injection of opioids into striatum amplifies 'liking' responses to and/or motivation for rewards. In humans, opioid agonists can induce euphoria, whereas antagonists reduce food reward. Brain regions implicated in reward processing such as the mesolimbic reward system are rich in μ-opioid receptors. We investigated the role of the μ-opioid receptor system in human reward processing using systemic manipulation with a μ-opioid receptor agonist (morphine). In a functional magnetic resonance imaging (fMRI) pilot study we developed test procedures to measure reward related brain activity to anticipation and delivery of rewards, and to minimise potential confounds related to the pharmacological manipulation. We predicted activity in in the ventral striatum during anticipation and delivery of reward in a modified monetary incentive delay (MID) task, and that this activation would be higher in the morphine condition compared to placebo. In a within-subjects, counter-balanced, placebo-controlled, double-blind design, 11 healthy volunteers (4 females, mean age 26 ± 3 years) were tested on a battery of reward tasks on two separate days. In line with previous research, our version of the MID task yielded significant activation in the ventral striatum during anticipation and delivery of rewards, compared to baseline. We also observed an indication of higher activation in the morphine condition compared to placebo in the left ventral putamen during reward delivery. Control measures (subjective effects, motor coordination, physiological measures, and a visual fMRI paradigm) revealed minimal confounding effects of drug manipulation on task results. These results validate our test procedures and are in line with the hypothesis that systemic stimulation of the μ-opioid receptor system modulates activity in the ventral striatum during reward processing. The methods developed in this thesis will be used in the future study investigating the role of the μ-opioid receptor system for reward and motivation in the healthy human brain.

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Introduction

Being able to navigate an environment relies on an individual's ability to evaluate and predict future rewards and punishments, and use these predictions as well as past experiences to direct behaviour (O'Doherty, 2004). A reward can be defined as an event or a stimulus "for which an animal will perform an operant response" (Koob, 1992). Rewards elicit approach behaviour whereas punishments suppress behaviour and lead to avoidance (Porcelli & Delgado, 2009). When a value is associated with a rewarding stimulus, this leads to predictions of similar rewards in the future through learning mechanisms (Schultz, Dayan, & Montague, 1997). To integrate information about rewards and punishments to guide behaviour, the brain must have a way of evaluating rewards and attributing values to different stimuli (Porcelli & Delgado, 2009). These processes are attributed to a complex reward system in the brain (Berridge & Kringelbach, 2008). Disruptions in the brain reward system can have debilitating consequences, as evidenced in psychiatric disorders such as substance abuse, mood disorders, attention deficit/hyperactivity disorder (ADHD), and schizophrenia (Gold, Waltz, Prentice, Morris, & Heerey, 2008; Luman, Tripp, & Scheres, 2010; Naranjo, Tremblay, & Busto, 2001; Nestler, 2005).

Reward as a process can be divided into different components and phases. One framework for dissociating the different reward components was proposed by Berridge and colleagues. They proposed that the major components of reward are (i)'liking', referring to hedonic impact and subjective pleasure of a reward; (ii) 'wanting', incentive salience, or motivation to approach a reward; and (iii) learning, the predictive associations and cognitions relating to reward (see e.g. Berridge, 2003, 2009). Other researchers have investigated reward events in terms of separate temporal phases, by decomposing the reward event into (i) anticipation/prediction and (ii) outcome/consummation (Knutson, Fong, Adams, Varner, & Hommer, 2001b). These two ways of parsing reward represent different perspectives on the reward process, but ultimately try to explain the same underlying idea. The two temporal phases can also be viewed in terms of the psychological components, as the anticipation phase is dominated by motivation to approach and the wanting of the reward, whereas the consummation of the reward itself is where the hedonic value (liking) is determined (Kringelbach, Stein, & van Hartevelt, 2012). Learning can occur throughout the cycle of reward based on integration of motivational and hedonic value aspects of rewarding experiences.

Extensive research in both animals and humans has been conducted to understand the underlying neurophysiological mechanisms of the brain reward system. Accumulating

information from affective neuroscience has begun to reveal an interconnected brain circuitry involving several neurotransmitter systems. Further understanding of causal mechanisms and nuances of these systems and phases of reward processing is a major aim for current reward research.

Reward circuitry in the brain

A number of cortical and subcortical brain regions have been implicated in reward processes (see Figure 1). An influential early contribution to the field of affective neuroscience came from electrophysiology studies in the 1950s. Using intracranial electrodes, Olds and Milner (1954) found evidence that electrical stimulation in 'lower areas of the brain' caused rats to repeatedly self-stimulate for pleasure. Following these experiments, numerous animal studies using methods such as single-cell-recordings and intracranial stimulation combined with classical conditioning paradigms have identified dopaminergic neurons in the nucleus accumbens (NAc) and the ventral tegmental area (VTA) at the centre of this reward system (e.g. Kelley & Berridge, 2002; Schultz, 2000; R.A. Wise & Bozarth, 1985).

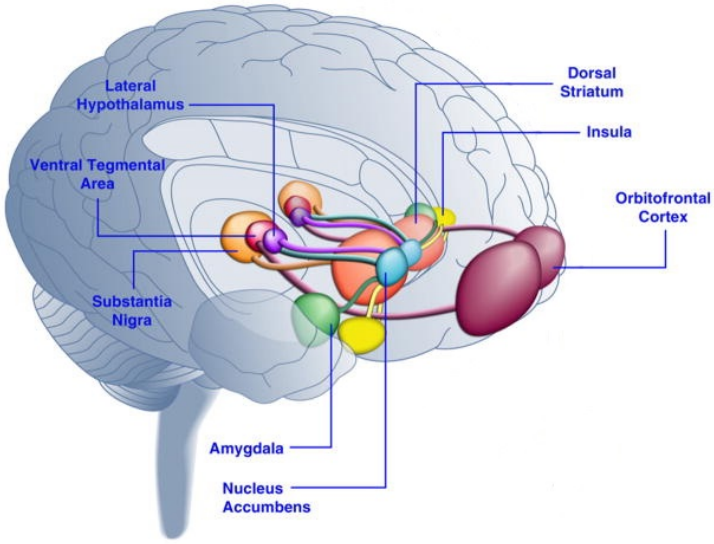

Figure 1. Selected brain regions associated with reward processing in humans. Adapted from (Kenny, 2011).

The striatum, including the NAc in the ventral striatum (VS), and its projection sites are believed to be at the core of the brain reward network in both animals and humans (Berridge & Kringelbach, 2008; Haber & Knutson, 2010; Porcelli & Delgado, 2009). The VS receives inputs from the orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC) as well as midbrain dopamine (DA) neurons, and projects to the ventral pallidum (VP) and the VTA and substantia nigra (SN). The VTA and SN in turn project back to the prefrontal

cortex (PFC) and regions of the thalamus. In humans, the targets of VTA neurons in striatal and limbic regions have been studied using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to investigate the involvement of these regions in anticipation and prediction of rewards (Martin-Soelch et al., 2001; Porcelli & Delgado, 2009). The ventral striatum has also been implicated in processing of both positive and negative outcomes, and in subsequent reward related decision making (Delgado, 2007; Delgado, Nystrom, Fissell, Noll, & Fiez, 2000).

Neutrotransmitters of reward. Many neurotransmitter systems have been implicated in reward processes including endocannabinoids, serotonin, DA and endogenous opioids (Kranz, Kasper, & Lanzenberger, 2010; Laurent, Morse, & Balleine, 2015; Mahler, Smith, & Berridge, 2007; Schultz, 2002). DA has a central role in the traditional reward research, but there is now a growing body of evidence from animal and human studies supporting a key role for the μ-opioid receptor system in several aspects of reward processing.

Dopamine. Dopamine is the most widely studied neurotransmitter in reward research, and has been implicated in both motivation and reward learning (Berridge & Robinson, 1998; Björklund & Dunnett, 2007b; Schultz, 2007a, 2007b). A large distribution of DA neurons are located within the regions implicated in reward processing (illustrated in Figure 1), forming central DA pathways such as the mesostriatal, mesocortical and mesolimbic pathways (Björklund & Dunnett, 2007a).

The mesolimbic DA pathway, consisting of dopaminergic neurons projecting from the VTA to the NAc, is consistently implicated in the processing of both natural and drug-induced rewards in non-human animals (Kelley & Berridge, 2002; Nestler, 2005). Both types of reward are associated with an increase in extracellular DA in mesolimbic areas (Di Chiara & Imperato, 1988; Schultz, 2000). Self-administration is an established method used for studying the reinforcing effects of drugs, and animals will consistently self-administer a range of drugs that are commonly abused in humans (Koob, 1992). Changes in the mesolimbic DA system affect the motivation to work for rewarding drug stimuli. An early animal study showed that lesions in the NAc or VTA (resulting in reduced DA firing) were associated with decreases in self-administered cocaine in rodents (Roberts, Corcoran, & Fibiger, 1977; Roberts & Koob, 1982). More recent research has shown that mice lacking the most common DA receptor in the central nervous system (D1 'knock out' mice) do not self-administer cocaine at all (Caine et al., 2007; Thomsen, Hall, Uhl, & Caine, 2009). DA antagonists have been found to influence self-administration of MDMA (Brennan, Carati,

Lea, Fitzmaurice, & Schenk, 2009) and methylphenidate (Botly, Burton, Rizos, & Fletcher, 2008) in rats.

DA has also been implicated in reinforcement learning and is involved in coding of prediction errors depending on the size and value of rewards (Roesch, Calu, & Schoenbaum, 2007; Schultz, 2007a). Prediction errors refer to a mismatch between predicted and actual event outcome, and are important for learning based on experience (Salamone & Correa, 2012). DA neurons fire in response to salient stimuli. When an association is learned between a cue and a subsequent rewarding stimulus, the (phasic) burst of DA is transferred to the cue instead of the reward delivery (Schultz, 2007a). Once learned, the absence of a reward leads to a negative prediction error and stops the dopamine firing to a rewarding stimulus. Electrophysiological studies in rodents and non-human primates have demonstrated this conditioning in single DA neurons, providing a neural basis for cellular learning believed to underlie neurophysiological mechanisms such as long-term depression and long-term potentiation (Schultz, 2007b, 2010). In all, animal research supports a key role for DA in initial encoding of rewarding stimuli. In humans, DA-dependent prediction errors have been shown to underpin reward seeking behaviour (Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006). Increased DA activity in OFC, PFC and ACC has also been observed during engagement in reward tasks in humans, corroborating DA-involvement in reinforcement learning across species (Vrieze et al., 2013).

In human clinical populations, imbalances in the DA systems are associated with disruptions in reinforcement learning and motivation. DA dysfunction has been implicated in the aetiology of ADHD, which is characterised by a range of learning and motivational deficits (Sagvolden, Johansen, Aase, & Russell, 2005; Volkow et al., 2012b). The primary treatment for ADHD is methylphenidate, a DA agonist that has been shown to increase available DA specifically in the VS and cause increased motivation, task engagement, and enhanced salience of stimuli (Groom et al., 2010; Volkow et al., 2001; Volkow et al., 2012b). The DA system is also implicated in the development of substance abuse. For example, studies have shown reduced reward sensitivity and reduced reward related activity in the mesolimbic DA pathway in substance dependent populations (e.g. Koob & Le Moal, 2001; Martin-Soelch et al., 2001; Volkow, Fowler, & Wang, 2002).

Although evidence supports the involvement of DA in several reward-related processes, the exact role of DA in reward has been debated (e.g. Berridge, 2007; Koob, 1996; Volkow, Fowler, Wang, & Goldstein, 2002; R.A. Wise, 1980; R.A. Wise, 1982). Some research suggests that DA is essential for all aspects of reward, including hedonic liking of

rewarding stimuli (Koob, 1996; R.A. Wise, 1982). This idea has been challenged. For example, within Kent Berridge and colleagues' reward component framework, DA has a primary role in 'wanting', through mediation of incentive salience mechanisms (Berridge, 2007; Berridge & Robinson, 1998). This notion is consistent with the incentive sensitisation theory of drug addiction proposed by the same authors (Robinson & Berridge, 1993, 2008). The incentive sensitisation theory posits that repeated drug use changes the incentive salience associated with taking drugs, subsequently increasing 'wanting' of the drug to a disproportional level (Berridge, Robinson, & Aldridge, 2009).

Together, evidence from animal research and studies on different clinical populations show the important role of DA in reward processing. Processing of both primary rewards and drug rewards is modulated by changes in DA activity, and regions in the mesolimbic DA pathway have been implicated in reward system deficiencies in many substance dependent populations (Daglish & Nutt, 2003; Nestler, 2005; Volkow, Wang, Fowler, & Tomasi, 2012a). However, there is evidence that DA is not solely responsible for all aspects of reward, and accumulating evidence suggests that dopaminergic neurotransmission is not necessary for hedonic liking or pleasure per se (Berridge & Kringelbach, 2015). Recent research has also questioned the causal role of DA in motivation and approach behaviour, and argued for a more interconnected network involving other neurotransmitter systems also in this aspect of reward (e.g. Laurent et al., 2015).

μ-opioid receptor system. In addition to being central in dopaminergic pathways, mesolimbic brain areas are rich in μ-opioid receptors (Mansour, Khachaturian, Lewis, Akil, & Watson, 1988). μ-opioid neurotransmission, particularly in the VTA-NAc pathway, has been shown to modulate multiple aspects of reward experience and behaviour (Nestler, 2005; Peciña, Smith, & Berridge, 2006; Wassum, Ostlund, Maidment, & Balleine, 2009). Opioids are essential in hedonic experience, or 'liking', of natural rewards such as high caloric food (Barbano & Cador, 2007; Leknes & Tracey, 2008; Nathan & Bullmore, 2009; Peciña & Berridge, 2000), the euphoric effects of drugs (Kreek, LaForge, & Butelman, 2002; Levran, Yuferov, & Kreek, 2012; Volkow, Fowler, & Wang, 2004), and social rewards and attachment (Burkett, Spiegel, Inoue, Murphy, & Young, 2011; Trezza, Damsteegt, Achterberg, & Vanderschuren, 2011) across species. Opioid agonist drugs increase dopamine release through inhibition of GABA (γ-Aminobutyric acid) inter-neurons, but also act directly on opioid receptors on NAc neurons (Johnson & North, 1992). Some evidence suggests that the rewarding effects of opioid drugs are independent of dopamine release (Daglish et al., 2008). Hnasko, Sotak, and Palmiter (2005) demonstrated intact opioid

agonist-induced reward in dopamine deficient mice. Similarly, a human PET study by Watson et al. (2013) found no detectable increase in striatal DA levels to either heroin reward or the expectation of heroin in a sample of opioid dependent patients.

