

# The Role of the Opioid System in Reward Responsiveness

Marie Eikemo



Master of Philosophy in Psychology,  
Cognitive Neuroscience

Department of Psychology  
University of Oslo

May 2012

© Marie Eikemo

Year: 2012

Title: The Role of the Opioid System in Reward Responsiveness

Author: Marie Eikemo

<http://www.duo.uio.no/>

Print: Reprosentralen, University of Oslo

## **Acknowledgements**

First and foremost I want to thank my supervisor Dr. Siri Leknes for invaluable guidance and moral support throughout every stage of the journey that has now become a thesis. I owe thanks to my co-supervisor Dr. Guido Biele for constructive guidance in particular during the development of the test paradigm and data analysis.

The experiment on which this thesis is based is part of a comprehensive research project led by Siri Leknes which aims to investigate the role of the human opioid system in motivation for and experience of reward. Five other tests were administered together with the task I developed, and I am grateful to Jeppe K. Riegels, Guro E. Løseth, Emilie W. Guttormsen, Ingunn Olsen, Liv Lohne Knudsen and Olga Chelnocova for helping with part of the data collection. I also want to thank Dr. Vigdis Vindenes and Dr. Liliana Bach, Professors from the Norwegian Institute of Public Health, and Cand. Psychol. Ingunn Olsen, for helping with decisions concerning the drug dosages used in this study. I would also like to thank Guro E. Løseth for analysing motor-coordination control data for the project. Finally, special thanks go to my boyfriend Even for helping me with MatLab and providing priceless moral support.

# Table of Contents

Introduction.....	1
The neuroscience of reward .....	2
The Current Study.....	10
Methods and Materials.....	12
Participants.....	12
Study Design.....	13
Procedures.....	14
Reward Responsiveness Task .....	16
Data Analysis.....	22
Variable Computation.....	22
ANOVAs.....	23
Regression analysis.....	23
Results.....	24
Data Exclusion .....	24
Effects of Task Manipulation.....	24
Effects of Drug Manipulation .....	26
BRAIN .....	30
Discussion.....	30
Limitations .....	34
Implications.....	35
Conclusion .....	36
References.....	37

## Abstract

Studies of non-human animals have provided ample evidence that opioids are involved in reward processes. Less is known about the role of the opioid system for human reward processes. We hypothesized that an opioid agonist would increase, and an opioid antagonist would decrease responsiveness to rewards in healthy humans. We tested 30 healthy male participants on a reward responsiveness task using a randomized, placebo-controlled double-blind design. Participants received oral treatment with a  $\mu$ -opioid agonist (morphine, 10mg), a non-selective opioid antagonist (naltrexone, 50mg) or placebo on three separate days. One-two hours after drug administration, one of three locally developed versions of a reward responsiveness task was completed. The task was a two-alternative signal detection task with a skewed reward schedule. Stimuli were schematic faces which differed in the size or position of the mouth. Reward responsiveness was operationalized as a bias towards choosing the most frequently rewarded response option. In line with our hypothesis, the results showed that morphine significantly increased and that naltrexone significantly decreased bias relative to placebo. These effects could be due to either a direct effect of opioid agonism/antagonism on the neural reward system, or due to indirect effects, e.g. via opioid effects on striatal dopamine functioning. Notably, although our naltrexone condition would be expected to block endogenous opioid function, reward responsiveness was not completely eliminated. This could indicate that opioids may be involved in, but not necessary for, this effect. Overall, our findings confirm a role for the opioid system in human reward responsiveness.

## Introduction

The events and objects that motivate human beings to act are many. Whether they pull us towards something or push us away, these are at the heart of things that govern our behaviour. Reward can be defined as “(...) anything for which an animal will work” (Rolls, 2006, p. 3). The term positive reinforcer is closely related to reward, but refers specifically to an event in which a response is followed by a stimulus and the probability for that response increases (Skinner, 1938). This stimulus is consequently defined as appetitive. The terms reward and positive reinforcer will be used interchangeably in this thesis.

A variety of different stimuli constitute positive reinforcers to animals and human beings. Some of these are rewarding without prior exposure or learning (primary/unconditioned reinforcers); other stimuli can acquire a positive value via its link to a primary reward, e.g. money and other tokens (secondary/conditioned reinforcers) (Dinsmoor, 2004; Skinner, 1938). The reinforcing value of a stimulus depends on the state of the individual and the properties of the stimulus itself (Berridge & Kringelbach, 2008). For instance, the value of food for an organism at a given time depends on whether food is abundant, or sparse (Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001). Many other aspects of the reward, or the environment, can affect the reinforcing value of a stimulus, such as the palatability of a type of food, or the safety of the environment (Rolls, 2005; Stevenson, Bilsky, & Negus, 2006)

Advances in affective neuroscience over the last decades have unravelled a range of partly dissociable and highly interrelated phenomena that constitute, and support, reward processing in humans and animals (Berridge & Kringelbach, 2008; Kelley, 2004). Berridge has divided the reward construct into three subcomponents (Berridge & Robinson, 1998; Berridge, Robinson, & Aldridge, 2009): (1) ‘Liking’, which refers to the positive affective response to a rewarding stimulus; (2) ‘Wanting’, which describes the motivation to obtain, ‘work for’, and approach rewarding stimuli; and (3) Learning, which entails obtaining and integrating reward relevant knowledge that allows for prediction of reward events, and updating this information as new information becomes available. ‘Wanting’, ‘liking’ and learning may be subconscious processes, or explicitly experienced by the agent (Berridge, 2007). Notably, in ecological situations positive reinforcers are normally both liked and wanted, and stimulate learning and anticipation (Barbano & Cador, 2007; Berridge & Kringelbach, 2008; Smith, Berridge, & Aldridge, 2011). However, during the last decades, evidence has accumulated to support the notion that different interrelated

## The Role of the Opioid System in Reward Responsiveness

neurotransmitter systems and networks of brain regions contribute to the different aspects of reward (Kranz, Kasper, & Lanzenberger, 2010). Dissociating these have been a major concern in neuroscience research on reward-related phenomena (Berridge et al., 2009; Der-Avakian & Markou, 2012).

### **The neuroscience of reward**

One of the first research findings that inspired the study of neurobiological correlates of reward mechanisms came from an experiment conducted by Olds and Milner (1954), in which rats with electrode implants were observed in a Skinner box. The rats were given the opportunity to self-administer electric stimulation to a certain brain area by pressing a lever. The researchers reported that electric stimulation to many areas in the “lower centres of the brain” appeared to be very rewarding to the subject, measured by the frequency of subsequent lever presses (Olds & Milner, 1954, p. 426). The areas that correlated with reinforcement behaviour have later been identified as striatal areas (Berridge, 2007). By the use of a wide variety of methods, the neural underpinnings of reward-related processes have been studied vigorously ever since (Haber & Knutson, 2010). This has led to a better understanding of the biological substrates of motivated behaviour, and valuable knowledge that has been used to develop treatments for drug addiction and dependence. However, there are still many unanswered questions in regards to reward-related behaviour and experience (Der-Avakian & Markou, 2011).

Much of what is known about the neural underpinnings of reward processes has been derived from non-human animal research, often using palatable food as positive reinforcer. There are advantages of using animal subjects in this type of research; it allows scientists to use strict experimental control the animal’s environment and stimulus exposure over an extended period of time. In addition to behavioural measures such as indexes of effort, preference towards a stimulus and stimulus reactivity, several methodologies allow for detailed investigation of the underlying neural processes of animals reward behaviour.

Intracranial self-stimulation (ICSS) is used to assess the motivation behaviour an animal has to stimulate regions in its own brain with electricity or substances. Single neuron recording is used in animal research and can inform us about neural activity on a very small scale in real-time (e.g. Roesch, Singh, Brown, Mullins, & Schoenbaum, 2009). Animal reward researchers also use direct microinfusions of neuromodulating substances into specific brain regions to look for area-

## The Role of the Opioid System in Reward Responsiveness

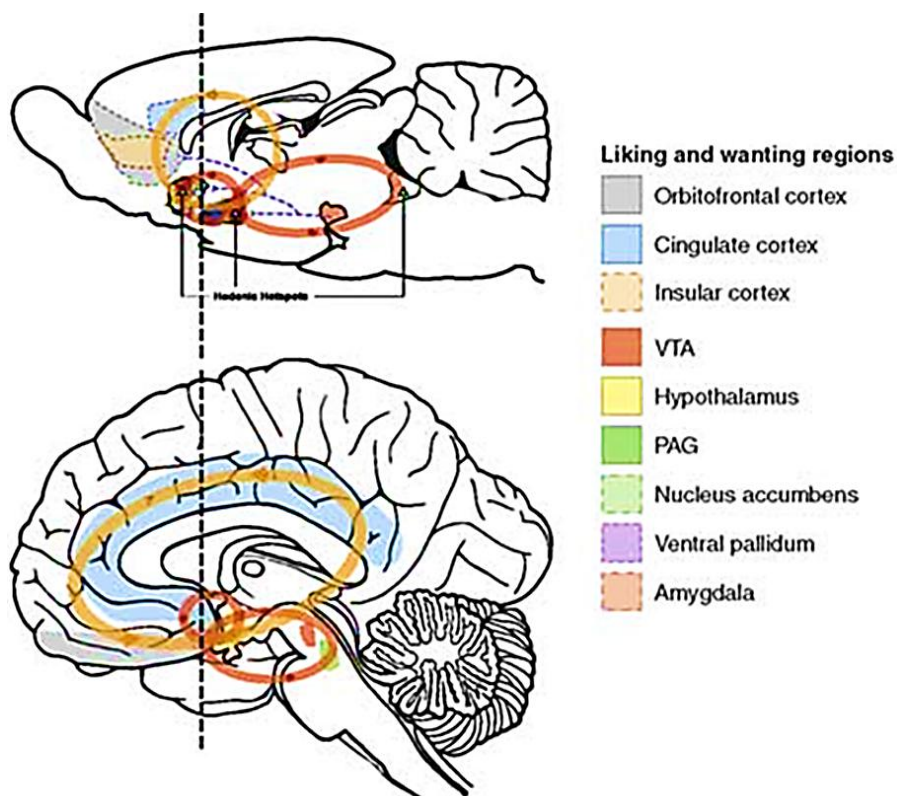
specific functions of the substances in question (e.g. Pecina & Berridge, 2005). Microdialysis and voltammetry are methods that allow for *in vivo* analysis of extracellular neurotransmitter levels on a very small scale (see Marsden, 2006). In addition to these methods, genetic knock-out (KO) studies have become available in animals. These permit manipulation of the availability of different neurotransmitters via genetic alterations which may reveal functional correlates of neurotransmitters (e.g. Hnasko, Sotak, & Palmiter, 2005). The methods used in animal research provide a level of detail and control still unobtainable in human research; this literature has also guided studies on the human reward system.

The question remains of how much of the results from these studies can be generalized to humans. Some research indicates that there are commonalities between the reward systems in human and other mammal brains (Haber & Knutson, 2010; Kringelbach & Berridge, 2009; McClure, York, & Montague, 2004) (see Figure 1 for an illustration of the reward circuits in the rodent and human brain. Nevertheless, because the methods available for studying human neural systems are more limited this relationship still remains somewhat elusive. The animal research finding on reward-related behaviour has to some extent been replicated, and complemented, in recent years by neuroimaging studies with human subjects (Porcelli & Delgado, 2009). Studies using human participants also allow us to look at the involvement in complex brain areas (that are less frequently studied in animal research) and to study more abstract, aesthetic and social rewards. In addition, human participants may report on the subjective experience of positive reinforcers, which is important in order to understand the relationship between brain activity, behavioural measures and hedonic experience. Nevertheless, there are important strengths and weaknesses to the methods available for studying reward processing in the human brain. Functional magnetic resonance imaging (fMRI) and Positron Emission Tomography (PET) are currently the neuroimaging methods of choice in the study of reward related phenomena (McClure et al., 2004). While fMRI blood-oxygen level-dependent (BOLD) signal is believed to reflect energy usage and ultimately neural activity in the brain (Logothetis, 2003), PET can provide information about receptor occupancy of neuromodulators (Spreckelmeyer et al., 2011). Both these methods offer temporal resolutions much slower than the specific neural events causing the activity measured, but have been much used in recent years in this line of research (Boecker et al., 2008; Ernst et al., 2004; Kringelbach, O'Doherty, Rolls, & Andrews, 2003; McClure et al., 2004; Pizzagalli et al., 2009; Porcelli & Delgado, 2009). The temporal and spatial



## The Role of the Opioid System in Reward Responsiveness

restrictions limit the inferences that can be made on the basis of neuroimaging results. While fMRI and PET may provide information about primary regions of activity associated with a stimulus or behaviour, the range of neurotransmitters involved and whether the neural activity is excitatory or inhibitory remains unknown. Electroencephalography (EEG) is less used in reward research, due to the difficulty in localizing the origin of electric activation in deeper regions of the brain. However, event-related fMRI, combining the resolution advantages of fMRI and EEG, is emerging as a viable method for studying reward-related activity in the brain (McClure et al., 2004).



*Figure 1.* Displaying reward related brain areas in the rodent and human brain. The figure is adapted from Kringelbach and Berridge (2009). In addition to these regions, the substantia nigra (SN), hippocampus (HC), ventromedial prefrontal cortex (vmPFC) and some brainstem areas have also been implicated in human and animal reward studies (Haber & Knutson, 2010).

The use of systemic pharmacological manipulation is also a viable option for studying causal relationships between neurotransmitter activity and behavioural, affective and cognitive measures in humans and animals (Rogers, 2011). This method allows us to stimulate, reduce or block the workings of endogenous neural modulators. Psychopharmacological studies can

potentially provide knowledge about which transmitter systems that are necessary for a certain psychological processes, and which transmitters that are involved in it, by blocking and stimulating endogenous neural transmission. Importantly, the neural mechanisms through which the substances work cannot be elucidated by this method alone because, for the time being, we cannot monitor or locally control neurotransmission like we can do in animal subjects. However, inferences can be made based on findings from animal and human reward research. Triangulating research from different methods or combining several methodologies (e.g. pharmacological fMRI), is perhaps the best chance we have of elucidating the neural substrates of the human brain.

**Dopamine reward.** Through the use of diverse methodologies, a complex interaction of many brain regions involved in reward has been revealed (Kelley, 2004; O'Doherty, 2004). Figure 1 displays some of the most commonly implicated brain regions in reward research, both in humans and animals (Kelley, 2004; Kelley & Berridge, 2002; Kringelbach, 2005; Rolls & Xiang, 2005; McClure et al., 2004; O'Doherty, 2004; Peciña & Berridge, 2005).

