Opioid-blunted cortisol response to stress is associated to increased negative mood and wanting of social reward

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Abstract

Animal research suggests a central role of the μ-opioid receptor (MOR) system in regulating affiliative behaviors and in mediating the stress-buffering function of social contact. However, the neurochemistry of stress-related social contact seeking in humans 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 a standardized psychosocial stress induction, participants engaged in a social reward task, in which the motivation to obtain skin-to-skin social touch and the hedonic reactions elicited by such touch were assessed.

Morphine prevented the increase of salivary cortisol typically observed following acute stress exposure. Notably, this altered HPA axis responsivity was associated with increased negative affect in response to psychosocial stress, and with enhanced subjective wanting of highly rewarding social contact.

These findings 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., 1,2], highlighting the importance to better understand the neurobiological basis of social motivation.

Based on pharmacological evidence in isolated animals showing that exogenous μ-opioids administration (e.g. morphine) reduces separation distress and contact seeking, the Brain Opioid Theory of Social Attachment [3] pinpoints the μ-opioid receptor (MOR) system as a key mediator of bonding and affiliation. Extending this model, Løseth and colleagues propose that the MOR system regulates social behaviors in a manner that is context-dependent [4]. Specifically, in contexts of distress (such as the social isolation described above), social stimuli are sought because they stimulate endogenous μ-opioids release, which in turn reduces pain and negative affect. If opioids are exogenously provided, this will result in alleviated distress, and therefore reduced need for social contact. On the other hand, in contexts of comfort, endogenous MOR activity mediates the rewarding properties, and associated pleasure, of social stimuli. In this case, exogenous MOR stimulation will result in increased pleasure and motivation to seek for social contact.

In the last decade, preliminary confirmatory evidence on the state-dependent MOR regulation of affiliation and social reward processing in humans has been provided. During states of comfort, MOR blockade decreases wanting and/or liking of different social stimuli [5–7, but see also 8], as well as feelings of social connection [9–11], interpersonal closeness and social reward expectation [12], while MOR enhancement increases wanting and liking of attractive faces [6]. Using PET,

62 Hsu and colleagues [13] showed that endogenous MOR activity during social rejection is
63 positively associated with reduced negative affect, while during social acceptance it predicts
64 greater desire for social interaction. However, to date, causal evidence of MOR regulation of social
65 motivation and contact seeking during distress is lacking.

66 Here, we aimed at filling this knowledge gap by investigating the effect of MOR agonist
67 administration (morphine) on social motivation and social pleasure following stress exposure.
68 Using a double-blind, placebo-controlled, randomized, between-subjects design, female
69 participants (N = 80) were orally administered with either 10 mg morphine sulfate (a highly
70 selective μ-opioid agonist) or placebo. Following a psychosocial stress induction procedure, the
71 motivation to obtain social touch (wanting) and the pleasure elicited by receiving it (liking) were
72 assessed (see Figure 1 for a detailed description). To enhance the comparability with animal
73 research, i) a stressor of social nature was employed; ii) in addition to self-reports of wanting and
74 liking, we assessed real physical effort and hedonic facial reactions, approximating the
75 motivational and hedonic (i.e., wanting and liking) measures used in animal studies; iii) to parallel
76 grooming in animals, skin-to-skin touch was employed as a social reward.

77 As recent human evidence showed that exogenous MOR stimulation alleviates stress, as indicated
78 by decreased cortisol response and threat/challenge appraisal [14,15], we expected reduced
79 responses to stress after morphine administration, compared to placebo. Based on previous
80 theoretical models and animal evidence [3,4], we hypothesized that this opioid-blunted stress
81 response would be associated to decreased social motivation. Given that previous studies showed
82 an effect of stress on wanting rather than on liking of touch [16] or food [e.g. 17] reward, we did
83 not expect changes in the hedonic reactions to social contact. Finally, given that MOR
84 manipulations have been shown to induce the strongest effects on the best reward option available

[6,18], and considering the stress buffering function of C tactile (CT)-optimal touch [19], we expected the predicted effects to be stronger for the most valuable social reward (i.e., touch at 6 cm/s, which is in the CT-optimal range stroking speed of 1 to 10 cm/s).

