Exploring variation of mu-opioid receptor gene and subjective opioid effects in healthy men

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Abstract

G-carriers of the functional SNP A118G of the $\mu$-opioid receptor gene OPRM1 have been associated with decreased pain sensitivity in men, and in alcoholic G-carriers a greater effect of naltrexone treatment has been found. This study investigated differences in subjective effects of $\mu$-opioid manipulation between A homozygotes and G carriers in a group of healthy males. We hypothesised that G-carriers would show higher sensitivity to naltrexone and lower sensitivity to morphine.

In a randomized double blind cross-over study, 49 healthy males received morphine (10 mg), naltrexone (50 mg) or placebo per-orally on three separate days. They completed a 21 variable questionnaire on four different time intervals (0 min, 60 min, 120 min, 150 min). Based on a principal component analysis, the variables were sorted into four components: “Lethargy”, “Wellbeing”, “Discomfort” and “Other”. The data were based on a principal component analysis scores, and run through a separate 3x3 repeated measure ANOVA for all the components.

Significant main effects of drug and time in the components ‘Lethargy’ and ‘Other’ were observed. For ‘Lethargy’, both naltrexone and morphine gave increased ratings compared to placebo, but the increase was only significant for naltrexone. No significant main effects of genotype or interaction effects between drug and genotype was observed in any of the four components.

This might be due the low doses of morphine, but may also be an indication of differences in the effect of drug when the system is disturbed, like during pain or addiction.
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Introduction

General function of the opioid system
The qualities of the juice from the opium poppy seedpod have been known for a long time in the history of men. As early as three thousand years B.C., the Samaritans cultivated the plant and isolated the opium from its pods (Brownstein, 1993). In honor of Morpheus, the god of dreams, the German pharmacist Sertrümer named his isolated opium alkaloid Morphine. Morphine stands today as the golden standard of analgesic for moderate to severe pain (Vindenes, Handal, Ripel, Boix, & Mørland, 2006). In 1942, Weijlard and Eriksen produced a drug that could reverse the hypoventilation caused by morphine and rapid withdrawal in addicts. They had produced nalorphine, the first opioid antagonist (Brownstein, 1993).
Several antagonists have followed. Among these is naltrexone, frequently offered as treatment for opiate addiction. Although naltrexone binds the opioid receptor with a higher affinity than the agonist morphine, it does not activate the receptor. It is therefore an effective way to block the receptor, inhibiting the body from responding to opiates. Naltrexone is the antagonist used for this study.

Beckett and Casy theorized the existence of a specific opiate receptor already in 1954. Differences in the modes of drug-receptor interactions caused Portoghese (1965) to theorize the existence of an opiate receptor ‘dualism’. By incubating mammalian brains in a nuclear naloxone solution, which bound to a special film that was exposed to the brain (³H-naloxone), Pert and Snyder (1973) were the first to publish a detailed study of opioid receptor binding. As can be seen on Figure 1, the dark areas contain more receptors (Source: Snyder, 1986, p. 44). Martin, Eades, Thompson, Huppler, & Gilbert (1976) finally demonstrated the existence of several opioid receptors when their study on dogs showed that the reactions to withdrawal varied depending on the opioids introduced. In their study they suggested that opioids had the ability to activate three different receptors. These receptors were named after the opioid agonists used in the study: μ for morphine, κ for ketocyclazocine and σ for N-allylnormetazocine.

Figure 1: From Snyder, 1986.
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Other substances than opiates will also activate the mu opioid receptor (MOR) through indirect routes, Colasanti et al. (2012) found that oral amphetamine administration also stimulates endogenous opioid release. Positron emission tomography (PET) has demonstrated MOR-mediated signalling in the central nervous system (CNS), and is the only method to quantitatively measure this in humans *in vivo* (Henriksen & Willoch, 2008). In a PET study Mitchell et al. (2012) concluded that alcohol consumption induced opioid release in the nucleus accumbens (NAc) and the orbitofrontal cortex. This is also found for nicotine (Ray et al., 2011).

Some PET studies have also found increased MOR activity from placebo analgesia, which indicates a shared neural network (Petrovic, Kalso, Petersson, & Ingvar, 2002). The idea of a shared neural network is supported by Zubieta et al. (2003), who found that administrated placebo with expected analgesic effects resulted in reduced pain ratings. It is therefore plausible that these placebo effects are mediated by the mu-opioid receptors, and that the expectation of analgesic effects are capable of controlling physical and emotional states.

**The opioid system of reward**

By implanting electrodes in the brains of mice and let them self administrate the electric stimulation (by pressing a lever in a Skinner box), Olds and Milner (1954) concluded that the lower centres of the brain appeared to activate rewards in the mice. In some cases the mice even preferred electric stimuli to food. These findings was replicated in both primates and humans (Bishop, Elder, & Heath, 1963; Bursten & Delgado, 1958; Heath, 1972). Over the last decades the research in affective neuroscience have presented results that support reward processing in humans and animals (Berridge & Kringelbach, 2008). Berridge has divided the reward construct into three categories (Berridge, Robinson, & Aldridge, 2009; Berridge & Robinson, 1998):

- *‘Liking’*: the positive affective response to a rewarding stimulus
- *‘Wanting’*: the motivation to obtain, ‘work for’, and approach rewarding stimuli.
- *Learning*: locating and integrating reward-relevant knowledge, useful for prediction of reward and updating information of reward events.

‘Wanting’, 'liking’ and learning may be subconscious processes, or explicitly experienced (Berridge, 2003). In the subcortical structures (see Figure 2) anatomically restricted areas,
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the so-called ‘hotspots’, have been identified. MOR activation in these areas activates ‘liking’, defined as reactions of ‘liking’ that not necessary needs to be conscious (Berridge & Kringelbach, 2008). When mu-opioid receptors in hotspots in NAc are stimulated by DAMGO injections (MOR selective agonist [D-Ala2, N-MePhe4, Gly-ol]-enkephalin), these hotspots increase ‘liking’, while the remaining areas around NAc have demonstrated to increase ‘wanting’ without a further boost of ‘liking’ when stimulated (Mahler & Berridge, 2012). Localised release of endogenous opioids is also involved in the regulation of positive emotions in humans (Koepp et al., 2009). Euphoria experienced with ‘runner's high’ was correlated with opioid receptor activation, supporting the theory of frontolimbic hotspots involvement in affective state and mood (Boecker et al., 2008).