A significant portion of the relatively few areas of dopaminergic neurons in the brain are located in the brain areas referred to in figure 1 (Björklund & Dunnett, 2007). More than any other neurotransmitter system, dopamine (DA) has been the key suspect for reward processes (Schultz, 2002). DA appears to be important for reward processes that are linked to both primary and drug reinforcers (Nestler, 2005). There is now ample evidence that DA is has a central role in processes such as anticipation of reward, motivated behaviour ('wanting') and reward learning (Daw, 2007; Der-Avakian & Markou, 2012; Kelley, 2004; Salamone, 2007; Schultz, 2007; Sugam, Day, Wightman, & Carelli, 2012; Tobler, Fiorillo, & Schultz, 2005). In particular, the midbrain DA pathways have been suggested to play a crucial role in reward processing (Barbano & Cador, 2007; Berridge, 2012; O'Doherty et al., 2004).

The ventral tegmental area (VTA) and substantia nigra (SN) are key DA producing nuclei, both located in the midbrain, these project to many other brain regions (Björklund & Dunnett, 2007). The axons of DA neurons in the SN project to the caudate and putamen in the dorsal striatum, forming the mesostriatal (or nigrostriatal) DA pathway while dopaminergic axons in the VTA project both to the NAc in the limbic system also called the mesolimbic DA pathway, and to the prefrontal cortex, forming the mesocortical DA pathway (Björklund & Dunnett, 2007). Among these, it is primarily the mesolimbic pathway that has been implicated in reward studies.

## The Role of the Opioid System in Reward Responsiveness

This pathway will receive a primary focus in this thesis although the mesostriatal and mesocortical pathways have also received attention in relation to motivated behaviour (e.g. Marsden, 2006; Palmiter, 2008).

The nucleus accumbens (NAc) and the VTA are key regions in the mesolimbic dopamine reward circuitry (Haber & Knutson, 2010). This network has received considerable interest in research on reward-related mechanisms, related to both primary and drug reinforcers (Baldo & Kelley, 2007; Chefer, Denoroy, Zapata, & Shippenberg, 2009; Iordanova, 2009; Nestler, 2005; Palmiter, 2008; Smith & Berridge, 2007). It has been proposed that the NAc integrates many types of reward related input from different brain areas, such as affective information from the amygdala and homeostatic information from the hypothalamus, in order to control reward-related behaviour (Kelley, 2004).

Single cell recordings of dopaminergic neurons in animals have demonstrated that DA transmission has several time courses. *Tonic* steady firing and transient *phasic* bursts of increased firing (Schultz, 2010). It is in particular the phasic activity of midbrain DA neurons that have been implicated in response to external salient and rewarding stimuli (Schultz, 2010). These typically follow a stimulus by 60-200 milliseconds, and have been suggested to code reward value as it differs from prediction, namely the prediction error (Schultz, 2007). Evidence for phasic DA transmission during anticipation and learning of rewards was provided in a study by Ljungberg and colleagues (Ljungberg, Apicella, & Schultz, 1992). In this experiment transient DA neuron firing was studied in monkeys using single-cell recordings. The results showed transient increases in DA cell firing *during* reward administration in the stimulus-response learning, but only in response to the cue predicting reward *after learning* the task. The VTA-NAc complex has also been implicated in the prediction error signal (Iordanova, 2009). Dopaminergic projections to the dorsal striatum appear to play an important role in stimulus-response-reward associations (Montague, Dayan, & Sejnowski, 1996), and DA is involved the process in which incentive salience is assigned to reward cues in particular (Flagel et al., 2011). NAc DA involvement in reward prediction error and reinforcement learning received additional support in a recent study by Sugam et al. (2012). Fast-scan cyclic voltammetry<sup>1</sup> was used to measure NAc

---

<sup>1</sup> An electrochemical technique that can be used to monitor release and uptake dynamics of endogenous dopamine, serotonin, and noradrenaline in vivo and in vitro (John & Jones, 2007).

## The Role of the Opioid System in Reward Responsiveness

core DA activity in rodents, during a risky choice decision task. The authors found that all cues predicting a reward elicited increased DA release in the NAc.

In regards to human homologues, a recent review of psychopharmacological research on DA and decision making lends support to the notion that dopamine also plays a crucial role in value-based decision making and reinforcement learning in humans (Rogers, 2011). Further, in a fMRI experiment conducted by Knutson and Wimmer (2007) reward prediction and reward prediction error were modeled separately, these were found to correlate with BOLD activity in the medial prefrontal cortex and NAc respectively. Both these regions are targets for dopamine projections from the VTA, suggesting dopaminergic involvement (Knutson & Wimmer, 2007). A study of patients with Parkinson's disease (a disease directly linked to NS dopamine deficit) by Frank, Seeberger, and O'Reilly (2004) revealed a special role for DA in learning from positive reinforcement (as opposed to negative reinforcement).

Many studies support a role for DA as a causal factor in reinforcement learning, and phasic dopamine involvement prediction error coding. However, these are still topics of hot debate (Berridge, 2007; Robinson, Sandstrom, Denenberg, & Palmiter, 2005).

**Is dopamine necessary for all reward processes?** The 'DA hypothesis of reward' has dominated the research on reward to the extent that there might be a research bias towards DA functioning in the related neuroscience literature (Salamone & Correa, 2002; Salamone, 2007). Dopamine was thought to directly mediate all reward functions, including the mediation of the pleasurable ('liking') properties of rewarding stimuli (Koob & Moal, 1997; Salamone, 2007; Wise, 1982). However, during the last decades, this idea has been challenged by several researchers and findings from experimental research (Berridge & Robinson, 1998; Smith et al., 2011). Results from studies on ingestive behaviour suggest that the pleasure element of reward ('liking') is mediated mainly by the  $\mu$ -opioid system, rather than dopaminergic circuit. (see reviews by Baldo & Kelley, 2007; Berridge et al., 2009). This notion receives support from studies demonstrating that dopamine antagonism fails to reduce food intake, whereas direct infusions and systemic administration of  $\mu$ -opioid receptor (MOR) agonists increase food intake (Bodnar, 2004). Another line of evidence supporting a key role of  $\mu$ -opioids for positive affect in reward ('liking') comes from research seeking to make objective measures of 'pleasure-behaviour' in different animals. Although pleasure as a concept is often understood as an experienced subjective state, some research indicates that pleasure can be measured in animals

## The Role of the Opioid System in Reward Responsiveness

and non-verbal humans (infants) by analysing cross-species equivalents in body reactions to appetitive stimuli (Berridge, 2007). Common denominators in how humans and other animals (e.g. apes and rodents) act when presented with a natural reward such as sucrose (Berridge, 2000), enable researchers to investigate homologous affective reactions across species. Positive taste reactivity is significantly increased by opioid manipulation, in particular by direct infusion of agonists to the NAc (Peciña & Berridge, 2000; Peciña, Smith, & Berridge, 2006).

Regarding evidence implicating opioids in human reward,  $\mu$ -opioid receptor binding has been found to correlate with the euphoria associated with physical activity ('runner's high') (Boecker et al., 2008). Also, opioid antagonism has been found to reduce the pleasantness of food (Yeomans & Gray, 1996), the pleasurable effects of amphetamine (Jayaram-Lindström, Wennberg, Hurd, & Franck, 2004) and the euphoria associated with runner's high in humans. The opioid system may also mediate the pleasure associated with alcohol and nicotine (Hnasko et al., 2005; Oslin et al., 2003).

In addition, some animal research indicate that positive reinforcement from food and is not completely dependent on DA functioning. Cannon and Palmiter (2003) tested sucrose naïve mice, that could not produce their own dopamine (knockout mice), with a sucrose reward task. They found the DA deficient (DD) mice, alike the control mice, preferred a sucrose solution to water. Based on previous ingestion and taste studies, the authors interpret this preference to be mediated by opioids rather than DA (Cannon & Palmiter, 2003). Results from this study indicate that dopamine may not be necessary for preferring a natural reward. Further, Hnasko et al. (2005) investigated the rewarding effects of morphine in DA deficient mice using a place-preference paradigm. They found that even though the morphine-associated hyperlocomotion associated with reward was attenuated, it was not eliminated in mice unable to produce endogenous dopamine.

Together these findings speak for a central role of opioids in mediating the affective aspect of reward ('liking'). Evidence for the involvement of a second neurotransmitter in reward-related processes, namely opioids, implies that some reward mechanisms may be supported by interactions of these two neuromodulators, a notion that has received solid support through animal research.

**The link between opioids and dopamine in reward mechanisms.** Opioid and dopamine systems interact during reward-related processes (Burkett, Spiegel, Inoue, Murphy, & Young, 2011; Spreckelmeyer et al., 2011). There is now ample evidence that opioid manipulation, either systemic or injected directly into striatal areas, can modify DA activity in the NAc of rodents (Maisonneuve, Warner, & Glick, 2001; Spanagel, Herz, & Shippenberg, 1992; Vindenes et al., 2009). One interpretation of these findings is that opioids can modulate the amount of DA released in response to a certain rewarding stimulus (Nestler, 2005). This mechanism may be mediated by GABA-containing interneurons ( $\gamma$ -aminobutyric acid) in the VTA, as shown in an *in vitro* rodent study by Johnson and North (1992). Tonic GABAergic activity in the VTA inhibits dopaminergic signal transmission to the NAc (Nestler, 2005). The idea is that opioid manipulation will excite or inhibit GABAergic interneurons in the VTA, which further leads to dopaminergic NAc activity (see Figure 2). Johnson and North (1992) also reported that it was mainly the  $\mu$ -opioid receptors (MOR) that mediated this effect. MOR agonists such as morphine and heroin can disinhibit these GABAergic cells consequently increasing phasic DA signalling to the NAc (Chefer et al., 2009). It has been postulated that opioid antagonists such as naltrexone or naloxone induce an opposite pattern (e.g. Nathan & Bullmore, 2009), namely a MOR blockade

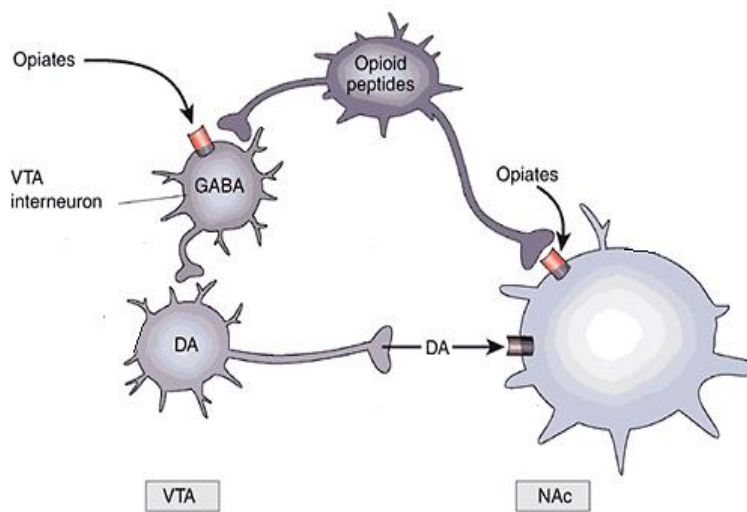


Figure 2. An illustration of the dopamine-opioid interaction in the VTA-NAc network. Adapted from Nestler, 2005. Nature Neuroscience.

increasing the inhibitory transmission of GABAergic neurons, leading to reduced DA transmission in the NAc. Also, Spanagel et al. (1992) demonstrated that MOR agonism in the VTA increase DA release in the NAc whereas the blockade of MORs results in a decrease in DA release in rodents. Notably, DA increase was not found when injecting this agonist directly into the NAc, speaking for an *indirect* effect of  $\mu$ -opioids on dopaminergic activity in the NAc.

## The Role of the Opioid System in Reward Responsiveness

As for systemic opioid manipulation, Vindenes et al. (2009) found increased levels of DA in the NAc of rodents following a morphine injection.

Less is known about a possible opioid-DA VTA-NAc mechanism in human beings. However, some indirect evidence, mainly from drug addiction research, suggests that this network and its functions can be found in human beings as well. Addictive behaviour related to both psychostimulant drugs and natural rewards diminish during opioid antagonist treatment (Bachs & Waal, 2003; Brauer, Behm, Westman, Patel, & Rose, 1999; Drewnowski, Krahn, Demitrack, Nairn, & Gosnell, 1995). One example of opioid-dopamine-interaction in human beings is found in a study conducted by Jayaram-Lindström and colleagues on healthy non drug-abusing men (2004). They studied the effect of naltrexone (50 mg) on the subjective, physiological and cognitive effects of amphetamine, a drug known to exert its effects on the DA system (2004). The authors reported a significant attenuation of the subjective effects of amphetamine, such as ratings of drug 'high' and drug liking, in the naltrexone condition compared with placebo. Also,  $\mu$ -agonism increases endogenous DA levels (Spreckelmeyer et al., 2011). Taken together, these studies make a good case for a possible interaction of DA and opioids in reward related neurotransmission in both humans and non-human animals.

### **The Current Study**

The ability to alter behaviour in order to obtain a reward is adaptive (O'Doherty et al., 2004). Being sensitive to reinforcement helps an organism in determining appropriate behaviour for fulfilling primary and secondary needs. The lack of this behaviour is associated with psychopathology, e.g. major depression, eating disorders and schizophrenia (Gorwood, 2008; Schultz, 2007). Asymmetric reward schedules can induce systematic biases in sensorimotor choices. This bias is sometimes termed 'reward responsiveness', and can be defined as the degree to which behaviour is modulated as a function of positive reinforcement. This process requires integrating reinforcement history over time (Gorwood, 2008). The evidence for opioid involvement in the affective aspects of natural rewards taken together with studies showing opioid-dopamine interaction in the VTA-NAc network, led us to hypothesize that systemic opioid agonism and antagonism would modulate reward responsiveness.

One parsimonious paradigm that has been used to study the degree to which behaviour is modulated as a function of rewards received in human beings is based on signal-detection

## The Role of the Opioid System in Reward Responsiveness

methodology. The task requires a participants to indicate which of two ambiguous schematic face stimuli (S1 or S2) that has been presented by making the appropriate response (B1 or B2, respectively) (Tripp & Alsop, 1999). Unknown to the participant, the ratio of reward outcome for correct B1 and B2 responses used in this task is skewed, so that correct B1 is rewarded three times more frequently than correct B2. This paradigm provides an objective laboratory measure of behaviour modification as result of positive reinforcement. Healthy participants ordinarily show a clear response bias towards the more frequently rewarded stimuli (e.g. Pizzagalli et al., 2009). This reward responsiveness bias has been replicated in a range of diverse contexts, including studies with different patient groups, psychopharmacological studies and neuroimaging studies (Barr, Pizzagalli, Culhane, Goff, & Evins, 2008; Bogdan & Pizzagalli, 2006; Pizzagalli, Evins, et al., 2008; Pizzagalli, Goetz, Ostacher, Iosifescu, & Perlis, 2008; Pizzagalli, Iosifescu, Hallett, Ratner, & Fava, 2008; Pizzagalli, Jahn, & O'Shea, 2005; Tripp & Alsop, 1999).