Areas of the brain where μ-opioid receptor stimulation/agonism significantly increases the hedonic valuation of sweet taste rewards have been identified as hedonic 'hot spots' (Peciña et al., 2006). Two such 'hot spots' are found in the NAc and the VP (Peciña et al., 2006). In addition, there is evidence that opioid activation influences motivational impact and incentive salience of rewards in animals. In a study by Mahler and Berridge (2012), μ-opioid stimulation of the central amygdala enhanced incentive salience of both learned and unlearned incentive stimuli in rats. Microdialysis studies have shown a surge in endogenous opioids (enkephalin) in the striatum during consumption of palatable foods in rodents (DiFeliceantonio, Mabrouk, Kennedy, & Berridge, 2012). Interestingly, the same study demonstrated that microinjections of a μ-opioid receptor agonist in the same area led to a 250% increase in chocolate intake. This provides convincing evidence of opioid involvement in the 'wanting' of rewards. In a series of experiments, Wassum et al. (2009) found distinct neural circuits related to changes in hedonic impact and incentive salience of rewards in rodents. While opioid antagonists infused into the NAc or VP decreased sucrose palatability, the incentive salience of sweet rewards was affected only after infusions into the amygdala (Wassum et al., 2009). In addition, the opioid receptor system has been implicated in the integration of reinforcement values with instrumental learning to guide decision-making (Laurent et al., 2015). These studies support the role of opioids in several aspects of reward processing in animals. There is also evidence suggesting separable roles of the μ-opioid receptor system in different brain regions, which reflects a complex brain system responsible for coding and processing of rewarding stimuli.

Some of the evidence from animal studies implicating opioids in reward processes has been extended to human research. Naltrexone, a non-selective opioid receptor antagonist, has been shown to reduce food intake and subjective appetite (Yeomans & Gray, 1997), and decrease the rewarding effects of sugar in several clinical populations (Laaksonen, Lahti, Sinclair, Heinälä, & Alho, 2011; Langleben, Busch, O'Brien, & Elman, 2011). Antagonism specifically at the μ-opioid receptor has been shown to decrease intake as well as hedonic liking of high-value food (typically sweet or fatty) in both animals (Parker, Maier, Rennie, & Crebolder, 1992; Taha et al., 2006) and humans (Nathan et al., 2012; Ziauddeen et al., 2013). Opioid antagonism also affects value-based decision making, as evidenced, for instance, by a study that demonstrated reduced preference for immediate (small) rewards over delayed

(larger) rewards (delay discounting) following naltrexone administration (Boettiger, Kelley, Mitchell, D'Esposito, & Fields, 2009). In addition, a study found that naltrexone administration reduced the reinforcing effects of amphetamine in healthy volunteers (Jayaram-Lindström, Wennberg, Hurd, & Franck, 2004). This shows interactions between DA and opioid systems in drug rewards in humans, consistent with findings of such interactions in animals (Johnson & North, 1992; Spreckelmeyer et al., 2011; Vindenes et al., 2009).

A small amount of psychopharmacology studies using opioid receptor agonism in healthy humans have shown reduced fear recognition sensitivity (Ipser et al., 2013), increased pleasantness ratings of neutral emotional images (Gospic et al., 2008), and increased attractiveness ratings and motivation to view beautiful faces (Chelnokova et al., 2014).

Further research is needed to understand the mechanisms through which DA and opioid systems interact, and how the NAc-VTA pathway is implicated in the different components and phases of human reward processing.

Neuroimaging studies of reward

Blood-oxygen-level-dependent (BOLD) fMRI studies in humans have investigated different aspects of reward processing, including anticipation and prediction of reward, delivery/consummation of rewards and punishment, and rewards of different valence and magnitude. Although fMRI does not provide the best temporal resolution, it can be used to identify brain areas involved in cognitive and affective processes. fMRI does not measure neuronal activity per se, but the BOLD signal is considered to reflect energy usage and therefore approximate neural activity (Logothetis, 2002).

The regions identified in animal studies as part of the reward circuit, such as the OFC and PFC, striatum, and the amygdala, are commonly activated in human fMRI studies on reward (Porcelli & Delgado, 2009). Liu et al. (2007) used a reward decision-making task and found activation in the striatum and medial PFC during positive reward anticipation and outcome, whereas negative events (punishments) activated the lateral OFC, anterior insula, superior temporal pole and dorso medial frontal cortex (dmFC). A more recent meta-analysis revealed that the NAc was commonly activated during both positive and negative events across all stages of reward processing, while other areas were preferentially activated either to rewards (medial OFC and posterior cingulate cortex (PCC)) or punishments (ACC, anterior insula and lateral PFC) (Liu, Hairston, Schrier, & Fan, 2011). These studies illustrate that it can be valuable to separate anticipation and outcome stages of reward, and to study both rewards and punishment in fMRI paradigms.

To study sub-components of human reward processes, Knutson, Westdorp, Kaiser, and Hommer (2000) designed an fMRI task intended for parsing brain activity during anticipation and delivery of rewards. The Monetary Incentive Delay (MID) task is a simple reaction time task. A typical trial consists of a cue signalling either the opportunity to win or avoid losing a given amount of money (see Figure 2). Following a short delay, the target appears and the participant has to quickly press a button. Feedback is then presented on the screen stating whether the participant was successful ('reward' or 'save'), or unsuccessful ('miss' or 'loss') depending on their accuracy (responding quickly enough to the target) and the type of trial. In the MID task, the reward is delivered or withheld based on the operant action (pressing the button) performed by the participant.

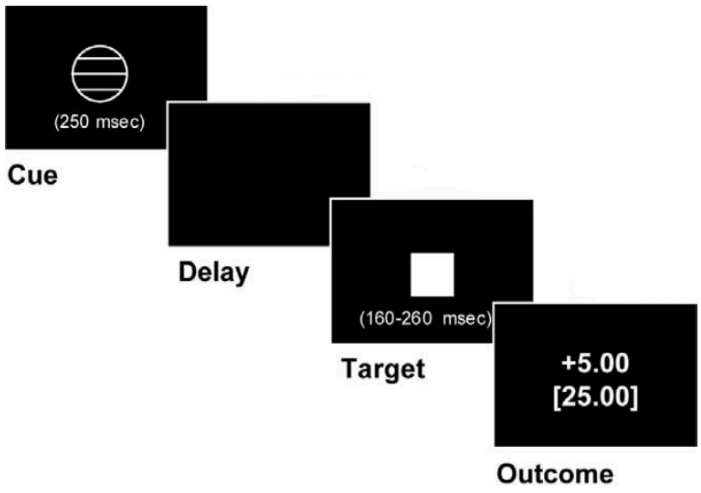

Figure 2. Illustration of a typical trial in the monetary incentive delay (MID) task. Values in the outcome stage represent task earnings for the trial (top) and total in the task (bottom) in US dollar. Figure from Knutson et al. (2003).

The MID task has been used in many studies to investigate brain activity during reward prediction and outcome in both healthy and patient populations. Striatal activation associated with anticipation and delivery of rewards in the MID task also correlates with other reward measures such as reward sensitivity (Santesso et al., 2008) and anhedonia scores (Wacker, Dillon, & Pizzagalli, 2009). In the original study by Knutson et al. (2000) and in many following studies (e.g. Furukawa et al., 2014; Knutson, Adams, Fong, & Hommer, 2001a; Kumar et al., 2014; Pizzagalli et al., 2009; Rademacher et al., 2010; Wrase et al., 2007), activation in NAc was observed in healthy individuals during anticipation of monetary gain. In addition, Knutson et al. (2001a) showed that activity in the NAc during reward anticipation was correlated with self-reported happiness. While initial reports indicated that the NAc was exclusively activated by anticipation of gain only (Knutson et al., 2001a; Knutson et al., 2001b), many later studies show NAc and other striatal activation during

anticipation of both gain and loss as well as during delivery of reward (Bjork, Smith, & Hommer, 2008b; Bustamante et al., 2014; Nestor, Hester, & Garavan, 2010; Scheres, Milham, Knutson, & Castellanos, 2007; Wrase et al., 2007). Some of the variation in results may be due to insufficient temporal separation of the reward event stages to accurately dissociate activity related to each stage separably in early versions of the MID task (Bjork et al., 2008b; Dillon et al., 2008). This issue has been addressed in more recent implementations of the paradigm.

The MID task has also been used to investigate reward related brain activity after pharmacological interventions. In healthy populations, DA agonism has been associated with increased NAc activity to anticipation of rewards (Knutson et al., 2004; Ye, Hammer, Camara, & Münte, 2011) while reductions in available DA have been associated with decreased activation to reward anticipation (da Silva Alves et al., 2011; Saji et al., 2013). In clinical populations, the MID task has been used to study brain reward during treatment with drugs acting on neurotransmitter systems implicated in reward, such as neuroleptics (Juckel et al., 2006; Schlagenhauf et al., 2008), selective serotonin reuptake inhibitors (SSRI's) (Stoy et al., 2012), and D9-tetrahydrocannabinol (THC) (Jansma et al., 2013; van Hell et al., 2012).

Together, the studies presented above show that BOLD signal changes during the MID task are sensitive to pharmacological manipulations of systems known to be involved in reward processing. However, it is important to note that reward activation in the MID task is not always consistent across studies and populations. One study found a reduced VS response to anticipation of rewards in healthy controls but not in substance dependent individuals following a single dose of a DA agonist (Schouw et al., 2013).

Despite some inconsistencies in reported results, the ability to disentangle brain activity to anticipation and outcome stages of reward renders the MID task useful for studying pharmacological effects on different facets of reward processing. To our knowledge, no previous studies have investigated the effect of opioid manipulation on different aspects of reward separately, such as anticipation and outcome.

Neuroimaging with opioid receptor drugs. While animal studies have the advantage of more extensive experimental control and the use of methods such as microinjections directly into opioid receptors, human research has to rely on more inferential methods to study the role of the opioid system. In recent years, advances in neuroimaging with methods such as pharmacological MRI are promising for studying drug effects on human brain processes by measuring drug related changes in BOLD signal (Colasanti, Lingford-Hughes, & Nutt, 2013).

The endogenous opioid system is heavily implicated in pain regulation, and μ-opioid receptor agonists are widely prescribed for pain relief due to their analgesic effects (Eidson & Murphy, 2013; Leknes & Tracey, 2008; Zubieta et al., 2001). The majority of fMRI studies using systemic opioid receptor manipulation in healthy volunteers come from the domain of pain and analgesia research. Opioid agonists such as morphine and remifentanil have been associated with increased resting-state BOLD activation in reward related regions such as NAc, amygdala, OFC, hippocampus, ACC, and insula during and following drug administration (Becerra, Harter, Gonzalez, & Borsook, 2006; Leppä et al., 2006). Wanigasekera et al. (2012) found that activity in the OFC, NAc and VTA also predicted analgesic effects of remifentanil in healthy volunteers. Furthermore, opioid agonists have been associated with a dose-dependent modulation of pain-induced BOLD response in limbic regions and reduced activation to pain in the insula and ACC (Upadhyay et al., 2012; R.G. Wise et al., 2002). A study by Atlas et al. (2012) corroborated these results by showing that reduction in pain related activation was independent of expectancy effects. A resting state fMRI study of healthy volunteers found reduced functional connectivity between the ACC and insula, and the ACC and putamen, after administration of the opioid agonist oxycodone (Gorka, Fitzgerald, de Wit, Angstadt, & Phan, 2014). The authors suggested that this could be a possible mechanism for the analgesic effects of opioids, by impairing both perception and appraisal of internal pain states (Gorka et al., 2014).

Opioid antagonists, such as naloxone or naltrexone, are used more frequently in fMRI studies with healthy volunteers than agonists. In a pain simulation study by Borras et al. (2004), naloxone induced increased pain intensity ratings and BOLD response to pain in cortical and subcortical regions in the reward circuit, including NAc and OFC. In another study, naloxone infusion enhanced fear acquisition and was associated with sustained amygdala response to fear, while the amygdala response to the conditioned stimulus was decaying rapidly in the placebo condition (Eippert, Bingel, Schoell, Yacubian, & Büchel, 2008).

Opioid antagonism has also been associated with decreased activation to rewarding food images in the amygdala, ACC and caudate (Murray et al., 2014; Rabiner et al., 2011). Murray et al. (2014) found that naltrexone increased activation in the amygdala and insula in response to aversive food stimuli. Using a gambling task, Petrovic et al. (2008) showed that naloxone attenuated neural response to rewards of increasing magnitude in the ACC while increasing neural activity to losses of all magnitudes in the insula and caudal ACC. These

findings are consistent with the idea that endogenous opioid release attenuates the negative aspects of losses (Colasanti et al., 2013).

Very few imaging studies have investigated the effects of μ-opioid receptor agonists on affective measures in healthy volunteers, likely due to high abuse potential of this type of drugs. In one recent fMRI study, BOLD response to emotional stimuli was measured following acute oxycodone administration in healthy volunteers (Wardle et al., 2014). Contrary to the hypotheses, oxycodone did not alter emotional processing in the primary regions of interest NAc and amygdala (Wardle et al., 2014). Notably, the study did not replicate drug-related effects in regions that have previously been associated with increased activity during resting state fMRI following opioid-receptor agonism (Becerra et al., 2006; Leppä et al., 2006). This highlights the need for further research into the effects of opioid receptor agonists on brain processes in the absence of pain.

The current study

To address central questions concerning opioid system involvement in reward in the healthy human brain, we designed a pharmacological fMRI study. Pharmacological fMRI requires a range of considerations that regular task-fMRI do not, and thus the current thesis has focused on preparing appropriate study procedures. To date, opioid agonists have primarily been used in fMRI studies of pain, analgesia, and drug addiction treatment. Opioid receptor agonists are widely used for pain relief (Vindenes, Handal, Ripel, Boix, & Mørland, 2006), yet little is known about how opioids influence processes in the healthy human brain (Fields, 2007). Administering opioids to healthy, non-addicted, pain-free individuals has been approached with apprehension, partly due to abuse potential and methodological concerns. Notably, in healthy individuals, moderate doses of 'slow-acting' μ-opioid receptor agonists produce very few subjective effects (Chelnokova et al., 2014; Hanks, O'Neill, Simpson, & Wesnes, 1995; O'Neill et al., 2000; Zacny & Lichtor, 2008).

Combining pharmacological manipulations with fMRI has the potential to elucidate mechanisms involved in reward processing in healthy humans in a non-invasive matter (Iannetti & Wise, 2007; Knutson & Gibbs, 2007; Nathan, Phan, Harmer, Mehta, & Bullmore, 2014). However, studies using this method are often limited by small sample sizes and lack of appropriate control measures (Becerra et al., 2006; Leppä et al., 2006; Wardle et al., 2014).