**The link between opioids and dopamine in reward mechanisms**

The mesolimbic dopamine system is considered a part of the brain sites encoding for pleasure. Although once regarded as a pleasure-neurotransmitter, recent findings question this description of dopamine (see review Berridge & Kringelbach, 2008). Opioid and dopamine systems do interact during reward-related processes (Burkett, Spiegel, Inoue, Murphy, & Young, 2011), and opioids may modulate the amount of dopamine released in response to a certain rewarding stimulus (Nestler, 2005). Vindenes et al. (2009) found increased levels of dopamine in the NAc of rodents following a morphine injection. But dopamine is not activated by direct reward, but by the expectancy or motivation of reward (see review Salamone et al, 2007), and dopamine is also activated by stimuli that are not regarded as rewarding. By failing to be activated by direct pleasure (Ferrari, Van Erp, Tornatzky, & Miczek, 2003; Scott, Heitzeg, Koepe, Stohler, & Zubieta, 2006), it is hypothesized that dopamine plays a bigger role in motivation and prediction of reward than in direct experience of reward (Berridge & Kringelbach, 2008).
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Figure 2: Hedonic brain circuits in rodent and human brain, from Berridge and Kringelbach, 2009

Natural variation of opioid system function: OPRM1
Genetic variability in the human opioid system has received some attention the last decades (see review by Kasai & Ikeda, 2011), especially one single-nucleotide polymorphism (SNP) of the mu-opioid receptor gene (OPRM1), the A118G. This functional SNP appears to affect some aspects of human behaviour. The G allele of the A118G polymorphism has been connected to increased risk of drug abuse (Bart et al., 2004; Deb, Chakraborty, Gangopadhyay, Choudhury, & Das, 2010), alcoholism (Gavin Bart et al., 2005; van den Wildenberg et al., 2007), sex differences in pain perception (Olsen et al., 2012) and increased sensitivity for social rejection (Copeland et al., 2011).
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**Pain and opioid genetics**
The 118G SNP variant receptor binds beta-endorphins three times more tightly than the more common A118 variation, and beta-endorphins are three times more potent at the 118G than the A118 (Bond et al., 1998). Beta-endorphins are endogenous opioids found in great concentration in the reward system. A tighter binding and higher potency of the endogenous opioids may cause differences in functions mediated by beta-endorphins, such as pain perception (Bond et al., 1998). Oertel et al. (2009) demonstrated that even though the binding is tighter the signal efficacy is weaker in brain regions important to the experience of pain. These findings support previous reports that higher dosages of opioid agonists are necessary for 118G to achieve analgesia and a decreased effect of the opioid agonist (Lötsch et al., 2002; Romberg et al., 2005; Skarke, Darimont, Schmidt, Geisslinger, & Lötsch, 2003). A study investigating post-surgical pain in women found that the patients carrying the 118G consumed significantly more fentanyl than women with the more common genetic variation (Zhang et al., 2010). Another study suggests gender differences in pain perception, as female carriers of the 118G SNP reported significantly more pain 6 months after surgery than their male counterpart. In the common allele group, there was no significant difference between male and female pain perception (Olsen et al., 2012). Similar results were also found by Sia et al. (2013) who reported higher post operative pain ratings and higher morphine use in the A118G SNP group compared to the common variation in women who had a hysterectomy performed. But even in this field the results are conflicting. Kolesnikov, Gabovits, Levin, Voiko, & Veske (2011), found that the 118 G carriers with a G1947A polymorphism of the COMT-gene (catechol-O-methyl transferase) self-administrated less morphine after surgery and experienced less nausea.

**Substance abuse and opioid genetics**
Several studies claim that an increased risk of substance abuse is conferred by the 118G SNP of OPRM1 (G Bart et al., 2004; Bergen et al., 1997; Bond et al., 1998; Deb et al., 2010; Drakenberg et al., 2006; Szeto, Tang, Lee, & Stadlin, 2001). Szeto, Tang, Lee, & Stadlin (2001) found an association between opioid dependence and the 118G allele in a Chinese population, where the 118G carriers were significantly more represented in a heroin-dependent group than the controls. For a Han-Chinese population, the number of G-carriers in a population is close to 50 % (Kasai & Ikeda, 2011). The findings were, however, replicated in a Swedish population by Bart et al. (2004), where the functional 118G SNP was associated with an increased risk for heroin addiction. Bart et al. (2005) also found an increased risk for alcohol dependence in Swedish G-carriers.
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On the other hand, three meta-analyses challenge these findings. Arias, Feinn, & Kranzler (2006) conclude that the 118G do not appear to affect the risk of substance dependence. Glatt et al. (2007) found no significant association between genotype and heroin dependence in Han Chinese (N=1208) heroin addicts and Coller et al. (2009) concluded with no association between 118G and opioid dependence, based on 5000 subjects. In the same study they point at a significant heterogeneity between the studies as a possible reason for the conflicting results. Being aware of the heterogeneity issue, a more recent meta-analysis challenge these findings. By investigating the correlation between substance abuse and genotype with a dominant (A/A+A/G vs. G/G), recessive (A/A vs. A/G+G/G) and codominant (A/A vs. G/G and A/G vs. GG) model, this analysis found a significant association between the polymorphism and susceptibility to opioid dependence in the overall studies under a codominant model (Haerian & Haerian, 2013). The G allele frequency is between 15% and 25% in a Caucasian population (Crowley et al., 2003), so recruitment of participants carrying more than one is scarce.

Several studies report a stronger treatment effect of the opioid antagonist naltrexone for abstinence in alcoholics of the 118G genotype (Anton et al., 2008; Kim et al., 2009; Oslin et al., 2003). Anton et al. (2008) found that in a group of alcoholics undergoing naltrexone treatment, the 118G-carriers showed a longer abstinence from alcohol than the A118 group. They also found a significantly decreased percentage of heavy drinking days, compared with the AA-carriers (see Figure 3).

![Figure 3: Naltrexone vs placebo treatment in alcoholic AA and G-carriers, from Anton et al, 2008](image)
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These results were supported by a meta analysis claiming that alcoholic G-carriers would have a greater effect of naltrexone treatment than the A-carriers (Chamorro et al., 2012).

