Enhanced negative response to stress following morphine administration increases wanting of social reward

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Abstract

Animal research suggests a central role of the μ-opioid receptor (MOR) system in mediating contact seeking and the stress-buffering function of social touch. However, the human neurochemistry of social motivation in aversive situations is still poorly understood.

In a randomized, double-blind, between-subject design, healthy female volunteers (N = 80) received either 10 mg of the μ-opioid agonist morphine sulfate or a placebo. Following psychosocial stress induction, participants engaged in a social reward task, in which the motivation (subjective ratings of wanting and physical effort) to obtain skin-to-skin social touch and the hedonic reactions (subjective ratings of liking and facial electromyography) elicited by it were assessed.

Morphine administration prevented the increase of salivary cortisol, usually observed in response to acute stress exposure. The dampened physiological reaction to the psychosocial stress was associated with increased negative mood and subsequent higher subjective wanting of the most pleasurable touch. Furthermore, participants administered with morphine displayed greater activity of the corrugator muscle during reward anticipation, possibly tracking enhanced attention toward the social stimuli.

Overall, the results provide novel evidence on the effect of exogenous opioids administration on the reactions to psychosocial stress and point to a state-dependent regulation of social motivation.

Introduction

Social behaviors such as bonding and affiliation are crucial for the survival and wellbeing of many species. By providing fundamental benefits (e.g., promoting safety and enhancing stress resilience) and by generating comfort and pleasure, social stimuli (e.g., social contact) gain rewarding value, inducing approach motivation. Inability to form and maintain social bonds contributes to a range of psychiatric and physical pathologies (e.g., Cacioppo & Cacioppo, 2014; Uchino, 2006), therefore highlighting the importance to better understand the neurobiological basis of social motivation.

The Brain Opioid Theory of Social Attachment (BOTSA), originally inspired by the analogies between opioid addiction and social dependence, pinpoint the μ-opioid receptor (MOR) system as a key mediator of bonding and affiliation (Panksepp, 1998; Panksepp et al., 1980). Specifically, BOTSA predicts that social isolation results in reduced levels of basal opioid levels, which in turn motivate social contact seeking, and that rewarding social interactions elicit release of endogenous opioids, associated with euphoria and contentment. The two-fold role of the endogenous opioid system in reward and pain (Leknes & Tracey, 2008) therefore seems to apply also to experiences of social nature. In contexts of comfort, the activity of the MOR system during social contact mediates the rewarding properties, and associated pleasure, of social stimuli, positively reinforcing exploration and affiliation. In contexts of distress, the release of endogenous opioids via social contact reduces pain and negative affect, negatively reinforcing social behaviors (Loseth et al., 2014).

In line with this model, animal studies have shown that μ-opioids are released following physical social contact, such as grooming and playing (Keverne et al., 1989; Panksepp & Bishop, 1981; Vanderschuren et al., 1995). Pharmacological studies on separation-distress in animals further

confirmed BOTSA predictions, showing that administration of MOR agonists reduces, and of MOR antagonists increases distress responses and motivation for social contact, such as grooming (Keverne et al., 1989; Martel et al., 1995; Panksepp et al., 1980; Fabre-Nys et al., 1982; Meller et al., 1980; for a review see Machin & Dunbar, 2011; see Loseth et al., 2014 for a model accounting for divergent findings in different species).

In the last decade, preliminary confirmatory evidence on the μ-opioid mediation of the rewarding properties of social stimuli in humans have been provided. These studies mainly focused on two fundamental components: wanting, namely the motivational drive to obtain the social stimulus, and liking, namely the pleasure elicited once it is received. Results showed that pharmacological challenge of the MOR system affects wanting and/or liking of different kind of social stimuli, such as erotic pictures (Buchel et al., 2018), attractive faces (Chelnokova et al., 2014), and social touch (Korb, Götzendorfer, et al., 2020; but see also Løseth et al., 2019). MOR activation has been also associated with social laughter (Manninen et al., 2017), and MOR blockade was shown to reduce feelings of social connection (Inagaki et al., 2015, 2016, 2020), as well as of interpersonal closeness and social reward expectation (Tchalova & MacDonald, 2020). Using PET, Hsu et al. (2013) observed MOR activation in response to both social rejection and social acceptance. In the first case, MOR activity was correlated with reduced negative affect, while in the latter it was associated with increased desire for social interaction. These findings are consistent with a "protective" and an "affiliative" role of the MOR system during social distress and social comfort, respectively. Regarding the role of the MOR system in stress regulation, a recent study showed that MOR agonist administration reduces individuals' physiological stress response (as measured by cortisol), as well as the perceived difficulty of the stress task (Bershad et al., 2018). However,

89 direct evidence of an involvement of the MOR system in downregulating stress and how this
90 affects social contact seeking, as shown in non-human animal species, is still lacking.

91 Here, we aimed at filling this knowledge gap by investigating the effect of MOR agonist
92 administration (morphine) on social motivation and social pleasure following stress exposure.
93 Using a double-blind, placebo-controlled, randomized, between-subjects design, female
94 participants (N = 80) were orally administered with either 10 mg morphine sulfate (a highly
95 selective μ-opioid agonist) or placebo. Following a psychosocial stress induction procedure, the
96 motivation to obtain social touch (wanting) and the pleasure elicited by receiving it (liking) were
97 assessed (see Figure 1 for a detailed description). In order to enhance the translational value of the
98 study, the following steps were taken: i) as in previous animal research, which entailed social
99 separation, a stressor of social nature (via the Trier Social Stress Test, Kirschbaum et al., 1993)
100 was employed; ii) in addition to self-reports of wanting and liking, we assessed real physical effort
101 (via a hand-dynamometer) and hedonic facial reactions (via facial electromyography of corrugator
102 and zygomaticus muscles), approximating the motivational and hedonic (i.e., wanting and liking)
103 measures used in animal studies; iii) to parallel grooming in animals, skin-to-skin touch
104 administered at different speeds (6, 21, 27 cm/s, corresponding to high, low, and very low reward
105 levels) was employed as a social reward.

106 Based on the existing human and animal literature, we hypothesized that participants administered
107 with morphine, compared to placebo, would show reduced physiological and subjective responses
108 to stress, and that this would result in decreased social motivation. As previous studies had shown
109 an effect of stress on wanting rather than on liking of touch (Massaccesi et al., 2021) or food (e.g.
110 Pool et al., 2015) reward, we did not expect changes in the hedonic reactions to social contact.
111 Given that MOR manipulations have been shown to induce the strongest effects on the best reward

option available (Chelnokova et al., 2014; Eikemo et al., 2016), and considering the stress buffering function of C tactile (CT)-optimal touch (i.e., touch with stroking speed of 1 to 10 cm/s; Morrison, 2016), we expected the predicted effects to be stronger for the most valuable social reward (touch at 6 cm/s).

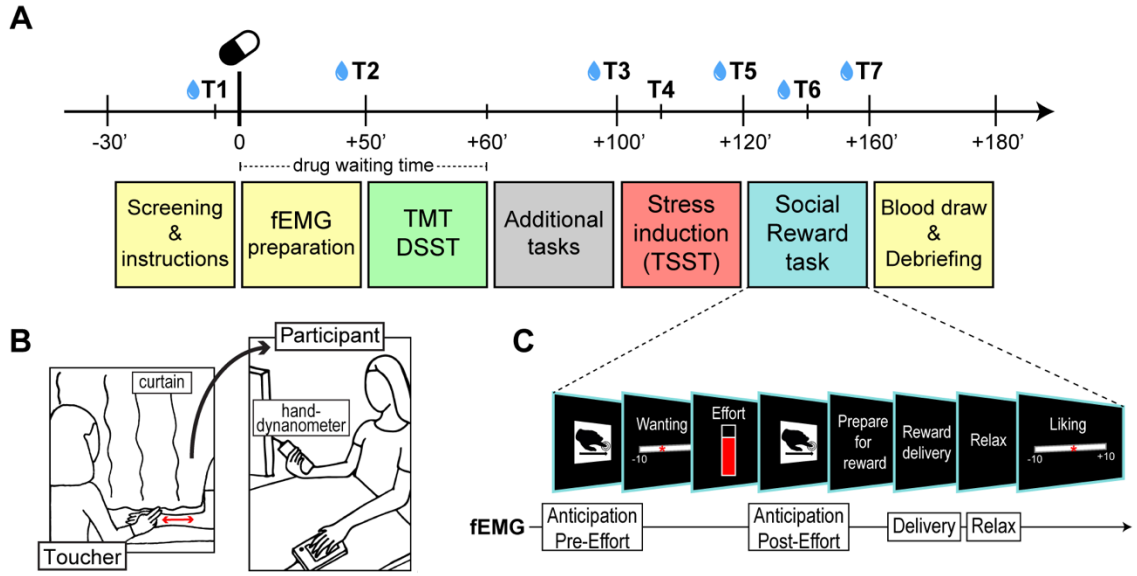


Figure 1. **Overview of the experimental session, set-up, and trial structure of the Social Reward task.** (A) Overview of the experimental session. T1 – T7 represent the time points at which subjective and/or physiological measures were obtained. Blue drops indicate saliva sample collection. (B) Set-up of the Social Reward task (based on Korb, Götzendorfer, et al., 2020; Massaccesi et al., 2021). Participants were seated in front of the monitor, holding the hand-dynamometer in the right hand. The left arm was rested on a cushion, next to a keyboard used to express judgements during the task. The toucher was seated on the other side of a curtain used to limit the participants' field of view to the monitor. Touch was administered on the participants' left forearm using the index and middle finger, at 3 stroking speeds corresponding to 3 levels of reward (high = 6 cm/s, low = 21 cm/s, very low = 27 cm/s). (C) Trial structure of the Social Reward task: i) announcement of the best attainable social reward (high or low); ii) rating of subjective wanting; iii) effort task requiring to squeeze a hand-dynamometer to obtain the announced reward. Participants' force exerted was indicated in real-time by a vertical bar filling in red. The top of the displayed vertical bar corresponded to their previously measured maximum voluntary contraction (MVC); iv) announcement of the reward attained (high, low, or – if insufficient effort had been exerted – very low); v) skin-to-skin touch administration; vi) relax phase; vii) rating of subjective liking. Facial electromyography (fEMG) was recorded during the whole task and analyzed in reward anticipation (Anticipation Pre-Effort and Post-Effort) and consumption (Delivery and Relax). TMT, Trail Making Test; DSST, Digit Symbol Substitution Test; TSST, Trier Social Stress Test.

Results

Drug blinding

After completing the session, 50% of the participants correctly guessed they received morphine, indicating successful blinding. Overall, 55% of the total sample believed to have been administered with placebo, 29% with morphine, and 16% with naltrexone[1].