Aims and hypotheses. To gain further understanding of the involvement of the opioid system in anticipation and delivery of rewards, we used a previously validated paradigm to probe activity in relevant mesolimbic brain areas. Pharmacological fMRI was deemed an appropriate method to investigate functional differences in brain activity

following μ-opioid receptor manipulation. By developing appropriate control tasks and measures, this pilot study will precede a larger pharmacological study aimed at studying μ-opioid receptor mechanisms in healthy volunteers. We decided to use the MID task (Knutson et al., 2001a; Knutson et al., 2000) to assess neural activity in response to monetary rewards and punishments in healthy individuals receiving a μ-opioid receptor agonist (morphine, 10mg per oral) and placebo in two separate sessions. For this purpose, we designed a modified version of the MID task enabling analysis of brain activity associated with both anticipation and outcome of reward as well as punishment. We used (BOLD) fMRI signal changes in the brain to approximate neural activity in response to task stimuli. The aims of this thesis were to develop a study procedure to measure brain reward processes and potential pharmacological effects on these. To achieve the goal, this pilot study was planned to (1) choose, develop and validate a reward task suitable for measuring reward related brain activity, (2) test whether a μ-opioid receptor agonist, morphine, would increase reward related brain activity compared to placebo, and (3) find appropriate control measures for confounding pharmacological effects.

We hypothesised that:

1. Participants would show activation in the ventral striatum (specifically NAc) during anticipation and delivery of monetary gains.
2. The activation in reward-related regions would be higher after administration of a selective μ-opioid receptor agonist, morphine, compared to the placebo condition, particularly during reward outcome (liking).

Support for these hypotheses would validate the current task procedures for the main study of opioid effects on reward and motivation, and provide support for the involvement of opioids in reward processing in the healthy human brain.

Methods

Participants

14 healthy volunteers were recruited for the current study from the University of Oslo and via acquaintances. Two participants were only tested once due to breakdown of the scanner head coil causing a delay in data collection. Two participants experienced aversive side effects. One participant felt discomfort during fMRI in the placebo condition and withdrew prior to the second session. Another participant felt nauseous in the morphine condition, but is included in the study sample since the aversive effects did not occur until after completion of the study tasks. This resulted in a final sample of 11 participants (4 females, age range 21-33 years, $M \pm SD = 26 \pm 3$ years). All participants received written

information by email and underwent a medical screening per phone prior to testing. Grounds for exclusion were: contraindications to morphine, current prescription of opioids, antidepressant, or antipsychotic medications, current medical or neurological illness, history of alcohol or substance abuse, claustrophobia, and other contraindications to MRI such as metal implants or pacemaker. All participants were morphine-naïve (defined as no morphine in the last 2 years, as per Becerra et al. (2006)), right handed, and had normal or corrected to normal vision. Each participant underwent two separate testing sessions of approximately 3 hours separated by a minimum of 1 day to avoid drug carry-over effects. Of the four female participants, two used hormonal contraception. The other two were unsure about timing since last menstruation and we were thus unable to assess phase of cycle. We tried to complete both test sessions within one hormonal phase by testing twice within a short time interval. In each session the participants received either morphine (10 mg per oral) or placebo prior to performing the tasks. Participants received a total monetary compensation of ~500 NOK (Norwegian Kroner), which included total earnings from the MID tasks ($M \pm SD$=184±16 NOK). Before testing commenced, all participants provided written informed consent and the study was approved by the Regional Health and Ethics Committee (REK: 2011/1337/ Helse Sør-Øst).

Design

This study was conducted in a double-blind, placebo-controlled, counter-balanced manner with repeated-measures within subjects. The MID task was administered as part of a battery of reward tasks. Participants also completed a food wanting regulation task, an emotion recognition task, and selected trait and state questionnaires. Only results from the MID task and relevant control measures are reported in this thesis. Participants answered questions about mood and "subjective state" at three different time points during each session (see Figure 3). fMRI data collection commenced ~60 minutes after drug administration to coincide with stable and high plasma concentrations. Trait questionnaires were completed prior to drug administration to avoid potential influence of opioids on test answers.

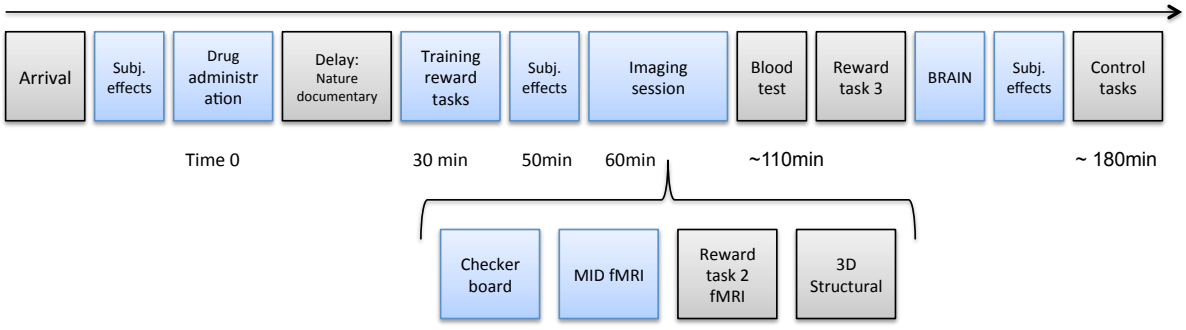

Figure 3. Timeline for session 1; procedures discussed in the current thesis are marked in blue.

Drug administration

Morphine is a selective μ-opioid receptor agonist, and is a widely prescribed analgesic for acute pain (Eidson & Murphy, 2013). In the current study we used pills of 10mg morphine (Morfin®, Nycomed Pharma). This dosage was chosen to activate μ-opioid receptors without causing sedation or euphoria, and to limit subjective effects that could influence task behaviour (Chelnokova et al., 2014; Walker & Zacny, 1998; Zacny & Lichtor, 2008). We chose oral administration, as it is less invasive for the participants than intravenous drugs. Orally ingested morphine reaches maximal effect 1-2 hours after intake and has a half-life of 2-4 hours (Lugo & Kern, 2002). Per oral morphine bioavailability is on average 20-30% but varies substantially between individuals (Hoskin et al., 1989).
Placebo pills were cherry-flavoured breath mints chosen to visually resemble morphine pills. To avoid recognition by flavour, a small amount of the placebo pills were added to the morphine drug dose. To ensure successful blinding of participants and experimenter, the participants were instructed not to chew or visually inspect the pills (presented in a small black cup).

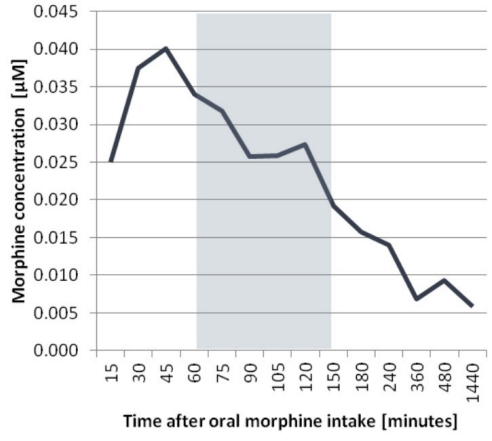

Figure 4. Time line showing blood concentration of morphine as a function of time after oral intake. The area in grey is the time interval chosen for fMRI testing in the current study. Reprinted with permission from Eikemo (2011).

Time line. Participants provided written consent, and then completed a state-relevant questionnaire. Following drug administration, participants waited for 60 minutes before testing commenced. The 60-minute delay was chosen in a previous study to allow for stable and high morphine concentrations (see Figure 4; Chelnokova et al., 2014; Eikemo, 2011). During the delay, participants watched a nature documentary for 30 minutes before completing practice runs for the reward tasks. ~50 minutes after drug administration, participants completed the subjective effects questionnaire again inside the scanner prior to the tasks. After the imaging session (~110 minutes post-drug administration), participants provided a blood sample, and completed the remaining questionnaires, reward tasks, and a motor-coordination test (Giovannoni, Van Schalkwyk, Fritz, & Lees, 1999).

Control measures

Checkerboard paradigm. Opioids can have a depressing effect on respiration, which in turn can cause increased overall BOLD signal (K. Pattinson, 2008; K.T. Pattinson, Rogers, Mayhew, Tracey, & Wise, 2007) and a decrease in relative stimulus-induced BOLD signal (Cohen, Ugurbil, & Kim, 2002). To ensure that any differences in activation between the morphine and the placebo conditions were not due to overall changes in BOLD signal caused by physiological effects, we included a control task in the scanner.

The visual cortex, and the occipital cortex generally, is low in μ-opioid receptors (Baumgärtner et al., 2006; Colasanti et al., 2013). Potential differences in activations in these areas are therefore a viable indication of drug effects not caused by opioid receptor stimulation by itself. We used a blocked visual checkerboard paradigm consisting of alternating blocks of a 1 second flickering checkerboard stimulus followed by 20 seconds rest (crosshair presented in the middle of the screen) for 4 minutes. This basic paradigm was chosen to induce consistent activation in the visual cortex and enable direct comparison of stimulus-induced BOLD signal between the two drug conditions. Participants were instructed to keep their eyes on the screen and not to blink while the checkerboard was presented. The paradigm was presented using E-prime 2.0 Professional software.

Physiological measures. As a second measure of respiratory effects of morphine, pulse and respiration were recorded during the functional scans to assess potential physiological changes between drug conditions. Heart rate and respiration recordings were also intended for modelling of physiological changes in fMRI analysis, but this was not performed on the small sample in the current pilot study. Pulse was measured with a pulse-oximeter on the left middle finger, and respiration was measured with a pneumatic belt strapped on the left side of the abdomen.

Motor coordination task. Previous studies have found that DA modulation can influence motor coordination, and measuring this effect is important for interpretation of drug effects on behavioural measures (Pizzagalli et al., 2008). Drugs acting on the μ-opioid receptor system can interact with the DA system and affect DA release (Johnson & North, 1992; Nestler, 2005). The Bradykinesia Akinesia Incoordination test (BRAIN test; Giovannoni et al., 1999) was included as a control task in the current study to ensure that any differences observed in the reward tasks were not due to changes in motoric functioning caused by sedation or morphine effects on DA. The test is a computerised finger-tapping test used to objectively assess upper limb motor function.

To complete the task, the participant was instructed to use their dominant index finger to press the buttons 's' and 'ø' (15 cm apart) alternately on a keyboard as fast and accurately as possible for 60 seconds. The task was administered directly in a browser window on a computer and required no further software (Giovanni & Noyce, https://predictpd.appspot.com/).

The BRAIN test generates four main outcome variables; 1) kinesia score (KS): total number of alternating key strokes in 60 seconds, 2) akinesia time (AT): total amount of time that keys are pressed, 3) dysmetria score (DS): a weighted index of incorrectly pressed keys corrected for speed, and 4) incoordination score (IS): a measure of rhythmicity based on the variance of time intervals between key strokes. The main variable of interest for the current study was the dysmetria score, as it provides an overall performance measure while also taking into account speed-accuracy trade-off strategies.

Questionnaires. Previous studies have shown that opioids can affect subjective experiences and induce euphoria in healthy participants (Becerra et al., 2006; Walker & Zacny, 1998; Zacny & Lichtor, 2008). Although the morphine dose in the current study was chosen to limit such influences (Chelnokova et al., 2014), we wanted to control for subjective effects relating to the drug dose. Participants completed a questionnaire about mood, and somatic and subjective effects of opioid drugs (e.g. feeling good, dizziness, feeling 'high'). The questionnaire was used in Chelnokova et al. (2014), and was based on scales developed previously (Walker & Zacny, 1998; Zacny & Lichtor, 2008). The scale included items about direct drug effects such as 'Do you feel an effect of the pills?', 'How much do you like/dislike the effect?' and 'Would you take the pills again?'. Items were rated on a visual analogue scale (VAS) from "Not at all" to "Very much/Extremely". The participants completed the questionnaire pre-drug administration and at two later time points (~50 and ~120 minutes post-drug administration). At the second time point, the questionnaire was administered while the participant was inside the scanner, before fMRI acquisitions, to measure subjective effects as close as possible to the reward tasks and the time of peak morphine blood concentration. Questionnaires were presented using MATLAB outside the scanner and E-Prime Professional inside the scanner. Items and rating scales were identical in both versions of the questionnaire.

Blood test. Levels of neurochemical compounds, such as opioids, can be measured in small amounts of blood (Johnsen, Leknes, Wilson, & Lundanes, 2015). To measure levels of relevant neurotransmitters and their metabolites in our current sample of healthy volunteers, we collected a blood sample following the fMRI tasks. The sample was taken approximately

110 minutes after drug administration. We used a finger prick blood test, and collected 120µl from each participant at each session. Blood samples will be analysed using the methods developed by Johnsen et al. (2015). Uptake of oral morphine varies between individuals, and data from the blood test will provide individual measures of opioid levels that can be entered in the reward task analyses. The results from this analysis was not finalised at the time of this thesis.

Monetary incentive delay task development

The MID task uses monetary incentives as reinforcement, and relies on the ability of these incentives to possess a stable value and elicit reward related brain activity. A benefit of using a secondary reward (money) in this task is that it allows for the investigation of loss/punishment as well as winning. The MID task has been shown to reliably probe neural activity in brain regions associated with reward, such as the striatum and OFC, during reward anticipation and delivery of reward feedback (Beck et al., 2009; Knutson et al., 2001a; Knutson, Fong, Bennett, Adams, & Hommer, 2003), and is a validated and widely used method for studying reward processing.

The test protocol developed in this study is intended for use in a larger pharmacological fMRI study and in a clinical study of heroin dependent patients. The MID task has low cognitive demand as it does not require participants to make decisions or learn complicated rules, and has therefore been the chosen task in many previous studies of substance abuse (see review by Balodis & Potenza, 2014). Another benefit of the task is that it is designed to have constant accuracy across participants (Knutson et al., 2001a), and is therefore not dependent on individual performance.

A literature review made it clear that previous implementations of the MID task differ on a range of parameters. There were variations both in designs and analyses in terms of reward modality used, reward type and amount of cues and rewards, inter-stimulus intervals and inter-trial intervals, task length and jittering (variable time intervals between task events). One of the main aims of the current pilot study is to develop appropriate testing protocols for the larger study. Therefore, we considered a number of aspects before deciding on the parameters to be used in the current version of the MID task. These will be discussed in the following paragraphs before moving on to task development. An overview of previous versions of the task is presented in Table 1.

Table 1

A non-exhaustive selection of different versions of the MID task used in previous literature.