**Subjective effects and side effects of opioid medications**
Opioid agonists are well known for their analgesic effect; currently approximately 90% of American patients with chronic pain receive opioid agonist treatment (Manchikanti, Whitfield, & Pallone, 2005; Trescot et al., 2006). Although opioid agonists are considered the gold standard in pain relief, they are associated with a risk of addiction (Benyamin et al., 2008). Over time, treatment with opioid agonists can lead to tolerance, such that increasingly large doses are necessary to provide adequate pain relief.

There are also some side effects associated with the use of opioids, both agonists and antagonists, which may discourage compliance of the drug treatment. Opioid agonists in larger doses may cause respiratory depression, nausea and drowsiness (Benyamin et al., 2008). For all opioid agonists, a common side effect is constipation. The distress and demoralising effects of such a state can make some patients prefer pain over the opioids (Wein, 2012).

In treatment of drug abuse, both opioid agonist and antagonist are used, e.g. methadone treatment for heroin addiction and naltrexone treatment for alcoholism. Unfortunately a lot of the side effects connected with general opioid use are also an issue with these treatments. In agonist treatment methadone and buprenorphine are the most common treatment options. Alongside the side effects already mentioned, the cost of the treatment itself has been reported as a reason for noncompliance in treatment of opioid addiction (Tkacz, Severt, Cacciola, & Ruetsch, 2011). There are also strong indications of cognitive impairment as a result of long term use of methadone (Mintzer & Stitzer, 2002).

In oral naltrexone administration, patients have reported side effects like nausea, vomiting, and muscle twitches (Oncken, Van Kirk, & Kranzler, 2001), and limited compliance in oral naltrexone is a major disadvantage in treatment of opioid addiction, highly increasing the risk of overdose (Chick et al., 2000; Minozzi et al., 2006).

The research reported in this thesis is a part of a larger study assessing the role of the human opioid system for various reward behaviours, such as motivation for and hedonic experience of sweet tastes, attractive faces or monetary reward. This was explored in a placebo-controlled crossover design. Participants received oral treatment with a µ-opioid agonist (morphine, 10mg), a non-selective opioid antagonist (naltrexone, 50mg) or placebo on
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three separate days. The drug dosages were calculated to minimize subjective effects of the
drugs that might interfere with task performance in the larger study. 10 mg of morphine is
considered to be a small dose compared to similar studies (Setnik & Sokolowska, 2014;
Walker & Zacny, 1998; James P Zacny & Lichtor, 2008), and 50 mg of naltrexone is
considered a standard dosage with minor side effects (Yeomans & Gray, 2002). The timing of
testing was chosen based on peak plasma concentration, which is reached after approximately
an hour for per-oral administration for both morphine and naltrexone (Glare & Walsh, 1991;
Verebey, Volavka, Mulé, & Resnick, 1976).

Importantly, the purpose of the larger study was to investigate the effects of the opioid
system, not of the specific drugs administered to participants or the side effects of the drugs.
Therefore, subjective state measures were collected each session before drug administration
and at three time points throughout testing as a control measure in the larger study. Higher,
per-oral doses, or injected doses of morphine have previously been reported to increased
feelings of being sedated, high and stimulated (Walker & Zacny, 1998; J P Zacny, Lichtor,
Flemming, Coalson, & Thompson, 1994) and positive subjective drug effect in men (Sandra
D. Comer et al., 2009). When given higher dosage of naltrexone (100mg), healthy subjects
reported decreased desire to drink alcohol, increased feelings of discomfort, nausea,
sleepiness and dizziness compared with placebo (McCaul, Wand, Eissenberg, Rohde, &
Cheskin, 2000). We wished to avoid strong subjective effects in the larger study, because of
the risk of affecting the experience or behaviour during the execution of the reward tasks.
Some subjective effects appear to be connected to a higher risk of drug addiction. Zacny &
Lichtor (2008) define abuse liability-related subjective effect as ‘those measures that are
considered pleasant in nature and have apparent face validity in predicting abuse liability’.
Subjective effect measures that include the rating of drug liking, feelings of drug effect and
whether you want to take the drug again, are classified as abuse liability-related subjective
effects (S.D. Comer, Sullivan, Vosburg, Kowalczyk, & Houser, 2010; James P Zacny &
Drum, 2010; James P Zacny & Lichtor, 2008). High ratings of abuse liability effects have
been significantly increased in both high sensation-seeking individuals and opioid addicts,
compared to control groups (James P Zacny, 2010). Compared with placebo, abuse liability
related effects have been found in other substances which cause opioid release, like nicotine
(Lindsey et al., 2013), alcohol (Evans & Levin, 2004) and amphetamine (Wardle & de Wit,
2012).
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The research questions
In this thesis we explore the subjective effects elicited by 10 mg per-oral morphine, 50 mg naltrexone or placebo over the course of approximately 150 minutes post-administration. Subjective effects were measured through a custom made questionnaire. The subjective drug effect questionnaire was a 21-item visual analogue scale (VAS) based on previous studies investigating subjective effects of morphine, naltrexone and codeine. The adjectives describing subjective states were collected from the studies by Comer et al. (2010), Comer et al. (2002), Walker & Zacny (1998) and Zacny & Lichtor (2008). The questions regarding drug effects, drug liking and whether they wished to take the pills again were borrowed from the Drug Effect/Drug Liking/Take Again (DEL/TA) questionnaire by Zacny & Lichtor (2008).

In studies using a well-known analgesic like morphine, expectation effects are likely to occur (Petrovic et al., 2002; Zubieta et al., 2005). Placebo effect, or a positive behavioural, physiological or emotional effect based on the expectation of effect where there is none, is an important aspect of pharmacological research (“Placebo,” snl.no, 2014). If the participants expect to get a sensation of being “high” this might trigger a positive experience and a placebo effect of positive outcome on the reward tasks. In a pharmacological study nocebo also has to be accounted for (Colloca, Sigaudo, & Benedetti, 2008). Nocebo is the opposite of placebo, a negative effect based on an expectation. Nausea is previously stated as a common side effect of both morphine and naltrexone, and a feeling of sickness and discomfort can negatively affect the results of the reward tasks and the subjective effect. To control for expectation effects and nocebo, a placebo condition is also included in this experiment.