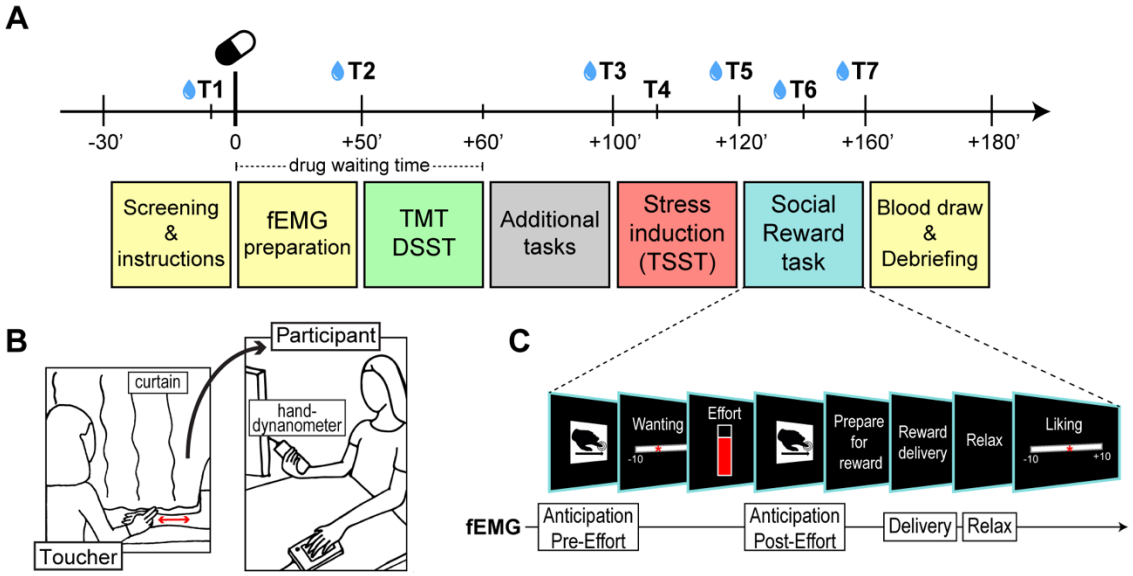

Figure 1. **Overview of the experimental session, set-up, and trial structure of the Social Reward task.** (A) Overview of the experimental session. T1 – T7 represent the time points at which subjective and/or physiological measures were obtained. Blue drops indicate saliva sample collection. (B) Set-up of the Social Reward task. 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. Facial electromyography (fEMG) was recorded during the whole task and analyzed in reward anticipation (Anticipation Pre-Effort and Post-Effort) and consumption

100 (Delivery and Relax). TMT, Trail Making Test; DSST, Digit Symbol Substitution Test; TSST,
101 Trier Social Stress Test.

102 Materials and Methods

103 Study design

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

106 Participants

107 Based on previous work that had investigated the effects of stress on social reward processing [16]
108 and the effects of similar compounds on stress responses [14], we aimed at collecting data from
109 40 participants per group. The study sample included 82 participants, of which 42 received
110 morphine (MORPH) and 40 received a placebo (PLB). Two participants (MORPH) did not
111 complete the session and were therefore not included in data analysis, yielding to a final sample
112 size of 80 participants (40/group)[1]. Only female participants were included due to i) gender
113 differences in opioid pharmacokinetics [20,21] and stress response [22], ii) expected higher
114 preference of same-gender touch in females than males [23,24]. Participants were tested during
115 the luteal phase of their menstrual cycle, as determined by self-report of their last menstruation
116 and average cycle length. All participants reported to be right-handed, to smoke less than ten
117 cigarettes per week, to have no history of current or former drug abuse, to have a BMI between 17
118 and 35, and to be free of psychiatric or neurological disorders. Other exclusion criteria were: single
119 or repeated use of any strong opioids in the last two years, use of hormonal contraceptives, regular

[1] 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.

120 intake of medications, current pregnancy or breastfeeding, suffering from impaired respiratory
121 functions, respiratory weakness or lung disease, injury/disease of the arms (making it impossible
122 to squeeze with the right hand and to be caressed on the left forearm). Participants were instructed
123 to refrain from eating, brushing their teeth and consuming caffeinated beverages, juices, and
124 chewing gum in the two hours preceding the test, as well as from smoking, doing physical activity
125 and intaking alcohol and medications in the 24 hours preceding the test. The two experimental
126 groups did not differ significantly in terms of age, BMI, autistic traits (short version of the German
127 Autism Spectrum Quotient, AQ-k) [25], general (State-Trate Anxiety Inventory, STAI) [26] and
128 social (Liebowitz Social Anxiety Scale, LSAS) [27] anxiety, and social touch appreciation (Social
129 Touch Questionnaire, STQ) [28] (see Table 1). The study was approved by the Ethics Committee
130 of the Medical University of Vienna (EK 1393/2017) and was performed in line with the
131 Declaration of Helsinki [29]. All participants signed a consent form before taking part in the study.
132 *Table 1.* Demographic and self-reported substance use characteristics of the participants.