**Neuromodulators for Reward Responsiveness.** Some research has been conducted in order to understand the neuromodulatory underpinnings of human reward responsiveness. For example, there is evidence for involvement of dopaminergic transmission. A very small dosage of the DA agonist, pramipexole acts as antagonist on the phasic DA response and caused reduced reward responsiveness (Pizzagalli, Evins, et al., 2008). Compared to the placebo control group, the pramipexole group showed reduced reward learning. Further, Pizzagalli and colleagues have employed the same task in a PET study to investigate endogenous DA release in relation to reward responsiveness (Vrieze et al., 2011). The authors found indirect evidence for endogenous dopamine release in the OFC, anterior CC and ventromedial prefrontal cortex associated with reward responsiveness.

One recent study has begun to address the question of opioid involvement in reward responsiveness in humans. This study was conducted recently by Lee and colleagues (2011), and investigated the role of two polymorphisms of the mu-opioid receptor (MOR) gene OPRM1 in reward responsiveness, applying the task used by Pizzagalli and colleagues. The results of this study showed that carriers of specific gene variant, G allele carriers, displayed reduced reward responsiveness. Carriers of the most common variant of the OPRM1-gene (AA homozygotes) showed typical reward responsiveness.

To measure reward responsiveness in the present study we employed the same behavioural paradigm used in the studies described in the previous paragraph, with some modifications. This



paradigm provides an index of behaviour modification as a function of rewards received, and is suitable for evaluating how drugs affect our reactions to external stimuli (Wardle & de Wit, 2012). Based on research indicating a dopamine-opioid interaction in reward processes and recent studies implicating a role of DA in reward responsiveness, we hypothesized that: Systemic opioid manipulation would alter reward responsiveness in healthy participants. More specifically we hypothesized that:

- a) A  $\mu$ -opioid receptor agonist, morphine, would lead to increase in reward responsiveness compared to a placebo control condition.
- b) A non-selective opioid antagonist, naltrexone, would reduce reward responsiveness compared to a placebo control condition.

## Methods and Materials

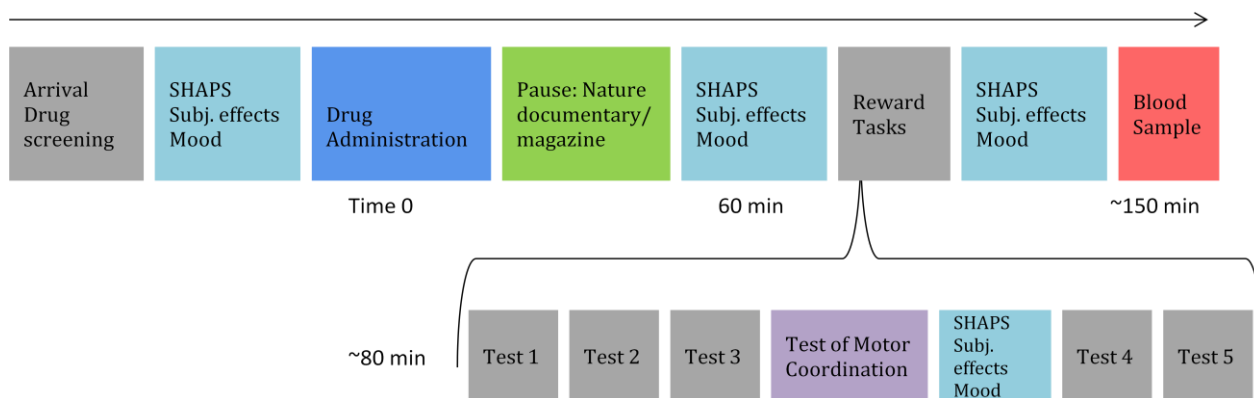
### Participants

We recruited 30 healthy male volunteers for this study. Two participants were excluded from analysis: one tested positive on the opiate urine screening, the other participant only completed one session. The final number of participants was 28, aged 20 to 36 years ( $M = 26.7$ ,  $SD = 4.7$  years, 26 right-handed). In a pre-testing telephone screening interview, none of the participants reported a history of depression or other major psychiatric illness, none were currently suffering from psychiatric or medical illness, none were currently on medication and none had multiple complex allergies. None of the participants reported prior drug dependence or addiction. All participants were morphine naïve, i.e. had not taken morphine in any form for at least two years prior to testing (Becerra, Harter, Gilberto Gonzalez, & Borsook, 2006). Participants had normal or corrected-to-normal vision. The participants were tested on three different days with a minimum inter-session interval of seven days. Each session lasted approximately three hours and the participants were reimbursed 400-500 NOK per session, depending on task performance. The experimental procedures were approved by the Regional Ethics Committee (2011/1337/REK sør-øst D).

## Study Design

The reward responsiveness task was administered as part of a larger psychopharmacological study with several tasks investigating the role of the endogenous opioid system in human reward experience and motivation. The tasks involved 1) A reward responsiveness task; 2) Evaluation of and motivation for receiving soft brush strokes on the forearm; 3) Evaluation of and motivation for looking at faces of differing attractiveness; 4) Social decision making with money; and 5) Evaluating sweet sucrose solutions. In addition to these tests we collected data about subjective experience related to hedonic capacity, mood ratings and possible drug related effects at four time points during each session: 1) before drug intake; 2) before testing, 3) during testing and 4) at the end of the session. Mid-way through the experiments each session, the participants completed a test of motor-coordination. At the end of every session a blood sample was collected.

The study had a repeated-measures within-subject design and was conducted in a double-blind, placebo-controlled manner. The tasks and the drug order were counterbalanced. At the end of session three participants were debriefed and asked to guess the identity of the drug received in each session. At the end of data collection, this measure showed that the participants on average identified the drug received correctly 34% of the time, indicating a successful blinding of the participants. The Reward Responsiveness Task was completed between 70 and 120 minutes after drug administration (See *Figure 3* for an example of a time line for one participant). The order of the tasks was counterbalanced between participants, but did not vary across sessions within-subject.



*Figure 3* Outline of an experiment session.

### Procedures

**Consent and General Instructions.** Prior to the first testing session participants received information about the study and a consent form by email. The participants were informed about the collection of biological material, i.e. urine- and blood sample. They were asked to sustain from eating an hour before testing, and advised not to drive a vehicle for 6 hours after drug administration. Further, participants were given brief information about the drugs and possible side-effects in the consent form, and were told that they would receive all three drugs in the course of three experimental sessions, but that the order would be unknown to them and the experimenter.

**Drug administration.** Morphine is a selective  $\mu$ -opioid receptor agonist. Morphine is the most widely chosen analgesic for moderate to severe pain (Vindenes, Handal, Ripel, Boix, & Mørland, 2006). In this study we used pills of 10mg morphine (Morfin®, Nycomed Pharma). We chose an oral administration as it is less invasive than giving drugs intravenously or intramuscularly. The bioavailability of oral morphine is on average 30-40%, but varies considerably between individuals. Morphine has maximal effect ( $t_{max}$ ) at 1-2 hours after oral administration, and a half-life of 2-4 hours (Lugo & Kern, 2002). To minimise subjective effects we chose a low dosage of morphine compared to similar studies (Walker & Zacny, 1998; Zacny & Lichtor, 2008). Opioids have been shown to interact with female sex hormones at different times of the hormone cycle (Ribeiro-Dasilva et al., 2011); for this reason we tested males only in this study.

Naltrexone is a non-selective opioid antagonist with a high affinity to  $\mu$ - and  $\kappa$ -opioid receptors. It is used in the treatment of drug and alcohol addiction to block the effects of exogenous opioids (e.g. heroine) or to reduce drug/alcohol craving. Naltrexone acts on the brain's opioid receptors, and therefore also blocks the binding of naturally occurring opioids: endorphins (Bachs & Waal, 2002; Bachs & Waal, 2003). The maximal plasma concentration of naltrexone is reached after one hour (Verebey, Volavka, Mule, & Resnick, 1976). The half-life of naltrexone is described as occurring in three phases: the first three hours; and intermediary phase of 10-12 hours; and the late phase of 24-72 hours (Verebey et al., 1976). In this study we used pills of 50 mg naltrexone (Adepend, Orpha-Devel), a standard dosage that has been used with only minor side-effects in several previous studies (see reviews by Bachs & Waal, 2002; Yeomans & Gray, 2002).

## The Role of the Opioid System in Reward Responsiveness

Placebo pills were cherry-flavoured breath mints that were visually matched to morphine and naltrexone pills. A small amount of the flavoured placebo pills were added to the drug dosages, in order to avoid any recognition of medication taste. The participants were asked to swallow, rather than chew, the pills.

*Test Interval.* The test interval between 1 and 2.5 hours after drug intake was deduced by comparing the time of maximal bioavailability of oral morphine and naltrexone. We also consulted data based on measures of morphine and its major metabolites in the blood from The Norwegian Institute of Public Health (The Division of Forensic Medicine and Drug Abuse Research, see Figure 4). The maximal plasma concentration of both morphine and naltrexone occurs at approximately one hour. While naltrexone levels take a long time to decrease, morphine levels decrease quite rapidly, however, morphine levels are quite high between 1 and 2.5 hours after oral ingestion.

**Time Line.** After giving written consent, participants were asked to submit a urine sample for opiate screening (MOP Opiate300 Test Strip; SureScreen Diagnostics Ltd). After completing state-relevant questionnaires, participants received one of three drugs if the drug toxicology was negative. To ensure blinding of the experimenter and the participants, the participants were instructed not to inspect the drugs visually, but to consume the contents directly from a small black box together with some water.

After drug consumption the participant spent one hour waiting for drug uptake; and were given a choice of watching a nature documentary or reading from a selection of magazines. Participants were not allowed to bring their own reading material or to work or study while waiting for the drug to act. Sixty minutes after ingesting the drug the participant completed state relevant questionnaires before moving on to a different room for testing. The test session

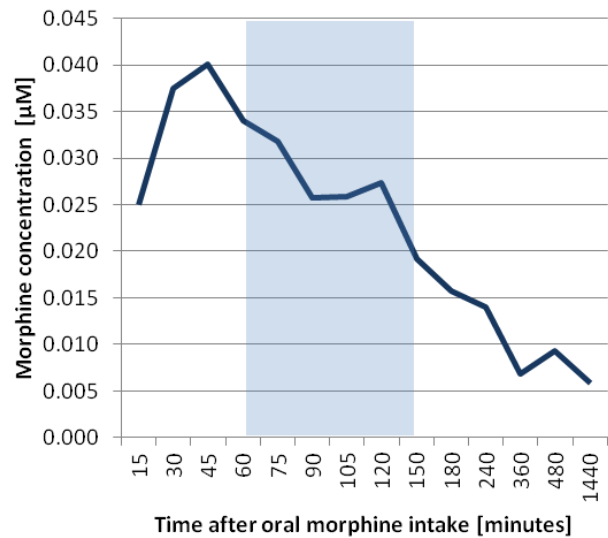


Figure 4. Illustration of the plasma concentration of morphine after oral administration at different time intervals. The shaded area displays the test interval chosen for the current study.

## The Role of the Opioid System in Reward Responsiveness

duration was approximately 80 minutes, and the test order was pseudo randomized and counter balanced across participants.

The Reward Responsiveness Task was completed between 70 and 120 minutes after drug administration and always at the same time across sessions within-subject.

### **Reward Responsiveness Task**

We wished to assess whether the opioid agonist and antagonist would increase and decrease (respectively) the degree to which we adapt our behaviour when presented with rewards. To achieve this, we adapted a test paradigm used by Diego A. Pizzagalli and his colleagues in a series of studies of reward sensitivity in various groups of patients and healthy controls using a range of different methods (Barr et al., 2008; Bogdan & Pizzagalli, 2006; Liu et al., 2011; Nikolova, Bogdan, & Pizzagalli, 2012; Pizzagalli, Evins, et al., 2008; Pizzagalli, Iosifescu, et al., 2008; Pizzagalli et al., 2005; Vrieze et al., 2011). The paradigm was originally developed by Tripp and Alsop (1999) to study reward processing in children with attention deficit hyperactivity disorder. The test used in the current study resembles the Pizzagalli-lab task more closely both in task structure and type of reinforcer (monetary).

The task, labelled “The Response Bias Probabilistic Reward Task”, measures an individual’s tendency to modulate his or hers behaviour as a function of reward. We operationalize it as a “measure of reward responsiveness”. In short, this is a signal detection task in which the participant is presented with one of two ambiguous stimuli for a very brief time. The task is to identify the stimulus using one of two corresponding buttons. A key element of the task is that the correct responses can lead to monetary reward, and unknown to the participant, there is a differential reinforcement schedule. One of the two stimuli is associated with more frequent reward *when the correct answer is provided* than the other stimulus. The stimulus with high reward value is often referred to as ‘rich’ as opposed to the less rewarded ‘lean’ stimulus; I will be adopting this terminology for the remainder of the thesis. In this task a correct response to the ‘rich’ stimulus is rewarded 75% of the time while correct responses to the ‘lean’ stimulus are rewarded 25% of the time. This “skewed” reward ratio has been shown to result in a *response bias*, favouring the more frequently rewarded stimulus (Barr et al., 2008; Bogdan & Pizzagalli, 2006; Liu et al., 2011; Nikolova et al., 2012; Pizzagalli, Evins, et al., 2008; Pizzagalli, Iosifescu, et al., 2008; Pizzagalli et al., 2005; Vrieze et al., 2011). In the task used by Pizzagalli and

## The Role of the Opioid System in Reward Responsiveness

colleagues the participant is presented with a schematic face with no mouth followed by a very brief presentation of a short or a long mouth (100ms). The participant's job is to decide whether the mouth presented was short or long, by pressing a corresponding button. The task consists of 300 trials divided into three equal blocks, the two conditions (e.g. short mouth and long mouth) being equiprobable in a random sequence within each block. Incorrect and unrewarded trials were followed by a fixation cross.