Effects of morphine on cognitive functions and drug side-effects

There were no significant differences in the Digit Symbol Substitution Test (DSST) and Trail Making Test (TMT) scores across groups, indicating that drug administration did not have negative effects on attention, psychomotor and processing speed, and visuo-perceptual functions (See Table S1 in *Supplementary Material*). Morphine administration significantly increased self-reported weakness (MORPH vs. PLB at T2: $t(69.9) = 2.64$, $p = 0.01$, and at T7: $t(55.96) = 2.19$, $p = 0.03$) and dry mouth (MORPH vs. PLB at T7: $t(57.70) = 2.56$, $p = 0.01$). For all side-effect measures, group average scores were generally low, and no side-effect was on average rated as moderate or strong (see Figure S2 in *Supplementary Material*).

Effects of morphine on stress response

Subjective measures

Morphine administration resulted in significantly higher scores of the Profile of Mood States (POMS) subscale "Displeasure" after stress induction (Drug*Time: $F(4,312) = 2.97$, FDR $p = 0.03$; MORPH vs. PLB at T5: FDR $p < 0.01$) (Figure 2A). A similar pattern, though not reaching statistical significance, was observed for negative mood (Drug*Time: $F(6,468) = 2.16$, FDR $p = 0.068$) (Figure 2A). No significant group differences were observed for positive mood or for the

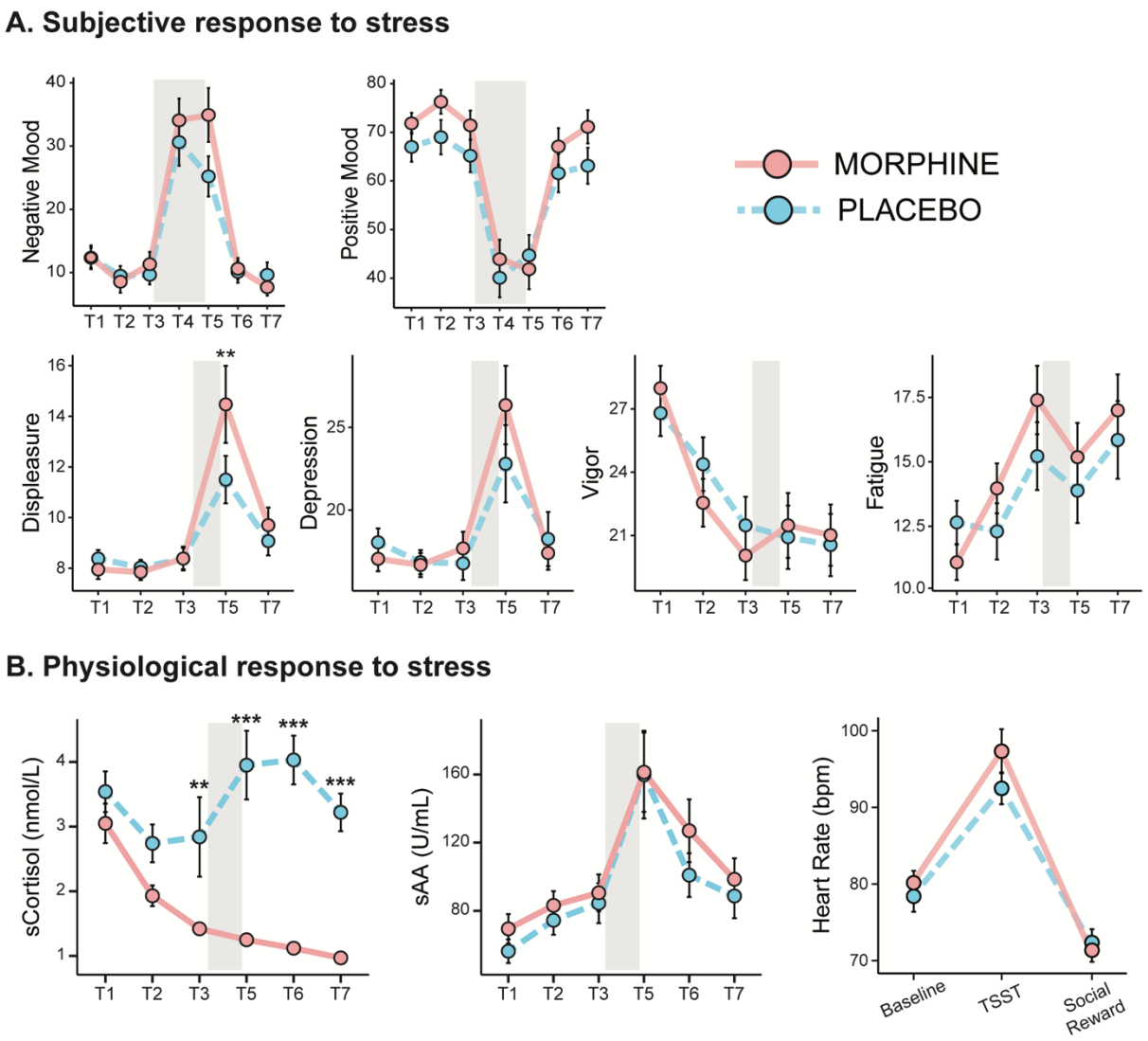
[1] To reduce drug-related expectancy, participants were told they might receive an opioid agonist (morphine), antagonist (naltrexone) or placebo (but they could receive only morphine or placebo).

158 POMS subscales “Depression”, “Vigor”, and “Fatigue” (all FDR $p > .15$). Furthermore, no drug
159 effects were observed in anticipatory stress (PASA primary and secondary appraisal at T4; both t
160 < 0.60, $p > 0.55$), nor in the participants’ satisfaction towards their performance expressed after
161 TSST completion (T5; $t(75.2) = -0.63$, $p = 0.53$).

162 ***Physiological measures***

163 As physiological stress biomarkers, salivary cortisol (hypothalamic–pituitary–adrenal [HPA] axis
164 activity), salivary alpha-amylase and heart rate (autonomic nervous system [ANS] activity) had
165 been assessed at different time-points during the session (see Figure 1).

166 Morphine administration suppressed cortisol response to the TSST (Drug*Time: $F(5,375) = 7.68$,
167 FDR $p < 0.001$, Figure 2B). Specifically, the morphine group showed reduced salivary cortisol
168 compared to the placebo group at T2 (FDR $p = 0.079$), T3 (FDR $p < 0.01$), T5 (FDR $p < 0.001$),
169 T6 (FDR $p < 0.001$) and T7 (FDR $p < 0.001$) (Figure 2A). No drug effects were observed in
170 salivary alpha-amylase (all $F < 0.38$, all FDR $p > 0.62$) or in heart rate (all $F < 2.83$, all FDR $p >$
171 0.09) (Figure 2B).



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173 *Figure 2.* **Effects of morphine administration on subjective and physiological stress**
174 **responses.** (A) Morphine administration increased the subjective negative response to stress, as
175 shown by elevated negative mood (measured via visual analog scales) and higher scores at the
176 "Displeasure" subscale (POMS) immediately after the Trier Social Stress Test (TSST). No
177 significant drug effects were observed in positive mood or in any other POMS subscales
178 (depression, vigor, fatigue). (B) Morphine administration suppressed the hypothalamic–pituitary–
179 adrenal (HPA) axis activity, as shown by blunted salivary cortisol before and after stress induction.
180 No significant drug effects were observed in the autonomic nervous system (ANS) response to
181 stress, assessed via salivary alpha-amylase (sAA) and heart rate. Grey bars represent the TSST
182 time window (anticipation, speech, and arithmetic task); error bars represent standard error of the
183 mean; asterisks indicate significant differences between drug groups (* p <.05, *** p < .001).

Correlations between physiological and subjective measures of stress

Given the observed opposite effect of the drug on cortisol and mood, we conducted a correlation analysis to investigate the association between physiological and subjective measures of stress. To this aim, we first computed the area under the curve in respect to increase ((AUCi, from T3 to T7; Pruessner et al., 2003) of the cortisol levels and of the negative mood ratings (VAS and POMS). Results showed a negative correlation between salivary cortisol and negative mood (VAS; r_s = -.36, *p bonferroni* = .048) as well as between cortisol and the Displeasure subscale of POMS (r_s = -.37, *p bonferroni* = .044), suggesting an inverse relationship between HPA axis and mood responses to stress.

Effects of morphine on social reward processing

Using a recently developed Social Reward task (Korb, Götzendorfer, et al., 2020; Massaccesi et al., 2021), we assessed subjective wanting and liking of social reward in different levels. Participants were announced with either high (6 cm/s, CT-optimal touch[2]) or low (21 cm/s, non-CT-optimal touch) social reward and rated their wanting of the stimulus. Based on an effort task, they could then receive the announced or the very low (27 cm/s, non-CT-optimal touch) social reward and had to rate their liking of the received stimulus.

We first assessed whether the number of high, low and very low rewards obtained during the task was similar for the two groups. A linear model on number of trials was fitted, including Drug (MORPH, PLB) and Reward Level (high, low, and very low). We observed only a main effect of Reward Level ($F(2,369.5) = 11.28$, $p < 0.001$), indicating that participants obtained more often high rewards as compared to low and very low rewards. We also tested for group differences in

[2] Touch administered at a velocity falling into the range of 1 to 10 cm/s activates CT afferents, a special class of nerve fibers found in human hairy skin involved in signaling the affective aspects of social touch (Morrison et al., 2010), and was shown to elicit greater pleasure, compared to touch administered at faster velocities.

the average maximum voluntary contraction (MVC), assessed before and after the Social Reward task and used to calibrate the effort task. No significant differences emerged (all $t < -0.84$, all $p > 0.40$), indicating no drug effects on participants' grip force at rest. Last, we tested for group differences in the baseline activity of the corrugator and zygomaticus muscles. No significant differences emerged (all $t < 1.27$, all $p > 0.21$).

Effects of morphine on subjective ratings of wanting and liking

To investigate the effects of morphine administration on subjective wanting and liking of social touch under stress, we fitted two linear mixed-effects models (LMM) on the ratings of wanting and liking, including Drug (MORPH, PLB) and Reward Level (high, low for wanting ratings, and high, low, very low for liking ratings) as fixed effects, and by-subject random intercepts and slopes for Reward Level.

Ratings of Wanting. Participants administered morphine expressed significantly greater wanting of the high social reward (CT-optimal touch) compared to the placebo group (Drug*Reward Level: $F(1,78) = 10.56$, FDR $p = 0.003$; high social reward MORPH vs. PLB: FDR $p = 0.035$) (Figure 3A). No drug effects emerged for the low social reward (FDR $p = 0.72$).

Ratings of Liking. Participants in both groups expressed greater liking for high compared to low social rewards, which in turn were more liked than the very low social rewards (Reward Level: $F(2,72.9) = 20.93$, FDR $p < 0.001$; high vs. low vs. very low: all FDR $p < 0.001$). Further, participants administered morphine generally liked the interpersonal touch (across all reward levels) more compared to the placebo group, but the effect did not reach significance (Drug: $F(1,78) = 4.23$, FDR $p = 0.065$) (Figure 3B).

Effects of morphine on force exerted and hedonic facial reactions

We further assessed wanting of social rewards in terms of force exerted to obtain the tactile stimuli, as well as hedonic facial reactions during reward anticipation (Anticipation Pre- and Post-Effort phases). Hedonic facial reactions during and after consumption (Delivery and Relax phases) of the social reward were employed as a measure of liking. Hedonic facial reactions were assessed via facial electromyography (fEMG) of the corrugator (frowning) and zygomaticus (smiling) muscles.