Version	Examples of articles	No. of cues	Reward magnitude	Win trials	Loss trials	Accuracy	Jittering*	Limitations	Strengths	Comments
1	Knutson, Adams, et al. 2001 Beck et al., 2009 Knutson et al., 2004 Wrase et al., 2007 Scheres et al., 2007	7 (or 8 with two $0 cues)	- $ 0.20 , 1, 5 + $ 0.20, 1, 5 $ 0	x	x	≈ 66%	ISI: 2-2.5s	Insufficient jitter to separate anticipation and outcome	Can parametrically analyse activity to increasing magnitude	Report changes due to increasing amounts.
2	Bjork,Knutson & Hommer, 2008	9	+ $0.20, 1, 5, (unknown) -$ 0.20, 1.5, (unknown) $0	x	x	≈ 66%	ISI:2-2.5s	Insufficient jitter to separate anticipation and outcome		Variation of version 1 (Knutson and colleagues).
3	Dillon et al., 2008 Pizzagalli et al., 2009 Santesso et al., 2008 Admon et al., 2014	3	+ $ 1.96- 2.34 (m= 2.15) - $1.81-2.19 (m=2.0)	x	x	Fixed rate 50% (actual responses not relevant)	ISI: 3-7.5s, 4.4-8.9s. ITI: 3-12 s	Actual responses not relevant. Low success rate. Cues do not indicate magnitude so cannot use parametric analysis for anticipation	Jittering , can separate anticipation and outcome	Separating anticipation and outcome, jittering and predetermined for balanced task. No feedback on cumulative earnings, common in other versions to present this with feedback.
4	Simon et al., 2010	3	+ € 1, 0.20, 0	x	-	Fixed rate (40/100 reward)	ITI: 1-8s (*M*=3.5)	No ISI jitter- problem for separating outcome from anticipation. Also no separate loss trials, lose when no response given.	Probabilistic element in task, different from most other versions.	Target presented either left or right, press corresponding button. Probabilistic task, so not all correct responses give reward.
5	Knutson,Fong, et al., 2001	3	+$1, $0 (response) $0 (no response)	x	-	≈ 66%	ISI: 2-2.5s	Insifficient jitter to separate anticipation and outcome. No loss trials		Variation of version 1, but no loss condition. Both a zero-incentive 'response' and a 'no-response' cue (inhibition of motor response).
6	Bjork,Smith & Hommer, 2008	4	High, low reward ($5, $0.50) $5 loss $0	x	x	≈ 66%	ISIs + ITI: 2,4,6 s	Less loss-trials than win-trials	Added jitter between all elements in each trial	Also double response trials.
7	Nestor et al., 2010	3	Win, lose, no incentive	x	x	Aimed for 50%	ISI: 2-8 s, ITI: 2-8s	Low success rate. No magnitude variation, only one level of win/loss.	Added temporal jitter ISI and ITI	
8	Bustamente et a.,l 2013	5	+€ 0.20, 3 -€ 0.20, 3 No response cue	x	x	≈ 75%	ISI: 0.2-2.25s, ISI2: 2-4s, ITI: 0.2-4s (random)		Jittered ISIs and ITI to separate events. Two level each valence.	Two levels win/loss, plus non-response trial. Higher predictabilidty of outcome than other versions.
9	Jia et al., 2010	6	+$0, 1, 5 -$0, 1, 5	x	x	≈ 66%	ISI: 3-5s, ISI2: 3-5s, ITI: 13s-(time of trial)		Use word cues to avoid having to remember cue meanings	All trials same length (13s), ITI is the time "leftover" after other task events
10	Andrew et al., 2011 Patel et al., 2013	6	+$0, 1, 5 -$0, 1, 5	x	x	≈ 66%	ISI: 3-5s, ISI2: 3-5s, ITI: 13s-(time of trial)		Separation of pre-target and post-target anticipation	Modified from version 1: separate phase 1 (anticipation of motor response:before target) from phase 2 (anticipation of reward: after target)
11	Kumar et al., 2014	2	+ (range $0.95-1.15, m=$1.05) $0	x	-	≈ 66%	ISI: 3, 4.5, 6, ISI2: 2.8,4.3,5.8, ITI: 3, 4.5, 6	No loss trials. Cue not indicative of amount, only valence		No feedback on cumulative earnings during task, common in other versions to present this with feedback.

Notes: Version numbers in column 1 are arbitrary and refer to different versions of the task identified by the author, and does not reflect order of importance or when it was published. "x" denotes yes, "-" denotes not present or not reported. *Jittering: variable time intervals between task events. ISI= inter-stimulus-interval, ITI=inter-trial-interval, time information in seconds (s).

Variation in MID task design.

Trial types. Many MID paradigms include cues signalling monetary gain and loss as well as neutral (no-incentive) cues (Knutson et al., 2000). However, results from both types of trials are not always discussed, and many studies do not report results from loss trials (e.g. Jia et al., 2011). Some studies do not include loss-trials at all, potentially influencing processing of the incentive trials as the participants become accustomed to receiving rewards (Balodis & Potenza, 2014). The studies that do report results from both types of trials have found inconsistent results. Some have found activation in the VS during both anticipation of winning money (win-trials) and anticipation of losing money (loss-trials) (Bjork et al., 2008b; Scheres et al., 2007; Wrase et al., 2007), while other studies find striatal activation in win-trials only (Bjork, Knutson, & Hommer, 2008a; Knutson et al., 2003). Notably, few studies have directly contrasted anticipation of winning versus losing, a contrast that would allow for further dissociation of positive and negative incentive processing (Balodis & Potenza, 2014).

Dissociating anticipation from outcome. Previous studies vary the length and variability in time between anticipation and outcome phases in the MID task, and this affects the ability to analyse anticipation and outcome periods separately in the fMRI analysis. Bjork et al. (2008b) discussed the issue of insufficient variation in time between events in early versions of the MID task (e.g. Knutson et al., 2001a; Knutson et al., 2001b), and suggested adding variable delays (jitters) between task events (anticipation and outcome) to allow for separation during analysis. More recent studies have incorporated different variable delays into the task structure (e.g. Jia et al., 2011; Kumar et al., 2014; Pizzagalli et al., 2009). A consequence of this is that the definition of "anticipation period" also varies between task implementations. Some previous studies have defined anticipation as the delay between cue and target (Knutson et al., 2004; Knutson et al., 2001b; Knutson et al., 2003; Saji et al., 2013) while others define anticipation as only the actual cue presentation (Admon et al., 2014; Bjork et al., 2008b; Dillon et al., 2008; Kumar et al., 2014; Nestor et al., 2010; Pizzagalli et al., 2009; Simon et al., 2010). The latter method allows for separation of the two phases of reward in the analysis, but can measure anticipation activation only to the information of reward (cue) and not during the following delay.

Accuracy. Most MID paradigms use a practice run and a genetic algorithm to titrate task difficulty and obtain an accuracy rate of approximately 66%. Keeping a consistent success rate across participants has been found to maintain the motivation to respond and expectation of reward (Balodis & Potenza, 2014). 66% is based on the success rate required to keep DA neurons firing in monkeys while performing similar reward tasks (Fiorillo,

Tobler, & Schultz, 2003). In other versions of the task, the accuracy rate has been controlled using a predetermined schedule of successful and non-successful trials (Admon et al., 2014; Dillon et al., 2008; Pizzagalli et al., 2009; Santesso et al., 2008). In this alternative method the actual responses of the participants do not matter, but participants still believe that outcomes rely on their performance. Studies using this version have reported similar activation patterns as with the original design.

Cue type. MID paradigms also use different types of cues (words, symbols, colours) that can influence the working memory load required to remember the different meanings (Jia et al., 2011). The number of cues also varies. Some studies have only one magnitude of reward and/or loss (Knutson et al., 2001b; Nestor et al., 2010), while other studies vary the amount of money to be won or lost or divide trials into high reward and low reward (Beck et al., 2009; Knutson et al., 2001a; Knutson et al., 2004). In addition, some studies include a 'non response cue' (instructing participants not to respond on the upcoming trial) as a measure of response inhibition (e.g. Scheres et al., 2007). As such, the number of cues and their meaning vary greatly between task implementations.

Despite the variation in task design, previous studies using the MID task have consistently found activation in the brain regions relevant for rewards, such as the VS and OFC. The different versions of the MID task each enable a range of different hypotheses and analyses depending on the number of trials in each condition and magnitude of reward. In the current study we wanted to include anticipation of both winning and losing as well as delivery of both reward and punishment (loss), and analyse brain activation associated with each of these conditions.

Task development. The MID script used in this study is adapted from a paradigm used in an ongoing multi-platform addiction study at several Universities in the United Kingdom (ICCAM Platform Study). This task version uses arrow symbols to indicate win or loss, as opposed to different geometric shapes (e.g. Knutson et al., 2004; Knutson et al., 2003), colours (e.g. Nestor et al., 2010), or words (e.g. Andrews et al., 2011; Jia et al., 2011; Patel et al., 2013). We chose these symbols as they provide intuitive meaning to the cues (arrow up for potential win, arrow down for potential loss, see Figure 5) and therefore require less effort from the participants in terms of remembering meaning of cues. This was especially important since the task developed in this pilot study will be used in a clinical sample of heroin dependent individuals.

To allow for analysis of both win and loss trials, we included an equal number of trials for each incentive condition. We also included two levels of each incentive cue (win: high, low; loss: high, low) as well as neutral cues to allow for parametric analyses.

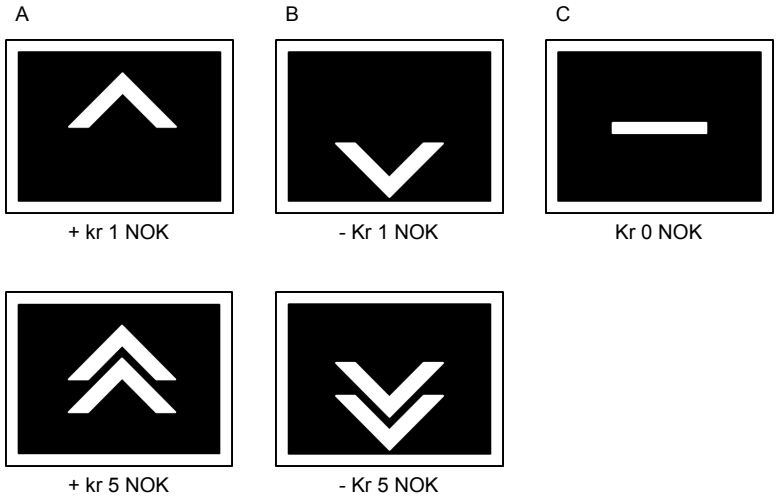

Figure 5. The different cues signalling win trials (A), loss trials (B), and neutral trials (C) with the associated magnitude of potential win/loss. Values in NOK (Norwegian Kroner).

Design and jittering. One important consideration for the current pilot study was designing the MID task in a way that would make it possible to separate brain activity related to anticipation and outcome of reward. While Knutson et al. (2001a) and many following articles presented results from anticipatory periods only, other studies included both anticipation and outcome in their analyses. Pizzagalli et al. (2009) found that anhedonic patients with major depressive disorder did not significantly differ from controls in the anticipation of reward in the MID task, but they observed a difference in NAc and caudate activity to rewarding outcomes. This study, among others, shows that groups can differ in reward related activity in specific phases of reward processing.

The MID task has been optimised for presentation in an event-related design, and the two phases of reward under investigation, anticipation and outcome, occur within a short period of time (Knutson et al., 2004). Event-related (rapid) fMRI is preferable to block designs if the intent is to isolate separate psychological events and maximise number of trials within a short period of time to avoid long and tedious tasks (Wager & Nichols, 2003). To separate brain activity associated with anticipation and outcome of reward, the MID task was modified from its original design. The original task by Knutson et al. (2000) did not include a contrast of outcome related activity, an addition that was implemented in Knutson et al. (2001b). More recent studies have concluded that the inter-stimulus intervals (ISIs) were too

short and/or not sufficiently jittered (having varying durations) to successfully disentangle the associated activity from the two events in this version of the task (Bjork et al., 2008b). One of the primary challenges with using rapid event-related fMRI is that the BOLD signal follows the slow time course of the haemodynamic response in the brain, peaking at approximately 5-8 seconds after a stimulus is presented (Kao, Temkit, & Wong, 2014; Logothetis, 2002; Serences, 2004). On the other hand, the psychological events we are making inferences about are often over in a few hundred milliseconds. The statistical power of effects in rapid event-related designs relies heavily on the sequence of events and timing parameters and how these interact (Wager & Nichols, 2003). A solution for disentangling activity from separate conditions that require a rapid presentation rate is to add jitters between events within a trial (ISIs) and between different trials (ITIs; inter-trial-intervals). This means that the sampled time points for the events are distributed along the BOLD response curve, thus enabling deconvolution analysis (estimating the haemodynamic response function (HRF) for each event type separately) (Serences, 2004). Introducing sufficient jittering between events in the MID task will facilitate deconvolution analysis of anticipation and outcome separately (Dillon et al., 2008). Jittering in the MID task has been done in different ways in previous studies (Bjork et al., 2008b; Pizzagalli et al., 2009).

When defining the anticipation and outcome periods in the MID task, a decision had to be made regarding how to dissociate these two phases of reward processing. To allow for separation of anticipation and outcome phases with jitters, we modelled only the presentation of the incentive cue as "anticipation" in the current study, (Bjork et al., 2008b; Dillon et al., 2008). This means that anticipation refers to the neural response to cue presentation, and not the anticipation of a predicted reward that may last until the onset of target (during the delay). While this could be a limitation, previous studies have found anticipatory brain activation using this method before (e.g. Dillon et al., 2008; Kumar et al., 2014). Modelling anticipation as the whole period until target onset makes it difficult to separate brain activation to anticipation and outcome due to the lack of variable timing, and the benefit of being able to investigate the separate temporal phases of reward was considered to outweigh the potential limitations of this approach. In the following method description and analysis, anticipation will therefore refer to the presentation of the incentive cue (see Figure 6).