Finally we also assessed whether the 118G-allele would affect the subjective effects caused by the drugs. Based on the research indicating an association between the 118G SNP and increased effect of naltrexone in drug addiction treatment, we expect an increased sensitivity to naltrexone in the 118G group compared to the A118 group. From the research claiming a reduced effect of morphine on pain, we except to find a decreased sensitivity to morphine in the 118G group compared to the A118 group.
Methods and materials

Participants
This is a pharmacological crossover study, investigating the role of the endogenous mu-opioid system for processing reward in humans. The participants were recruited through posters at student houses, recruitment e-mails and through interested contacts. Most of the participants were students at the University of Oslo. Because of a possible interaction between opioids and varying hormonal levels at different phases of the female hormone cycle, only men were tested in this study (Ribeiro-Dasilva et al., 2011).

To participate in the study the subjects had to report no history of depression or other major psychiatric illnesses, and no current suffering from psychiatric or medical illness. None of the participants were currently on medication or had any multiple complex allergies. None of the participants reported prior drug dependence or addiction. All of the participants were morphine naïve i.e. had not taken morphine in any form for at least two years prior to testing (Becerra, Harter, Gonzalez, & Borsook, 2006). Participants reported to have normal or corrected-to-normal vision. The study was completed over two separate segments.

Part 1
In the first part, 32 subjects were tested and some results from that group has already been published (Chelnokova et al., 2014). During testing two of the participants were excluded; one participant only completed the first session and one participant tested positive on the opiate urine screening. From this first group we collected blood samples during testing, and received a signed consent with permission to analyse their blood for variants of the functional OPRM1 118 SNP. The blood samples were analysed at the National Institute of Occupational Health. Five of the 30 participants were carriers of the 118G-allele on OPRM1.

Part 2
As we wanted to investigate possible genotype differences in opioid manipulation response, we extended the study to include close to equal numbers of AA- and AG-carriers. Because of the minor frequency of the G allele variant of the OPRM1 polymorphism (Crowley et al., 2003), participants with this genetic variation were oversampled to obtain close to equal group sizes. In the second part we recruited 136 new subjects for genotype screening, collecting saliva though a self-collection kit (Oragene-DNA OG-500, DNA genotek). The
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DNA samples were analysed at the National Institute of Occupational Health. From this large group, 20 subjects (18 AG-carriers and two AA-carriers) were recruited, leaving an n of 50 subjects.

From the second part of the study one participant was excluded due to lack of attendance after the first session. Including the new participants, 49 subjects completed the study and were divided into genetic groups (mean age: AA: 26.6, SD=4.5. AG: 22.7, SD=3.17). The AA group was significantly older than the AG group (p<.001). Since drinking and drug experimenting habits are related to age and life situation (Naimi et al., 2003), the groups were matched for age for the purpose of the subjective effects analysis. The sample analysed in this thesis includes 38 participants, 19 subjects in each genetic group, mean age 23.6 (SD=2.8) with a range from 19 to 30 years old. For further demographics about the participants included in this thesis see Table 1.

Experimental procedures

Drug administration

Morphine: Morphine is a selective µ-opioid receptor agonist. For the current study we chose the pills of 10 mg of morphine (Morfin®, Nycomed Pharma), to minimize subjective drug effects. This is considered to be a small dose compared to similar studies (Setnik & Sokolowska, 2014; Walker & Zacny, 1998; James P Zacny & Lichtor, 2008). Bioavailability of oral morphine is between 30-50% and it has a half-life of 2-4 hours (Glare & Walsh, 1991). Peak plasma concentration is expected within the first hour after administration (Hoskin et al., 1989).

Naltrexone: Naltrexone is a non-selective opioid antagonist. Meta-analysis has proved naltrexone to be an efficient tool in treatment of alcoholism and opioid addiction (Johansson, Berglund, & Lindgren, 2006; Streton & Whelan, 2001). Naltrexone blocks the opioid receptors and reduces craving for alcohol and opiates. It also blocks endogenous opioids. For this study we used pills of 50 mg of naltrexone (Adepent, Orpha-Devel), which is considered a standard dosage with minor side effects (Yeomans & Gray, 2002). For naltrexone, the peak plasma concentration is expected after the first hour (Verebey et al., 1976). It has a half-life of 4 to 10 hours, with a slow terminal elimination phase half-life of 96 hours (Crabtree, 1984).

Placebo: For placebo-condition a cherry flavored breath mint was used. The portion was matched to the naltrexone and morphine condition to get a similar “mouthfeel”. The
Table 1: Demographic characteristics*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AA (n=19)</th>
<th>AG (n=19)</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>24.15 ±2.77</td>
<td>23.21 ±2.95</td>
<td>.315</td>
</tr>
<tr>
<td>Weight</td>
<td>83.61 ±13.3</td>
<td>76.57 ±7.43</td>
<td>.053</td>
</tr>
<tr>
<td>Height</td>
<td>1.85 ±0.05</td>
<td>1.82 ±0.06</td>
<td>.137</td>
</tr>
<tr>
<td>BMI</td>
<td>24.30 ±2.91</td>
<td>23.18 ±2.95</td>
<td>.253</td>
</tr>
<tr>
<td>Alcoholism in family?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 alcoholic</td>
<td>15 %</td>
<td>10 %</td>
<td>.426</td>
</tr>
<tr>
<td>2 or more alcoholics</td>
<td>15 %</td>
<td>8 %</td>
<td>.676</td>
</tr>
<tr>
<td>Total</td>
<td>42 %</td>
<td>37 %</td>
<td>.740</td>
</tr>
<tr>
<td>How often do you drink alcohol?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>0 %</td>
<td>16 %</td>
<td>.071</td>
</tr>
<tr>
<td>Monthly</td>
<td>5 %</td>
<td>11 %</td>
<td>.547</td>
</tr>
<tr>
<td>2-4 times per month</td>
<td>42 %</td>
<td>47 %</td>
<td>.744</td>
</tr>
<tr>
<td>2-3 times per week</td>
<td>47 %</td>
<td>21 %</td>
<td>.087</td>
</tr>
<tr>
<td>4 timer per week or more</td>
<td>5 %</td>
<td>5 %</td>
<td>1</td>
</tr>
<tr>
<td>How often do you drink 6 or more items?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>5 %</td>
<td>21 %</td>
<td>.150</td>
</tr>
<tr>
<td>Monthly</td>
<td>47 %</td>
<td>10 %</td>
<td>.012</td>
</tr>
<tr>
<td>2-4 times per month</td>
<td>47 %</td>
<td>57 %</td>
<td>.516</td>
</tr>
<tr>
<td>2-3 times per week</td>
<td>0 %</td>
<td>10 %</td>
<td>.146</td>
</tr>
<tr>
<td>4 timer per week or more</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
<tr>
<td>Have you ever taken:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>37 %</td>
<td>37 %</td>
<td>1</td>
</tr>
<tr>
<td>Ever</td>
<td>32 %</td>
<td>42 %</td>
<td>.501</td>
</tr>
<tr>
<td>Within the last year</td>
<td>32 %</td>
<td>21 %</td>
<td>.461</td>
</tr>
<tr>
<td>Amphetamines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>89 %</td>
<td>95 %</td>
<td>.547</td>
</tr>
<tr>
<td>Ever</td>
<td>11 %</td>
<td>5 %</td>
<td>.547</td>
</tr>
<tr>
<td>Within the last year</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
<tr>
<td>Cocaine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>89 %</td>
<td>89 %</td>
<td>1</td>
</tr>
<tr>
<td>Ever</td>
<td>11 %</td>
<td>11 %</td>
<td>1</td>
</tr>
<tr>
<td>Within the last year</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
<tr>
<td>Opiates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>100 %</td>
<td>100 %</td>
<td>1</td>
</tr>
<tr>
<td>Ever</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
<tr>
<td>Within the last year</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
<tr>
<td>Hallucinogens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>84 %</td>
<td>89 %</td>
<td>.631</td>
</tr>
<tr>
<td>Ever</td>
<td>16 %</td>
<td>0 %</td>
<td>.071</td>
</tr>
<tr>
<td>Within the last year</td>
<td>0 %</td>
<td>11 %</td>
<td>.146</td>
</tr>
<tr>
<td>Solvents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>100 %</td>
<td>100 %</td>
<td>1</td>
</tr>
<tr>
<td>Ever</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
<tr>
<td>Within the last year</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
<tr>
<td>GHB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>95 %</td>
<td>95 %</td>
<td>1</td>
</tr>
<tr>
<td>Ever</td>
<td>5 %</td>
<td>5 %</td>
<td>1</td>
</tr>
<tr>
<td>Within the last year</td>
<td>0 %</td>
<td>0 %</td>
<td>1</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± standard deviation or %.