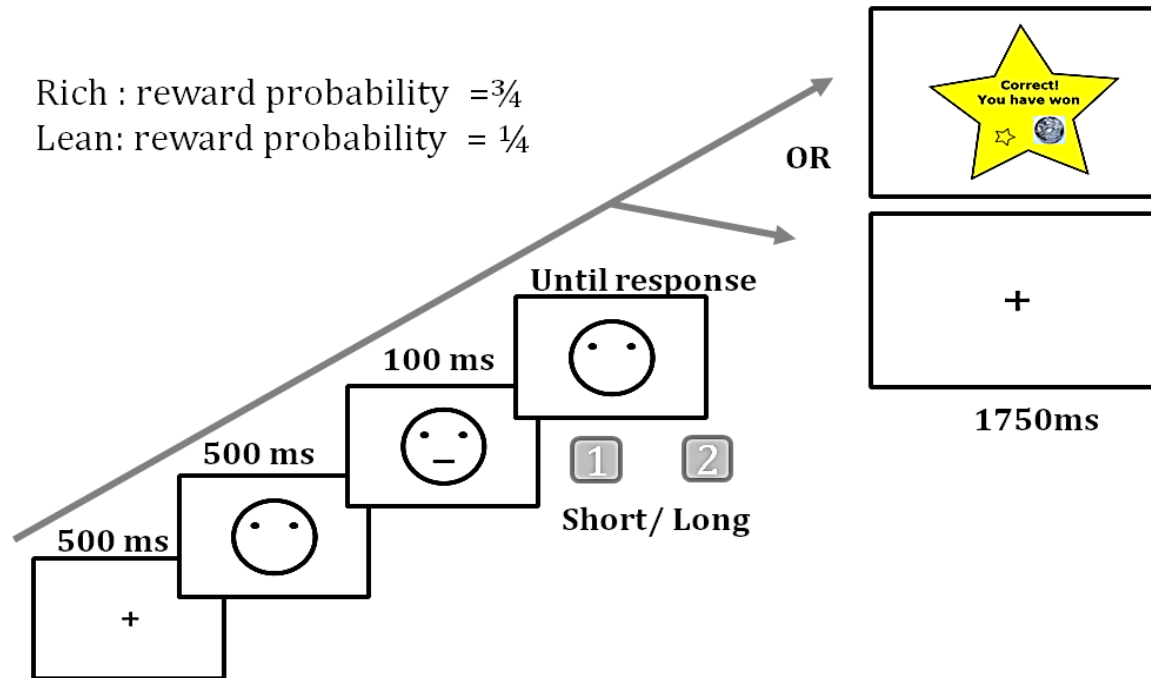


Figure 5. Example of a trial, participants used their dominant hand and the 1 & 2 keys of the numpad to respond.

**Task Development.** The tasks made to measure reward responsiveness in this study are modelled after the task described above, but differ in some aspects. Firstly, we adapted the task to suit our cross-over design, with three repeats per participant. Three equivalent stimulus pairs were created in order to avoid learning effects across sessions. Test-retest reliability of the original task has been assessed by Pizzagalli and colleagues (2005), but the test interval assessed (> one month) was much larger than our design allowed. Also, the dimensions of the stimuli used in the tests differed slightly as we adapted the task to our test environment, as has been done by several other laboratories using this paradigm (Heerey, Bell-Warren, & Gold, 2008; Lee et al., 2011; Liu et al., 2011). The three versions of the task are identical; the only feature differing is the pairs of stimuli (see Figure 5 for an example of a trial). The three versions created for this

## The Role of the Opioid System in Reward Responsiveness

study will be referred to as task A, B and C. Stimulus pair A mirrored the original task and the mouth was either short or long. In stimulus pair B the mouth appeared slightly to right or the left of the middle of the face. Stimulus pair C was slightly angled up towards the left or the right (the exact measurements will be described in detail after presentation of the task development studies). The stimuli were adapted through the task development phase to ensure that the two stimulus alternatives were so similar that it would be difficult to tell them apart at a brief glance, but not impossible. In line with previous studies using this task we opted for an average accuracy of 75-85% to ensure that the stimuli were sufficiently ambiguous.

Three sub-studies were conducted during the task development phase. These studies were used to (1) assess whether the three stimuli-pairs were equivalent in difficulty (2) look for possible carry-over effects between the sessions, and (3) further adjust the task difficulty.

(1) *Testing the three stimulus pairs.* The first versions of the picture pairs were made using approximations of stimuli ratios from the studies conducted by Pizzagalli and colleagues. To test the three pairs of stimuli, and assess the difficulty level of each task, eight participants (mean age: 28 years; age range: 22-42) completed 100 trials of each of the three tasks in one session, divided into three blocks. In this first investigation the reward schedule was not implemented and a fixation cross served as the only feedback. The results showed that all three tasks were too easy (see Table 1). One participant reported that the left-right task (B) was very difficult, and indeed performed quite poorly (mean accuracy = 61%). The remaining participants had high accuracies on all tests.

Table 1.  
*Hit rates for the three tasks*

Stimulus	Mean hit rate (%)	Range (%)
A	92	88-96
B	90	61-100
C	94	88-97

The descriptive statistics displayed in Table 1 indicated a ceiling effect, suggesting the stimuli were not ambiguous enough. Adjustments were made to all three picture pairs to increase the difficulty levels. We also decided that the participants should be seated at a fixed distance of 80cm distance from the screen. After modifications a more thorough study was conducted in order to assess possible carry-over effects between the sessions and to further validate the test stimuli with a full-length test.

## The Role of the Opioid System in Reward Responsiveness

(2) *Assessment of test-retest reliability.* Nine right-handed men (age range: 20-40 years, mean age: 29) completed the three tasks (300 trials per session) in a pseudo-randomized order, at intervals of one week. The participants received reward feedback, and also received the total amount of money won at the end of session three. In this study we did not administer any drugs, given that we wanted to look

for possible carry-over effects, and further evaluate the three stimulus-pairs in order to minimize the differences between them.

Despite the low  $n$ , the data was explored, and some analyses were performed to look for robust differences. When comparing the three sessions, we found no main effect of session, and no trend indicating learning or boredom effects in session 2 and 3 ( $F(2,22) = 0.345, p = .712$ ) compared to the first session. Task equivalence was assessed by using a repeated-measures ANOVA of Accuracy for each task type (A, B or C). This revealed a significant main effect of task ( $F(2,48) = 5.977, p = .006$ ). This was due to the fact that task A and B were easier than C (see Figure 6). Further, some of the participants in the pilot had very high accuracies (three participants had accuracy scores of  $>93\%$  on all three tests), indicating that the stimuli had not been altered enough after the first pilot to avoid a ceiling effect. However, in line with earlier results from studies on reward responsiveness, we did find a behavioural preference towards the rich stimuli. This preference was reflected in increasing response bias across blocks, higher accuracies and shorter reaction times for the rich condition compared to the lean. The findings from this study highlighted the necessity to further alter the stimulus pairs, both in order to make the differentiation between every two faces more difficult, and to make this differentiation as equal in every pair as possible.

(3) *Manual thresholding study.* Sixteen participants (age range 22-44 years, mean age: 30) completed a short version of the experiment, consisting of 100 trials from each of the three tasks (A, B, C), divided into three blocks. In this version of the task, the reward feedback schedule was implemented; a yellow star with the text “correct!”, but the participants received no money for participation. The overall hit rate per block and session was evaluated after every three

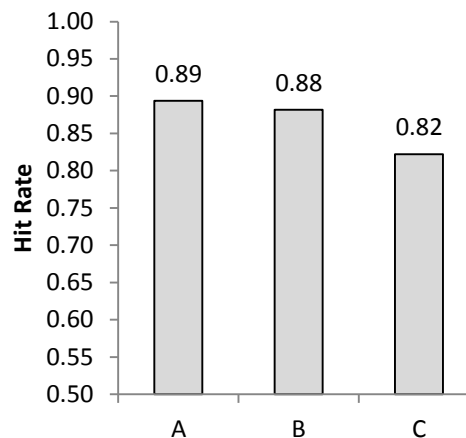


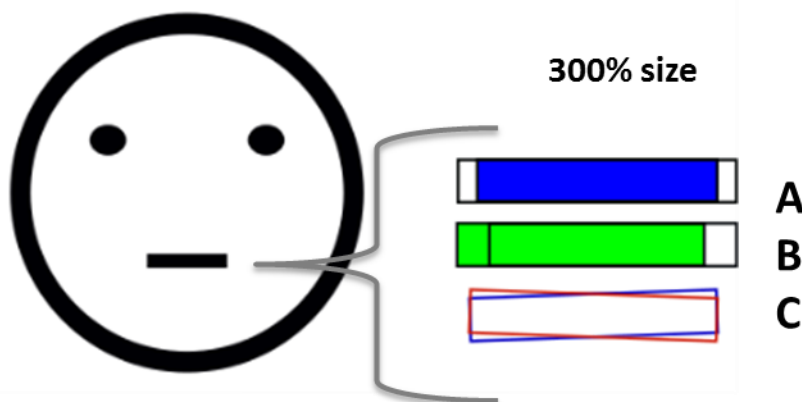
Figure 6. Average hit-rates for the test-retest pilot study for each task.



## The Role of the Opioid System in Reward Responsiveness

participants, and the length, angle and positioning of the test stimuli were altered with very small adjustments ( $\pm \sim 0.2$  mm). This manual thresholding of the stimuli size ratio was used to make the three tasks as equal as possible, and to fit the desired accuracy level of 75-85%. The last six participants completed the same task, and none of the participants had hit rates lower than 74% or higher than 88% on any of the tasks. Mean accuracy task A: 78%; task B: 80%; task C: 77%: These stimulus pairs were used in the final experiment.

**Final task stimuli.** The first set of stimuli (Task A) consisted of faces with two horizontal lines (mouths) of different length: 11mm and 12mm (0.788 and 0.859 degrees of visual angle respectively). This task mirrored the original task developed by Pizzagalli and colleagues, and used in previous literature. In the second stimulus-pair (Task B) the positioning of the mouth (11.5mm: 0.624 degrees of visual angle) varied along the horizontal axis; and appeared slightly to the left or right of the centre. The difference between the two stimuli was 1 mm (0.072 degrees of visual angle), each mouth positioned 0.5mm from the centre of the face. In the final pair of faces (Task C), the line (11,5mm) was given a slight angle (1 degree) either upwards to the right or to the left (see Figure 7 for illustrations of the mouth properties in the three stimulus pairs). The face size and eyes were the same across tasks. The face had a diameter of 5.3 cm (degree of visual angle:  $3.794^\circ$ ), and the person was always sitting 80 cm from the monitor.



*Figure 7.* Illustration of the stimuli differences. The mouths from each stimulus pair are superimposed on each other and inflated. The coloured areas are the overlaps between the two stimuli in pair A and B. The angled stimulus pair (C) is demonstrated with a blue and red outline.

The asymmetric reinforcement schedule applied was identical across the three tasks versions. A correct response to a presentation of the *rich* stimulus meant a  $\frac{3}{4}$  probability of reward. For the *lean* stimulus the reward probability was  $\frac{1}{4}$ . The identity of the stimulus with *rich* value was counterbalanced and randomized across participants. Each session contained 3 blocks of

## The Role of the Opioid System in Reward Responsiveness

randomized stimulus presentations each consisting of 100 trials. After each block the participant had the opportunity to take a short break. The average test duration was 16.4 minutes (range: 15.2 - 18.9 minutes).

**Instructions.** Before starting the task, the participants were given verbal and written instructions about the stimulus properties of the two faces and the corresponding response buttons. Ten practice trials (five rich and five lean trials) preceded the test, in which they received feedback about their accuracy after each trial, but no money. Following the practice, the participants were instructed that only correct responses *could* lead to a reward. Further, participants were encouraged to try to make as much money as possible by answering quickly and accurately. The verbally given instructions were repeated in writing on the computer screen, and participants were asked to indicate whether they clearly understood the instructions before commencing the task. The participants completed the experiment alone in a lab-room.

**Equipment.** The tasks were presented on a 20" PC monitor with a resolution of 1600×1200 pixels using E-prime software (version 2.0; Psychology Software tools, Inc, Pittsburg Pennsylvania). Participants were positioned in a chair with a neck rest with an 80 cm distance from the eyes to the screen.

### **BRAIN Test**

To ensure that experiment effects were not due to reduced motor functions in either of the drug conditions, the participants completed the BRAIN test (Bradykinesia Akinesia Incoordination task, Giovannoni, Van Schalkwyk, Fritz, & Lees, 1999) mid-way through each session. In this test participants use their dominant index finger to alternate between two keyboard keys, 15 cm apart, as quickly and accurately as possible. This test results consist of four indexes of upper limb motor function: (1) A kinesia score (KS) which provides the number of keystrokes in 60 seconds; (2) an akinesia score (AS): cumulative time the keys a depressed; (3) a dysmetria score (DS) providing a weighted score of number of incorrect presses corrected for speed; and (4) an incoordination score (IS) a measure of rhythmicity. This test was originally made to assess upper-limb function in patients with Parkinson's disease who have dopamine deficiency and motor coordination problems. We were primarily interested in assessing the dysmetria score, which is an index for overall task performance that takes into account that different people may choose

## The Role of the Opioid System in Reward Responsiveness

different strategies when completing this test (i.e. prioritizing speed over accuracy or *vice versa*). However, all scores were analysed for drug differences.

### Data Analysis

Our main dependent variable was the measure of response bias, which was our method of assessing reward responsiveness. Based on other studies using this paradigm, we also analysed general measures of task performance: discriminability, accuracy and reaction time.

Discriminability is used in signal detection methodology to assess a participant's ability to distinguish between the stimuli in question. Discriminability is a log transformed accuracy score across the two stimulus-types; I have included accuracy as an additional measure only in order to assess possible differences in accuracy within the rich and lean condition. Further, to evaluate the general effectiveness of the task in inducing reward responsiveness (overall task manipulation), analyses on the placebo condition data are presented before the analyses comparing drug conditions.

#### Variable Computation

The response bias ( $\log b$ ) was computed using this formula:

$$\log b = \frac{1}{2} * \log \left( \frac{rich_{correct} * lean_{incorrect}}{lean_{correct} * rich_{incorrect}} \right)$$

The  $\log b$  gives us the log transformed ratio of presses on the rich button versus presses on the lean button, which can be used as a measure for preference towards the rich stimulus. The Response Bias was calculated using mean accuracy scores for each block.