	MORPHINE	PLACEBO	*p* value
N	40	40	---
Age (years)	23.1 ± 3.1	23.9 ± 3.9	0.31
BMI (kg/m^2)	21.4 ± 3.4	22.3 ± 2.7	0.19
Autism (AQ-k)	6.5 ± 3.8	5.7 ± 3.6	0.34
Anxiety (STAI)	38.1 ± 9.4	35.7 ± 8.3	0.23
Social Anxiety (LSAS)	36.7 ± 17.9	33.2 ± 19.0	0.39
Social Touch Preferences (STQ)	25.3 ± 9.5	27.1 ± 7.7	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	---

133

134 **Procedure**

135 The study was conducted at the Department of Psychiatry and Psychotherapy of the Medical
136 University of Vienna. After completing an online survey to assess their eligibility, potential
137 participants were first invited to a health screening (~45'), including blood examination,
138 electrocardiogram, blood pressure measurement, and psychiatric interview (Mini-International
139 Neuropsychiatric Interview) [30]. Eligible participants were then invited to the experimental
140 session (~210'), which always started between 11:30 and 12:30 in order to control for cortisol
141 diurnal fluctuations [31]. At the beginning of the session, urine drug and pregnancy tests were
142 administered. After baseline mood and physiological measures were collected, participants
143 received a standardized snack, and took the assigned capsule. Throughout the session, mood and
144 physiological measures were obtained at regular intervals. The experimental tasks were completed
145 between 60 and 160 min after drug administration, and included economic decision making, facial
146 mimicry, emotion recognition, stress induction and social reward processing (see Figure 1A for an
147 overview of the session timeline). Here we will focus on the last two, while the others will be
148 reported elsewhere. Approximately 180 min after pill administration, a blood sample was taken to
149 confirm drug uptake (see Table S2 of the *Supplementary Material*). After completing the
150 experimental session, participants were debriefed and received a financial compensation of 65€.
151 Half of the sample was tested before and half during the COVID-19 pandemic (see *Supplementary*
152 *Material* for details regarding the employed safety measures and additional statistical analyses to
153 assess possible effects of the pandemic and safety measures on the results).

154 **Drug administration**

155 Ten mg of morphine sulfate (Morapid®) were orally administered, with the specific dosage chosen
156 so as to stimulate the activity of the MOR system with minimal subjective (side-)effects. Morphine

157 is a selective MOR agonist and, for oral administration, has an average bioavailability of 30–40%,
158 a maximal effect (t-max) at 1–2 h after administration, and a half-life of 2–4 h [32]. Placebo
159 consisted of capsules containing 650 mg of mannitol (sugar), visually identical to the ones
160 containing morphine.

161 **Stress induction**

162 In order to induce a stress reaction in the participants, the Trier Social Stress Test (TSST) [33] was
163 employed. In the TSST, participants were given 3 minutes to prepare a 5 min speech for a mock-
164 job interview, followed by a 5 min arithmetic task, in which they were asked to count backwards
165 from 2043 in steps of 17 as fast and as accurate as possible. The speech and arithmetic tasks were
166 completed in front of an evaluating panel (one male and one female confederate). Participants were
167 told that these tasks would be video recorded via a camera located next to the examiners (no video
168 was actually taped).

169 **Social Reward task**

170 ***Stimuli***

171 Pleasantness of touch is highest between 1 and 10 cm/s, and decreases between 10 and 30 cm/s
172 [34]. Gentle caresses in three different speeds (CT-optimal: 6 cm/s, non-CT-optimal: 21 cm/s and
173 27 cm/s) were thus used as social rewards of different levels of pleasantness (high, low, very low).
174 The suitability of these stroking speeds has been confirmed in three previous independent studies
175 from our group [7,16,35]. Caresses were delivered over a previously-marked (from the wrist
176 towards the elbow) 9 cm area on the participant's left forearm by a female experimenter, moving
177 her index and middle fingers back and forth in the marked area (Figure 1B). Touch delivery was
178 guided by auditory rhythms, which matched the frequency of the stimulation, over headphones.
179 The experimenter administering the touch was seated on the other side of a curtain, used to limit

180 the participant's field of view to the monitor (Figure 1B). All experimenters were presented as
181 trained masseurs, wore standardized clothes (white t-shirt and beige trousers) to minimize
182 differences in their appearance, and underwent extensive training on the tactile stimulation
183 delivery.