**Calculated by T-test or chi-test, uncorrected p values
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breath mints were also included in the morphine and naltrexone condition to avoid any recognition of medication taste.

**Blinding of participants:** Task and drug administration orders were pseudo-randomized and counterbalanced between participants. At the end of the last session, participants were debriefed and asked to guess the identity of the drug received in each session. The participants were able to identify the drug correctly 34% of the time for part 1 and 32% for part 2, which indicates a successful blinding of the participants.

**Study design**
Reports of subjective effects of pharmacological manipulation on mood, hedonic capacity and other possible drug related effects were collected. These were a part of a battery of tasks from a double-blind, placebo-controlled psychopharmacological crossover study that investigated the role of the endogenous opioid system in the processing reward and motivation in humans. The battery included:

- A task measuring hedonic evaluation of, and motivation for, receiving soft brush strokes on the forearm
- A task measuring hedonic evaluation of, and motivation for, looking at faces of varying attractiveness levels
- A reward responsiveness task
- A social decision making game
- A task measuring perceived sweetness and palatability/pleasantness of sweet sucrose solutions

A control measure of motor-coordination test was performed halfway through each session. The data on the subjective effects were collected at four different time points during each session; before drug administration, before testing, during testing and at the end of the session. After each session a blood sample was collected.

The subjective drug effects questionnaire was administered four times each session, at 0 min, 60 min, ~90 min and ~ 120 min after drug ingestion. The first was received before drug administration the second an hour after drug administration, the third halfway through the reward tasks and the last after the motivation/reward tasks were completed. This did not vary across the sessions (see Figure 4 for an overview of a typical session). The test interval was calculated to coincide with the peak plasma concentrations for both morphine and
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naltrexone, which are reached around an hour after per oral drug administration, and still is quite high 1-2 hours after peak levels.

The participants were tested on three different days with a minimum interval of seven days between every session. Each session lasted approximately three hours and the participants were compensated 400-500 NOK per session, depending on their performance in a monetary reward task carried out as a part of the test battery.

**General Instructions**

The experimental procedures were approved by the Regional Ethics Committee (2011/1337/REK sør-øst D).

**Prior to testing:** Prior to the first session all the participants received an informational email about the collection of urine and blood sample. The participants were also instructed to sustain from eating an hour before testing, avoid eating foods containing poppy seeds at least 3 days before testing (Fritch & Prescott, 1985; Moeller, Hammer, & Engel, 2004) and advised to not drive a vehicle for at least 6 hours after the testing. They were also given information about possible side effects of the drugs, that they would receive all of the drugs during the 3 sessions and that the study had a double-blind design (neither the participants or the experimenter knew which drug was received at any certain session). The same information was given in the consent form at the first session, which had to be signed by both participant and experimenter before starting the session.

**Testing:** After the written consent was signed, the participants were asked to submit a urine sample. The sample was screened with an opiate sensitive test strip (MOP Opiate300 Test Strip; SureScreen Diagnostics Ltd), and a negative result had to be obtained to continue
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testing. In the first session the participants went through a PowerPoint presentation with the
experimenter. This presentation briefed the participants on the different tasks they would go
through in the next hours. At the following sessions, they were offered to go through this
presentation again if they needed a reminder. Before the drug administration they had to
complete the first subjective drug effect-questionnaire and a hedonic capacity questionnaire
(Snaith-Hamilton Pleasure Scale, SHAPS, Snaith et al., 1995). After completing the
questionnaires participants received one of the three drugs. Regardless of the drug condition
participants were always given the pills in a small black box, so they were unable to see the
pills inside. They were resolutely told to not inspect the drugs visually, and to swallow the
pills in one gulp. Afterwards they were offered a glass of water.

After the drug administration, participants were asked to rest in a room for an hour. They were not allowed to use their phone or bring their own reading materials, but were offered to watch either a nature documentary or read a selection of magazines. During the resting period the experimenter would check on them at least three times. When the resting time was over the participants went through the subjective drug effect- and SHAPS questionnaires again, before moving over to the battery of reward tasks. Midway through the reward tasks the subjective drug effect- and SHAPS responses were collected again, and then the last time before ending the testing. After the testing, trained experimenters drew a blood sample, and biscuits, tea or coffee was offered. In the last session a short debriefing was conducted with questions regarding participation and experienced drug effects.