Force exerted. Participants overall exerted greater force to obtain the high compared to the low social reward (Reward Level: $F(1,78) = 14.23$, FDR $p < 0.001$). We also observed greater difference in terms of force exerted for the high compared to the low reward in the morphine than in the placebo group, but the effect did not reach significance (Drug*Reward Level interaction: $F(1,78) = 4.13$, FDR $p = 0.068$) (Figure 3C).

Corrugator (frowning). During the announcement of the highest attainable social reward (Anticipation Pre-Effort), we observed greater corrugator activity following morphine administration, compared to placebo (Drug: $F(1,791.3) = 6.15$, FDR $p = 0.036$) (Figure 3D). Opposite to previous evidence (e.g., Korb, Götzendorfer, et al., 2020; Korb, Massaccesi, et al., 2020; Ree et al., 2020), in this phase greater frowning was associated with greater subjective wanting in both groups (Wanting: $F(1,1203) = 7.94$, FDR $p = 0.036$) (Figure 3D). No significant effects were observed in other phases of the task.

Zygomaticus (smiling). During the announcement of the obtained social reward (Anticipation Post-Effort), activity of the zygomaticus was positively associated with subjective wanting of the social reward (Wanting: $F(1,73.2) = 10.31$, FDR $p < 0.01$) (Figure 3E). No significant effects of drug were observed in the activity of this muscle.

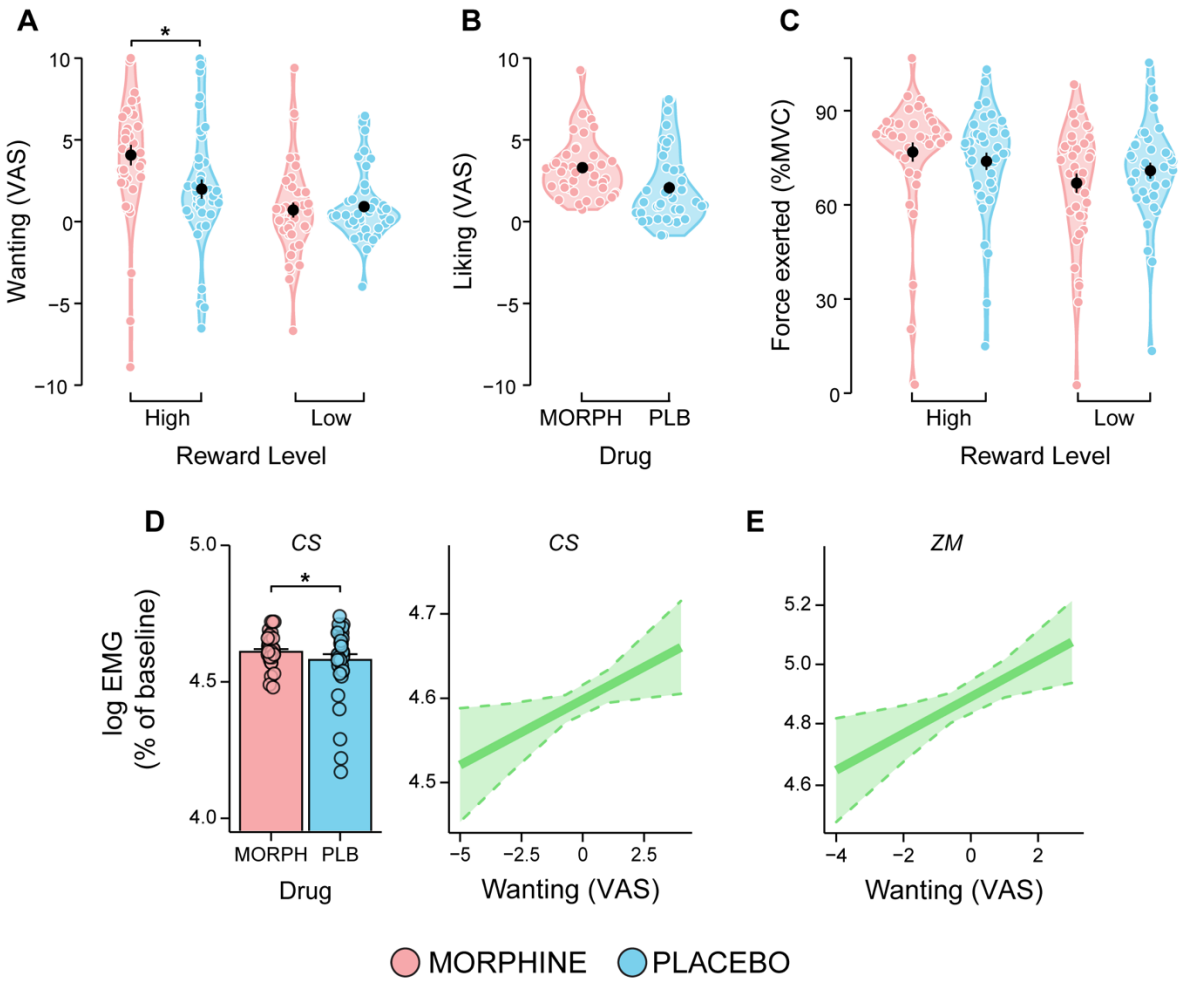

Figure 3. **Effects of morphine administration on social reward processing after stress exposure.** Morphine administration resulted in (A) significantly greater wanting for the high social reward (6 cm/s, CT-optimal touch), (B) greater liking of the social stimuli regardless of reward level (marginally significant drug main effect), and (C) greater differentiation of the high and low reward levels in terms of effort exerted (marginally significant drug by reward level interaction effect), compared to placebo. Regarding facial hedonic reactions, (D) during the announcement of the highest attainable reward (Anticipation Pre-Effort), the morphine group showed greater corrugator (CS) activity, which was generally positively associated with subjective wanting. (E) During the announcement of the gained reward (Anticipation Post-Effort), greater zygomaticus (ZM) activity was associated with greater subjective wanting, but no significant drug effects were observed for this muscle. Error bars represent standard error of the mean; black dots represent group means; colored dots represent individual means; asterisks indicate significant differences between conditions (* $p < .05$). VAS, Visual Analogue Scale; MORPH, morphine group; PLB, placebo group.

Discussion

Social motivation is a powerful force guiding behavior, as social rewards (e.g., social contact, bonding, affiliation) are fundamental to the individual's physical and psychological well-being. Despite the important role of social contact in stress resilience, the neurochemical mechanisms underlying social contact seeking following stress exposure in humans are still poorly understood. In this study, we pharmacologically challenged the μ-opioid receptor (MOR) system to investigate its role in the regulation of the motivational and hedonic components of social reward processing following stress induction. To parallel previous animal research, participants were exposed to a stressor of social nature and interpersonal touch was used as a social reward. Further, force exerted to obtain the reward as well as hedonic facial reactions during its anticipation and consumption were assessed, together with subjective ratings of wanting and liking. Following the enhancement of the MOR system activity via administration of its agonist morphine, we observed suppression of the HPA axis activity (as indicated by a reduced cortisol response) and increased negative affect in response to psychosocial stress. Notably, this increased negative response to stress following morphine administration was associated with enhanced motivation for the most pleasurable social reward.

μ-opioid agonist morphine dampens cortisol and increases negative affect in response to stress

In this study, single administration of the μ-opioid agonist morphine prior to TSST exposure resulted in blunted salivary cortisol response, indicating suppression of the HPA axis reactivity to stress. This is in line with previous animal and human evidence indicating an inhibitory role of μ-opioids on cortisol release following stress exposure (Pechnick, 1993). Recently, two studies investigated the effects of partial (buprenorphine) and full (hydromorphone) MOR agonists on psychosocial stress, induced via TSST (Bershad et al., 2016, 2018). Akin to the present findings,

reduced cortisol responses to stress were observed. Buprenorphine and hydromorphone also reduced the perceived threat and the appraisal of how challenging the participants found the stress task, respectively. While the authors interpret the findings on cortisol and stress appraisal as indicators of a reduced stress response, no mood-buffering effects of the drug were observed. This is in contrast with the current study, where we find that the dampened cortisol response is accompanied by an enhanced aversive reaction to stress, as shown by higher negative affect following morphine compared to placebo administration. Human cortisol permeates the blood-brain barrier to feedback the central nervous system, reducing HPA axis activity and promoting emotion regulation (Het et al., 2012; McEwen, 1998). The observed inverse relationship between the HPA axis activity and negative affect possibly indicates a disruption of this feedback loop by morphine administration and is in line with a mood-buffering effect of cortisol (Het et al., 2012; Het & Wolf, 2007). Accordingly, it was recently shown that pharmacological HPA axis suppression, by means of dexamethasone administration, blunts the cortisol response to stress and increases negative mood, especially in women (Ali et al., 2017, 2020).

Our results are opposite to our *a priori* hypothesis based on previous evidence from animal studies on separation distress indicating a reduction of stress indices, such as distress vocalizations, following MOR agonist administration (Panksepp et al., 1980). The paradoxical morphine effect of enhanced aversive stress reaction observed in the current study vs. the previous animal literature may be explained by experimental differences, such as route and timing of administration of the opioid compounds. For instance, in animal research morphine is typically delivered intravenously after stress induction, possibly allowing the system to prepare to face the stressor via an initial mounting of the physiological stress response. In this study, morphine was administered orally to minimize the invasiveness of the administration procedure. Unlike intravenous administration,

per-oral morphine has a slow pharmacokinetic profile and requires an average time of 60 min to reach the peak blood concentration. As the subjective response to acute stress, especially its effect on mood, typically lasts only for short periods of time after laboratory stress induction, orally administering the drug and waiting for it to reach peak concentration after the TSST would not have been feasible. For these reasons here the compound was administered prior to stress exposure. However, the resulting suppression of the HPA axis activity before the beginning of the stress induction may have led to the observed difference in the mood response. Accordingly, the raising of cortisol levels, via cortisol administration, prior to stress induction has been shown to have a protective role in women, lowering the negative subjective reaction to stress (Het & Wolf, 2007).

Morphine-induced increased negative reaction to stress is associated with enhanced social motivation

BOTSA posits that when facing stressors, individuals seek physical social contact with others in order to down-regulate the negative state and re-store comfort, via endogenous release of beta-endorphins, unless opioids are externally provided. Contrary to our initial hypotheses, morphine increased, rather than reduced, participants' negative reactions to the TSST, and such increased stress responses were accompanied by enhanced social motivation. Specifically, we observed greater subjective wanting of the most pleasurable social reward (CT-optimal touch) following morphine administration, compared to placebo. This enhanced subjective wanting was also accompanied by greater differentiation of the high and low social rewards (CT-optimal and non-CT-optimal touch) in terms of effort exerted to obtain them in the morphine group compared to placebo, but the effect did not reach statistical significance.