184 ***Task***

185 The Social Reward task [7,16,35] consisted of two blocks of 16 trials, separated by a 5 min break.
186 To avoid habituation to the touch, the site of application (left or right area of the forearm) was
187 alternated within the two blocks, in a counter-balanced order. Before starting the task, participants
188 experienced each type of touch once and performed two training trials. Each trial was structured
189 as follow (see Figure 1C): i) announcement of the best attainable reward (high or low, 16 trials
190 each, 3 s); ii) rating of subjective wanting via a VAS ranging from -10 (not at all) to +10 (very
191 much) (no time limit); iii) effort task (4 s), requiring to squeeze a hand-dynamometer (HD-BTA,
192 Vernier Software & Technology, USA) with the right hand, in order to obtain the announced
193 reward – the applied force, displayed via an online visual-feedback, was expressed as percentage
194 of the participants' maximum voluntary contraction (MVC), measured immediately before the
195 task, and translated into the probability of obtaining the announced reward (0–100%); iv)
196 announcement of the reward obtained (high, low, or – if insufficient force had been exerted – very
197 low; 2 s); v) reward delivery (6 s); vi) relax phase (5 s); vii) rating of subjective liking via a VAS
198 ranging from -10 (not at all) to +10 (very much) (no time limit). At the end of the task, participants'
199 MVC was measured again.

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

Facial electromyography (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). Participant's face areas were prepared using alcohol, water and an abrasive paste. Reusable Ag/AgCl electrodes were then attached bipolarly according to guidelines [36] on the left corrugator supercilii and zygomaticus major muscles. A ground electrode was attached to the participant's forehead and a reference electrode on the left mastoid. The EMG data were sampled at 1200 Hz with impedances below 20 kΩ. Data preprocessing included filtering with a 20 to 400 Hz bandpass filter, rectification and smoothing with a 40 Hz low-pass filter.

Each trial was divided in 4 epochs: Anticipation Pre-Effort (announcement of best attainable reward, 3 s), Anticipation Post-Effort (announcement of attained reward, 2 s), Delivery (touch administration, 6 s), and Relax (relax phase after reward delivery, 5 s). EMG was first averaged over 1 s 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, 2 s). Outliers in baseline values (defined as values more than 3 SDs away from the subjects' average baseline) were substituted with the average amplitude of the baseline preceding and following that trial. The extracted epochs were visually inspected to identify signal artifacts which were then removed (33 epochs for corrugator and 51 epochs for zygomaticus across 5 participants). Because of data skewness and to reduce the effect of non-experimental movements, for each subject, epochs over the subject's mean ± 3 SD were removed (average removed epochs per subject: corrugator: $M = 2.4$, $SD = 1.2$; zygomaticus: $M = 3.1$, $SD = 1.1$), and the remaining data were transformed using natural logarithmic transformation.

224 **Subjective measures of stress and mood**

225 During the session, participants completed self-report questionnaires assessing their mood and
226 subjective state at regular intervals. Happiness, calmness, relaxation, feeling good, as well as
227 stress, tension, anxiety and feeling bad were assessed using VAS ranging from "not at all" (+1) to
228 "very much" (+101) at 7 time points (T1-T7; see Figure 1A). Positive and negative mood items
229 were averaged to constitute the "Positive mood" and "Negative mood" scales used for statistical
230 analyses. The German short version Profile of Mood States (POMS) [37], consisting of 4 subscales
231 for current mood (depression, vigor, fatigue, and displeasure), was administered at 5 time points
232 (T1, T2, T3, T5, T6; see Figure 1A). During the preparation phase of the TSST (T4), anticipatory
233 cognitive appraisal (PASA) [38] was also assessed. Lastly, after completion of the TSST (T5),
234 participants' satisfaction towards their performance at the speech and math tasks was assessed on
235 a VAS scale ranging from "not at all" (+1) to "very much" (+101).