**Measure of subjective drug effects**
The subjective drug effect-questionnaire is a 21-item visual analogue scale (VAS) based on
previous studies looking at subjective effects of morphine, codeine and naltrexone. The
adjectives describing subjective states were collected from the studies by Comer et al., (2002),
Comer, Sullivan, Vosburg, Kowalczyk, & Houser (2010), Walker & Zacny (1998) and Zacny & Lichtor (2008). The questions regarding drug effects, drug liking and whether they wished to take the pills again were adapted from the Drug Effect/Drug Liking/Take Again (DEL/TA) questionnaire by Zacny & Lichtor (2008). The questionnaire was administrated on a computer
*Table 2* shows the list of items in the questionnaire.
Table 2: Items in Subjective drug effect questionnaire with abbreviations.

<table>
<thead>
<tr>
<th>The 21 items in the Subjective drug effect-questionnaire</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ‘Right now I feel high (pharmaceutical high)’</td>
<td>‘high’</td>
</tr>
<tr>
<td>2 ‘Right now I feel spaced out’</td>
<td>‘spaced out’</td>
</tr>
<tr>
<td>3 ‘Right now I feel dizzy’</td>
<td>‘dizzy’</td>
</tr>
<tr>
<td>4 ‘Right now I feel dull’</td>
<td>‘dull’</td>
</tr>
<tr>
<td>5 ‘Right now I feel numb’</td>
<td>‘numb’</td>
</tr>
<tr>
<td>6 ‘Right now I don’t feel like myself’</td>
<td>‘don’t feel like’</td>
</tr>
<tr>
<td>7 ‘Right now I feel tired’</td>
<td>‘tired’</td>
</tr>
<tr>
<td>8 ‘Right now I feel nauseous’</td>
<td>‘nauseous’</td>
</tr>
<tr>
<td>9 ‘Right now I feel irritable’</td>
<td>‘irritable’</td>
</tr>
<tr>
<td>10 ‘Right now I feel happy’</td>
<td>‘happy’</td>
</tr>
<tr>
<td>11 ‘Right now I feel good’</td>
<td>‘good’</td>
</tr>
<tr>
<td>12 ‘Right now I feel confident’</td>
<td>‘confident’</td>
</tr>
<tr>
<td>13 ‘Right now I feel discomfort in muscles and joints’</td>
<td>‘discomfort in’</td>
</tr>
<tr>
<td>14 ‘Right now I feel hungry’</td>
<td>‘hungry’</td>
</tr>
<tr>
<td>15 ‘Right now I feel red/warm in my face’</td>
<td>‘red/warm in’</td>
</tr>
<tr>
<td>16 ‘Right now I feel dry in my mouth’</td>
<td>‘dry in mouth’</td>
</tr>
<tr>
<td>17 ‘Right now I feel anxious’</td>
<td>‘anxious’</td>
</tr>
<tr>
<td>18 ‘Do you feel an effect of the tablets?’</td>
<td>‘effect’</td>
</tr>
<tr>
<td>19 ‘Do you like the effect of the tablets?’</td>
<td>‘liking’</td>
</tr>
<tr>
<td>20 ‘Do you dislike the effect of the tablets?’</td>
<td>‘disliking’</td>
</tr>
<tr>
<td>21 ‘How much do you agree on the following statement? I would take these tablets again on a later occasion’</td>
<td>‘take again’</td>
</tr>
</tbody>
</table>

The task was operated on two different computers; the first two blocks were conducted on a 15” PC monitor with 1600x1200 pixels, the two last blocks on a 20” PC monitor, also 1600x1200 pixels. Due to the drug administration the first part had to be conducted in a separate lab. Each of the 21 items was presented on the screen with a VAS ranging form ‘not at all’ to ‘very much’. Participants were then asked to drag a vertical mark, using a computer mouse, on each VAS to indicate how much they agreed with the statement of a given item at that exact time (see Figure 5). Then they where asked to press “next” and a new statement would appear on the screen.
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Data analysis

In previous studies investigating subjective effects of drugs (S.D. Comer et al., 2010; Walker & Zacny, 1998; James P Zacny & Lichtor, 2008) most report how the average ratings on each item vary with drug dose and type.

Part 1
Our data was baseline corrected, by subtracting the score from the first block (before the participants had received any drugs) with each value in the other blocks. As can be seen from Figure 6, there are great differences between the items, but few items with great variation within the item itself. Gathering items that cluster together in a principal component analysis (PCA) may shed light on variables that were not observed within the single item.

![Figure 5: example of screen-shot from the questionnaire](image)

Figure 5: example of screen-shot from the questionnaire

![Figure 6: baseline corrected means, 30 first participants.](image)

Figure 6: baseline corrected means, 30 first participants.
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The baseline corrected scores from the 21 items on time points 2-4 were analysed with an explorative PCA using an oblique rotation. The Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis, KMO=.784. An initial analysis was run to obtain eigenvalues for each component in the data, which yielded 6 components with an eigenvalue over Kaiser’s criterion of 1, and together these 6 explained 64.826% of the variance. A parallel analysis was then conducted, comparing the eigenvalues obtained in the initial factor analysis with those from 100 randomly generated datasets sharing the same characteristics with the original data sets. Four of the initial eigenvalues exceeded the corresponding eigenvalues from the parallel analysis, and this is the number of components that were retained in the final analysis. Items that cluster on the same components suggest a principal component, and the four principal component identified were: Lethargy, Wellbeing, Discomfort and Other (see Table 3).

Table 3: Principal components identified from component loadings.

<table>
<thead>
<tr>
<th>Lethargy</th>
<th>Wellbeing</th>
<th>Discomfort</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘high’</td>
<td>.855</td>
<td>.736</td>
<td>.781 ‘hungry’</td>
</tr>
<tr>
<td>‘spaced out’</td>
<td>.854</td>
<td>.734</td>
<td>.695 ‘red/warm’</td>
</tr>
<tr>
<td>‘effect’</td>
<td>.722</td>
<td>.700</td>
<td>.497</td>
</tr>
<tr>
<td>‘don’t feel like’</td>
<td>.759</td>
<td>.678</td>
<td>.407</td>
</tr>
<tr>
<td>‘dizzy’</td>
<td>.675</td>
<td>.453</td>
<td>.393</td>
</tr>
<tr>
<td>‘tired’</td>
<td>.608</td>
<td>-.440</td>
<td></td>
</tr>
<tr>
<td>‘numb’</td>
<td>.502</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘dry in mouth’</td>
<td>.452</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The principal components were then analysed for drug and time effects in a 3 (drug: morphine, placebo and naltrexone) x3 (time points) repeated measure analysis of variance (rmANOVA).