Discriminability ( $\log d$ ) provides the log transformed ratio of hits and misses, and was computed using this formula:

$$\log d' = \frac{1}{2} * \log \left( \frac{rich_{correct} * lean_{correct}}{rich_{incorrect} * lean_{incorrect}} \right)$$

Percentage values were used in the calculations of response bias and discriminability. To avoid log-transformation of scores of zero, 0.5 was added to every cell before calculating the  $\log b$  and  $\log d$  variables. Finally, accuracy scores for the two stimulus conditions (rich and lean) and

## The Role of the Opioid System in Reward Responsiveness

reaction time values were considered. These also reflected the behavioural bias towards the most frequently rewarded stimulus in previous studies.

### **ANOVAs**

Overall task manipulation (placebo data) was analysed using repeated-measures ANOVAs. Also, for the drug comparison analyses of the secondary dependent variables (Accuracy, Reaction Time and Discriminability) were assessed using repeated-measures ANOVAs. Statistical Package for the Social Sciences 18 (SPSS INC., Chicago, IL, USA) was used for these analyses.

### **Regression analysis**

We used a repeated measures design in which each participant performed the experiment all drug conditions, each time using a different version of the task. Order of drug condition and association of drug conditions with task types were counterbalanced or randomized. To test our main hypothesis, i.e. the effect of drug on reward responsiveness, we used a multiple linear regression model (using MatLab R2011a, Mathworks, Natic, USA). With a multiple regression model the response bias measure can be assessed while modelling out the variance from nuisance variables. The multiple regression was set up as a 3 x 3 factorial design of the three drug conditions and the three blocks. This design closely resembles a standard repeated-measures ANOVA, but permits more flexibility in inclusion of nuisance variables. Included nuisance variables were one dummy variable for each participant to account for differences in overall bias in participants, *Session number*, *Task Type*, and *Discriminability*. The 9 main regressors of the model allowed us to test the main effects and interactions relevant for our reward responsiveness hypothesis by using planned contrasts. Each regressor corresponds to the average response bias for a particular block and drug condition (block-wise bias). Table 2 displays the planned contrasts, i.e. comparisons of average effects with 0 or with each other, used to test the hypotheses. For each planned contrast t-values were calculated, these were compared with the relevant t distributions to test for statistical significance.

Table 2.

*Matrix for response bias contrasts.*

Contrast	<u>Morphine</u>			<u>Placebo</u>			<u>Naltrexone</u>			Nuisance variables
	b1	b2	b3	b1	b2	b3	b1	b2	b3	
M > P	1	1	1	-1	-1	-1	0	0	0	0
P > N	0	0	0	1	1	1	-1	-1	-1	0
M > N	1	1	1	0	0	0	-1	-1	-1	0
All b3 > b1	-1	0	1	-1	0	1	-1	0	1	0
Mb3 > Mb1	-1	0	1	0	0	0	0	0	0	0
Pb3 > Pb1	0	0	0	-1	0	1	0	0	0	0
Nb3 > Nb1	0	0	0	0	0	0	1	0	-1	0

**Note.** *M = morphine, P = placebo, N = naltrexone. b = block and numbers signify block.*

## Results

### Data Exclusion

Across all participants, two sessions and six blocks were excluded from the analysis due to very low hit rates (below chance level), or failure to follow task instructions, i.e. pressing only one button for more than 25 consecutive trials, or misidentifying the response buttons (three blocks). Further, trials with reaction times shorter than 250 ms or longer than 2500 ms were excluded. In addition, for each session, for every subject, trials falling out of range of mean reaction time  $\pm 3SD$  (after natural log transformation) were considered as outliers and hence excluded from further analysis. Overall, 3.41 % of the remaining trials were excluded.

Data from the placebo condition were first analysed separately. This was done to ensure that our tasks indeed did induce the response bias and that our control data are comparable to those of previous studies. Repeated-measures ANOVAs were used. Analyses were conducted on the four measures described in the analysis section.

### Effects of Task Manipulation

**Response Bias.** In line with previous findings, a one way repeated-measures ANOVA of *Block* on placebo response bias scores showed a main effect of Block  $F(1,52) = 6.699, p = .005$ , partial  $\eta^2 = .205$ . Contrasts revealed that response bias in block 1,  $F(1,26) = 8.603, p = .007$ , and block 2,  $F(1,26) = 7.418, p = .011$ , were significantly lower than response bias in block 3 (see Figure 8 a).

**Discriminability.** A repeated-measures ANOVA of the discriminability scores per Block revealed a significant main effect of Block,  $F(2, 52) = 6.698$ ,  $p = .004$ , partial  $\eta^2 = .169$ . Contrasts showed that discriminability in block 1,  $F(1,26) = 10.803$ ,  $p = .003$ , and block 2,  $F(1,26) = 5.409$ ,  $p = .028$ , were significantly lower than discriminability in block 3 (see Figure 8 b)

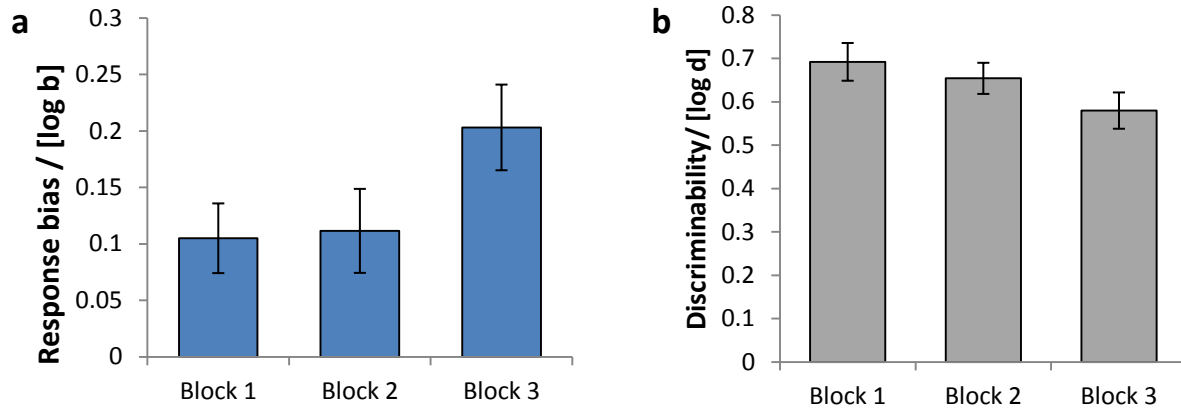


Figure 8. Mean response bias (a) and discriminability (b) per block in the placebo condition. Error bars show standard error of the mean.

**Accuracy.** A two-way repeated measures ANOVA of Block  $\times$  Stimulus Condition (rich and lean) for the placebo accuracy data only revealed significant main effects of Block ( $F(2,52) = 6.411$ ,  $p = .003$ ,  $\eta^2 = .198$ ) and Stimulus Condition ( $F(1,26) = 20.091$ ,  $p = .000$ , partial  $\eta^2 = .436$ ). Replicating findings from earlier studies, the interaction of Block  $\times$  Stimulus Condition was also significant  $F(2,52) = 6.715$ ,  $p = .005$ , partial  $\eta^2 = .205$ . Separate ANOVAs for each stimulus level indicated that the interaction was due to a significant decrease in lean accuracy in from block 1 to block 3. There was no significant difference in rich accuracy across blocks (see Figure 9 a).

## The Role of the Opioid System in Reward Responsiveness

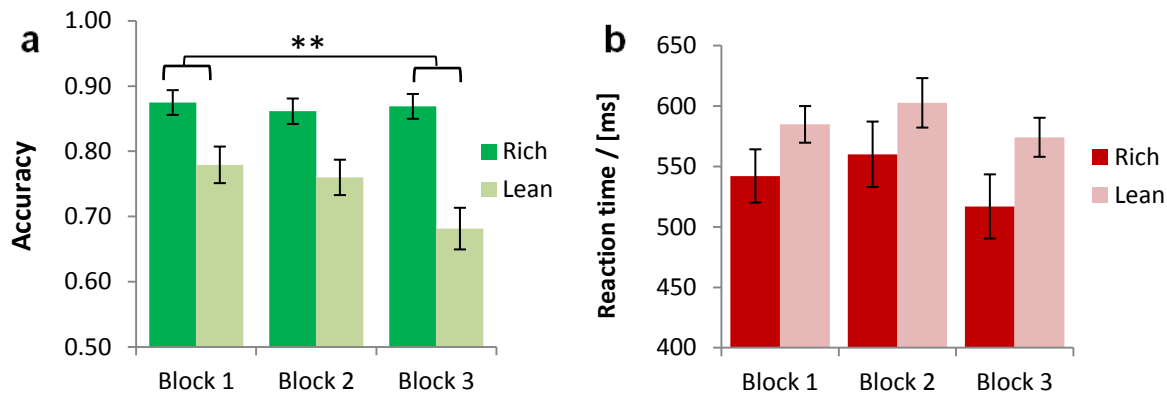


Figure 9. (a) Mean accuracy for and (b) mean reaction time for the rich and lean stimuli in the placebo condition. Error bars show standard error of the mean. The asterisks \*\* represent p-values <.01 and refer to the contrast of the difference between rich and lean in block 1 versus block 3.

**Reaction Time.** The ANOVA on Reaction Time data of Block  $\times$  Stimulus Condition revealed main effects of Block  $F(2,52)=3.557, p=.038$ , partial  $\eta^2=.120$ ) and Stimulus Condition ( $F(1,26)=14.104, p=.001$ , partial  $\eta^2=.352$ ). However there was no significant interaction, i.e. no significant increase in this difference over the course of the three blocks.

Together, the accuracy and reaction time results indicate that the task induces a behavioural preference towards the rich stimulus; the participants correctly identify the rich stimulus more often than the lean, and that they respond faster to it.

### Effects of Drug Manipulation

**Response Bias.** The main effects of interest were the contrasts that allowed us to assess (a) whether there are significant differences in the degree of Response Bias between the three drug conditions; (b) whether there is an *increase* in Response Bias across the three blocks; and (c) whether the pattern of increase in bias was different between in the three drug conditions. The planned contrasts showed that the bias in the morphine condition was significantly higher than bias induced in the placebo and naltrexone conditions. Further, the overall bias was significantly lower in the naltrexone condition than in both placebo and morphine conditions (see Figure 10 and Table 3). The  $R^2$  for the model was .4305, adjusted  $R^2=.3166$  (see Table 3 for statistics).

## The Role of the Opioid System in Reward Responsiveness

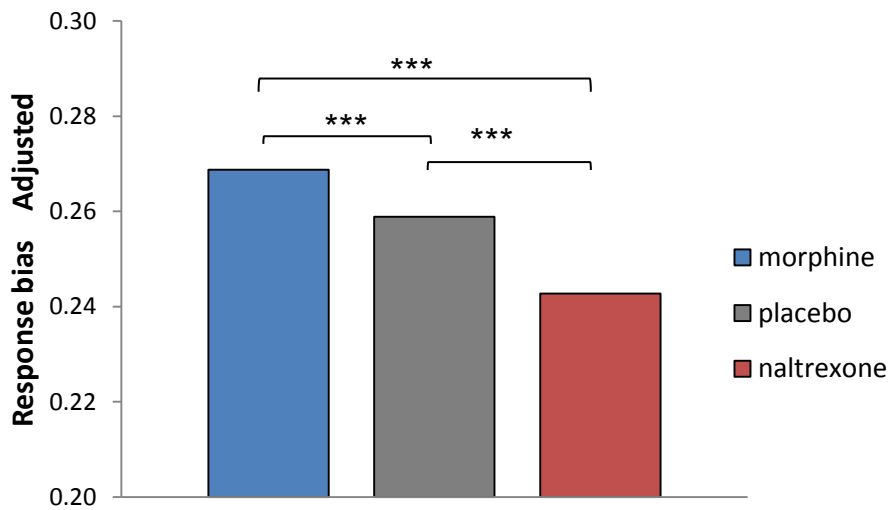


Figure 10. Adjusted Beta-values from the GLM for the bias in each drug condition. \*\*\* = p values lower than .0001. Significance value notations refer to drug-specific contrasts shown in Table 3.

Table 3.

*Planned contrasts of Response Bias in all Drug conditions and Beta values for the Task and Session regressors.*

<u>Contrasts</u>	<u>Standardized Coefficients</u>			
	$\beta$	Std. Error	t	p
Morphine > Placebo	0.089	0.0139	6.37	<0.0001
Morphine > Naltrexone	0.234	0.0144	16.24	<0.0001
Placebo > Naltrexone	0.145	0.0145	10.01	<0.0001
<u>Nuisance regressors</u>				
Task A – Task C	0.0029	0.0288	0.10	0.9194
Task A – Task B	0.0143	0.0280	0.51	0.6102
Session 1 – Session 2	0.0750	0.0279	2.68	0.0078
Session 1 – Session 3	0.0165	0.0282	0.58	0.5600

Model  $R^2 = .4181$ , Adjusted  $R^2 = .2982$ ,  $F(41,199) = 3.7362$ ,  $p < .0001$

**Note.** Standardized values are shown:  $\beta$  values, Standard error of the mean, t-statistic and significance level (respectively).

*Response bias over time.* The planned contrasts in the multiple regression revealed a significant increase in overall response bias regardless of drug condition from block 1 to block 3



## The Role of the Opioid System in Reward Responsiveness

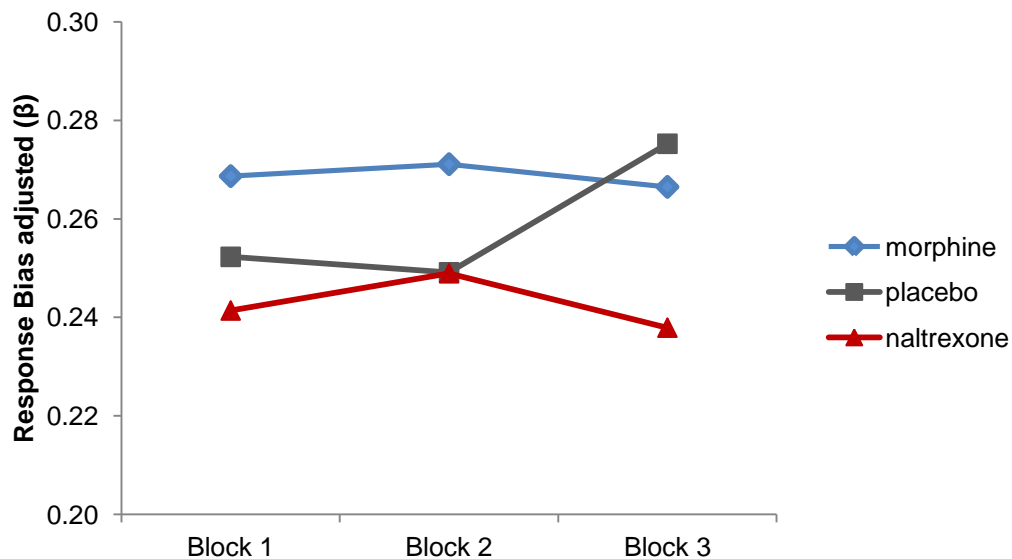
(see Table 4). Contrasts for each drug condition separately showed that the increase in bias from block 1 to block 3 was only significant for the placebo condition. We ran a post hoc contrast to see whether the response bias was significantly higher in block 1 for morphine than placebo, - this was confirmed (see Figure 11 and Table 4). Response bias was high in block 1 of the morphine condition and remained high throughout the task.