236 **Physiological measures of stress**

237 As physiological stress biomarkers, salivary cortisol (hypothalamic–pituitary–adrenal [HPA] axis
238 activity), salivary alpha-amylase and heart rate (autonomic nervous system [ANS] activity) were
239 assessed. Heart rate was recorded using a chest strap (Polar H10; Polar Electro Oy, Kempele,
240 Finland). Data were collected over a 10 min period at baseline, during the TSST, and during the
241 Social Reward task. Values in each time window were then averaged for statistical analyses.
242 Saliva samples were collected via passive drool method using Salicaps (IBL, Hamburg, Germany)
243 at 6 time points (T1, T2, T3, T5, T6, T7; see Figure 1A). Free cortisol concentration in saliva was
244 determined by using commercial luminescence immunosorbent assay (LUM; IBL, Hamburg,
245 Germany). Salivary alpha-amylase activity was measured using a kinetic colorimetric test and
246 reagents obtained from DiaSys Diagnostic Systems (Holzheim, Germany). For heart rate, salivary

247 cortisol and alpha-amylase analyses, outliers were defined as subjects with a baseline value (T1)
248 3 SDs over the mean baseline of the sample. This procedure led to the exclusion of two participants
249 for cortisol (1 MORPH, 1 PLB) and of one participant for alpha-amylase (MORPH).

250 **Drug effects on cognitive functions and side-effects**

251 To assess potential drug effects on cognitive functions, participants completed the Trail Making
252 Test (TMT) [39] and the Digit Symbol Substitution Test (DSST) [40] 55 min after drug
253 administration. Regarding subjective drug effects, participants filled out a self-report questionnaire
254 assessing nausea, dry mouth and other 24 possible side-effects on a 4-point Likert scale (with the
255 anchors 1 = "not at all" and 4 = "very much") at baseline (T1), as well as 50 min (T2) and 160 min
256 (T7) after drug administration (see Figure 1A).

257 **Statistical analyses**

258 Statistical analyses were conducted in R [41]. Drug effects on subjective and physiological stress
259 measures were analyzed using linear mixed effects models (LMM) with Drug (MORPH, PLB)
260 and Time as fixed effects and by-subject random intercepts. Drug effects on ratings of wanting
261 and liking, and force exerted were analyzed with LMMs including Drug (MORPH, PLB) and
262 Reward Level (high, low, and very vow in the case of liking) as fixed effects, and by-subject
263 random intercepts and slopes for Reward Level. For EMG data, LMMs for each muscle and task
264 phase (anticipation, consumption) were fitted, including Drug (MORPH, PLB), trial-by-trial
265 Wanting (for anticipation) or Liking (for consumption) and Epoch (Anticipation Pre-Effort and
266 Post-Effort for anticipation, Delivery and Relax for consumption) as fixed effects, and by-subject
267 random intercepts and slopes for Wanting/Liking, Epoch, and their interaction. In case of model
268 unconvergence or singularity, random effects with the lowest cumulative variance were removed
269 and, in case of categorical variables, transformed into the corresponding complex random intercept

270 [42]. Group differences in age, BMI, and personality traits, as well as drug effects on executive
271 functions, PASA, satisfaction for the TSST performance and side-effects were assessed using
272 independent two-sided t-tests.
273 LMMs were computed using the *lmer* function of the lme4 package [43]. Type-III F-tests were
274 computed with the Satterthwaite degrees of freedom approximation, using the *anova* function of
275 the lmerTest package [44]. Significant interactions were further analyzed with multiple
276 comparisons using the function *emmeans* from the homonymous package [45]. Results from all
277 LMMs and multiple comparisons were controlled for the false discovery rate (FDR) associated
278 with multiple testing using the Benjamini–Hochberg method [46].
279 The data and analysis scripts supporting the article are available at https://osf.io/gbd24/.

280 Results

281 Drug blinding

282 After completing the session, 50% of the participants who had actually received morphine
283 correctly guessed to have received the drug. 55% of the total sample believed to have been
284 administered with placebo, 29% with morphine, and 16% with naltrexone[2]. Overall, these numbers
285 indicate successful blinding.