**Part 2**

In order to explore whether these effects would interact with genotype, a weighted average was calculated for all the datapoints in the second part of this larger study. The weighted average is calculated by adding the principal component loading for the item with the item score (see formula).

$$\bar{x} = \sum x_i$$
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In addition, rmANOVAs were conducted for each of the four components. For the four items related to abuse liability (drug liking, drug disliking, effect of drug and ‘want to take the drug again’), rmANOVAs was executed for each item. Demographic variables and effect size was calculated with PAWS statistics 18.0 (SPSS). To estimate the power of our results, the power analytic program G*Power was operated (Faul, Erdfelder, Lang, & Buchner, 2007)

Results

Part 1
For the first 30 participants, we observed significant main effects of drug and time on components labeled ‘Lethargy’ and ‘Other’ only. For ‘Lethargy’, both naltrexone and morphine gave increased ratings compared to placebo, but this was only significant for naltrexone treatment (Drug main effect: $F(2, 28) = 8.372, p=.001$, naltrexone > placebo $p=.001$, naltrexone > morphine $p=.004$, morphine > placebo $p=.139$), see Figure 7a. For the component ‘Other’, both naltrexone and morphine treatment gave significantly lower ratings than the placebo (Drug: $p=.007$, N<P $p=.007$, M<P $p=.034$), see Figure 7d. Neither morphine nor naltrexone treatment significantly affected ‘Wellbeing’ although there was a weak pattern in the expected direction, i.e. increased ‘Wellbeing’ with morphine and decreased with naltrexone. See Figure 7b

‘Lethargy’ decreased linearly over time with placebo, whilst ratings peaked mid-session with both naltrexone and morphine, before decreasing significantly at the end of the session (Main effect Time: $F(2, 28) = 14.222, p=.000$), see Figure 8a. Ratings on ‘Other’ increased with time over all, mostly due to increases in hunger (Time: $F(1.513, 28.487) = 3.565, p=.049$). See Figure 8d.
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Figure 7: A) main effect of drug in Lethargy. Significant increase for naltrexone treatment compared to morphine ($p=.004$) and placebo ($p=.001$). B) Drug effect in Wellbeing. C) Drug effect in Discomfort. D) Drug effect Other. Significant effect of naltrexone ($p=.007$) and morphine ($p=.034$) compared to placebo.
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Figure 8: A) Drug and time effect of Lethargy, significant decrease in both morphine and naltrexone at the end of the session ($p=.000$). B) Drug and time effect on Wellbeing. C) Drug and time effect of Discomfort. D) Drug and time effect of Other, overall effect of time ($p=.049$).
Part 2

To test whether genotype affected the subjective experience of the drugs, we analyzed the ratings from each of the three sessions. A 2 (genotype: AA-carriers vs. AG-carriers) x 3 (drug: morphine, placebo and naltrexone) repeated measure analysis of variance (ANOVA) was conducted for each of the four components. For Lethargy, no drug and genotype interaction was found, $F(2,72) = .931, p = .399$. For the other components, no significant drug/genotype interaction was identified. Wellbeing, $F(2,72) = .505, p = .606$; Discomfort, $F(2,72) = .316, p = .730$; Other, $F(2,72) = .081, p = .922$. See Figure 9.

The drug effect differs at different time points during the testing. This could result from differences in drug uptake or differences in drug action at the receptor, but to assess if there are any possible differences in the subjective effects as a factor of time after drug administration a 2 (genotype: AA-carriers vs. AG-carriers) x 3 (drug: morphine, placebo and naltrexone) x 3 (Time point: block 1, block 2 and block 3) repeated measure ANOVA was conducted. The interaction effect of drug, block and genotype factors was not significant: Lethargy, $F(4,144) = 1.170, p = .327$; Wellbeing, $F(4,144) = 1.394, p = .239$; Discomfort, $F(4,144) = 1.101, p = .358$; Other, $F(4,144) = 1.193, p = .317$. See Figure 10.
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- **Figure 9**: A, B, C and D: Interaction effect of drug and genotype, no significant drug/genotype interaction was identified.
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Figure 10: A, B, C and D: interaction effect of drug, block and genotype, no significant interaction was found.
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To test whether genotype specifically affected the abuse liability effect scores in the different drug conditions, we analyzed the rating from the abuse liability items in the VAS-scale for each of the three sessions. A 2 (genotype: AA-carriers vs. AG-carriers) x 3 (drug: morphine, placebo and naltrexone) repeated measure ANOVA was conducted for each of the four items. For drug and genotype interaction no significant effect was found: Effect: $F(2,72) = .937, p=.397$; Liking: $F(2,72) = .524, p=.584$; Disliking: $F(2,72) = .326, p=.723$; Take again: $F(2,72) = .859, p=.428$. See Appendix 1.

As with the components, we also assessed whether the abuse liability scores would differ at different time points during the testing. A 2 (genotype: AA-carriers vs. AG-carriers) x 3 (drug: morphine, placebo and naltrexone) x 3 (Time point: block 1, block 2 and block 3) repeated measure ANOVA was conducted for each of the items. The interaction effect of drug, block and genotype was not significant: Effect: $F(4,144) = .593, p=.668$; Liking: $F(4,144) = .861, p=.489$; Disliking: $F(4,144) = 1.316, p=.267$; Take again: $F(4,144) =1.460, p=.218$. See Appendix 2.

**Discussion**

This study took aim to examine the subjective effects caused by 10 mg per-oral morphine, 50 mg naltrexone or placebo over the course of 150 minutes post-administration. We also wished to assess whether the A118G polymorphism would affect the subjective effects caused by drugs. Morphine and naltrexone both led to increased scores of ‘Lethargy’, naltrexone significantly more so than morphine. Both drugs supressed feelings of hunger, as indicated by drug effects on ‘Other’, which is reasonable due to the extent of the session. Interestingly, no main effects of drug or drug/time interaction effects were found on ‘Wellbeing’, which contained all the abuse liability related-items. The results reported here also demonstrate that there was no significant difference between the different carriers of the A118G polymorphism in subjective experience of either naltrexone or morphine, in any of the four components. Also no significant difference was found between the two genetic groups concerning the items labelled as ‘abuse liability related’.