This pattern of results is consistent with previous findings from our group indicating that, as already shown for other kinds of reward like food and money (e.g. Kumar et al., 2014; Lewis et al., 2014; Pool et al., 2015), stress increases wanting of rewards of social nature, such as

interpersonal touch (Massaccesi et al., 2021). Differently from the present findings, in Massaccesi et al. (2021) the effect of enhanced wanting for the social reward was independent from reward magnitude. In other words, participants under stress expressed higher wanting ratings for all types of social touch compared to non-stressed individuals, regardless of reward magnitude. Here we observed an effect of morphine and stress on the most pleasurable touch only (high reward level), characterized by slow stroking velocity (CT-optimal touch).

This potentiation of wanting for the most pleasurable touch may be due to a specific influence of the enhanced MOR system activity. Indeed, previous research has shown that pharmacological challenges of the MOR system have strongest effects on rewards of greatest magnitude (e.g., Chelnokova et al., 2014; Eikemo et al., 2016). Therefore, it can be hypothesized that while stress alone may result in increased motivation for all available rewarding stimuli, an additional potentiation of motivation for the most valuable stimulus is seen for the best reward due to MOR stimulation. This would align well with the observation that participants administered with morphine also exerted more effort in order to obtain the high reward, as compared to the low reward, and that this difference in force exerted was greater than in the placebo group (but with FDR $p = 0.068$ did not reach the statistical significance). Alternatively, a reason for the partially different result pattern might be that, differently from the current study, in Massaccesi et al. (2021) participants were allowed to individually rank the three types of touch as high, low and very low. The high reward was therefore not necessarily the CT-optimal touch. This could have masked a possible impact of the type of touch. While Massaccesi et al. 2021 included different state manipulations, this study focused on the effects of stress. Since slow CT-optimal touch is more likely to convey affiliative intentions such as social support compared to faster touch (Kirsch et

al., 2017), here the slow CT-optimal touch was kept as fixed high reward to avoid possible confounding effects related to the speed of stroking.

During reward anticipation (announcement of the best attainable reward), the increased subjective wanting of social touch in the morphine group was accompanied by greater activity of the corrugator muscle. Previous research indicates that individuals relax the corrugator during anticipation and consumption of rewarding stimuli, showing an inverse relationship between corrugator activity and reward wanting/liking (Korb, Götzendorfer, et al., 2020; Korb, Massaccesi, et al., 2020; Massaccesi et al., 2021; Mayo et al., 2018; Ree et al., 2020; Sato et al., 2020). Here, in contrast, we observed greater frowning for most wanted stimuli. Activity of the corrugator is commonly associated with emotional processing, but it has been shown to be related also to processes without emotional content, such as mental and physical effort, alertness, perceptual attention, task engagement and difficulty (Cacioppo et al., 1985; Cohen et al., 1992; de Morree & Marcora, 2010; Van Boxtel & Jessurun, 1993; Waterink & van Boxtel, 1994). As individuals are more attentive to highly rewarding stimuli (e.g. Hickey et al., 2010; Hickey & van Zoest, 2012; Theeuwes & Belopolsky, 2012) and, since morphine - at a similar dose - has already been shown to enhance attentional processes such as set-shifting (Quednow et al., 2008), it is possible that the observed pattern in the corrugator activity reflects enhanced attention/preparation towards the most wanted stimuli. Together with the higher subjective of wanting, the greater saliency of the anticipated social rewards, reflected in greater frowning, suggests an enhancement of social motivation following stress exposure in the morphine group, compared to placebo.

Based on previous evidence, we predicted that the effects of morphine administration on social touch processing under stress would be specific for the motivational component, without affecting the hedonic aspect. Accordingly, we did not observe a significant effect of drug on the ratings of

liking or in the facial hedonic reactions during the reward consumption phase. However, we nevertheless observed that the ratings of liking were generally higher in the morphine group compared to the placebo group (FDR $p = 0.065$), and we therefore refrain to exclude an effect of the manipulation on social pleasure.

Study limitations

Some limitations of the study should be considered. First, while the use of a within-subject design is usually preferable in pharmacology, in this study we employed a between-subjects design. The choice was mainly motivated by the fact that repeated exposure to the TSST, especially with a short time interval, can lead to habituation of the stress response, resulting in low test re-test reliability (e.g., Gerra et al., 2001; Petrowski et al., 2012) and thus outweighing by far the possibly higher statistical power a within-subject design might have had in principle. Second, despite our efforts in enhancing the social properties of the administered touch (e.g., skin-to-skin administration), the absence of a social relationship between the toucher and the participant, as well as other methodological aspects (e.g., use of a curtain separating participant and toucher), might have affected our results. Nevertheless, in humans an involvement of the MOR system in bond formation, rather than just maintenance, has also been observed (Tchalova & MacDonald, 2020). Third, the current study tested a sample of healthy female participants, therefore a generalization to male individuals is not possible, especially given the known sex differences in stress reactivity and MOR system functioning. Finally, it is unlikely that a complex behavior such as social motivation can be fully explained by the activity of a single neurochemical system only. For instance, the neuropeptides oxytocin and vasopressin are well-known for their crucial role in bonding and reproductive functions (Donaldson & Young, 2008). Stress exposure induces a potent activation of the dopaminergic system (Bloomfield et al., 2019; Holly & Miczek, 2016), and it has

been shown that MOR stimulation disinhibits dopaminergic neurons in the ventral tegmental area and increases dopamine release in the nucleus accumbens (Pan, 1998). To investigate how these systems interact in driving social contact seeking during aversive states seems therefore necessary to understand the neurobiology of social motivation.

Conclusions

To conclude, our findings show that enhancing μ-opioid signaling before psychosocial stress exposure increases, rather than reduces, the aversive reaction to stress, leading to an increased subjective motivation for the attainment of highly rewarding social contact. Specifically, we showed that morphine administration blunted cortisol reactivity to stress and increased the negativity of the adverse experience, in line with a mood-buffering effect of cortisol. Further, the results indicate that this enhanced morphine-induced stress response was followed by increased motivation to attain social rewards, both in terms of subjective wanting as well as of facial reactions during reward anticipation.

Overall, the results provide novel evidence on the modulation of the physiological and subjective responses to social stress by the MOR system and indicate a state-dependent regulation of social motivation. To better understand the role of μ-opioids in the potentiation of social motivation during aversive states, further investigation of the effects of MOR agonists and antagonists administered after stress induction, as well as of the interaction with other neurochemical systems, is needed.

Materials & Methods

Study design

The between-subject, double-blind, placebo-controlled study consisted of one experimental session in which participants received either 10 mg morphine sulfate or a placebo (60 mg mannitol).

Participants

Based on previous work that had investigated the effects of stress on social reward processing (Massaccesi et al., 2021) and the effects of similar compounds on stress responses (Bershad et al., 2018), we aimed at collecting data from 40 participants per group. The study sample included 82 participants, of which 42 received morphine (MORPH) and 40 received a placebo (PLB). Two participants (MORPH) did not complete the session and were therefore not included in data analysis, yielding to a final sample size of 80 participants (40/group)[3]. Only female participants were included due to i) gender differences in opioid pharmacokinetics (Fillingim & Gear, 2004; Zubieta et al., 1999) and stress response (Kelly et al., 2008), ii) expected higher preference of same-sex touch in females than males (Stier & Hall, 1984; Suvilehto et al., 2015). Participants were tested during the luteal phase of their menstrual cycle, as assessed by self-report. All participants reported to be right-handed, to smoke less than ten cigarettes per week, to have no history of current or former drug abuse, to have a BMI between 17 and 35, and to be free of psychiatric or neurological disorders. Other exclusion criteria were: single or repeated use of any strong opioids in the last two years, use of hormonal contraceptives, regular intake of medications,

[3] Due to technical problems, EMG data from 6 (4 MORPH) participants and HR data from 1 participant (MORPH) were not recorded. Saliva samples from one participant (MORPH) are also missing.

current pregnancy or breastfeeding, suffering from impaired respiratory functions, respiratory weakness or lung disease, injury/disease of the arms (making it impossible to squeeze with the right hand and to be caressed on the left forearm). Participants were instructed to refrain from eating, brushing their teeth and consuming caffeinated beverages, juices, and chewing gum in the two hours preceding the test, as well as from smoking, doing physical activity and intaking alcohol and medications in the 24 hours preceding the test. The two experimental groups did not differ significantly in terms of age, BMI, autistic traits (short version of the German Autism Spectrum Quotient, AQ-k; Freitag et al., 2007), general (State-Trate Anxiety Inventory, STAI; Spielberger et al., 1970) and social (Liebowitz Social Anxiety Scale, LSAS; Heimberg et al., 1999) anxiety, and social touch appreciation (Social Touch Questionnaire, STQ; Wilhelm et al., 2001) (Table 1). The study was approved by the Ethics Committee of the Medical University of Vienna (EK 1393/2017) and was performed in line with the Declaration of Helsinki (World Medical Association, 2013). All participants signed a consent form before taking part in the study.

Table 1. Demographic and self-reported substance use characteristics of the participants.

	MORPHINE	PLACEBO	*p* value
N	40	40	---
Age (years)	23.08 (3.08)	23.88 (3.86)	0.31
BMI (kg/m^2)	21.37 (3.38)	22.26 (2.66)	0.19
Autism (AQ-k)	6.45 (3.81)	5.65 (3.58)	0.34
Anxiety (STAI)	38.13 (9.43)	35.73 (8.27)	0.23
Social Anxiety (LSAS)	36.73 (17.87)	33.18 (19.02)	0.39
Social Touch Preferences (STQ)	25.33 (9.53)	27.10 (7.67)	0.36
Drug use (% lifetime – last year)			
Cannabis	47.5 – 25	60 – 32.5	---
Tranquilizers	7.5 – 5	5 – 2.5	---
Stimulants	25 – 10	17.5 – 7.5	---
Opiates	7.5 – 0	2.5 – 0	---
Hallucinogens	12.5 – 2.5	12.5 – 2.5	---

Other	5 – 2.5	2.5 – 0	---

Procedure

The study was conducted at the Department of Psychiatry and Psychotherapy of the Medical University of Vienna. After completing an online survey to assess their eligibility, potential participants were first invited to a health screening (~45'), including blood examination, electrocardiogram, blood pressure measurement, and psychiatric interview (Mini-International Neuropsychiatric Interview; Sheehan et al., 1998). Eligible participants were then invited to the experimental session (~210'), which always started between 11:30 and 12:30 in order to control for cortisol diurnal fluctuations (Labuschagne et al., 2019). At the beginning of the session, urine drug and pregnancy tests were administered. After baseline mood and physiological measures were collected, participants received a standardized snack, and took the assigned capsule. Throughout the session, mood and physiological measures were obtained at regular intervals. The experimental tasks were completed between 60 and 160 min after drug administration, and included economic decision making, facial mimicry, emotion recognition, stress induction and social reward processing (see Fig.1A for an overview of the session timeline). Here we will focus on the last two, while the others will be reported elsewhere. Approximately 180 min. after pill administration, a blood sample was taken to confirm drug uptake (see Table S2 of the *Supplementary Material*). After completing the experimental session, participants were debriefed and received a financial compensation of 65€. Half of the sample was tested before and half during the COVID-19 pandemic (see *Supplementary Material* for details regarding the employed safety measures and additional statistical analyses to assess possible effects of the pandemic and safety measures on the results).