Table 4

*Planned and post hoc contrasts for block-wise bias scores.*

Contrasts	Standardized Coefficients			
	$\beta$	Std. Error	t	p
Overall: Block 3 > Block 1	0.052	0.0142	3.65	<0.0001
Morphine: b3 > b1	-0,0080	0,0078	-1,0242	0.3069
Placebo: b3> b1	0,0681	0,0080	8,4881	<0.0001
Naltrexone b3 > b1	-0,0067	0,0082	-0,8116	0.4180
M1 > P1*	0.049	0.008	6.18	<0.0001

**Note.** Standardized values are shown:  $\beta$  values, Standard error of the mean, t-statistic and significance level (respectively). M= morphine, P=placebo and numbers signify block. \* A contrast of special interest, testing whether the difference between response bias in block 1 was significantly higher than response bias in block 1 for the placebo condition.



*Figure 11.* Response bias per block and drug. Numbers on the Y-axis are the adjusted Beta weights from the multiple correlation.

## The Role of the Opioid System in Reward Responsiveness

**Accuracy.** As expected from the uniform findings in earlier studies, a repeated-measures ANOVA on accuracy data of Drug  $\times$  Stimulus Condition revealed a significant main effect of stimulus condition ( $F(1,25)= 36.712, p < .0001, \eta^2 = .595$ ). The main effect of drug condition was not significant, but the linear contrast did approach significance ( $F(1,25) = 3.810, p = .062$ , partial  $\eta^2 = .132$ ) Indicating that accuracy in the morphine condition was somewhat higher than accuracy in the placebo condition and that accuracy for the placebo condition was higher than the accuracy for the naltrexone condition (see Figure 12 a). Separate ANOVAs for each stimulus condition showed a significant main effect of drug for the rich stimuli accuracy  $F(2,50)= 3.853, p = .028$ , partial  $\eta^2 = .134$ . The linear trend for rich stimulus accuracy was also significant  $F(1,25)=8.830, p = .006$ , partial  $\eta^2 = .261$  (see 12 a). There was no significant effect of drug on accuracy in the lean condition.

**Reaction Time.** A repeated-measures ANOVA of reaction time data on Drug  $\times$  Block  $\times$  Stimulus Condition showed a significant main effect of stimulus condition ( $F(1,20) = 20.498, p < .0001$ , partial  $\eta^2 = .506$ ) (see Figure 12 b) and a main effect of block ( $F(2,40)= 8.805, p = .002$ , partial  $\eta^2 = .306$ ). The interaction between drug and stimulus reaction time was not significant.

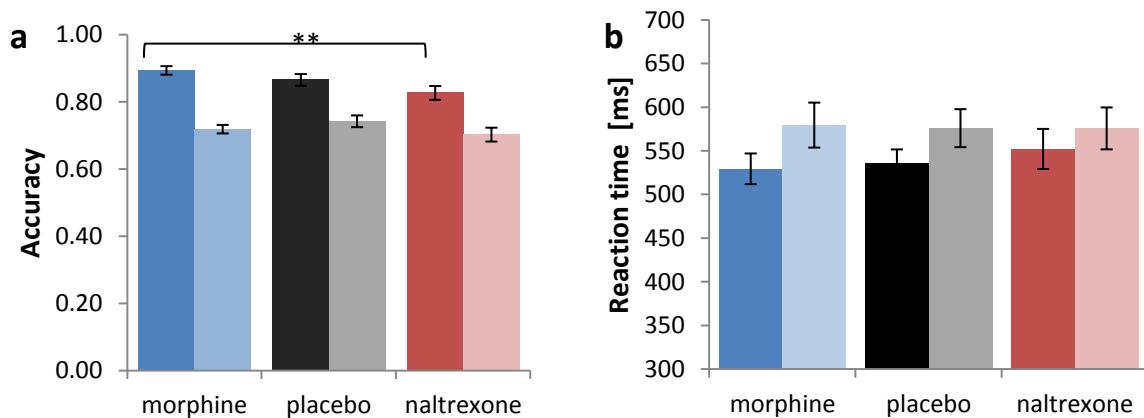


Figure 12. Graph of Rich and lean reaction time data for each drug condition. Rich values are illustrated with strong colours and displayed first in the pair of columns. Mean accuracy for the rich and lean stimulus condition for each drug. Rich stimuli accuracy is depicted in strong colours and lean in a lighter shade. Error bars show standard error of the mean. \*\* signify  $p < .01$  and refers to the main effect of drug on rich accuracy.

**Discriminability** A two-way repeated measures ANOVA of drug  $\times$  block on the  $\log d$  scores revealed no significant main effect of drug,  $F(2,40) = .924, p = .390, \eta^2 = .045$ ). There was however a significant main effect of block,  $F(2,40) = 7.085, p = .004, \eta^2 = .262$ . Contrasts showed

## The Role of the Opioid System in Reward Responsiveness

that this was due to a decrease in discriminability from block 1 to block 3 ( $F(1,29)=9.654$ ,  $p=.006$ ,  $\eta^2=.326$ ). Separate ANOVAs for each drug condition showed that the main effect of block on discriminability was only significant for the placebo condition (see overall task manipulation section).

**Task and Session.** Data from the regression model allowed us to assess whether the two new task types (B and C) contributed significantly less or more to the response bias than the original short-long task (A) from which the tasks were adapted. Neither of these turned out to be significant contributors to the response bias (Task B:  $\beta = .0029$ ,  $p = .9195$ ; Task C  $\beta = .0143$ ,  $p = .6103$ ). The  $\beta$  weights reflecting the contribution of session order (session 2 and 3 compared to session 1) showed that session 2 ( $\beta = .0750$ ,  $p = .0078$ ), but not session 3 ( $\beta = .0165$ ,  $p = .5600$ ) contributed significantly to the response bias.

## BRAIN

The repeated-measures ANOVAs for the four measures of motor coordination showed no significant effects of drug. Dysmetria:  $F(1,25) = .037$ ,  $p < .964$ ,  $\eta^2 = .003$ . Kinesia:  $F(1, 25) = .128$ ,  $p < .881$ ,  $\eta^2 = .011$ . Incoordination:  $F(1,25) = .155$ ,  $p < .857$ ,  $\eta^2 = .013$ . Akinesia:  $F(1,25) = 2.404$ ,  $p < .112$ ,  $\eta^2 = .167$ . These results indicate that the differences in performance on the test across drug conditions were not due to significant decreases in motor or eye-hand coordination.

## Discussion

We measured reward responsiveness in thirty healthy male participants across three different drug conditions (morphine, naltrexone and placebo) to investigate whether reward responsiveness is modulated by systemic opioid manipulation. We predicted a linear relationship between the drug conditions, with opioid agonist treatment leading to higher reward responsiveness than the placebo condition, and opioid antagonist treatment causing lower reward responsiveness than the placebo treatment. This hypothesis was supported by the results from our regression analysis of response bias. In addition to our reward responsiveness measure, the analysis of the accuracy in the rich condition across drugs confirms a stronger preference towards the most frequently rewarded stimuli in the morphine condition compared to naltrexone. In line with previous findings (Pizzagalli, Evins, et al., 2008) we found no significant differences in reaction time and discriminability measures between the three drug conditions. The results for

## The Role of the Opioid System in Reward Responsiveness

the placebo condition alone show that the tasks modified for this study successfully induced the response bias found in earlier studies using this paradigm. These results give reason to believe that we can validly compare the effects of our measures across the drug conditions. Also, the analyses of motor-coordination showed that motor abilities were not significantly different across drug conditions.

The reward responsiveness task does not allow us to dissociate between the three reward components proposed by Berridge (Berridge, 1996; Berridge & Robinson, 1998). The process mediating reward responsiveness is likely to involve all three reward components (i) hedonic evaluation of the reward; (ii) prediction and anticipation of a reward following a given choice and (iii) association learning of the reward information following a trial, and updating this association as the task progresses and the information on which to base a decision increases. These constructs are however useful for interpreting the results and hypothesizing about the underlying mechanisms of the results.

*Indirect dopamine modulation of reward responsiveness?* The drug effects found in the current study could be caused by several different mechanisms. The involvement of the dopaminergic system in reward related processes is well documented and two studies have assessed DA involvement in this paradigm specifically (Pizzagalli et al., 2008; Vrieze et al., 2011). Systemic opioid manipulation has been shown to modulate the release of DA in the brain via GABAergic mechanisms (Corbett, 2006). It is therefore possible that systemic agonism of the opioidergic system could have an indirect effect on reward responsiveness by increasing the endogenous DA transmission during the task. The opposite effect could be elicited by opioid antagonism with naltrexone, namely an inhibition of DA transmission between the VTA and the NAc by increasing GABAergic activity in the VTA (Johnson & North, 1992; Nestler, 2005). If the observed drug effects on reward responsiveness are due to DA increase and decrease, we could hypothesize that they are due to enhancement and reduction of DA dependent motivational or 'wanting' aspects of the reward process as proposed by Berridge, Robinson and colleagues (Berridge, 2007; Robinson et al., 2005). On the other hand, DA modulation may also have directly affected the efficiency of positive reinforcement learning (Schultz, 2010). There is evidence to support the role of dopamine reinforcement learning in humans and animals, in particular when it comes to learning from *positive* reinforcement (Ljungberg et al., 1992; Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006; Stevenson et al., 2006). The current data

## The Role of the Opioid System in Reward Responsiveness

also align with a previous study using the same reward responsiveness paradigm to test the influence of a polymorphism of the  $\mu$ -opioid receptor gene (OPRM1) on reinforcement learning (Lee et al., 2011). Notably, the authors of this study interpreted their findings as due to an indirect effect of MOR-availability on dopaminergic mechanisms.

*Direct opioid modulation of reward 'liking'?* As outlined in the introduction, opioidergic modulation has been shown to influence the positive affective evaluation of natural rewards (Dreher, 2009; Langleben, Busch, O'Brien, & Elman, 2012; Peciña & Berridge, 2000; Yeomans & Gray, 1996), a process that may be independent of dopaminergic processes (Cannon & Palmiter, 2003). The observed increase in reward responsiveness in the morphine condition may be partly due to direct modulation of opioids on the 'liking' of the reward, i.e. the positive affective reaction. If this is the case, the bias is modulated by the subjective hedonic value that the 'reward message + monetary reward' has for the participant during the task in the different drug conditions. According to Berridge (2003), this type of hedonic modulation would not need to be consciously explicit for the agent. Nonetheless, systemic administration of MOR agonists has been shown to increase subjective rating of well-being/euphoria (Becerra et al., 2006; Vaupel, Lange, & London, 1993). Using pharmacological fMRI study Petrovic et al. (2008) found naloxone attenuation of pleasure ratings of larger rewards (compared to a relatively small reward), lending support to the idea of opioid modulation of subjective pleasure experience of a given reward. Also, studies using opioid-antagonism have demonstrated reduction in euphoria measures (Hollister, 1981; Janal, Colt, Clark, & Glusman, 1984). Furthermore, increased endorphin levels have shown to correlate with measures of euphoria on a conscious level in human beings (Boecker et al., 2008; Koeppe et al., 2009). Increased 'liking' of the reward could thus be a result of mood modulation related to opioid manipulation. These findings collectively suggest a key role for the opioid system in subjective well-being.

The drug effect presented here, showing morphine up-regulation and naltrexone reduction of reward responsiveness, could also be facilitated by a combination of several processes. It is possible that the effects are partly dopamine mediated via GABA perhaps affecting wanting and learning aspects required by the task, and partly caused by direct opioid modulation of reward value.

*Are opioids necessary for or merely involved in reward responsiveness?* In order to answer this question based on a data from a pharmacological study, it is crucial whether one can

## The Role of the Opioid System in Reward Responsiveness

efficiently block the entire population of receptors in question or not. The most cited study on receptor occupancy by oral naltrexone was conducted by Lee et al., in 1988. They used a radioactive ligand ( $[^{11}\text{C}]$ Carfentanil) to investigate the MOR availability after a single dosage of 50mg naltrexone in healthy participants at different time points. Their results showed a  $\mu$ -receptor blockade of  $91 \pm 6\%$  48 hours after oral naltrexone administration, and a maximum blockade at one hour post drug ingestion. The authors conclude that 50mg of naltrexone is more than what it needed to block the opioid receptors. The high receptor occupancy at 48 hours post administration indicated that a complete blockade at the time of our experiment (1-2.5h) is very likely. Unfortunately, the study does not report measures of receptor blockade at one hour. The efficacy of naltrexone in blocking opioid receptors is also demonstrated in a study using an agonist challenge (hydromorphone)(Schuh, Walsh, & Stitzer, 1999). In this study oral naltrexone 25mg completely blocked the effects of the agonist challenge.

If opioids are necessary for the modulation of behaviour as a function of reward outcomes, and naltrexone (50 mg) results in a complete blockade of endogenous opioid signalling, we might expect that a naltrexone blockade of endorphins would abolish this effect completely, and result in no bias at all. What we observe in the current study is that response bias is blunted, or down-regulated, but does not disappear.

*Enhanced speed of reinforcement learning by  $\mu$ -opioid agonism?* A curious finding from our study is the speed at which the participants acquire a high response bias in the morphine condition. The participants develop a high bias during block one in the morphine session, and maintain this level of preference for the rich stimulus across the remainder of the experiment. One possible interpretation for this is that opioid agonism may induce a primacy effect of reward when morphine is involved. The probability of the first reward following a “rich face” is 75 per cent. Of interest in relation to this effect, a reinforcement model put forward by (Frank, 2005) posits that phasic dopamine bursts during positive feedback strengthen the chosen response, which in turn promotes learning of this stimulus-response link that was followed by a positive reinforcer. If morphine indeed increases or potentiates the phasic dopamine bursts, this may explain the rapidity of the response bias development in this drug condition.