To find the optimal length and distribution of jitters that would allow for efficient separation of anticipation and outcome, without making the task too long or too difficult, we used the FSL design tool (FMRIB Software Library, http://www.fmrib.ox.ac.uk/fsl). A selection of jitter schedules used in similar designs were tested for relative power using FSL

fMRI Expert Analysis Tool (FEAT) full model set up. We chose to use a reversed exponential distribution of the jitter lengths, with a higher frequency of the short jitters than the long. This was done to maximise variation in time between events without sacrificing number of trials or experiment duration (Serences, 2004). Different combinations of jitters were combined in a random order with task events (anticipation and outcome) using MATLAB 8.3 (R2014a, The MathWorks, Inc., Natick, Massachusetts, United States). The generated event files were then entered as a general linear model (GLM) in FEAT and the power of the relevant contrasts was compared across the different variations. By comparing the correlation between predictors in each design, we estimated each predictor individually to get an approximation of the detection power of the full model (Wager & Nichols, 2003). Using this method, ISIs of 2,4, and 6 seconds, and ITIs of 1,3, and 5 seconds were chosen for the final task. This range of jitters gave the best estimated power to detect activation changes for both event types (anticipation and outcome), compared to the other jittering schedules tested (ISI/ITI: 2,4,6 seconds (Bjork et al., 2008b), ISI: 2,3,4 seconds, ITI: 3,4,5 seconds (ICCAM Platform study)). Figure 6 illustrates the distribution of jitters as well as the modelling of task events.

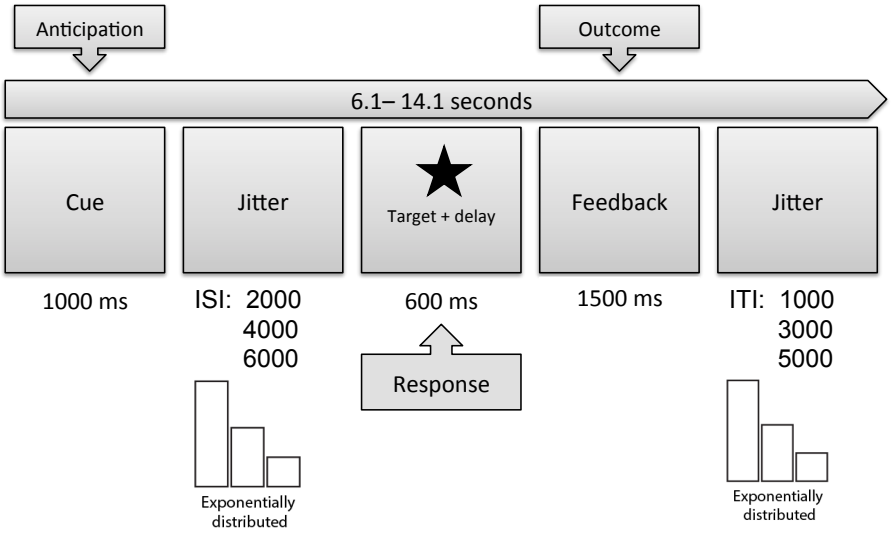

Figure 6. Depiction of the jittering distributions and the modelling of anticipation and feedback periods within the MID task design. ISI= inter-stimulus-interval, ITI= inter-trial-interval. Timing information in seconds and milliseconds (ms).

Following from the test of jittering schedules, we decided to have 100 trials in the MID task. Each cue type occurred 20 times, resulting in 40 win trials and 40 loss trials (collapsed across magnitude) for each session. This was similar to previous task implementations (e.g Beck et al., 2009; Pizzagalli et al., 2008; Santesso et al., 2009). The task

was split into two runs of equal length, each consisting of 50 trials. This would make it easier for participants to maintain concentration and remain still in the scanner throughout the task, and limit effects of fatigue. In the case of having to interrupt scanning due to unforeseen circumstances, each of the two runs could also be analysed separately. To obtain a balanced task with equal frequencies of trials evenly distributed throughout each run, we used a pre-determined pseudo randomisation of cue type and jitter (Kao, Mandal, Lazar, & Stufken, 2009). Choosing a pre-determined order of events has been shown to be preferable over a completely random stimulus presentation in event related experiments (Maus, Van Breukelen, Goebel, & Berger, 2010; Wager & Nichols, 2003). MATLAB was used to generate pseudo-randomised orders, with restrictions on the frequency of cues and proximity between cues of the same type. From a sample of 200 randomisations, the order with the most evenly distributed cues along the timeline (i.e. all cue types spaced out over the length of the task) was chosen and implemented in the final task design. This randomisation order was then reversed to make a second version of the experiment.

Final task design. The final task structure was decided based on the design considerations discussed. Each trial commenced with a cue signalling the possibility of winning (win trial; +1 or + 5 NOK), losing (loss trial; -1 or -5 NOK) or neither winning or losing money (neutral trial). Monetary values were based on previous studies using the task (Andrews et al., 2011; Jia et al., 2011; Simon et al., 2010). The cue was presented for 1 second (s), followed by a jittered delay (ISI; 2s, 4s, or 6s). A target was then briefly presented (150-500 ms), during which the participants were expected to respond to the stimulus, and followed by an ISI (100-450 ms). Feedback presentation (1.5s) followed immediately after, consisting of trial accuracy (and money won or lost depending on trial type) as well as the accumulated earnings thus far. Feedback was followed by a jittered ITI (1s, 3s, or 5s) during which a crosshair was presented before the beginning of the next trial (see Figure 7). The task was presented in two runs of fifty trials (10 trials per each cue type) and each run lasted approximately 7 minutes separated by a break.

Participants viewed the task stimuli on a projected screen (screen resolution 1920x1080 pixels) through a mirror mounted on the head coil and responded to the task by pressing a button with their index finger on a response grip in their right hand. E-Prime 2.0 Professional software (Psychology Software Tools, Pittsburgh, PA) was used to administer the task.

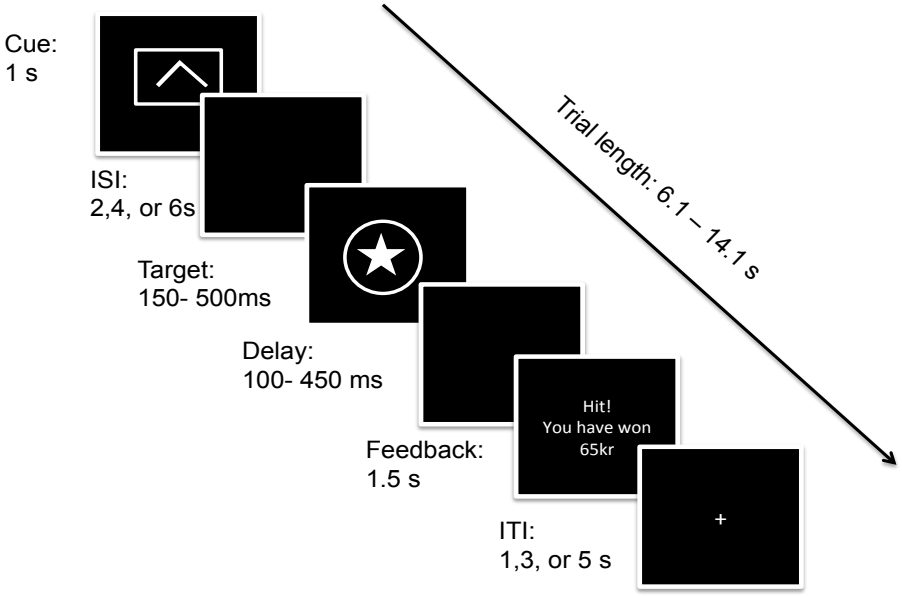

Figure 7. Typical trial structure for a win trial. ISI= inter-stimulus-interval. ITI= inter-trial-interval. Duration of each task event in seconds (s) and miliseconds (ms).

Prior to performing the MID task in the scanner, participants completed a practice run outside the scanner to ensure they understood the task completely and to collect mean response times (RTs). This participant's mean RT from the practice run was used to titrate the duration of target presentation in the main experiment to ensure ~66% success rate. In both the practice run and the main experiment, an adaptive algorithm tracked the participant's RTs and adjusted the allowable response window on a trial-by-trial basis to keep the task difficulty consistent. The response window was adjusted separately per valence condition to ensure similar accuracy across conditions.

The participants were told that they would accumulate money during the task (not including the practice run), and would receive the total amount won at the end of the second test session. The starting amount was 50 NOK, and for each trial the corresponding sum was added or deducted.

fMRI parameters

Images were acquired using a 3.0-Tesla Philips Ingenia MRI scanner equipped with a 32-channel SENSE head coil (Philips Medical Systems, Best, The Netherlands). High-resolution T1 weighted images were acquired for anatomical reference and co-registration (voxel size 1 x 1 x 1mm, TR/TE 4.7/2.3, 184 slices, field of view (FOV) 256x184, overcontiguous sampling). For the MID task, each participant underwent two gradient echo, echo-planar-imaging (EPI) functional scans, each lasting approximately 7.5 minutes with 200

volumes. For the checkerboard paradigm, 115 volumes were collected. The following parameters were used for all functional scans: 3 x 3 x 3mm voxels, TR= 2208 ms, TE= 30 ms, flip angle 80°, FOV 240 x 126mm. Fourty-two transverse slices were collected (phase encoding direction from anterior to posterior) parallel to the anterior-posterior-commissure (AC-PC) axis.

Slice acquisition. The current study was one of the first fMRI studies to be conducted on a new Phillips Ingenia 3T scanner at The Intervention Centre at Oslo University Hospital. It was of interest both for the current and future studies to optimise the scanner settings to ensure the best possible data recording. To find the acquisition method that would maximise BOLD signal sensitivity, we performed a test comparing level of activation and temporal signal-to-noise ratio between different slice acquisition schemes. To evoke high activity in visual and motor cortex we used a visual flickering checkerboard paradigm combined with finger-tapping alternating on the right and left hand during the checkerboard periods. This allowed us to compare BOLD activation between the different acquisition schemes.

Interleaved acquisition is more susceptible to spin history motion artifacts due to the temporal delays between acquisition of adjacent slices (Cheng & Puce, 2014). As the protocol designed for the current study will also be used in a clinical sample where head motion is a potential problem, we decided to focus mainly on sequential acquisitions in this test. The sequential acquisitions were collected in descending slice order.

60 volumes were collected using each of four slice acquisitions: sequential with 0mm, 0.3mm (10% of voxel size), and 0.5mm gaps, and interleaved acquisition (0mm gap). The other parameters were identical for the four scans: TR/TE = 2208/30, FOV 240 x 126, voxel size 3x3x3, 42 transverse slices. Data was acquired from a single subject during a blocked paradigm of 15s flickering checkerboards and 15 seconds rest. Each scan lasted 2 minutes, resulting in four checkerboard + finger-tap blocks per scan. The functional scans were pre processed using standard FSL FEAT settings. Based on visual inspection of the activation maps, it was decided to use sequential acquisition with 0.3 mm slice gap to maximise activation in frontal and subcortical regions. 0.5mm gap yielded a marginally larger Z-stat max score (Z= 15.9 versus 12.5), but the smaller gap was preferable in the absence of any larger differences.

Parallel imaging. Using parallel imaging (sensitivity encoding; SENSE) for functional scans has been shown to minimise susceptibility distortions and reduce influence of physiological noise, thus increasing spatial resolution as well as allowing data to be

collected in a shorter time period (Preibisch et al., 2003; Triantafyllou, Polimeni, & Wald, 2011). There have been concerns about using a SENSE factor higher than 2 in the past due to reductions in signal-to-noise ratio (SNR), especially in brain areas where the BOLD signal is usually weak and susceptible to artifacts (Preibisch, Wallenhorst, Heidemann, Zanella, & Lanfermann, 2008; Schmidt, Degonda, Luechinger, Henke, & Boesiger, 2005). However, these conclusions have been drawn based on data collected with 8-channel head coils. A higher number of channels in the head coil has been shown to increase SNR and allow for higher SENSE factors without compromising the signal (Triantafyllou et al., 2011). Since the scanner used in the current study was equipped with a 32-channel head coil, a SENSE factor of 3 was chosen to maximise signal and minimise signal distortions and physiological noise.

Analysis

Control measures.

MID task behavioural measures. The MID task is designed to minimise variation in performance, and we therefore predicted no significant effects of session or drug order on accuracy (percent correct responses within the response window). Response times were controlled by the adaptive algorithm used to titrate accuracy, and therefore no analysis was performed on response time data. Average accuracy rates were analysed using a 2 x 5 repeated measures analysis of variance (ANOVA) comparing drug (2: morphine, placebo) and cue type (5: positive valence (+5, +1 NOK), negative valence (-5, -1 NOK), and neutral). An ANOVA was also used to compare performance in the first and second session to ensure there were no learning effects. All statistics on behavioural data were analysed using Statistical Package for the Social Sciences (SPSS INC., Chicago, IL, USA).

Motor coordination task. To analyse the effect of drug on motor coordination, the results from the BRAIN test were entered as one-way repeated measures ANOVAs for each of the sub-scores (KS, AT, IS, DS) with drug (morphine, placebo) as the independent variable.

Subjective effects. To avoid potential false negatives due to correction for multiple comparisons, analysis of subjective effects was performed only on the three most relevant items ‘feeling drug effect’, ‘feeling high’, and ‘feeling good’ from the questionnaire. Paired t-tests were used to analyse difference between baseline ratings (pre-drug) and the post-drug ratings between morphine and placebo conditions.

Physiological measures. To compare pulse and respiration patterns between the two drug conditions we set up a frequency spectrum analysis in MATLAB using fast Fourier transform. Reported respiration rate and pulse correspond to the frequency of the highest

amplitude harmonic obtained for each measure, and the analysis was based on physiological data recorded from the first run of the MID task during fMRI. One-way repeated measures ANOVAs were then performed to compare morphine and placebo conditions. Due to technical problems setting up the protocol, physiological data was only available for six participants.

fMRI analysis. fMRI data processing was carried out using FEAT (fMRI Expert Analysis Tool) version 6.00, part of FSL. Registration of functional data to individual high-resolution and standard space images (Montreal Neurological Institute; MNI) was carried out using FLIRT (Jenkinson & Smith, 2001). Further pre-processing statistics included motion correction (Jenkinson, Bannister, Brady, & Smith, 2002), slice-timing correction using Fourier-space time-series phase-shifting, removal of non-brain tissue using BET (Smith, 2002), spatial smoothing with a Gaussian kernel 5mm, grand-mean intensity normalisation, and high pass temporal filtering. Statistical analysis of the time-series was carried out using FILM with local autocorrelation correction (Woolrich, Ripley, Brady, & Smith, 2001). Cluster-based Z statistic images were thresholded at $Z > 2.3$, and clusters significant at the $p < .05$ level after correction for multiple comparisons are reported as significant activations.

Checkerboard paradigm. The checkerboard time series' were analysed using a general linear model (GLM) design matrix with one explanatory variable (visual stimuli 1 second), which was convolved with a double-gamma HRF. Fixed effects (FE) analysis for each drug condition was run to get the average activation maps per condition. The two drug conditions were also contrasted using another high level analysis with the contrasts morphine > placebo, and placebo > morphine.