Drug administration

Ten mg of morphine sulfate (Morapid®) were administered per oral to stimulate the activity of the MOR system with minimal subjective effects. Morphine is a selective MOR agonist and, for oral administration, has an average bioavailability of 30–40 %, a maximal effect (t-max) at 1–2 h after administration, and a half-life of 2–4 h (Lugo & Kern, 2002). Placebo consisted of capsules containing 650 mg of mannitol (sugar), visually identical to the ones containing morphine.

Stress induction

In order to induce a stress reaction in the participants, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) was employed. In the TSST, participants were given 3 minutes to prepare a 5-minute speech for a mock-job interview, followed by a 5-minute arithmetic task, in which they were asked to count backwards from 2043 in steps of 17 as fast and as accurate as possible. The speech and arithmetic tasks were completed in front of an evaluating panel (one male and one female confederates). Participants were told that these tasks would be video recorded via a camera located next to the examiners (no video was actually taped).

Social Reward task

Stimuli

Gentle caresses in three different speeds (CT-optimal: 6 cm/s, non-CT-optimal: 21 cm/s and 27 cm/s) were used as social rewards of different level of pleasantness (high, low, very low). Pleasantness of touch is high between 1 and 10 cm/s, and decreases between 10 and 30 cm/s (Löken et al., 2009) and CT-optimal touch has been shown to have a stress buffering effects, reducing arousal (Pawling et al., 2017) and distress following social exclusion (von Mohr et al., 2018), and increasing heart rate variability (Triscoli et al., 2017). The suitability of these stroking speeds has been confirmed in three previous independent studies from our group (Korb,

Götzendorfer, et al., 2020; Korb, Massaccesi, et al., 2020; Massaccesi et al., 2021). Caressed were delivered over a previously-marked nine–cm area on participants' left forearm (measurement started from the wrist towards the elbow) by a female experimenter, moving her index and middle fingers back and forth in the marked area of the forearm (Figure 1B). Touch delivery was guided by auditory rhythms, which matched the frequency of the stimulation, over headphones. The experimenter administering the touch was seated on the other side of a curtain, used to limit the participants' field of view to the monitor (Figure 1B). All experimenters were presented as trained masseurs, wore standardized clothes (white t-shirt and beige trousers) to minimize differences in their appearance, and underwent extensive training on the tactile stimulation delivery.

Task

The Social Reward task (Korb, Götzendorfer, et al., 2020; Massaccesi et al., 2021) consisted of two blocks of 16 trials, separated by a 5-minute break. To avoid habituation to the touch, the site of application (left or right area of the forearm) was alternated within the two blocks, in a counter-balanced order. Before starting the task, participants experienced each type of touch once. Each trial was structured as follow (see Figure 1C): i) announcement of the best attainable reward (high or low, 16 trials each, 3s); ii) rating of subjective wanting via a VAS ranging from -10 (not at all) to +10 (very much) (no time limit); iii) effort task (4s), requiring to squeeze a hand-dynamometer (HD-BTA, Vernier Software & Technology, USA) with the right hand, in order to obtain the announced reward – the applied force, displayed via an online visual-feedback, was expressed as percentage of the participants' MVC, measured immediately before the task, and translated into the probability of obtaining the announced reward (0–100%); iv) announcement of the reward obtained (high, low, or – if insufficient force had been exerted – very low; 2s); v) reward delivery

(6s); vi) relax phase (5s); vii) rating of subjective liking via a VAS ranging from -10 (not at all) to +10 (very much) (no time limit). At the end of the task, participants' MVC was measured again.

The task was implemented in MATLAB 2014b (MathWorks, Inc) and presented on an LCD monitor with a resolution of 1280 × 1024 pixels.

Facial EMG

Facial EMG was recorded throughout the Social Reward task, using a g.USBamp amplifier (g.tec Medical Engineering GmbH) and the software Matlab (MathWorks, Inc). Participants' face areas were prepared using alcohol, water and an abrasive paste. Reusable Ag/AgCl electrodes were then attached bipolarly according to guidelines (Fridlund & Cacioppo, 1986) on the left corrugator supercilii (corrugator), and zygomaticus major (zygomaticus) muscles. A ground electrode was attached to the participants' forehead and a reference electrode on the left mastoid. The EMG data were sampled at 1200 Hz with impedances below 20 kΩ.

Each trial was divided in 4 epochs: Anticipation Pre-Effort (announcement of best attainable reward, 3s), Anticipation Post-Effort (announcement of attained reward, 2s), Delivery (touch administration, 6s), and Relax (relax phase after reward delivery, 5s). EMG was first averaged over 1s time-windows and then over the epoch total duration. For each trial, values in the four epochs were expressed as percentage of the average amplitude during the fixation cross at the beginning of that trial (baseline, 2s). Outliers in baseline (defined as values 3 SDs over the subjects' average baseline) were substituted with the average of the baseline preceding and following that trial. The extracted epochs were visually inspected in order to identify signal artifacts which were then removed (33 epochs for corrugator and 51 epochs for zygomaticus across 5 participants). As data were extremely skewed, for each subject, epochs over the subject's mean ± 3 SD were

543 removed (average removed epochs per subject: corrugator: M = 2.4, SD = 1.2; zygomaticus: M =
544 3.1, SD = 1.1), and the remaining data were transformed using natural logarithmic transformation.

545 **Subjective measures of stress and mood**

546 During the session, participants completed self-report questionnaires assessing their mood and
547 subjective state at regular intervals. Happiness, calmness, relaxation, feeling good, as well as
548 stress, tension, anxiety and feeling bad were assessed via visual analogue scales ranging from +1
549 ("not at all") to +101 ("very much") at 7 time points (T1-T7; see Figure 1A). Positive and negative
550 mood items were averaged to constitute the "Positive mood" and "Negative mood" scales used for
551 statistical analyses. The German short version Profile of Mood States (POMS; Albani et al., 2005),
552 consisting of 4 subscales for current mood (depression, vigor, fatigue, and displeasure), was
553 administered at 5 time points (T1, T2, T3, T5, T6; see Figure 1A). During the preparation phase
554 of the TSST (T4), anticipatory cognitive appraisal (PASA; Gaab et al., 2005) was also assessed.
555 Lastly, after completion of the TSST (T5), participants' satisfaction towards their performance at
556 the speech and math tasks was assessed on a VAS scale ranging from 1 ("not at all") to +101 ("very
557 much").

558 **Physiological measures of stress**

559 Heart rate was recorded using a chest strap (Polar H10; Polar Electro Oy, Kempele, Finland). Data
560 were collected over a 10-minute period at baseline, during TSST, and during the Social Reward
561 task. Values in each time window were then averaged for statistical analyses.

562 Saliva samples were collected via passive drool method using Salicaps (IBL, Hamburg, Germany)
563 at 6 time points (T1, T2, T3, T5, T6, T7; see Figure 1A). Free cortisol concentration in saliva was
564 determined by using commercial luminescence immunosorbent assay (LUM; IBL, Hamburg,

Germany). Salivary alpha-amylase activity was measured using a kinetic colorimetric test and reagents obtained from DiaSys Diagnostic Systems (Holzheim, Germany). For heart rate, salivary cortisol and alpha-amylase analyses, outliers were defined as subjects with a baseline value (T1) 3 SDs over the mean baseline of the sample. This procedure led to the exclusion of two participants for cortisol (1 MORPH, 1 PLB) and of one participant for alpha-amylase (MORPH).

Drug effects on cognitive functions and side-effects

To assess potential drug effects on cognitive functions, participants completed the Trail Making Test (TMT; Reitan, 1958) and the Digit Symbol Substitution Test (DSST; Wechsler, 1939) 55 min after drug administration. Regarding subjective drug effects, participants filled out a self-report questionnaire assessing nausea, dry mouth and other 24 possible side-effects on a 4-point Likert scale (with the anchors 1 = “not at all” and 4 = “very much”) at baseline (T1), as well as 50 min (T2) and 160 min (T7) after drug administration (see Figure 1A).

Statistical analyses

Statistical analyses were conducted in R (R Core Team, 2019). Drug effects on subjective and physiological state measures were analyzed using LMMs with Drug (MORPH, PLB) and Time as fixed effects and by-subject random intercepts. Drug effects on ratings of wanting and liking, and force exerted were analyzed with LMMs including Drug (MORPH, PLB) and Reward Level (high, low, and very vow in the case of liking) as fixed effects, and by-subject random intercepts and slopes for Reward Level. For EMG data, LMMs for each muscle and task phase were fitted, including Drug (MORPH, PLB) and trial-by-trial Wanting (for anticipation) or Liking (for consumption) as fixed effects, and by-subject random intercepts and slopes for Wanting/Liking. In case of model unconvergence or singularity, random effects with the lowest cumulative variance were removed and transformed into the corresponding complex random intercept (CRI; Scandola

& Tidoni, 2021). Group differences in age, BMI, and personality traits, as well as drug effects on executive functions, PASA, satisfaction for the TSST performance and side-effects were assessed using independent two-sided t-tests.

LMMs were computed using the lmer() function of the *lme4* package (Bates et al., 2015). Type-III F-tests were computed with the Satterthwaite degrees of freedom approximation, using the anova() function of the *lmerTest* package (Kuznetsova et al., 2017). Significant interactions were further analyzed with multiple comparisons using the function emmeans() from the homonymous package (Lenth, 2021). Results from all LMMs and multiple comparisons were controlled for the false discovery rate (FDR) associated with multiple testing using the Benjamini–Hochberg method (Benjamini & Hochberg, 1995). Data and analysis scripts are available online (https://osf.io/gbd24/).

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Authors contribution

CM: Conceptualization of the study, Data curation, Software, Formal analysis, Supervision, Investigation, Visualization, Methodology, Project administration, Funding acquisition, Writing - original draft, review and editing; MW: Contribution to the conceptualization of the design, Project administration, Medical testing, Funding acquisition, Writing - review and editing; BBQ: Contribution to the conceptualization of the design, Writing - review and editing; UMN: Contribution to the conceptualization of the design, Curation of saliva analysis, Writing - review and editing; CL: Contribution to the conceptualization of the design, Writing - review and editing; DM: Curation of blood analyses; GS: Conceptualization of the study, Supervision, Methodology, Funding acquisition, Project administration, Writing - review and editing.

All authors approved the final version of the manuscript.

Competing interests

No competing interests declared.