### **Limitations**

We did not directly control, or monitor, central dopaminergic neurotransmission, nor any other neurotransmitter systems in this study. Therefore we can only hypothesize about the possible interactions of different neuromodulator systems in the reward responsiveness effect. Also, we cannot be sure that there are no brain compensatory mechanisms that are in play when one transmitter system is 'down' due to antagonist treatment (Cannon & Palmiter, 2003). This thesis has exclusively focussed on the opioidergic and dopaminergic neurotransmitter systems, with a reference to GABAergic mechanisms. This choice is not due to their unique roles for reward-related processes, but rather owing to a major focus on these two transmitters in this line of research (Kranz et al., 2010). Substances such as serotonin, norepinephrine, cannabinoids, and glutamate are some of the neurotransmitters found to play a role in reward although the involvement of these are not well understood yet in humans (Harley, 2004; Kranz et al., 2010; Palminteri, Clair, Mallet, & Pessiglione, in press).

While we used a selective MOR agonist, the antagonist naltrexone was a non-selective opioid antagonist that also binds to  $\kappa$ - and to some extent  $\delta$ -opioid receptors. Different, and sometimes competing, functions have been found for these different receptor types. (Taha et al., 2006) There are no viable oral options for blocking  $\mu$ -opioids selectively as of today. Nevertheless, naltrexone has high affinity to  $\mu$ -receptors, and the dosage used in the current study is likely to have had a complete, or very high blockade of this receptor.  $\mu$ -opioid involvement is further supported by the opposite effects we have observed on response bias by agonist and antagonist treatment.

*By Carrot-or-stick?* In the current experiment we only assessed the effect of positive reinforcement on behaviour. Our study did not address how an opioidergic agonist and antagonist manipulation would affect our behavioural response to negative reinforcement, as has been done in some other studies (Frank et al., 2004; Pessiglione et al., 2006; Petrovic et al., 2008). Pessiglione and colleagues (2006) tested healthy participants treated with DA enhancing or reducing drugs on a probabilistic decision task with rewards and losses. Interestingly, they found an asymmetric effect of drug on gain and loss conditions, indicating a special role for dopamine in positive reinforcement. It would be interesting to test the effect of opioid agonism and antagonism on learning from negative reinforcement.

## The Role of the Opioid System in Reward Responsiveness

We found decreased discriminability across the three blocks in the placebo condition. This has not been reported in earlier studies using this task. The discriminability measure is assumed to reflect task performance, indicating ability to distinguish between the different stimuli but also possible failure to attend to the stimuli (Tripp & Alsop, 1999). However, response bias and discriminability scores are not independent of each other, and a high  $\log b$  score can decrease the  $\log d$  measure (Klein, 2001). This indicates that the discriminability effect need not be interpreted solely as a declined ability in telling the stimuli apart, but rather as an effect of a strong bias leading to more frequent misidentification of the lean stimulus. However, discriminability was included as a factor in our regression model, which allowed us to model out the variance due to differences in task performance.

### **Implications**

*Deficits in reward processing.* Reward responsiveness has been studied in several clinical populations. Symptoms of depression are correlated with a reduction in reward responsiveness in a group of healthy volunteers (Pizzagalli et al., 2005). In a subsequent study a group of individuals diagnosed with major depressive disorder (MDD) was shown to have reduced reward responsiveness compared to a matched group of healthy controls (Pizzagalli, Iosifescu, et al., 2008). Also patients with bipolar disorder (BPD) in a euthymic state (Pizzagalli, Goetz, et al., 2008) and children with attention deficit hyperactivity disorder (ADHD)(Tripp & Alsop, 1999) show reduced reward responsiveness. Reduced reward responsiveness can be an indicator of anhedonia; a symptom associated with MDD and BPD, which refers to a reduction or inability to experience pleasure (Der-Avakian & Markou, 2012). An understanding of the neural underpinnings of anhedonia may lead to better understanding of disorder that effect reward mechanisms, and may be used to make treatments for the symptom. To my knowledge, only one other study using this paradigm has shown enhancement of reward responsiveness. In this study, the effect of nicotine in non-smokers on reward responsiveness was tested with and without nicotine, revealing an increased bias in the nicotine condition (Barr et al., 2008). Curiously, and contrasting earlier studies, the control condition in this study did only induce a very small response bias. Response bias reduction has been found in some psychopathologies, in response to stress and with dopamine antagonism. It would be interesting to see whether opioid agonism could reverse the ‘reward responsiveness deficit’ in clinical populations. It would also be



## The Role of the Opioid System in Reward Responsiveness

interesting to know whether this reward responsiveness reduction can be inverted by standard pharmacological treatments for MDD, such as selective serotonin reuptake inhibitors (SSRIs).

There are still many questions left to answer in the quest for a better understanding of psychological and neural aspects of reward. Nevertheless, such knowledge may be crucial for elucidating the ontology of excessive reward seeking and consumption (or lack there-of) underlying several psychopathologies (e.g. major depressive disorder, sex-, food-, and gambling disorders). Opioid-reward directed research has already led to some successful opioid antagonist treatment studies. Grant, Suck, and Hartman (2008) used naltrexone to treat patients who suffered from pathological gambling and Drewnowski et al. (1995) found naloxone attenuation of binge eating. Naltrexone has also been shown to be successful in the treatment of alcohol dependence (Volpicelli, Alterman, Hayashida, & O'Brien, 1992).

In addition to being relevant for the understanding of reward-related psychopathology, gaining a better understanding of the mechanisms behind what drives us to act and makes us feel good has inherent value.

## Conclusion

The current study replicates findings from animal research revealing an important role for the opioid system in reward-related behaviour. Systemic manipulation of the opioidergic system modulates reward responsiveness in healthy young men. Morphine enhances, and naltrexone diminishes, this effect compared to a placebo control condition. The naltrexone condition did not eliminate the response bias, which implies that endogenous opioids are involved in, but may not be necessary, for the mediation of reward responsiveness. Although the exact neural mechanism that mediates this effect cannot be elucidated by the current experiment, findings from animal research indicate that the effect could be either dopamine mediated or directly mediated by  $\mu$ -opioids. Although little is known about the role of the human opioid system for related reward processes, findings from this study argue for a significant role for the  $\mu$ -opioid system in mediating reward responsiveness.

## References

- Bachs, L., & Waal, H. (2002). Naltrekson: klinisk bruk av en opiatantagonist – hva vet vi i dag? *Statens rettsstoksikologiske institutt, Universitetet i Oslo, RELIS Øst., Oslo: , .*
- Bachs, L., & Waal, H. (2003). Naltrexone in the treatment of addiction. *Behandling av opioidavhengige med naltrekson, 123*(12), 1665-1667.
- Baldo, B. A., & Kelley, A. E. (2007). Discrete neurochemical coding of distinguishable motivational processes: Insights from nucleus accumbens control of feeding. *Psychopharmacology, 191*(3), 439-459.
- Barbano, M. F., & Cador, M. (2007). Opioids for hedonic experience and dopamine to get ready for it. *Psychopharmacology, 191*(3), 497-506.
- Barr, R. S., Pizzagalli, D. A., Culhane, M. A., Goff, D. C., & Evins, A. E. (2008). A Single Dose of Nicotine Enhances Reward Responsiveness in Nonsmokers: Implications for Development of Dependence. *Biological Psychiatry, 63*(11), 1061-1065.
- Becerra, L., Harter, K., Gilberto Gonzalez, R., & Borsook, D. (2006). Functional magnetic resonance imaging measures of the effects of morphine on central nervous system circuitry in opioid-naive healthy volunteers. *Anesthesia and Analgesia, 103*(1), 208-216.
- Berridge, K. C. (1996). Food reward: Brain substrates of wanting and liking. *Neuroscience and Biobehavioral Reviews, 20*(1), 1-25.
- Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neuroscience & Biobehavioral Reviews, 24*(2), 173-198.
- Berridge, K. C. (2003). Pleasures of the brain. *Brain and Cognition, 52*(1), 106-128.
- Berridge, K. C. (2007). The debate over dopamine's role in reward: The case for incentive salience. *Psychopharmacology, 191*(3), 391-431.
- Berridge, K. C. (2012). From prediction error to incentive salience: Mesolimbic computation of reward motivation. *European Journal of Neuroscience, 35*(7), 1124-1143.
- Berridge, K. C., & Kringelbach, M. L. (2008). Affective neuroscience of pleasure: Reward in humans and animals. *Psychopharmacology, 199*(3), 457-480.
- Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Research Reviews, 28*(3), 309-369.
- Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: 'liking', 'wanting', and learning. *Current Opinion in Pharmacology, 9*(1), 65-73.
- Björklund, A., & Dunnett, S. B. (2007). Dopamine neuron systems in the brain: an update. *Trends in Neurosciences, 30*(5), 194-202.
- Bodnar, R. J. (2004). Endogenous opioids and feeding behavior: A 30-year historical perspective. *Peptides, 25*(4), 697-725.

## The Role of the Opioid System in Reward Responsiveness

- Boecker, H., Sprenger, T., Spilker, M. E., Henriksen, G., Koppenhoefer, M., Wagner, K. J., . . . Tolle, T. R. (2008). The runner's high: Opioidergic mechanisms in the human brain. *Cerebral Cortex*, *18*(11), 2523-2531.
- Bogdan, R., & Pizzagalli, D. A. (2006). Acute Stress Reduces Reward Responsiveness: Implications for Depression. *Biological Psychiatry*, *60*(10), 1147-1154.
- Brauer, L. H., Behm, F. M., Westman, E. C., Patel, P., & Rose, J. E. (1999). Naltrexone blockade of nicotine effects in cigarette smokers. *Psychopharmacology*, *143*(4), 339-346.
- Burkett, J. P., Spiegel, L. L., Inoue, K., Murphy, A. Z., & Young, L. J. (2011). Activation of opioid receptors in the dorsal striatum is necessary for adult social attachment in monogamous prairie voles. *Neuropsychopharmacology*, *36*(11), 2200-2210.
- Cannon, C. M., & Palmiter, R. D. (2003). Reward without Dopamine. *Journal of Neuroscience*, *23*(34), 10827-10831.
- Chefer, V. I., Denoroy, L., Zapata, A., & Shippenberg, T. S. (2009). Mu opioid receptor modulation of somatodendritic dopamine overflow: GABAergic and glutamatergic mechanisms. *European Journal of Neuroscience*, *30*(2), 272-278.
- Corbett, A. D. (2006). 75 Years of opioid research: The exciting but vain quest for the Holy Grail. *British Journal of Pharmacology*, *147*(S1), S153-S162.
- Daw, N. D. (2007). Dopamine: at the intersection of reward and action. [10.1038/nn1207-1505]. *Nat Neurosci*, *10*(12), 1505-1507.
- Der-Avakian, A., & Markou, A. (2012). The neurobiology of anhedonia and other reward-related deficits. *Trends in Neurosciences*, *35*(1), 68-77.
- Dinsmoor, J. A. (2004). THE etymology of basic concepts in the experimental analysis of behavior. *Journal of the Experimental Analysis of Behavior*, *82*(3), 311-316.
- Dreher, J. C. (2009). Variation in dopamine genes influences responsivity of the human reward system. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(2), 617-622.
- Drewnowski, A., Krahn, D. D., Demitrack, M. A., Nairn, K., & Gosnell, B. A. (1995). Naloxone, an opiate blocker, reduces the consumption of sweet high-fat foods in obese and lean female binge eaters. *The American journal of clinical nutrition*, *61*(6), 1206-1212.
- Ernst, M., Nelson, E. E., McClure, E. B., Monk, C. S., Munson, S., Eshel, N., . . . Pine, D. S. (2004). Choice selection and reward anticipation: an fMRI study. *Neuropsychologia*, *42*(12), 1585-1597.
- Flagel, S. B., Clark, J. J., Robinson, T. E., Mayo, L., Czuj, A., Willuhn, I., . . . Akil, H. (2011). A selective role for dopamine in stimulus-reward learning. *Nature*, *469*(7328), 53-59.
- Frank, M. J. (2005). Dynamic dopamine modulation in the basal ganglia: A neurocomputational account of cognitive deficits in medicated and nonmedicated Parkinsonism. *Journal of Cognitive Neuroscience*, *17*(1), 51-72.
- Frank, M. J., Seeberger, L. C., & O'Reilly, R. C. (2004). By carrot or by stick: Cognitive reinforcement learning in Parkinsonism. *Science*, *306*(5703), 1940-1943.

## The Role of the Opioid System in Reward Responsiveness

- Giovannoni, G., Van Schalkwyk, J., Fritz, V. U., & Lees, A. J. (1999). Bradykinesia akinesia inco-ordination test (BRAIN TEST): An objective computerised assessment of upper limb motor function. *Journal of Neurology Neurosurgery and Psychiatry*, *67*(5), 624-629.
- Gorwood, P. (2008). Neurobiological mechanisms of anhedonia. *Dialogues in clinical neuroscience*, *10*(3), 291.
- Grant, J. E., Suck, W. K., & Hartman, B. K. (2008). A double-blind, placebo-controlled study of the opiate antagonist naltrexone in the treatment of pathological gambling urges. *Journal of Clinical Psychiatry*, *69*(5), 783-789.
- Haber, S. N., & Knutson, B. (2010). The reward circuit: Linking primate anatomy and human imaging. *Neuropsychopharmacology*, *35*(1), 4-26.
- Harley, C. W. (2004). Norepinephrine and dopamine as learning signals. *Neural Plasticity*, *11*(3-4), 191-204.
- Heerey, E. A., Bell-Warren, K. R., & Gold, J. M. (2008). Decision-Making Impairments in the Context of Intact Reward Sensitivity in Schizophrenia. *Biological Psychiatry*, *64*(1), 62-69.
- Hnasko, T. S., Sotak, B. N., & Palmiter, R. D. (2005). Morphine reward in dopamine-deficient mice. *Nature*, *438*(7069), 854-857.
- Hollister, L. E. (1981). Aversive effects of naltrexone in subjects not dependent on opiates. *Drug and alcohol dependence*, *8*(1), 37-41.
- Iordanova, M. D. (2009). Dopaminergic modulation of appetitive and aversive predictive learning. *Reviews in the Neurosciences*, *20*(5-6).
- Janal, M. N., Colt, E. W. D., Clark, W. C., & Glusman, M. (1984). Pain sensitivity, mood and plasma endocrine levels in man following long-distance running: Effects of naloxone. *Pain*, *19*(1), 13-25.
- Jayaram-Lindström, N., Wennberg, P., Hurd, Y. L., & Franck, J. (2004). Effects of naltrexone on the subjective response to amphetamine in healthy volunteers. *Journal of Clinical Psychopharmacology*, *24*(6), 665-669.
- John, C. E., & Jones, S. R. (2007). Voltammetric characterization of the effect of monoamine uptake inhibitors and releasers on dopamine and serotonin uptake in mouse caudate-putamen and substantia nigra slices. *Neuropharmacology*, *52*(8), 1596-1605.
- Johnson, S. W., & North, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *Journal of Neuroscience*, *12*(2), 483-488.
- Kelley, A. E. (2004). Ventral striatal control of appetitive motivation: Role in ingestive behavior and reward-related learning. *Neuroscience & biobehavioral reviews*, *27*(8), 765-776.
- Kelley, A. E., & Berridge, K. C. (2002). The Neuroscience of Natural Rewards: Relevance to Addictive Drugs. *Journal of Neuroscience*, *22*(9), 3306-3311.
- Klein, S. A. (2001). Measuring, estimating, and understanding the psychometric function: A commentary. *Perception and Psychophysics*, *63*(8), 1421-1455.
- Knutson, B., & Wimmer, G. E. (2007) Splitting the difference: How does the brain code reward episodes? : *Vol. 1104* (pp. 54-69).