MID task. MID task fMRI data was analysed as a GLM with the following explanatory variables (EVs) entered in the design matrix: 5 incentive cues, 5 successful and 5 non-successful outcomes corresponding to cue, and 1 target (as well as the temporal derivatives of these EVs) convolved with a double-gamma HRF. In addition to the separate EVs, relevant contrasts entered in the first level analysis were: 1) anticipation of win versus neutral cue, 2) anticipation of loss versus neutral, and 3) successful outcome versus non-successful outcome. The contrasts were selected to test whether this task version was successful at inducing activity in areas associated with human reward processing.

Following first-level analysis using FEAT, the two runs for each person per session were combined using FE analysis to get a an average for each individual per drug condition. Relevant COPE images from these FEATs were entered in third-level FE analyses to get mean activation maps for each drug condition for each contrast of interest. Fixed effects

analysis was deemed appropriate due to the small sample size and preliminary nature of analyses in the current study. Finally, separate third-level repeated measures FE analyses were run contrasting the two drug conditions (contrasts: morphine > placebo, and placebo > morphine).

ROI analysis. Following the whole brain analysis, FSL's tool featquery was used to obtain mean signal change within a priori regions of interest (ROIs). We selected ROIs based on a recent unpublished ALE (activation likelihood estimation) meta-analysis of studies using the MID task (McGonigle, personal communication; ICCAM Platform Study). For the anticipation phase of the MID task, this review identified MNI weighted centre coordinates in the bilateral ventral putamen (right: 15, 9, -4, left: -15, 9, -6) and right insula (33, 23, -4). In the outcome stage, peak coordinates were identified in the bilateral NAc (right: 10, 18, -4, left: -12, 6, -10). To define ROIs for the current study we used these peak coordinates to create spheres of 6mm radius using FSLmaths, similar to the methods described in a previous MID task study (Cho et al., 2013). Upon visual inspection of these masks, it became clear that the left NAc and the left putamen masks were overlapping, and according to the Harvard Subcortical atlas (in FSL) this region corresponded better to the putamen than the NAc. Instead, we used the coordinates for the right NAc mask and created a new ROI for the left NAc based on these (-10, 18, -4). An illustration of the final ROIs can be seen in Figure 8.

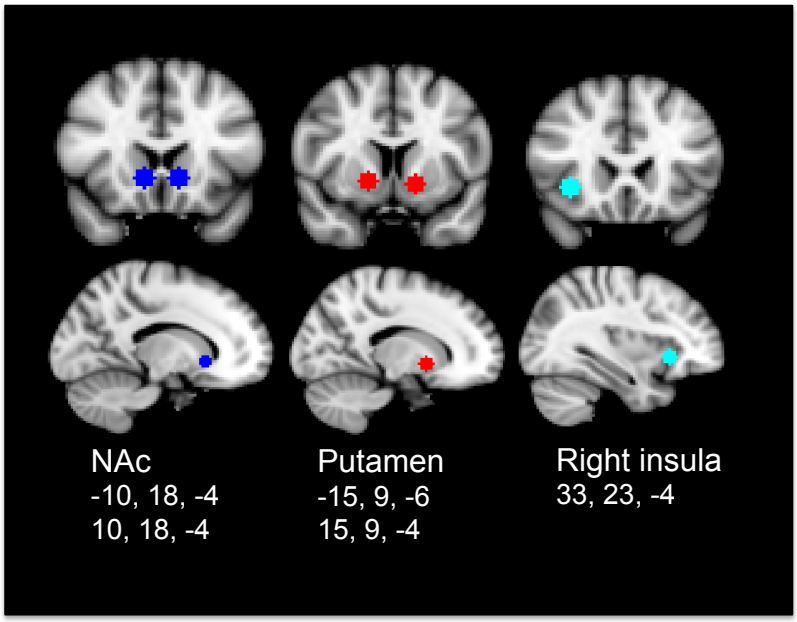

Figure 8. A priori region of interest (ROI) defined masks in the nucleus accumbens (NAc; blue), ventral putamen (red), and right insula (light blue) in coronal and sagittal view. All coordinates refer to MNI (Montreal Neurological Institute) standard space, and masks were created as 6mm spheres around the peak coordinates identified in the ICCAM Platform Study meta-analysis (McGonigle, personal communication). The left side of the coronal images correspond to the right side of the brain.

Results

Subjective effects and control data

MID task behavioural results. The average accuracy across participants and conditions was 65%, reflecting successful control of accuracy rate in the task design. To test whether the task titration ensured constant performance across conditions, a 2(drug) x 5(cue type) repeated measures ANOVA compared difference in performance between conditions. Because Mauchly's test of sphericity was significant ($p< 0.05$) for all comparisons, results are reported using the Greenhouse-Geisser correction. There was a significant main effect of cue type ($F(2.1, 21)= 10.17$, $p<.001$, $\eta^2= .504$), and pairwise comparisons (corrected for multiple comparisons using the Bonferroni correction) indicated that this was driven by the highest value positive cue being significantly different from the low value positive cue ($p=.029$) and the neutral cue ($p=.019$; see Figure 9). The main effect of cue was due to differences between magnitudes within one condition, thus yielding constant accuracy between valence conditions (as per task design). There were no significant effects of drug, drug*cue interaction, or session on accuracy (all $F< .67$, $p>.43$).

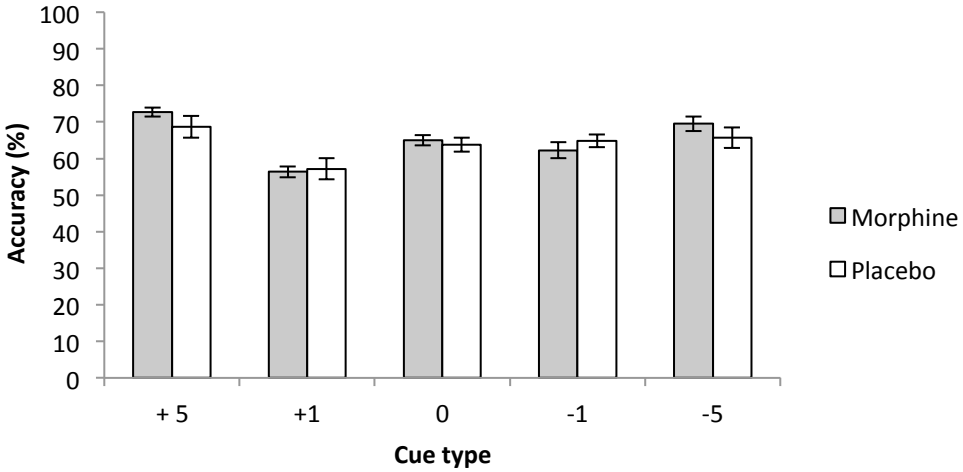

Figure 9. Accuracy (% correct responses inside response window) across all cue conditions (denoted by values in Norwegian Kroner (NOK)) in the monetary incentive delay (MID) task for morphine and placebo. When averaging accuracy rates within each cue valence, performance was constant at ~65 % for all conditions. Error bars represent within-subjects standard error of the mean (SEM).

Motor coordination task. Four one-way repeated measures ANOVAs were used to compare motor functioning between drug conditions. Data from one participant was excluded due to computer errors. For the main variable of interest, dysmetria score, there was no significant effect of drug ($F(1,9)=.27$, $p=.615$, $\eta^2=.272$). Neither were there differences between conditions on the other measures KS, AT and IS (all $F< .81$, $p>.391$).

Subjective effects. When asked at the end of the second session to guess the drug conditions, participants only correctly identified if they received placebo or morphine 64% of the time. Paired t-tests showed no significant difference between baseline corrected session average subjective ratings of feeling drug effect (p= .91), feeling 'high' (p= .47), or feeling good (p= .89). Thus, any potential drug effects in the reward tasks cannot be attributed to subjective feelings of being under the influence of drugs.

Physiological measures. Despite having data from both drug conditions for only six participants, analysis showed a significant difference between drug conditions on respiration ($F(1,5)=8.2$, $p=.035$, $\eta^2=.622$), but not heart rate (p= .435). On average, respiration rate was lower in the morphine condition (breaths per minute; M=19, SD=2.4) than placebo (M=20.5, SD=2). This is within the normal range, as average respiration is 14-20 breaths/minute in healthy adults (Lindh, Pooler, Tamparo, Dahl, & Morris, 2013). Average heart rate was 77 (SD=12) beats per minute for morphine and 75 (SD=10) for placebo.

fMRI results

Due to the low sample size (N=11) we visually inspected all individual first level FEAT analyses to check for outliers. Pre-processing parameters and results for each individual run were inspected to assure the quality of registration, and to evaluate movement and model fit. All functional images were successfully registered both to individual high-resolution images and MNI standard space. There was minimal movement in all time series, resulting in no excluded data points in the current analysis (absolute movement <0.76mm for all participants).

Checkerboard paradigm. Fixed effects analyses in the placebo and morphine conditions showed that the flickering checkerboards induced significant activation in visual areas. For both conditions, significant clusters were identified in the occipital cortex (maxium Z-scores: Z= 24.6 (morphine) and Z= 20.2 (placebo); see Figure 10). Contrasting the two conditions revealed a significantly higher activation in the fusiform gyrus of the temporal lobe in the morphine condition (Z= 3.37). Given the comparable activations in the task-relevant occipital lobe, we find no indication of drug effects on overall BOLD sensitivity.

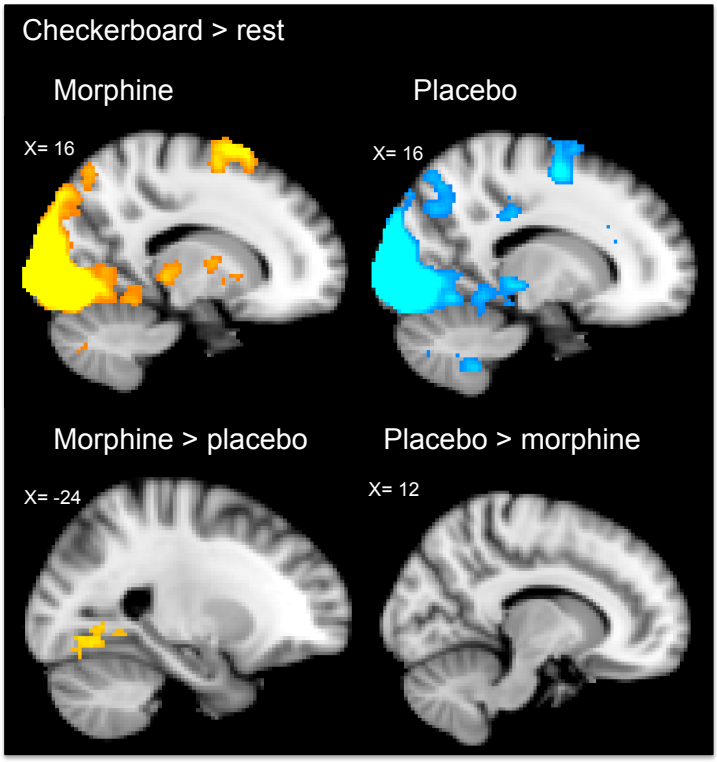

Figure 10. Significant activations in the checkerboard paradigm, with visual stimuli modelled against rest (baseline). Activation for the morphine condition is presented in red-yellow, placebo in blue. Bottom row shows significant activation in the contrasts morphine > placebo (left) and placebo > morphine. MNI slice coordinates are displayed on the left side of the images. Activation clusters are thresholded at Z> 2.3.

MID task.

Whole-brain analysis. All activation peaks and coordinates reported below are listed in Table 2.

Anticipation of gain. In the placebo condition, anticipation of monetary gain (contrast: positive cues > neutral cues) induced significant activation clusters in the striatum, occipital cortex and the frontal gyrus (Figure 11a). In the morphine condition, significant clusters were found in the striatum, the occipital cortex, and postcentral gyrus (Figure 11a). In the morphine > placebo contrast, no activations survived cluster thresholding. In the placebo > morphine contrast, there were significant clusters identified in the precuneus and cerebellum.

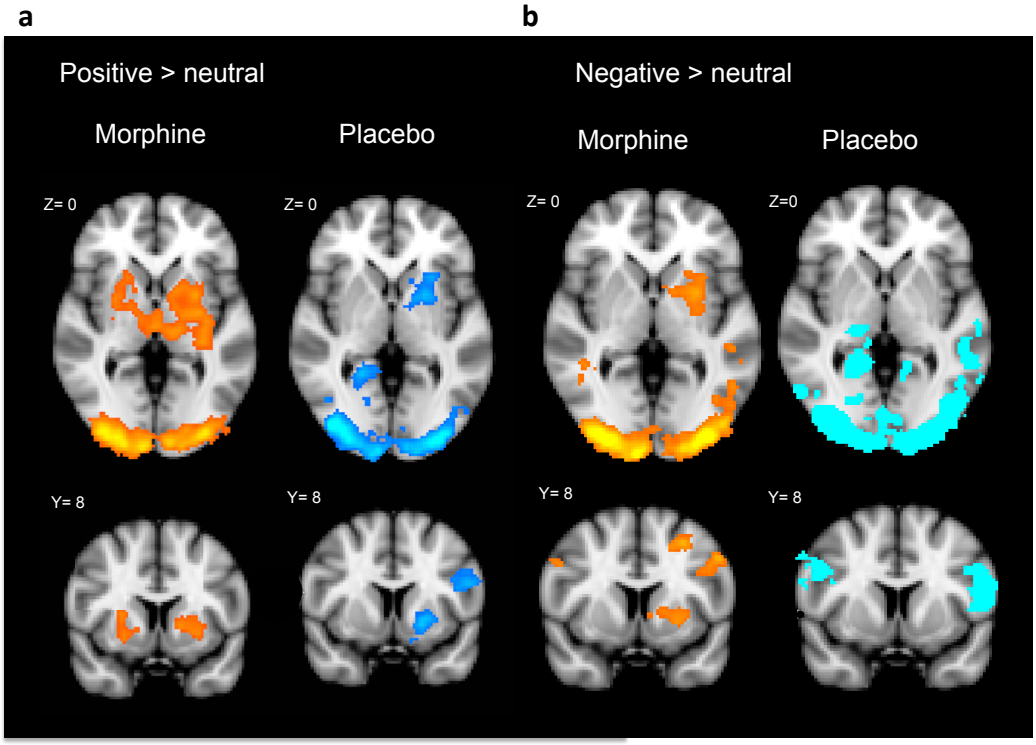

Figure 11. Brain activity elicited by visual cues indicating potential gain (a), and potential loss (b) in the monetary incentive delay task for morphine (red-yellow) and placebo (blue) conditions. Slice coordinates are displayed to the left of the figures (in MNI standard space), and the left side of the images correspond to the right side of the brain. Activation clusters are thresholded at Z>2.3.