References

Albani, C., Blaser, G., Geyer, M., Schmutzer, G., Brähler, E., Bailer, H., & Grulke, N. (2005). The German short version of "Profile of Mood States" (POMS): Psychometric evaluation in a representative sample. *PPmP - Psychotherapie · Psychosomatik · Medizinische Psychologie*, *55*(07), 324–330. https://doi.org/10.1055/s-2004-834727

Ali, N., Cooperman, C., Nitschke, J. P., Baldwin, M. W., & Pruessner, J. C. (2020). The effects of suppressing the biological stress systems on social threat-assessment following acute stress. *Psychopharmacology*, *237*(10), 3047–3056. https://doi.org/10.1007/s00213-020-05591-z

631 Ali, N., Nitschke, J. P., Cooperman, C., & Pruessner, J. C. (2017). Suppressing the endocrine and
632 autonomic stress systems does not impact the emotional stress experience after
633 psychosocial stress. *Psychoneuroendocrinology*, *78*, 125–130.
634 https://doi.org/10.1016/j.psyneuen.2017.01.015
635 Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models
636 Using lme4. *Journal of Statistical Software*, *67*(1), 1–48.
637 https://doi.org/10.18637/jss.v067.i01
638 Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and
639 Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B*
640 *(Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x
641 Bershad, A. K., Miller, M. A., Norman, G. J., & de Wit, H. (2018). Effects of opioid- and non-
642 opioid analgesics on responses to psychosocial stress in humans. *Hormones and*
643 *Behavior*, *102*, 41–47. https://doi.org/10.1016/j.yhbeh.2018.04.009
644 Bershad, A. K., Seiden, J. A., & de Wit, H. (2016). Effects of buprenorphine on responses to
645 social stimuli in healthy adults. *Psychoneuroendocrinology*, *63*, 43–49.
646 https://doi.org/10.1016/j.psyneuen.2015.09.011
647 Bloomfield, M. A., McCutcheon, R. A., Kempton, M., Freeman, T. P., & Howes, O. (2019). The
648 effects of psychosocial stress on dopaminergic function and the acute stress response.
649 *ELife*, *8*, e46797. https://doi.org/10.7554/eLife.46797
650 Buchel, C., Miedl, S., & Sprenger, C. (2018). Hedonic processing in humans is mediated by an
651 opioidergic mechanism in a mesocorticolimbic system. *ELife*, *7*, e39648.
652 https://doi.org/10.7554/eLife.39648

653 Cacioppo, J. T., & Cacioppo, S. (2014). Social Relationships and Health: The Toxic Effects of
654 Perceived Social Isolation. *Social and Personality Psychology Compass*, *8*(2), 58–72.
655 https://doi.org/10.1111/spc3.12087
656 Cacioppo, J. T., Petty, R. E., & Morris, K. J. (1985). Semantic, Evaluative, and Self-Referent
657 Processing: Memory, Cognitive Effort, and Somatovisceral Activity. *Psychophysiology*,
658 *22*(4), 371–384. https://doi.org/10.1111/j.1469-8986.1985.tb01618.x
659 Chelnokova, O., Laeng, B., Eikemo, M., Riegels, J., Løseth, G., Maurud, H., Willoch, F., &
660 Leknes, S. (2014). Rewards of beauty: The opioid system mediates social motivation in
661 humans. *Molecular Psychiatry*, *19*(7), 746–747. https://doi.org/10.1038/mp.2014.1
662 Cohen, B. H., Davidson, R. J., Senulis, J. A., Saron, C. D., & Weisman, D. R. (1992). Muscle
663 tension patterns during auditory attention. *Biological Psychology*, *33*(2–3), 133–156.
664 https://doi.org/10.1016/0301-0511(92)90028-s
665 de Morree, H. M., & Marcora, S. M. (2010). The face of effort: Frowning muscle activity reflects
666 effort during a physical task. *Biological Psychology*, *85*(3), 377–382.
667 https://doi.org/10.1016/j.biopsycho.2010.08.009
668 Donaldson, Z. R., & Young, L. J. (2008). Oxytocin, Vasopressin, and the Neurogenetics of
669 Sociality. *Science*, *322*(5903), 900–904. https://doi.org/10.1126/science.1158668
670 Eikemo, M., Løseth, G. E., Johnstone, T., Gjerstad, J., Willoch, F., & Leknes, S. (2016). Sweet
671 taste pleasantness is modulated by morphine and naltrexone. *Psychopharmacology*,
672 *233*(21–22), 3711–3723. https://doi.org/10.1007/s00213-016-4403-x
673 Fabre-Nys, C., Meller, R. E., & Keverne, E. B. (1982). Opiate antagonists stimulate affiliative
674 behaviour in monkeys. *Pharmacology Biochemistry and Behavior*, *16*(4), 653–659.
675 https://doi.org/10.1016/0091-3057(82)90432-4

676 Fillingim, R. B., & Gear, R. W. (2004). Sex differences in opioid analgesia: Clinical and
677 experimental findings. *European Journal of Pain*, *8*(5), 413–425.
678 https://doi.org/10.1016/j.ejpain.2004.01.007
679 Freitag, C. M., Retz-Junginger, P., Retz, W., Seitz, C., Palmason, H., Meyer, J., Rösler, M., &
680 von Gontard, A. (2007). Evaluation der deutschen Version des Autismus-Spektrum-
681 Quotienten (AQ)—Die Kurzversion AQ-k. *Zeitschrift Für Klinische Psychologie Und*
682 *Psychotherapie*, *36*(4), 280–289. https://doi.org/10.1026/1616-3443.36.4.280
683 Fridlund, A. J., & Cacioppo, J. T. (1986). Guidelines for Human Electromyographic Research.
684 *Psychophysiology*, *23*(5), 567–589. https://doi.org/10.1111/j.1469-8986.1986.tb00676.x
685 Gaab, J., Rohleder, N., Nater, U. M., & Ehlert, U. (2005). Psychological determinants of the
686 cortisol stress response: The role of anticipatory cognitive appraisal.
687 *Psychoneuroendocrinology*, *30*(6), 599–610.
688 https://doi.org/10.1016/j.psyneuen.2005.02.001
689 Gerra, G., Zaimovic, A., Mascetti, G. G., Gardini, S., Zambelli, U., Timpano, M., Raggi, M. A.,
690 & Brambilla, F. (2001). Neuroendocrine responses to experimentally-induced
691 psychological stress in healthy humans. *Psychoneuroendocrinology*, *26*(1), 91–107.
692 https://doi.org/10.1016/S0306-4530(00)00046-9
693 Heimberg, R. G., Horner, K. J., Juster, H. R., Safren, S. A., Brown, E. J., Schneier, F. R., &
694 Liebowitz, M. R. (1999). Psychometric properties of the Liebowitz Social Anxiety Scale.
695 *Psychological Medicine*, *29*(1), 199–212. https://doi.org/10.1017/S0033291798007879
696 Het, S., Schoofs, D., Rohleder, N., & Wolf, O. T. (2012). Stress-Induced Cortisol Level
697 Elevations Are Associated With Reduced Negative Affect After Stress: Indications for a

Mood-Buffering Cortisol Effect. *Psychosomatic Medicine*, *74*(1), 23–32. https://doi.org/10.1097/PSY.0b013e31823a4a25

Het, S., & Wolf, O. T. (2007). Mood changes in response to psychosocial stress in healthy young women: Effects of pretreatment with cortisol. *Behavioral Neuroscience*, *121*(1), 11–20. https://doi.org/10.1037/0735-7044.121.1.11

Hickey, C., Chelazzi, L., & Theeuwes, J. (2010). Reward Changes Salience in Human Vision via the Anterior Cingulate. *Journal of Neuroscience*, *30*(33), 11096–11103.

Hickey, C., & van Zoest, W. (2012). Reward creates oculomotor salience. *Current Biology*, *22*(7), R219–R220. https://doi.org/10.1016/j.cub.2012.02.007

Holly, E. N., & Miczek, K. A. (2016). Ventral tegmental area dopamine revisited: Effects of acute and repeated stress. *Psychopharmacology*, *233*(2), 163–186. https://doi.org/10.1007/s00213-015-4151-3

Hsu, D. T., Sanford, B. J., Meyers, K. K., Love, T. M., Hazlett, K. E., Wang, H., Ni, L., Walker, S. J., Mickey, B. J., Korycinski, S. T., Koeppe, R. A., Crocker, J. K., Langenecker, S. A., & Zubieta, J.-K. (2013). Response of the μ-opioid system to social rejection and acceptance. *Molecular Psychiatry*, *18*(11), 1211–1217. https://doi.org/10.1038/mp.2013.96

Inagaki, T. K., Hazlett, L. I., & Andreescu, C. (2020). Opioids and social bonding: Effect of naltrexone on feelings of social connection and ventral striatum activity to close others. *Journal of Experimental Psychology: General*, *149*(4), 732–745. https://doi.org/10.1037/xge0000674

719 Inagaki, T. K., Irwin, M. R., & Eisenberger, N. I. (2015). Blocking opioids attenuates physical
720 warmth-induced feelings of social connection. *Emotion (Washington, D.C.)*, *15*(4), 494–
721 500. https://doi.org/10.1037/emo0000088
722 Inagaki, T. K., Ray, L. A., Irwin, M. R., Way, B. M., & Eisenberger, N. I. (2016). Opioids and
723 social bonding: Naltrexone reduces feelings of social connection. *Social Cognitive and*
724 *Affective Neuroscience*, *11*(5), 728–735. https://doi.org/10.1093/scan/nsw006
725 Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., & Carpenter, L. L. (2008). Sex
726 differences in emotional and physiological responses to the Trier Social Stress Test.
727 *Journal of Behavior Therapy and Experimental Psychiatry*, *39*(1), 87–98.
728 https://doi.org/10.1016/j.jbtep.2007.02.003
729 Keverne, E. B., Martensz, N. D., & Tuite, B. (1989). Beta-endorphin concentrations in
730 cerebrospinal fluid of monkeys are influenced by grooming relationships.
731 *Psychoneuroendocrinology*, *14*(1), 155–161. https://doi.org/10.1016/0306-
732 4530(89)90065-6
733 Kirsch, L. P., Krahé, C., Blom, N., Crucianelli, L., Moro, V., Jenkinson, P. M., & Fotopoulou, A.
734 (2017). Reading the Mind in the Touch: Neurophysiological Specificity in the
735 Communication of Emotions by Touch. *Neuropsychologia*.
736 https://doi.org/10.1016/j.neuropsychologia.2017.05.024
737 Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'—A
738 tool for investigating psychobiological stress responses in a laboratory setting.
739 *Neuropsychobiology*, *28*(1–2), 76–81. https://doi.org/10.1159/000119004
740 Korb, S., Götzendorfer, S. J., Massaccesi, C., Sezen, P., Graf, I., Willeit, M., Eisenegger, C., &
741 Silani, G. (2020). Dopaminergic and opioidergic regulation during anticipation and

consumption of social and nonsocial rewards. *ELife*, *9*, e55797. https://doi.org/10.7554/eLife.55797

Korb, S., Massaccesi, C., Gartus, A., Lundström, J. N., Rumiati, R., Eisenegger, C., & Silani, G. (2020). Facial responses of adult humans during the anticipation and consumption of touch and food rewards. *Cognition*, *194*, 104044. https://doi.org/10.1016/j.cognition.2019.104044

Kumar, P., Berghorst, L. H., Nickerson, L. D., Dutra, S. J., Goer, F. K., Greve, D. N., & Pizzagalli, D. A. (2014). Differential effects of acute stress on anticipatory and consummatory phases of reward processing. *Neuroscience*, *266*(Supplement C), 1–12. https://doi.org/10.1016/j.neuroscience.2014.01.058

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(1), 1–26. https://doi.org/10.18637/jss.v082.i13

Labuschagne, I., Grace, C., Rendell, P., Terrett, G., & Heinrichs, M. (2019). An introductory guide to conducting the Trier Social Stress Test. *Neuroscience and Biobehavioral Reviews*, *107*, 686–695. https://doi.org/10.1016/j.neubiorev.2019.09.032

Leknes, S., & Tracey, I. (2008). A common neurobiology for pain and pleasure. *Nature Reviews. Neuroscience*, *9*(4), 314–320. https://doi.org/10.1038/nrn2333

Lenth, R. V. (2021). *emmeans: Estimated marginal means, aka least-squares means. R package version 1.5. 4*.