From the research claiming a reduced effect of morphine in pain (Sia et al., 2013; Zhang et al., 2010) we expected to find a decreased sensitivity to subjective effects of morphine in the 118G group compared to the A118 group. These results were not supported in this thesis.
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Based on previous findings indicating an association between the 118G SNP and increased effect of naltrexone in drug addiction treatment (Anton et al., 2008; Kim et al., 2009; Oslin et al., 2003), we expected an increased sensitivity to subjective effects of naltrexone in the 118G group compared to the A118 group. Our results did not support this effect.

Except for a significant increase in the Lethargy component in the naltrexone condition, and the increased sensation of hunger in the naltrexone and morphine condition after 3 hours of testing, few strong subjective drug effects were reported. The subjective effect of morphine has in previous studies been defined as a sedative drug effect, but several previous studies have operated with higher doses with oral morphine. To receive an increased experience of a euphoric “high” other opiates might give a higher score. In a study comparing the subjective effects of morphine and oxycodone, the morphine scored higher on opiate drug effects like ‘dry mouth’ and ‘flushing’, while oxycodone scored higher on abuse liability related effects (James P Zacny & Lichtor, 2008).

The blinding of the participants was considered successful with a percentage of correct guessing which time they received which drug between 34 - 32%. It is natural to think that the participants easily could mistake placebo for morphine and got an opioid-based placebo effect. Although the participants were opioid naïve, morphine would be the drug they had most experience with and most expectation towards (most of the participants had never heard about naltrexone before they participated in the study). A common statement when debriefed and asked to guess the identity of the drugs morphine was often based on the belief that morphine is connected to a feeling of sedation and comfort, and which day they had felt most sedated. This does not explain why they could not identify the naltrexone though. The overall correct guesses of naltrexone alone were only 22%.

One explanation for the lack of interaction between the genetic groups may be the dosage size of morphine. For the larger study we wanted to keep the dosage as low as possible to archive successful blinding of the participants and minimize the subjective drug effects. Similar studies have been operating with either similar dosage given intramuscularly, which will increase the overall bioavailability to 100% (Pöyhiä, Seppälä, Olkkola, & Kalso, 1992), or a considerably larger dose of 20-60mg (Walker & Zacny, 1998; James P Zacny & Lichtor, 2008). So what about the naltrexone condition? In this condition we did use a common dose, because of the reports of few subjective effects from this dose. Still, 50mg of naltrexone is a big enough dose to block the MOR, but no effect of genotype was reported from our study.
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To increase the dose of naltrexone may create a bigger gap between the genetic groups as done by other studies, like Anton et al. (2008) which gave their subjects 100mg of naltrexone.

To our knowledge, no previous studies have investigated subjective drug effects in A118G polymorphism in a healthy population. In our study, all of the participants reported no history of mental illness or drug dependence. Such disturbances of the opioid system might interact with genetic variation. Haerian & Haerian (2013) concludes that risk of addiction may be partly explained by a genetic – epigenetic interaction, and not genetics alone. Cox (2013) points to the complexity of the expression of the MOR gene, with environmental, genetic and epigenetic regulation factors. This is also supported in studies that have found an effect of genotype. In these studies participants have often either belonged to a clinical group, like psychiatric patients (Troisi et al., 2011, 2012), substance abusers (Bergen et al., 1997; Deb et al., 2010; R Ray et al., 2006) or a group with a difficult social bonding with close relatives, like the study by Copeland et al. (2011) where the effect of the 118G only was found in children who had a stressful relationship with their parents. In future research it would be interesting to include a clinical group in a similar study, to investigate possible epigenetic effects on the phenotype of the A118G polymorphism.

A recent meta-analysis by Haerian & Haerian (2013) found a significant association between substance abuse and the homozygote 118G polymorphism when the subjects were analysed as three separate groups, an association not found by previous meta-analyses excluding this factor (Arias et al., 2006; Coller et al., 2009). A reoccurring issue in the research of the A118G polymorphism is the infrequency of recruiting subjects that are homozygote 118G carries. According the International HapMap project, the homozygote frequency of the 188G allele in Caucasians is <3% (Gibbs, Belmont, Hardenbol, Willis, & Fuli, 2003). Although some studies look at the three options of the A118G (AA, AG and GG) as individual groups, the more common solution to this issue is to combine the heterozygote and the homozygote carriers of 118G into one group. In our study this choice did not become a concern, as none of our 118G carriers were homozygote.

Limitations
While we used a selective MOR agonist, the antagonist naltrexone was a non-selective opioid antagonist that also binds to κ- and to some extent δ-opioid receptors (Gerra, Fantoma, & Zaimovic, 2006). There are no viable oral options for blocking μ-opioids selectively as of today. Nevertheless, naltrexone has high affinity to μ-receptors (Gerra et al., 2006), and the
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dosage used in the current study is likely to have had a complete, or very high blockade of this receptor (Weerts et al., 2008). μ-opioid involvement is further supported by the opposite effects we have observed on social reward by agonist and antagonist treatment (Chelnokova et al., 2014).

The subjective drug effect questionnaire was originally developed as a control measure for the larger study. This might be viewed as a limitation for this thesis, as the questionnaire not is optimized to look at genetic differences in subjective drug effects. A recent study concludes that the field would benefit from a demonstration of validity of different subjective drug effect measures (Morean et al., 2013). Such a demonstration would also benefit future research exploring drug effect in a genetic group.

Conclusions
In this study, we reported that our participants experienced a significant effect of both naltrexone and morphine in the “Lethargy” component. We found no significant effect of genotype in subjective drug effect ratings, and no significant genotype effect in the “abuse liability” related effects. The absence of genotype effect may be a result of too small a dosage, but also of the fact that we only investigated heterozygote 118G allele carriers. The lack of effect of the opioid agonist, overall, may also be caused by the chosen drug, morphine, which report few subjective effects that are connected to the ‘Wellbeing’ or ‘Lethargy’ components.
Exploring variation of mu-opioid receptor gene and subjective opioid effects in healthy men

References:


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Lindsey, K. P., Bracken, B. K., Maclean, R. R., Ryan, E. T., Lukas, S. E., & Frederick, B. D. (2013). Nicotine content and abstinence state have different effects on subjective ratings


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Appendix 1: Abuse liability related items, drug and genotype interaction. A, B, C and D: no significant interaction between genotype and drug.
Appendix 2: Abuse liability related items, drug, block and genotype interaction. A, B, C and D: no significant interaction between genotype, block and drug