## The Role of the Opioid System in Reward Responsiveness

- Koepp, M. J., Hammers, A., Lawrence, A. D., Asselin, M. C., Grasby, P. M., & Bench, C. J. (2009). Evidence for endogenous opioid release in the amygdala during positive emotion. *NeuroImage*, *44*(1), 252-256.
- Koob, G. F., & Moal, M. L. (1997). Drug Abuse: Hedonic Homeostatic Dysregulation. *Science*, *278*(5335), 52-58.
- Kranz, G. S., Kasper, S., & Lanzenberger, R. (2010). Reward and the serotonergic system. *Neuroscience*, *166*(4), 1023-1035.
- Kringelbach, M. L. (2005). The human orbitofrontal cortex: linking reward to hedonic experience. *Nat Rev Neurosci*, *6*(9), 691-702.
- Kringelbach, M. L., & Berridge, K. C. (2009). Towards a functional neuroanatomy of pleasure and happiness. *Trends in Cognitive Sciences*, *13*(11), 479-487.
- Kringelbach, M. L., O'Doherty, J., Rolls, E. T., & Andrews, C. (2003). Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. *Cerebral Cortex*, *13*(10), 1064-1071.
- Langleben, D. D., Busch, E. L., O'Brien, C. P., & Elman, I. (2012). Depot naltrexone decreases rewarding properties of sugar in patients with opioid dependence. *Psychopharmacology*, *220*(3), 559-564.
- Lee, M. R., Gallen, C. L., Zhang, X., Hodgkinson, C. A., Goldman, D., Stein, E. A., & Barr, C. S. (2011). Functional polymorphism of the mu-opioid receptor gene (OPRM1) influences reinforcement learning in humans. *PLoS One*, *6*(9).
- Liu, W. H., Chan, R. C. K., Wang, L. Z., Huang, J., Cheung, E. F. C., Gong, Q. Y., & Gollan, J. K. (2011). Deficits in sustaining reward responses in subsyndromal and syndromal major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(4), 1045-1052.
- Ljungberg, T., Apicella, P., & Schultz, W. (1992). Responses of monkey dopamine neurons during learning of behavioral reactions. *Journal of Neurophysiology*, *67*(1), 145-163.
- Lugo, R. A., & Kern, S. E. (2002). Clinical pharmacokinetics of morphine. *Journal of Pain and Palliative Care Pharmacotherapy*, *16*(4), 5-18.
- Maisonneuve, I. M., Warner, L. M., & Glick, S. D. (2001). Biphasic dose-related effects of morphine on dopamine release. *Drug and alcohol dependence*, *65*(1), 55-63.
- Marsden, C. A. (2006). Dopamine: the rewarding years. *British Journal of Pharmacology*, *147*(S1), S136-S144.
- McClure, S. M., York, M. K., & Montague, P. R. (2004). The Neural Substrates of Reward Processing in Humans: The Modern Role of fMRI. *The Neuroscientist*, *10*(3), 260-268.
- Montague, P. R., Dayan, P., & Sejnowski, T. J. (1996). A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *Journal of Neuroscience*, *16*(5), 1936-1947.
- Nathan, P. J., & Bullmore, E. T. (2009). From taste hedonics to motivational drive: central  $\mu$ -opioid receptors and binge-eating behaviour. *The International Journal of Neuropsychopharmacology*, *12*(07), 995-1008.

## The Role of the Opioid System in Reward Responsiveness

- Nestler, E. J. (2005). Is there a common molecular pathway for addiction? *Nature Neuroscience*, 8(11), 1445-1449.
- Nikolova, Y., Bogdan, R., & Pizzagalli, D. A. (2012). Perception of a naturalistic stressor interacts with 5-HTTLPR/rs25531 genotype and gender to impact reward responsiveness. *Neuropsychobiology*, 65(1), 45-54.
- O'Doherty, J. P. (2004). Reward representations and reward-related learning in the human brain: Insights from neuroimaging. *Current Opinion in Neurobiology*, 14(6), 769-776.
- O'Doherty, J. P., Dayan, P., Schultz, J., Deichmann, R., Friston, K., & Dolan, R. J. (2004). Dissociable Roles of Ventral and Dorsal Striatum in Instrumental Conditioning. *Science*, 304(5669), 452-454.
- Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *The Journal of comparative and physiological psychology*, 47(6), 419.
- Oslin, D. W., Berrettini, W., Kranzler, H. R., Pettinati, H., Gelernter, J., Volpicelli, J. R., & O'Brien, C. P. (2003). A functional polymorphism of the  $\mu$ -opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. *Neuropsychopharmacology*, 28(8), 1546-1552.
- Palminteri, S., Clair, A.-H., Mallet, L., & Pessiglione, M. (in press). Similar Improvement of Reward and Punishment Learning by Serotonin Reuptake Inhibitors in Obsessive-Compulsive Disorder. *Biological Psychiatry*(0).
- Palmiter, R. D. (2008). Dopamine Signaling in the Dorsal Striatum Is Essential for Motivated Behaviors. *Annals of the New York Academy of Sciences*, 1129(1), 35-46.
- Peciña, S., & Berridge, K. C. (2000). Opioid site in nucleus accumbens shell mediates eating and hedonic 'liking' for food: Map based on microinjection Fos plumes. *Brain Research*, 863(1-2), 71-86.
- Peciña, S., & Berridge, K. C. (2005). Hedonic hot spot in nucleus accumbens shell: Where do  $\mu$ -Opioids cause increased hedonic impact of sweetness? *Journal of Neuroscience*, 25(50), 11777-11786.
- Peciña, S., Smith, K. S., & Berridge, K. C. (2006). Hedonic hot spots in the brain. *Neuroscientist*, 12(6), 500-511.
- Pessiglione, M., Seymour, B., Flandin, G., Dolan, R. J., & Frith, C. D. (2006). Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature*, 442(7106), 1042-1045.
- Petrovic, P., Pleger, B., Seymour, B., Klöppel, S., De Martino, B., Critchley, H., & Dolan, R. J. (2008). Blocking central opiate function modulates hedonic impact and anterior cingulate response to rewards and losses. *Journal of Neuroscience*, 28(42), 10509-10516.
- Pizzagalli, D. A., Evins, A. E., Schetter, E. C., Frank, M. J., Pajtas, P. E., Santesso, D. L., & Culhane, M. (2008). Single dose of a dopamine agonist impairs reinforcement learning in humans: Behavioral evidence from a laboratory-based measure of reward responsiveness. *Psychopharmacology*, 196(2), 221-232.

## The Role of the Opioid System in Reward Responsiveness

- Pizzagalli, D. A., Goetz, E., Ostacher, M., Iosifescu, D. V., & Perlis, R. H. (2008). Euthymic Patients with Bipolar Disorder Show Decreased Reward Learning in a Probabilistic Reward Task. *Biological Psychiatry*, *64*(2), 162-168.
- Pizzagalli, D. A., Holmes, A. J., Dillon, D. G., Goetz, E. L., Birk, J. L., Bogdan, R., . . . Fava, M. (2009). Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *American Journal of Psychiatry*, *166*(6), 702-710.
- Pizzagalli, D. A., Iosifescu, D., Hallett, L. A., Ratner, K. G., & Fava, M. (2008). Reduced hedonic capacity in major depressive disorder: Evidence from a probabilistic reward task. *Journal of Psychiatric Research*, *43*(1), 76-87.
- Pizzagalli, D. A., Jahn, A. L., & O'Shea, J. P. (2005). Toward an objective characterization of an anhedonic phenotype: A signal-detection approach. *Biological Psychiatry*, *57*(4), 319-327.
- Porcelli, A. J., & Delgado, M. R. (2009). Reward processing in the human brain: insights from fMRI. In J.-C. Dreher & L. Tremblay (Eds.), *Handbook of Reward and Decision Making*. USA: Academic Press.
- Ribeiro-Dasilva, M. C., Shinal, R. M., Glover, T., Williams, R. S., Staud, R., Riley Iii, J. L., & Fillingim, R. B. (2011). Evaluation of menstrual cycle effects on morphine and pentazocine analgesia. *Pain*, *152*(3), 614-622.
- Robinson, S., Sandstrom, S. M., Denenberg, V. H., & Palmiter, R. D. (2005). Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. *Behavioral Neuroscience*, *119*(1), 5-15.
- Roesch, M. R., Singh, T., Brown, P. L., Mullins, S. E., & Schoenbaum, G. (2009). Ventral Striatal Neurons Encode the Value of the Chosen Action in Rats Deciding between Differently Delayed or Sized Rewards. *The Journal of neuroscience*, *29*(42), 13365-13376.
- Rogers, R. D. (2011). The roles of dopamine and serotonin in decision making: Evidence from pharmacological experiments in humans. *Neuropsychopharmacology*, *36*(1), 114-132.
- Rolls, E. T. (2005). Taste and Related Systems in Primates Including Humans. *Chemical Senses*, *30*(suppl 1), i76-i77.
- Rolls, E. T. (2006) Brain mechanisms of emotion and decision-making. *Vol. 1291* (pp. 3-13).
- Salamone, J. D. (2007). Functions of mesolimbic dopamine: Changing concepts and shifting paradigms. *Psychopharmacology*, *191*(3), 389.
- Schuh, K. J., Walsh, S. L., & Stitzer, M. L. (1999). Onset, magnitude and duration of opioid blockade produced by buprenorphine and naltrexone in humans. *Psychopharmacology*, *145*(2), 162-174.
- Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron*, *36*(2), 241-263.
- Schultz, W. (2007). Multiple dopamine functions at different time courses. *Annual Review of Neuroscience*, *30*, 259-288.
- Schultz, W. (2010). Multiple functions of dopamine neurons. *Faculty of 1000 biology*, *2*(1).

## The Role of the Opioid System in Reward Responsiveness

- Skinner, B. F. (1938). *The behavior of organisms: an experimental analysis*. Oxford, England: Appleton-Century.
- Small, D. M., Zatorre, R. J., Dagher, A., Evans, A. C., & Jones-Gotman, M. (2001). Changes in brain activity related to eating chocolate: From pleasure to aversion. *Brain*, *124*(9), 1720-1733.
- Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: Interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *Journal of Neuroscience*, *27*(7), 1594-1605.
- Smith, K. S., Berridge, K. C., & Aldridge, J. W. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(27), E255-E264.
- Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(6), 2046-2050.
- Spreckelmeyer, K. N., Paulzen, M., Raptis, M., Baltus, T., Schaffrath, S., Van Waesberghe, J., . . . Grnder, G. (2011). Opiate-induced dopamine release is modulated by severity of alcohol dependence: An [ 18F]fallypride positron emission tomography study. *Biological Psychiatry*, *70*(8), 770-776.
- Stevenson, G. W., Bilsky, E. J., & Negus, S. S. (2006). Targeting Pain-Suppressed Behaviors in Preclinical Assays of Pain and Analgesia: Effects of Morphine on Acetic Acid-Suppressed Feeding in C57BL/6J Mice. *Journal of Pain*, *7*(6), 408-416.
- Sugam, J. A., Day, J. J., Wightman, R. M., & Carelli, R. M. (2012). Phasic nucleus accumbens dopamine encodes risk-based decision-making behavior. *Biological Psychiatry*, *71*(3), 199-205.
- Tobler, P. N., Fiorillo, C., D. , & Schultz, W. (2005). Adaptive coding of reward value by dopamine neurons. *Science*, *307*(5715), 1642-1645.
- Tripp, G., & Alsop, B. (1999). Sensitivity to Reward Frequency in Boys with Attention Deficit Hyperactivity Disorder. *Journal of clinical child and adolescent psychology*, *28*(3), 366-375.
- Vaupel, D. B., Lange, W. R., & London, E. D. (1993). Effects of verapamil on morphine-induced euphoria, analgesia and respiratory depression in humans. *Journal of Pharmacology and Experimental Therapeutics*, *267*(3), 1386-1394.
- Verebey, K., Volavka, J., Mule, S. J., & Resnick, R. B. (1976). Naltrexone: Disposition, metabolism and effects after acute and chronic dosing. *Clinical Pharmacology and Therapeutics*, *20*(3), 315-328.
- Vindenes, V., Handal, M., Ripel, Å., Boix, F., & Mørland, J. (2006). Conditioned place preference induced by morphine and morphine-6-glucuronide in mice. *Pharmacology Biochemistry and Behavior*, *85*(2), 292-297.
- Vindenes, V., Pettersen, B. S., Gottås, A., Christiansen, N. L., Boix, F., & Mørland, J. (2009). Different effects on dopamine release in nucleus accumbens in mice by the morphine



## The Role of the Opioid System in Reward Responsiveness

- metabolites morphine-6-glucuronide and morphine-3-glucuronide. *Basic and Clinical Pharmacology and Toxicology*, 105(5), 357-360.
- Volpicelli, J. R., Alterman, A. I., Hayashida, M., & O'Brien, C. P. (1992). Naltrexone in the treatment of alcohol dependence. *Archives of General Psychiatry*, 49(11), 876-880.
- Vrieze, E., Ceccarini, J., Pizzagalli, D. A., Bormans, G., Vandenbulcke, M., Demyttenaere, K., . . . Claes, S. (2011). Measuring extrastriatal dopamine release during a reward learning task. *Human Brain Mapping*.
- Wise, R. A. (1982). Neuroleptics and operant behavior: The anhedonia hypothesis. *Behavioral and Brain Sciences*, 5(01), 39-53.
- Yeomans, M. R., & Gray, R. W. (1996). Selective effects of naltrexone on food pleasantness and intake. *Physiology and Behavior*, 60(2), 439-446.
- Yeomans, M. R., & Gray, R. W. (2002). Opioid peptides and the control of human ingestive behaviour. *Neuroscience and Biobehavioral Reviews*, 26(6), 713-728.