Lewis, A. H., Porcelli, A. J., & Delgado, M. R. (2014). The effects of acute stress exposure on striatal activity during Pavlovian conditioning with monetary gains and losses. *Frontiers in Behavioral Neuroscience*, *8*. https://doi.org/10.3389/fnbeh.2014.00179

765 Löken, L. S., Wessberg, J., Morrison, I., McGlone, F., & Olausson, H. (2009). Coding of
766 pleasant touch by unmyelinated afferents in humans. *Nature Neuroscience*, *12*(5), 547–
767 548. https://doi.org/10.1038/nn.2312
768 Løseth, G. E., Eikemo, M., & Leknes, S. (2019). Effects of opioid receptor stimulation and
769 blockade on touch pleasantness: A double-blind randomised trial. *Social Cognitive and*
770 *Affective Neuroscience*, *14*(4), 411–422. https://doi.org/10.1093/scan/nsz022
771 Loseth, G. E., Ellingsen, D.-M., & Leknes, S. (2014). State-dependent μ-opioid modulation of
772 social motivation. *Frontiers in Behavioral Neuroscience*, *8*, 430.
773 https://doi.org/10.3389/fnbeh.2014.00430
774 Lugo, R. A., & Kern, S. E. (2002). Clinical Pharmacokinetics of Morphine. *Journal of Pain &*
775 *Palliative Care Pharmacotherapy*, *16*(4), 5–18. https://doi.org/10.1080/J354v16n04_02
776 Machin, A. J., & Dunbar, R. I. M. (2011). The brain opioid theory of social attachment: A review
777 of the evidence. *Behaviour*, *148*(9–10), 985–1025.
778 https://doi.org/10.1163/000579511X596624
779 Manninen, S., Tuominen, L., Dunbar, R. I., Karjalainen, T., Hirvonen, J., Arponen, E., Hari, R.,
780 Jääskeläinen, I. P., Sams, M., & Nummenmaa, L. (2017). Social Laughter Triggers
781 Endogenous Opioid Release in Humans. *Journal of Neuroscience*, *37*(25), 6125–6131.
782 https://doi.org/10.1523/JNEUROSCI.0688-16.2017
783 Martel, F. L., Nevison, C. M., Simpson, M. J. A., & Keverne, E. B. (1995). Effects of opioid
784 receptor blockade on the social behavior of rhesus monkeys living in large family groups.
785 *Developmental Psychobiology*, *28*(2), 71–84. https://doi.org/10.1002/dev.420280202

786 Massaccesi, C., Korb, S., Skoluda, N., Nater, U. M., & Silani, G. (2021). Effects of Appetitive
787 and Aversive Motivational States on Wanting and Liking of Interpersonal Touch.
788 *Neuroscience*, *464*, 12–25. https://doi.org/10.1016/j.neuroscience.2020.09.025
789 Mayo, L. M., Lindé, J., Olausson, H., Heilig, M., & Morrison, I. (2018). Putting a good face on
790 touch: Facial expression reflects the affective valence of caress-like touch across
791 modalities. *Biological Psychology*, *137*, 83–90.
792 https://doi.org/10.1016/j.biopsycho.2018.07.001
793 McEwen, B. S. (1998). Stress, adaptation, and disease. Allostasis and allostatic load. *Annals of*
794 *the New York Academy of Sciences*, *840*, 33–44. https://doi.org/10.1111/j.1749-
795 6632.1998.tb09546.x
796 Meller, R. E., Keverne, E. B., & Herbert, J. (1980). Behavioural and endocrine effects of
797 naltrexone in male talapoin monkeys. *Pharmacology Biochemistry and Behavior*, *13*(5),
798 663–672. https://doi.org/10.1016/0091-3057(80)90010-6
799 Morrison, I. (2016). Keep Calm and Cuddle on: Social Touch as a Stress Buffer. *Adaptive*
800 *Human Behavior and Physiology*, *2*(4), 344–362. https://doi.org/10.1007/s40750-016-
801 0052-x
802 Morrison, I., Löken, L. S., & Olausson, H. (2010). The skin as a social organ. *Experimental*
803 *Brain Research*, *204*(3), 305–314. https://doi.org/10.1007/s00221-009-2007-y
804 Pan, Z. Z. (1998). μ-Opposing actions of the κ-opioid receptor. *Trends in Pharmacological*
805 *Sciences*, *19*(3), 94–98. https://doi.org/10.1016/S0165-6147(98)01169-9
806 Panksepp, J. (1998). *Affective Neuroscience: The Foundations of Human and Animal Emotions*.
807 Oxford University Press.

808 Panksepp, J., & Bishop, P. (1981). An autoradiographic map of (3H)diprenorphine binding in rat
809 brain: Effects of social interaction. *Brain Research Bulletin*, *7*(4), 405–410.
810 Panksepp, J., Herman, B. H., Vilberg, T., Bishop, P., & DeEskinazi, F. G. (1980). Endogenous
811 opioids and social behavior. *Neuroscience & Biobehavioral Reviews*, *4*(4), 473–487.
812 https://doi.org/10.1016/0149-7634(80)90036-6
813 Pawling, R., Cannon, P. R., McGlone, F. P., & Walker, S. C. (2017). C-tactile afferent
814 stimulating touch carries a positive affective value. *PLOS ONE*, *12*(3), e0173457.
815 https://doi.org/10.1371/journal.pone.0173457
816 Pechnick, R. N. (1993). Effects of Opioids on the Hypothalamo-Pituitary-Adrenal Axis. *Annual*
817 *Review of Pharmacology and Toxicology*, *33*(1), 353–382.
818 https://doi.org/10.1146/annurev.pa.33.040193.002033
819 Petrowski, K., Wintermann, G.-B., & Siepmann, M. (2012). Cortisol response to repeated
820 psychosocial stress. *Applied Psychophysiology and Biofeedback*, *37*(2), 103–107.
821 https://doi.org/10.1007/s10484-012-9183-4
822 Pool, E., Brosch, T., Delplanque, S., & Sander, D. (2015). Stress increases cue-triggered
823 "wanting" for sweet reward in humans. *Journal of Experimental Psychology. Animal*
824 *Learning and Cognition*, *41*(2), 128–136. https://doi.org/10.1037/xan0000052
825 Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas
826 for computation of the area under the curve represent measures of total hormone
827 concentration versus time-dependent change. *Psychoneuroendocrinology*, *28*(7), 916–
828 931. https://doi.org/10.1016/S0306-4530(02)00108-7
829 Quednow, B. B., Csomor, P. A., Chmiel, J., Beck, T., & Vollenweider, F. X. (2008).
830 Sensorimotor gating and attentional set-shifting are improved by the μ-opioid receptor

agonist morphine in healthy human volunteers. *International Journal of Neuropsychopharmacology*, *11*(5), 655–669. https://doi.org/10.1017/S1461145707008322

R Core Team. (2019). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing.

Ree, A., Bendas, J., Pabel, L., Croy, I., & Sailer, U. (2020). Right between the eyes: Corrugator muscle activity tracks the changing pleasantness of repeated slow stroking touch. *Physiology & Behavior*, *222*, 112903. https://doi.org/10.1016/j.physbeh.2020.112903

Reitan, R. M. (1958). Validity of the Trail Making Test as an Indicator of Organic Brain Damage. *Perceptual and Motor Skills*, *8*(3), 271–276. https://doi.org/10.2466/pms.1958.8.3.271

Sato, W., Minemoto, K., Ikegami, A., Nakauma, M., Funami, T., & Fushiki, T. (2020). Facial EMG Correlates of Subjective Hedonic Responses During Food Consumption. *Nutrients*, *12*(4). https://doi.org/10.3390/nu12041174

Scandola, M., & Tidoni, E. (2021). *The development of a standard procedure for the optimal reliability-feasibility trade-off in Multilevel Linear Models analyses in Psychology and Neuroscience.* PsyArXiv. https://doi.org/10.31234/osf.io/kfhgv

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The Development and Validation of a Structured Diagnostic Psychiatric Interview for DSM-IV and ICD-10. *The Journal of Clinical Psychiatry*, *59*(suppl 20), 0–0.

853 Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the State-Trait Anxiety*
854 *Inventory*. http://ubir.buffalo.edu/xmlui/handle/10477/2895
855 Stier, D. S., & Hall, J. A. (1984). Gender differences in touch: An empirical and theoretical
856 review. *Journal of Personality and Social Psychology*, *47*(2), 440–459.
857 https://doi.org/10.1037/0022-3514.47.2.440
858 Suvilehto, J. T., Glerean, E., Dunbar, R. I. M., Hari, R., & Nummenmaa, L. (2015). Topography
859 of social touching depends on emotional bonds between humans. *Proceedings of the*
860 *National Academy of Sciences*, *112*(45), 13811–13816.
861 https://doi.org/10.1073/pnas.1519231112
862 Tchalova, K., & MacDonald, G. (2020). Opioid receptor blockade inhibits self-disclosure during
863 a closeness-building social interaction. *Psychoneuroendocrinology*, *113*, 104559.
864 https://doi.org/10.1016/j.psyneuen.2019.104559
865 Theeuwes, J., & Belopolsky, A. V. (2012). Reward grabs the eye: Oculomotor capture by
866 rewarding stimuli. *Vision Research*, *74*, 80–85.
867 https://doi.org/10.1016/j.visres.2012.07.024
868 Triscoli, C., Croy, I., Steudte-Schmiedgen, S., Olausson, H., & Sailer, U. (2017). Heart rate
869 variability is enhanced by long-lasting pleasant touch at CT-optimized velocity.
870 *Biological Psychology*, *128*, 71–81. https://doi.org/10.1016/j.biopsycho.2017.07.007
871 Uchino, B. N. (2006). Social Support and Health: A Review of Physiological Processes
872 Potentially Underlying Links to Disease Outcomes. *Journal of Behavioral Medicine*,
873 *29*(4), 377–387. https://doi.org/10.1007/s10865-006-9056-5