286 Effects of morphine on cognitive functions and drug side-effects

287 There were no significant differences in the DSST and TMT scores across groups, indicating that
288 drug administration did not have negative effects on attention, psychomotor and processing speed,
289 and visuo-perceptual functions (See Table S1 in *Supplementary Material*). Morphine

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

290 administration significantly increased self-reported weakness (MORPH vs. PLB at T2: $t(69.9) =$
291 2.64, $p = 0.01$, and at T7: $t(55.96) = 2.19$, $p = 0.03$) and dry mouth (MORPH vs. PLB at T7:
292 $t(57.70) = 2.56$, $p = 0.01$). For all side-effect measures, group average scores were generally low,
293 and no side-effect was on average rated as moderate or strong (see Figure S2 in *Supplementary*
294 *Material*).

295 **Effects of morphine on stress response**

296 ***Physiological measures***

297 Morphine administration suppressed the cortisol response to the TSST (Drug*Time: $F(5,375) =$
298 7.68, FDR $p < 0.001$, Figure 2A). Specifically, the morphine group showed reduced salivary
299 cortisol compared to the placebo group at T2 (FDR $p = 0.079$), T3 (FDR $p < 0.01$), T5 (FDR $p <$
300 0.001), T6 (FDR $p < 0.001$) and T7 (FDR $p < 0.001$) (Figure 2A). No drug effects were observed
301 for salivary alpha-amylase (all $F < 0.38$, all FDR $p > 0.62$) or heart rate (all $F < 2.83$, all FDR $p >$
302 0.09).

303 ***Subjective measures***

304 Morphine administration resulted in significantly higher scores of the POMS subscale
305 "Displeasure" after stress induction (Drug*Time: $F(4,312) = 2.97$, FDR $p = 0.03$; MORPH vs.
306 PLB at T5: FDR $p < 0.01$) (Figure 2B). A similar pattern, though not reaching the statistical
307 significance threshold, was observed for negative mood (Drug*Time: $F(6,468) = 2.16$, FDR $p =$
308 0.068) (Figure 2C). No significant group differences were observed for positive mood or for the
309 other POMS subscales (all FDR $p > .15$). Furthermore, no drug effects were observed in
310 anticipatory stress (PASA primary and secondary appraisal at T4; both $t < 0.60$, $p > 0.55$), nor in
311 the participants' satisfaction towards their performance expressed after TSST completion (T5;
312 $t(75.2) = -0.63$, $p = 0.53$).