Anticipation of loss. In the placebo condition, anticipation of monetary loss (contrast: negative cues > neutral cues) elicited significant activation in the occipital lobe and the superior parietal lobule (SPL)(Figure 11b). In the morphine condition, significant activation clusters were identified in the putamen, occipital lobe, SPL, temporal gyrus, and the cerebellum (Figure 11b). The contrasts morphine > placebo and placebo > morphine yielded no significant activation clusters.

Successful and non-successful outcome. For the placebo condition, successful outcomes (contrast: successful trials > non-successful trials) activated the striatum as well as frontal cortex, cerebellum, SPL, and frontal gyrus (Figure 12a). In the morphine condition, the striatum, frontal cortex, and occipital lobe were significantly activated (Figure 12a). In the drug contrasts, significant clusters were identified in the SPL and precuneus in the morphine > placebo contrast only. However, visual inspection of below-threshold activation maps indicated group differences in striatal areas that did not survive cluster thresholding. Using small volume correction (with voxel-based correction) within the a priori defined left putamen ROI mask, voxels in the ventral putamen survived correction for multiple comparisons at the p=0.05 level in the morphine > placebo contrast (but not in the placebo > morphine contrast; Figure 12b).

Table 2
Regions activated for the different contrasts in the whole-brain analysis.

					Peak voxel		
Contrast	Condition	Area	# of voxels	Max Z-score	Z	Y	Z
Positive cues > neutral cues	Morphine	Occipital cortex	5363	8.76	16	-92	-10
		Putamen (L), thalamus (L), Putamen (R)	2736	4.42	-26	-10	4
		Postcentral gyrus	1862	4.36	-44	-22	56
		Postcentral gyrus	1225	3.78	48	-22	44
	Placebo	Occipital cortex	16209	7.04	12	-94	-8
		Middle frontal gyrus	945	5.03	-40	20	24
		Pallidum, putamen (L), OFC (R)	459	4.34	-18	4	-4
Negative cues > neutral cues	Morphine	Occipital cortex	13804	7.69	12	-102	4
		Superior parietal lobule	1321	4.14	38	-40	54
		Middle temporal gyrus	651	3.31	42	-44	4
		Putamen (L)	511	3.82	-26	4	4
		Occipital cortex	508	3.3	10	-82	16
		Cerebellum	424	3.91	-38	-70	-48
	Placebo	Occipital pole	20537	7.54	14	-102	8
		Superior parietal lobule	1298	4.15	-38	-46	56
Successful > non-successful trials	Morphine	Occipital cortex	33228	6.64	46	-70	-40
		Frontal pole	3471	5.83	-2	64	8
		Putamen (L)	555	5.99	-20	8	-10
	Placebo	Frontal pole	5138	5.29	-46	48	14
		Cerebellum	2070	5.53	44	-72	-38
		Superior parietal lobule	1054	4.05	-46	-50	62
		Superior parietal lobule	964	5.22	-22	28	54
		Nucleus accumbens, putamen (R)	686	4.7	12	12	-8
		Inferior frontal gyrus	625	4.42	24	12	26
Drug contrasts							
Positive cues > neutral	Morphine > Placebo	-					
	Placebo > Morphine	Precuneus	2427	4.13	24	-52	26
		Cerebellum	1519	3.86	-2	-46	-20
Negative cues > neutral	Morphine > Placebo	-					
	Placebo > Morphine	-					
Successful > non-successful trials	Morphine > Placebo	Postcentral gyrus	1452	4.5	20	-42	68
		Precuneus	742	4.24	0	-62	48
	Placebo > Morphine	-					

Note. Cluster-based activations in the contrasts of interest for each drug condition, and the contrasts morphine > placebo and placebo > morphine. Peak coordinates in Montreal Neurological Institue (MNI). All activations are cluster thresholded at Z > 2.3 and corrected for multiple comparisons at the $p<0.05$ level.

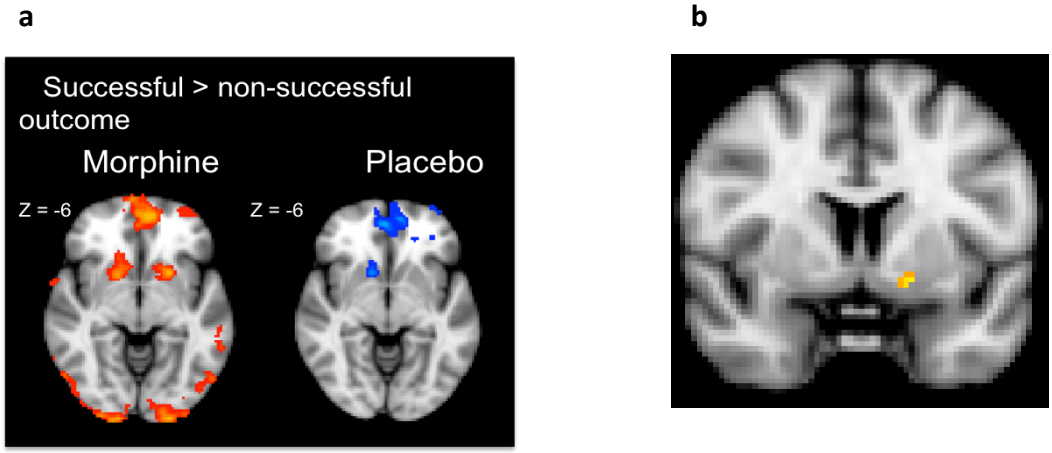

Figure 12. (a) Significant activation clusters in the morphine (red-yellow) and placebo (blue) conditions for successful versus non-successful outcomes regardless of associated trial valence. Slice coordinates are displayed to the left of the images (in MNI standard space), and the left side of the images correspond to the right side of the brain. Activations are cluster thresholded at Z> 3 for illustration purposes. *(b)* Voxels activated in the contrast morphine > placebo for successful versus non-successful outcome in the ventral striatum, identified using small volume correction inside the left putamen mask (MNI coordinates: -15, 9, -6; a priori ROI). Maximum Z-score = 3.2, $p<0.05$. Left side of image corresponds to right side of the brain.

ROI analysis. Analysis of mean signal change within the a priori defined ROIs showed a significant main effect of cue type in the left ($F(4, 40)= 2.63$, $p= .047$, $\eta^2=.209$) and right ($F(4,40)= 6.00$, $p= .001$, $\eta^2=.375$) putamen for the anticipation phase of the MID task (Figure 13). There were no significant effects of drug or drug*cue interactions in this or any other ROIs for the anticipation phase. In the outcome phase there were no significant effects of cue type or drug condition for successful trials. In the analysis of successful versus non-successful trials there was a significant main effect of outcome in the left putamen ($F(1,10)= 15.44$, $p=.003$, $\eta^2=.607$), right putamen ($F(1,10)= 12.51$, $p=.005$, $\eta^2=.556$), left NAc ($F(1,10)= 11.85$, $p=.006$, $\eta^2=.542$), right NAc ($F(1,10)= 16.40$, $p=.002$, $\eta^2=.621$), and right insula ($F(1,10)= 6.22$, $p=.032$, $\eta^2=.384$), illustrated in Figure 14. There was also a significant main effect of drug in the left putamen ($F(1,10)= 6.16$, $p=.032$, $\eta^2=.381$), but no significant drug*outcome interaction.

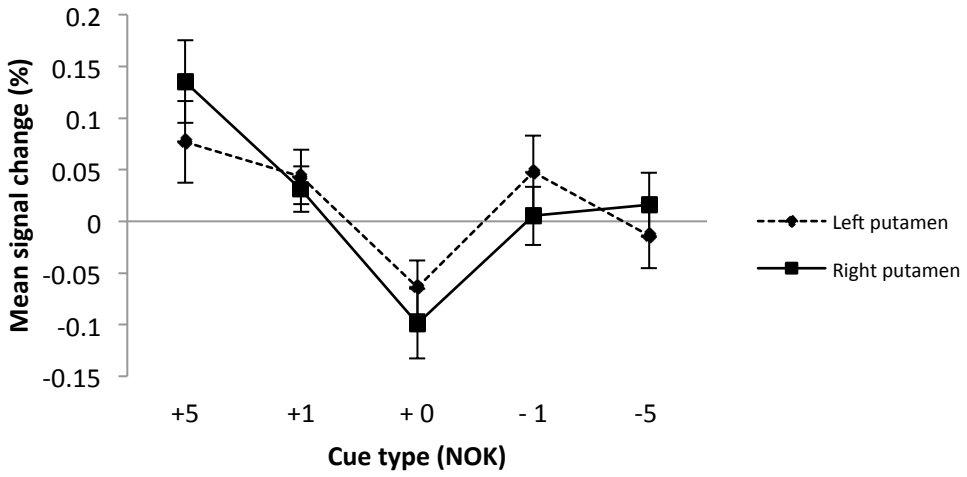

Figure 13. Main effect of cue type on mean signal change for each cue type during anticipation in the right and left putamen. Cue type is indicated by the monetary value associated with each cue in Norwegian kroner (NOK). Error bars represent within-subjects standard error of the mean (SEM).

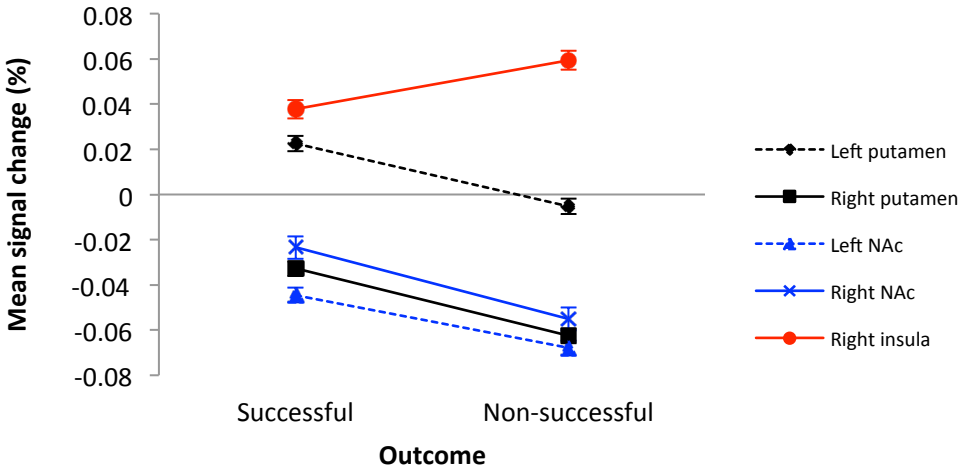

Figure 14. Main effect of MID task outcome in the five a priori ROIs (regions of interest). Successful refers to all trials, regardless of incentive cues, that the participants successfully responded to. Non-successful refers to trials where no response was detected, or was given outside of the response window. NAc= nucleus accumbens. Error bars represent within-subject standard error of the mean (SEM).

Discussion

The main aim for the current pilot study has been to develop a set of procedures to investigate the effect of opioid manipulation on reward behaviour and brain processes. Based on the special consideration pharmacological fMRI studies require, a test battery has been developed and data from 11 healthy participants under two drug conditions was collected and analysed. Our main experimental outcome measure was brain activation during the MID paradigm. We wanted to ensure that our paradigm could be successfully used to measure reward behaviour and ultimately to assess potential changes associated with μ-opioid receptor manipulation. A range of control tasks and measures were included to test for potential confounds introduced by the pharmacological fMRI design. Based on this data, a large pharmacological fMRI study will be founded (N= 50-60).

A large part of this thesis has been to adapt the MID task to assess opioid effects on BOLD responses to reward. Analyses validated that our MID task version did elicit activity in the ventral striatum during anticipation of both monetary rewards and losses as well as delivery of rewards in both drug conditions. In a priori defined ROIs in the bilateral ventral putamen, significant main effects of cue type were observed in anticipation of reward. The bilateral ventral putamen, NAc, and the right insula ROIs also showed significant difference in activation between successful and non-successful outcomes. These results are in line with previous studies regarding activity in striatal regions during reward processing, and validates the modified MID task used in this study. Control measures indicated no systematic effects of

morphine on general task performance or BOLD response, supporting the current drug administration protocol. In this pilot study, we also compared healthy volunteers on morphine and placebo to see if we could detect an effect of μ-opioid receptor agonism on BOLD activation in the MID task. The results showed few significant effects of morphine on task activation, but there was an effect of drug in the expected direction (morphine > placebo) in the left ventral putamen for successful outcomes. These preliminary results are promising for the main study of opioid effects on reward processing.

Choice of regions of interest

The choice of ROIs in the current study was based on regions identified in a meta-analysis of MID task results produced in relation to the ongoing ICCAM Platform Study. The brain regions identified were in line with an earlier MID task meta-analysis (Knutson & Greer, 2008), and studies using other reward tasks (Delgado, 2007; Liu et al., 2011). NAc and other striatal regions are often reported in reward studies using fMRI, but the exact location and definition of ROIs varies between studies. For this reason, it is often recommended to define ROIs based on meta-analyses of relevant data rather than single peak activations from one study (Poldrack, 2007). Hypothesis based ROIs are also less biased than defining areas of interest post hoc (Vul, Harris, Winkielman, & Pashler, 2009). We used the regions identified in the most recent of the two meta-analyses available on MID task data (McGonigle, ICCAM Platform Study meta-analysis, obtained through personal communication). We cross-referenced the locations of our chosen ROIs with other studies and found that our ROIs in the left and right putamen overlapped with more than half of reported striatum peak coordinates in a review by Balodis and Potenza (2014). This indicates that our choice of ROIs was consistent with previous literature, and our findings of significant activation in the putamen correspond to other studies that report activation in the ventral striatum. In future analyses it is possible to include other ROIs relevant for reward, such as the OFC. The OFC and the mPFC have been implicated especially in the outcome phase of the MID task (Knutson et al., 2003), and previous studies have found that these regions respond specifically to the highest and lowest valued rewards as compared to mid-range rewards (Elliott, Newman, Longe, & Deakin, 2003). In the current study we observed activations in the OFC in the whole-brain analyses. We did not investigate this further in the preliminary ROI analysis, as the primary goal for this thesis was to validate the task and protocol to be used in a larger study. This brain region is of interest for further exploration, especially in the context of parametric variation of reward values.

In addition to activation in reward-related areas, we also observed significant activation clusters in visual and sensory-motor areas in all contrasts of interest. This could be explained by increased salience or attention towards the incentive cues compared with the neutral trials, and more conscious effort exerted towards responding to the stimuli.