874 Van Boxtel, A., & Jessurun, M. (1993). Amplitude and bilateral coherency of facial and jaw-
875 elevator EMG activity as an index of effort during a two-choice serial reaction task.
876 *Psychophysiology*, *30*(6), 589–604. https://doi.org/10.1111/j.1469-8986.1993.tb02085.x
877 Vanderschuren, L. J. M. J., Stein, E. A., Wiegant, V. M., & Van Ree, J. M. (1995). Social play
878 alters regional brain opioid receptor binding in juvenile rats. *Brain Research*, *680*(1),
879 148–156. https://doi.org/10.1016/0006-8993(95)00256-P
880 von Mohr, M., Krahé, C., Beck, B., & Fotopoulou, A. (2018). The social buffering of pain by
881 affective touch: A laser-evoked potential study in romantic couples. *Social Cognitive and*
882 *Affective Neuroscience*, *13*(11), 1121–1130. https://doi.org/10.1093/scan/nsy085
883 Waterink, W., & van Boxtel, A. (1994). Facial and jaw-elevator EMG activity in relation to
884 changes in performance level during a sustained information processing task. *Biological*
885 *Psychology*, *37*(3), 183–198. https://doi.org/10.1016/0301-0511(94)90001-9
886 Wechsler, D. (1939). *The measurement of adult intelligence* (pp. ix, 226). Williams & Wilkins
887 Co. https://doi.org/10.1037/10020-000
888 Wilhelm, F. H., Kochar, A. S., Roth, W. T., & Gross, J. J. (2001). Social anxiety and response to
889 touch: Incongruence between self-evaluative and physiological reactions. *Biological*
890 *Psychology*, *58*(3), 181–202. https://doi.org/10.1016/S0301-0511(01)00113-2
891 Zubieta, J.-K., Dannals, R. F., & Frost, J. J. (1999). Gender and Age Influences on Human Brain
892 Mu-Opioid Receptor Binding Measured by PET. *American Journal of Psychiatry*,
893 *156*(6), 842–848. https://doi.org/10.1176/ajp.156.6.842
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Supplementary Material

Enhanced negative response to stress following morphine administration increases wanting of social reward

C. Massaccesi, M. Willeit, B. B. Quednow, U. M. Nater, C. Lamm, D. Mueller, G. Silani

Index

1. Effects of COVID-19 pandemic

Half of the sample was collected after the COVID-19 pandemic outbreak. The implemented safety measures, as well as the statistical analyses conducted to assess the possible effects of the pandemic on the study dependent variables are described below.

a. Safety measures

After the COVID-19 pandemic outbreak the following safety measures were implemented:

- All experimenters wore face masks. The evaluating panel of the TSST wore a special face mask with a clear plastic insert on the mouth region in order to allow the participant to see the "absence of facial feedback" (crucial for stress induction) during the stress paradigm.
- Participants were provided with clear mouth visors resting on the chin in order to minimize the contact with the face and avoid disturbances during facial electromyography (EMG).

In order to assess participants reaction towards these safety measures, they were asked to answer the following questions at the end of the experimental session:

1) "How well/comfortable did you feel during the study?" rated on a VAS ranging from 1 (not all) to 101 (very much).
2) "How high do you rate the risk of infection during the study?" rated on a VAS ranging from 1 (very high) to 101 (very low).
3) "Were you afraid of being infected with COVID-19 during the study?", Yes/No
4) "How comfortable did you feel with the research team wearing a mask during the study?" rated on a VAS ranging from 1 (not all) to 101 (very much).

As shown in Figure S1, participants did not report to feel threatened by COVID-19 infection during the study, and to feel comfortable during the session.

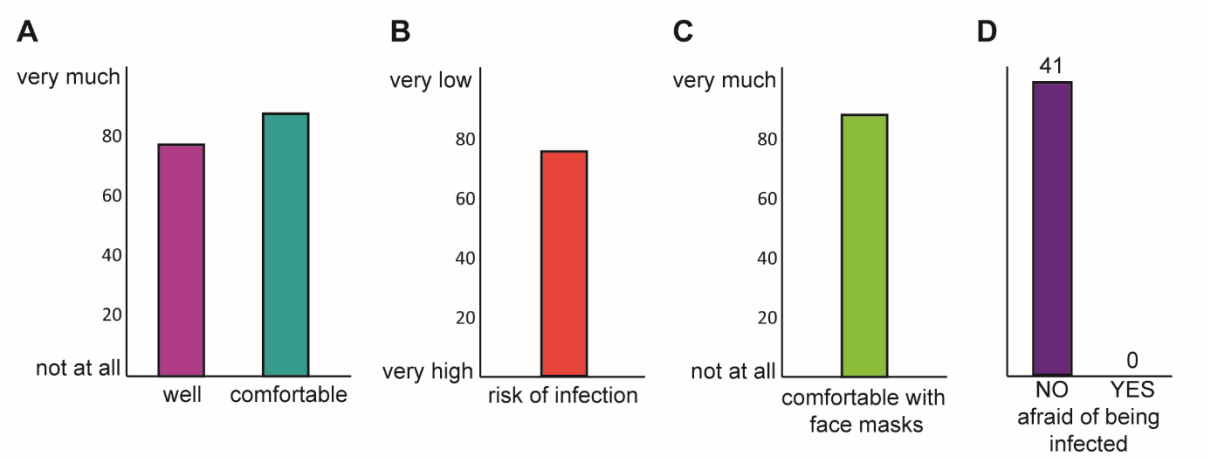

Figure S1. Participants' attitudes toward COVID-19 pandemic and safety measures during the study. (A) Ratings of wellness and comfort. (B) Perceived risk of COVID-19 infection during the study. (C) Ratings of comfort related to use of face masks during the study. (D) Perceived fear of having been infected with COVID-19 during the study.

b. Additional analyses to assess the effects of the COVID-19 pandemic and employed safety measures on responses to social rewards

In order to rule out possible effects of the pandemic and related implemented safety measures on subjective stress response (positive and negative mood, POMS subscales), as well as on the ratings of wanting and liking, on the force exerted to obtain the social rewards, and on facial EMG data, by adding the covariate "COVID-19" (2 levels: pre-covid19, covid19) to the analyses. No changes in the pattern of results were observed.

2. Drug effects on cognitive functions

Table S1. Mean (SD) scores at the Trial Making Test (TMT) part A and part B, and at the Digit Symbol Substitution Test (DSST) across drug groups.

	MORPHINE	PLACEBO	*p* value
TMT A	26.33 (8.02)	26.40 (8.60)	0.97
TMT B	57.25 (33.05)	58.93 (21.94)	0.79
DSST	54.20 (8.99)	54.10 (9.32)	0.96

3. Drug side-effects

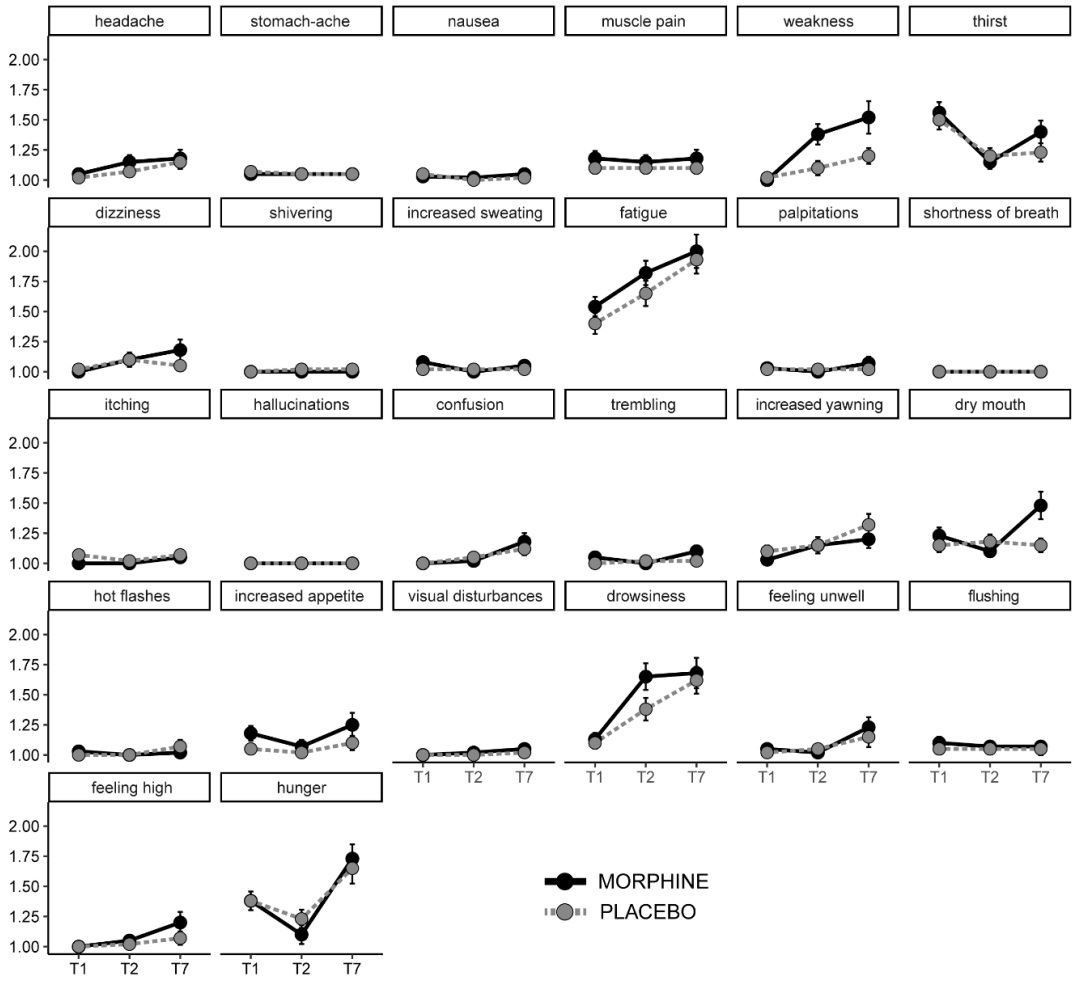

Figure S2. Drug side-effects assessed at baseline (T1), 60 min (T2) and 160 min (T7) after drug administration using a 4-point Likert scale (with the anchors 1 = "not at all" and 4 = "very much").

4. Serum levels of morphine and its metabolites

A blood sample was drawn at the end of the session (~180 min after drug administration). Analyses were performed at the Institute of Clinical Chemistry, University Hospital Zurich, using liquid chromatography coupled to mass spectrometry (LC-MS) to identify serum levels of morphine and its two major metabolites: morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Blood samples from 3 participants could not be obtained and 10 samples were lost because of

958 storage problems. Results from the available samples confirmed drug uptake, as shown in *Table*
959 *S2*.

960 *Table S2.* Serum levels (nmol/l) of morphine and its major metabolites at the end of the
961 experimental session (~180 min after drug administration). M3G & M6G = morphine-3/6-
962 glucronide.

	M	**SD**
Morphine	12.63	6.82
M3G	394.79	179.46
M6G	84.63	44.32

963