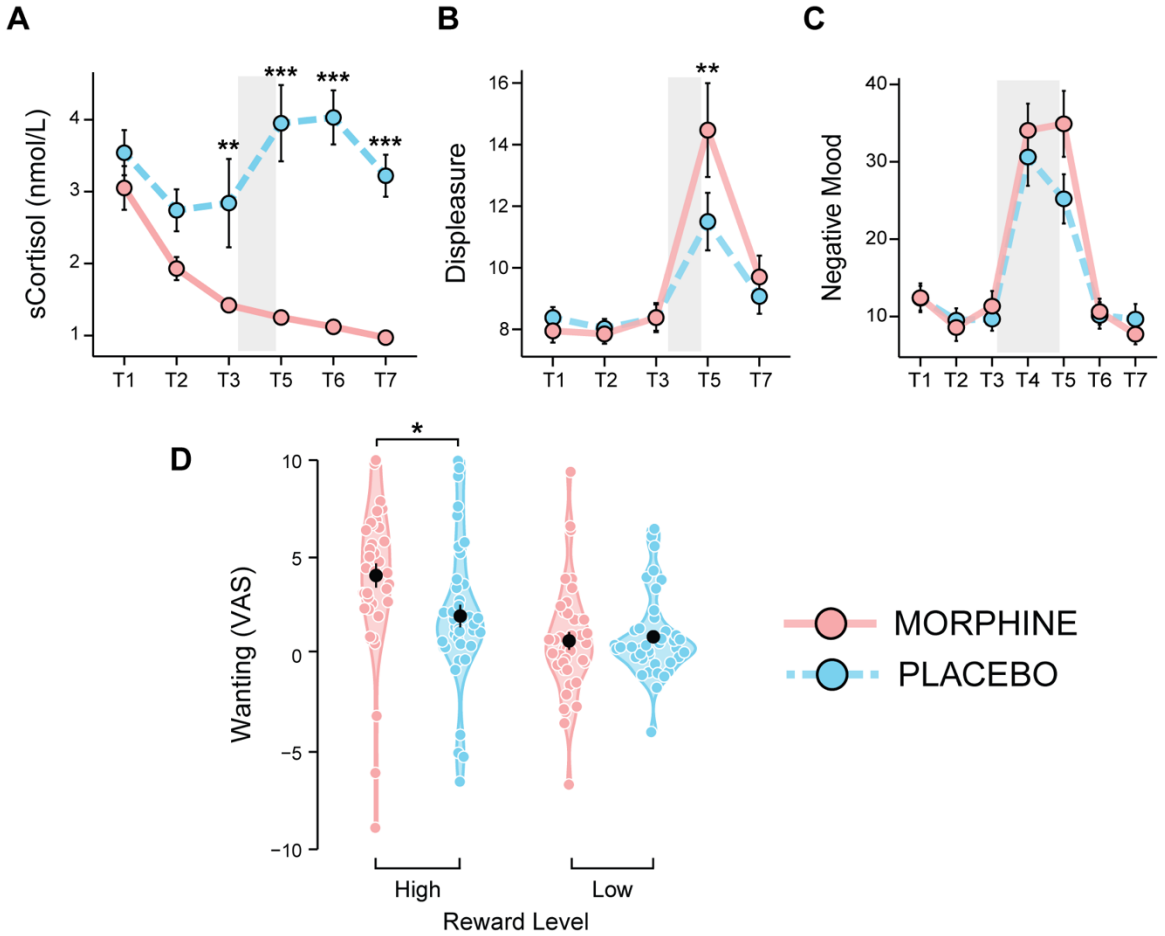

Figure 2. **Effects of morphine administration on stress responses and social reward processing.** (A) Morphine administration suppressed the hypothalamic–pituitary–adrenal (HPA) axis activity, as shown by blunted salivary cortisol (sCortisol) before and after stress induction. Morphine administration also increased the subjective negative response to stress, as shown by (B) higher scores on the "Displeasure" subscale (POMS) and (C) elevated negative mood (measured via visual analog scales) immediately after the Trier Social Stress Test (TSST). (D) Morphine administration resulted in significantly greater wanting for the high social reward (6 cm/s, CT-optimal touch), compared to placebo (black dots represent group means and colored dots represent individual means). Grey bars represent the TSST time window (anticipation, speech, and

323 arithmetic task); error bars represent standard error of the mean; asterisks indicate significant
324 differences between drug groups (* p <.05, *** p < .001).

325 ***Correlations between physiological and subjective measures of stress***

326 Given the observed opposite effect of the drug on cortisol and mood, we conducted a correlation
327 analysis to investigate the association between endocrine and subjective measures of stress. To
328 this aim, we first computed the area under the curve in respect to increase (AUCi, from T3 to T7)
329 [47] of the cortisol levels and of the negative mood ratings (VAS and POMS). Results showed a
330 negative correlation between salivary cortisol and negative mood (VAS; r_s = -.36, *p bonferroni* =
331 .048) as well as between cortisol and the Displeasure subscale of the POMS (r_s = -.37, *p bonferroni*
332 = .044), suggesting an inverse relationship between HPA axis and mood responses to stress.

333 **Effects of morphine on social reward processing**

334 We first examined whether the number of high, low and very low rewards obtained during the task
335 was similar for the two groups. A linear model on number of trials was fitted, including Drug
336 (MORPH, PLB) and Reward Level (high, low, and very low). We observed only a main effect of
337 Reward Level ($F(2,369.5) = 11.28$, $p < 0.001$), indicating that participants obtained more often
338 high rewards as compared to low and very low rewards. We also tested for group differences in
339 MVC, assessed before and after the Social Reward task and used to calibrate the effort task. No
340 significant differences emerged (all $t <$ -0.84, all $p > 0.40$), indicating no drug effects on
341 participants' grip force at rest. Last, we tested for group differences in the baseline activity of the
342 corrugator and zygomaticus muscles. No significant differences emerged (all $t < 1.27$, all $p > 0.21$).