Validation of the current MID paradigm

During the MID task development we considered a range of different options and psychometric properties of task designs used in earlier studies, each one influencing the possible analyses and comparisons that could be made (e.g. Bjork et al., 2008a; Knutson et al., 2001b; Pizzagalli et al., 2009). We modified the task to create a version that could be used to investigate anticipation of both gain and loss, would allow parsing of anticipation and delivery of reward, and could be adapted for use with different patient populations and other populations of varying cognitive ability. Therefore, we needed to ensure that both behavioural and imaging results would compare to previous studies.

As expected, the behavioural results yielded no significant differences across session or drug conditions, indicating that there was no learning effect or drug effect on task performance. This is consistent with previous studies (e.g. Beck et al., 2009; Jia et al., 2011; Knutson et al., 2001b; Scheres et al., 2007). These results show that the adaptive algorithm in the task itself, as well as the adjustment to individual reaction times, successfully kept accuracy rates stable across participants and conditions. We did observe higher accuracy rates for the high positive cue than the low positive cue, and this was expected since the task was designed to yield 66% accuracy for each valence condition and not per cue (meaning that the small and large incentive cues were combined). This was done to have enough repetitions of each trial type for effective task titration. In fact, the higher accuracy for the higher reward further supports the validity of the task design, as it can indicate that participants were more motivated to respond for high rewards than low rewards. The most important aspect of the behavioural results was to show comparable performance across all conditions, as an equal performance and high (and stable) success rate is necessary for the MID task to successfully elicit reward-related activity (K. Lutz & Widmer, 2014). Having established this in our current paradigm, the next step was to ensure that the task elicited activity in reward related brain regions identified in both the hypotheses and previous studies.

The results from this pilot study are based on a small sample with low detection power, and must therefore be considered preliminary. Using fixed effects analysis, we found significant activation in striatal areas for anticipation of both gains and losses, and to successful outcomes. This is consistent with previous studies using the MID task that have

found striatal activation to anticipation and outcome phases (Admon et al., 2014; Bustamante et al., 2014; Dillon et al., 2008; Kumar et al., 2014; Pizzagalli et al., 2009). The first studies by Knutson and colleagues (2001; 2003; 2004) focused primarily on the role of the NAc in anticipation of gain. Later studies have found that other areas related to the striatum/basal ganglia complex are also activated in the MID task, such as the caudate (Pizzagalli et al., 2009), putamen (Beck et al., 2009), and pallidum (Kumar et al., 2014). In addition, the insula has been implicated in anticipation and delivery of losses (Bjork et al., 2008b; Nestor et al., 2010), the OFC and mPFC in the outcome stages of the task (Bjork et al., 2008b; Knutson et al., 2001b; Simon et al., 2010), and the thalamus in anticipation (Cho et al., 2013; Jia et al., 2011).

Anticipation. In the current study we found significant activation the bilateral putamen, pallidum, thalamus, caudate and OFC in both drug conditions during anticipation of both gains and losses. In the ROI analyses we did not find a significant effect of cue type in the NAc ROIs in the anticipatory phase. We did find that cue type affected signal change in the ventral putamen, however, with higher signal change to gain and loss cues compared to the neutral cues. Notably, the ROIs defined in the current study are not identical to those used previously, as these also differ between studies. It is possible that activations in similar areas have been labelled differently due to close proximity of the NAc and putamen in the VS.

Activation of the ventral putamen in anticipation of reward is consistent with previous studies using the MID task (Beck et al., 2009; Knutson et al., 2001a; Knutson et al., 2004; Knutson & Greer, 2008; Kumar et al., 2014; Simon et al., 2010). This VS activation to anticipation of reward has been previously linked to motivational and saliency aspects of rewarding stimuli (Delgado et al., 2000; Liu et al., 2011), as well as prediction of reward in response to cues depending on valence and magnitude (K. Lutz & Widmer, 2014). The VS activation to positive and negative cues observed in the current study is therefore in line with the assumption that these incentive cues induce an expectation of a particular outcome (reward or punishment) that the neutral cues do not.

Outcome. A primary aim for the current task development was to allow for separate analysis of anticipation and outcome phases of reward. For the outcome, we were interested in brain areas that were activated more to successful than non-successful outcomes. This contrast included all cue-conditions to allow for a more robust analysis due to the lower number of non-successful trials (~33% vs ~66%). Voxels in the ventral putamen and NAc showed significantly higher activation in the successful trials compared to the non successful. In the ROI analysis, we identified significant differences in mean signal change between

correct and incorrect trials in all of the a priori regions of interest. This shows that a successful outcome is probing activity in reward related areas consistent with previous studies (Dillon et al., 2008; Pizzagalli et al., 2009; Simon et al., 2010). We observed that while the striatal ROIs were more activated for correct than incorrect outcomes, the right insula ROI showed the opposite pattern. This is consistent with previous findings using the MID task (Bjork et al., 2008b) and the literature implicating the insula particularly for negatively valenced rewards (Liu et al., 2011).

In sum, we observed activation clusters around the ventral/dorsal striatum in the whole-brain analysis for both anticipation and delivery of rewards, and we are satisfied that the current task measures up to previous versions.

Using the MID task to study morphine effects

To allow for interpretation of potential drug effects on brain activity or task performance, we included a range of control tasks and measures in the current study to rule out confounding variables related to the pharmacological manipulation. Having established that the task elicited detectable activation in reward-related regions in both drug conditions, we were satisfied that the task was appropriate for studying reward processing. We then investigated the effects of morphine on brain activity related to monetary gain and loss. μ-opioid receptor agonism has been found previously to increase reinforcing effects and decrease aversive effects of rewards and punishment (and pain) in humans and animals (Chelnokova et al., 2014; P.E. Lutz & Kieffer, 2013; R.G. Wise et al., 2002). In our pilot sample of 11 volunteers, we did not find significant effects of morphine that survived cluster thresholding and correction for multiple comparisons in the whole brain analysis. We did, however, find trends suggesting an increase in reward related brain activity for successful outcomes in the morphine condition in an exploratory analysis applying correction for multiple comparisons only within the a priori defined left ventral putamen mask. While this result is not sufficient to make inferences about the meaning of these effects, it is encouraging as there could be significant effects in a larger sample with sufficient power.

Drug dose and subjective effects. The morphine dose given in the current study was chosen to stimulate μ-opioid receptors without affecting task performance or give serious side effects. Previous studies in our laboratory have shown that the same dose of morphine influences response bias (Eikemo, 2011) and attractiveness ratings of faces (Chelnokova et al., 2014), while not affecting subjective experience. When we compared subjective ratings of feeling ‘high’ and feeling effects of drugs in the current study, we found no differences between drug conditions. This renders it unlikely that participants’ performance in the reward

task was influenced by expectation or drug effects on cognitive abilities. Previous studies have found that most people experience no serious side effects with morphine doses up to 60 mg (Walker & Zacny, 1998; Zacny & Lichtor, 2008). In the current study, one participant experienced aversive side effects at the end of the morphine session (included in study sample) and another during the placebo session (withdrew from study). This indicates no consistent side effects associated with the current drug dose.

Motor coordination. Opioids assert some of their effects in the brain through interactions with DA neurons in the mesolimbic system (Nestler, 2005). In addition to being implicated in reward and motivation, mesolimbic DA is also important for movement and execution of motoric responses (Obeso, Rodriguez-Oroz, Stamelou, Bhatia, & Burn, 2014). In a study by Pizzagalli et al. (2008), a low dose of DA agonist affected both reward sensitivity and motor speed. In the MID task, the delivery of reward is reliant on a fast response from participants at the right time (target presentation). While the task is titrated to an individual's average response time, there is a pre-determined window of time in which a response has to be made. We therefore included the BRAIN test (Giovannoni et al., 1999) as a control task to make sure that potential slowing of motoric responses caused by drug condition did not affect task performance and consequently delivery of rewards. Compared to placebo, morphine did not influence motor speed or coordination in the current study. This is in line with results from previous studies in our laboratory using the same morphine dose (Chelnokova et al., 2014; Eikemo, 2011), and behavioural results from the MID task showing no differences in accuracy or reaction times between the two drug conditions.

Effect of drug manipulation on BOLD response. One of the main concerns when planning a pharmacological fMRI study is to ensure that the pharmacological manipulation by itself does not alter the ability to detect significant activations. Opioids can cause respiratory depression and consequently reduce detectability of task-relevant BOLD signals (Cohen et al., 2002; MacIntosh et al., 2008; K. Pattinson, 2008). We recorded respiration and heart rate during fMRI scanning in both conditions drug, and observed a small but significant reduction in respiration rate in the morphine condition compared to placebo. This could be due to opioid effects on respiratory systems. This analysis was based on a sample of only six participants and the difference in means was relatively small. It is therefore not appropriate to draw conclusions based on these results, and it is not clear whether this difference in mean respiration would be enough to affect BOLD signal sensitivity. Correcting for physiological variance by including average heart rate and respiration rate in higher level fMRI analysis has been found to significantly improve BOLD results (Brooks et al., 2008; Khalili-Mahani et al.,

2013). In the main study following this pilot, physiological recordings will be included in the analyses. It is also worth noting that while it has been recommended to monitor breathing to make sure it does not drop below 6 breath/minute after morphine administration (Becerra et al., 2006), the mean for both conditions in the current study was closer to 20 breaths/minute. Breathing frequencies less than 8-10 breaths/minute is considered respiratory depression (Dahan, Aarts, & Smith, 2010), and we find no evidence of this in our participants who are all within normal range (Lindh et al., 2013). It is therefore too early to interpret the physiological findings, and we will need to address this at a later point in the data collection. It is possible to control for respiratory depression effects by supplying oxygen to participants, and monitor end-tidal CO^2 to get a more accurate measure of respiratory effects on BOLD signal (Khalili-Mahani et al., 2012).

We also included a visual fMRI paradigm in our test battery to examine the potential confound of respiratory depression. The visual checkerboard stimuli induced significant activation in visual areas for both morphine and placebo conditions. There was no difference between drug conditions in the visual cortex, and the only significant difference between morphine and placebo was an activation cluster in the fusiform gyrus (morphine > placebo) Considering the small sample size in the current study, this could be due to variation unrelated to drug condition. The visual cortex was the primary area of interest for the control task (checkerboard), and this area, unlike more temporal regions such as the fusiform, has a negligible distribution of opioid receptors (Baumgärtner et al., 2006; Frost et al., 1989; Liberzon et al., 2002). The occipital cortex is therefore a good point of reference for assessing opioid effects on BOLD response, and any general morphine effects would be expected to result in activations in the placebo > morphine contrast in this area.

We did not observe any such effects, and as such there are no indications that the current morphine dose has any effects on respiration and/or BOLD response that influences the ability to detect changes in reward task activation.

Limitations and future research

The current findings are based on preliminary data from a pilot study and as such there are limitations on what inferences can be drawn from the results. As the main aim was to validate the task and procedures, the finding of ventral striatum activation to anticipation and delivery of rewards was in line with previous literature and does not directly provide any new knowledge about the reward system. One of the problems with the fMRI research using the MID task, and with a lot of fMRI research in general, is that many published studies have low power due to small sample sizes. Therefore, just replicating findings of previous studies

using the MID task is good task validation as there are many inconsistencies in previous literature.

Although our sample of 11 is relatively small, we utilised a within-subjects design which makes it more powerful than a similar sample size tested between subjects. However, we did not have power to analyse gender effects in this pilot study. Morphine can interact with female sex hormones dependent on the time of the menstrual cycle (Ribeiro-Dasilva et al., 2011). We tried to keep conditions as consistent as possible for the females included in the sample, and all females were tested within a time frame of maximum four days to avoid testing at radically different phases in the menstrual cycle. However, we cannot be certain that no hormonal changes took place within this testing interval in the two participants who did not use hormonal contraception, since they were unfortunately unable to determine the timing since their last menstruation. In a larger sample it would be of interest to test for gender effects and control for influence of menstrual cycle and contraception on morphine effects.

The current study also used a pre-selected dose of morphine, and we did not individually titrate dosage to ensure similar blood concentrations of drug between participants. However, this is less important due to the within subjects design than it would be in a study comparing different groups of participants. Future studies could also investigate dose-response effects of morphine on reward related activity, while still monitoring and controlling for respiratory effects, or include results from blood samples in FEAT analysis.

In terms of the MID task, we developed a task that was powered to look at both wins and losses and allowed parsing of anticipation and outcome. While we did not fully explore all the contrasts available in the current pilot study, it is possible to investigate differences or similarities in activity elicited by winning and losing in the future.

This is also the first implementation of the MID task in Norwegian, and it could therefore be of interest to adjust the values associated with the different cues. The current values (1 and 5 NOK) were similar to studies using other currencies, but the value of these small monetary sums might not be sufficient to elicit strong effects in our sample. High income and cost of living in Norway may render these monetary sums relatively smaller than the corresponding US dollar or Euro values. The “real” value of money depends on subjective value attributions (Kable & Glimcher, 2007), and it could be that the subjective values of the cues in the current study do not cover a large enough range to obtain strong effects. This could also explain the weak parametric variation in brain activations to different anticipation cues within the striatum in the current study compared to earlier findings

(Knutson et al., 2001a; Knutson & Greer, 2008). In the continuation of the study we will include a measure of subjective ratings and happiness associated with the different MID cues that the participants will complete at the end of each session. This will give an indicator of how the participants value the different rewards, and it will allow for a comparison of subjective experience of winning versus losing that could be valuable for further analysis of these two conditions separately.

We did not apply B0 field map correction to the fMRI data in the current study. This could improve functional data quality as it reduces noise from magnetic field inhomogeneities (Jezzard, 2012). An alternative method to traditional B0 field map scans, which take several minutes, is to include short 'reversed-blip' scans and use these to correct for distortions (for a comprehensive explanation of this method see Andersson, Skare, & Ashburner, 2003). This can be implemented in analyses using the FSL TOPUP tool. We are currently in the process of setting up a protocol for the use of TOPUP in fMRI analysis, and this will be applied to the current data set (appropriate scans were recorded at the time of testing) and to the additional data collected in the continuation of the project.

Conclusion

In the current pilot study we developed a protocol for a pharmacological fMRI investigation of the role of the opioid system in reward processing in the healthy human brain. The MID task successfully activated areas implicated in previous research. Specifically, we observed ventral striatal activation to monetary rewards in both of our drug conditions, as well as indications of a possible morphine effect in the reward outcome contrast. This is consistent with the overarching research hypothesis that systemic manipulation of the μ-opioid receptor system can increase reward related brain activity in the ventral striatum to delivery of rewards. The reward task and control measures in the current pilot study will be used in the main pharmacological study. With a larger sample size and more definite results, the main study can provide further insight into the role of the μ-opioid receptor system in reward processing in the brain and have implications for our understanding of addiction and affective psychopathologies.

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