343 ***Effects of morphine on subjective ratings of wanting and liking***

344 *Ratings of Wanting.* Participants administered morphine expressed significantly greater wanting
345 of the high social reward (CT-optimal touch) compared to the placebo group (Drug*Reward Level:

346 $F(1,78) = 10.56$, FDR $p = 0.003$; high social reward MORPH vs. PLB: FDR $p = 0.035$) (Figure
347 2D). No drug effects emerged for the low social reward (FDR $p = 0.72$).
348 *Ratings of Liking.* Participants in both groups expressed greater liking for high compared to low
349 social rewards, which in turn were more liked than the very low social rewards (Reward Level:
350 $F(2,72.9) = 20.93$, FDR $p < 0.001$; high vs. low vs. very low: all FDR $p < 0.001$). No significant
351 effects of drug were observed (all $F < 4.23$, all FDR $p > 0.07$).

352 ***Effects of morphine on force exerted and hedonic facial reactions***

353 We further assessed wanting of social rewards in terms of force exerted to obtain the tactile stimuli,
354 as well as hedonic facial reactions during reward anticipation (Anticipation Pre- and Post-Effort
355 phases). Hedonic facial reactions during and after consumption (Delivery and Relax phases) of the
356 social reward were employed as a measure of liking.
357 *Force exerted.* Participants overall exerted greater force to obtain the high compared to the low
358 social reward (Reward Level: $F(1,78) = 14.23$, FDR $p < 0.001$). No significant drug effects were
359 observed (all $F < 4.13$, all FDR $p > 0.07$).
360 *Facial EMG.* No significant drug effects were observed on the activity of the corrugator and
361 zygomaticus muscles during reward anticipation and consumption. The analyses on the
362 zygomaticus revealed a significant Wanting by Epoch interaction ($F(1,3831) = 10.61$, FDR
363 $p < 0.01$), as smiling was positively associated to the ratings of wanting during the announcement
364 of the attained reward (Anticipation Pre-Effort) but not during the announcement of the attainable
365 reward (Anticipation Pre-Effort). The analyses on the corrugator revealed a significant main effect
366 of Epoch ($F(1,73.1) = 8.79$, FDR $p = 0.03$), as participants frowned less during the delivery of
367 social touch as compared to the relax period.

368 **Discussion**

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

384 *Morphine-blunted cortisol stress response is associated with increased negative affect*

385 Administration of the μ-opioid agonist morphine prior to TSST exposure resulted in blunted
386 salivary cortisol response, indicating suppression of the HPA axis reactivity to stress. This is in
387 line with previous animal and human evidence indicating an inhibitory role of μ-opioids on cortisol
388 release following stress exposure [48]. Recently, two studies investigated the effects of partial
389 (buprenorphine) and full (hydromorphone) MOR agonists on psychosocial stress, induced via
390 TSST [14,15]. Akin to the present findings, reduced cortisol responses to stress were observed.

391 Buprenorphine and hydromorphone also reduced the perceived threat and the appraisal of how
392 challenging the participants found the stress task, respectively. While the authors interpret the
393 findings on cortisol and stress appraisal as indicators of a reduced stress response, no mood-
394 buffering effects of the drug were observed. This is in contrast with the current study, where we
395 find that the dampened cortisol response is accompanied by an enhanced aversive reaction to
396 stress, as shown by higher negative affect following morphine compared to placebo administration.
397 Human cortisol permeates the blood-brain barrier to feedback the central nervous system, reducing
398 HPA axis activity and promoting emotion regulation [49,50]. The observed inverse relationship
399 between the HPA axis activity and negative affect possibly indicates a disruption of this feedback
400 loop by morphine administration and is in line with a mood-buffering effect of cortisol [49,51].
401 Accordingly, it was recently shown that pharmacological HPA axis suppression, by means of
402 dexamethasone administration, blunts the cortisol response to stress and increases negative mood,
403 especially in women [52,53].
404 Our results are opposite to our *a priori* hypothesis based on previous evidence from animal studies
405 on separation distress indicating a reduction of stress indices, such as distress vocalizations,
406 following MOR agonist administration [54]. The paradoxical morphine effect of enhanced
407 aversive stress reaction observed in the current study vs. the previous animal literature may be
408 explained by experimental differences, such as route and timing of administration of the opioid
409 compounds. For instance, in animal research morphine is typically delivered intravenously after
410 stress induction, possibly allowing the system to prepare to face the stressor via an initial mounting
411 of the physiological stress response. In this study, morphine was administered orally to minimize
412 the invasiveness of the administration procedure. Unlike intravenous administration, per-oral
413 morphine has a slow pharmacokinetic profile and requires an average time of 60 min to reach the

414 peak blood concentration. As the subjective response to acute stress, especially its effect on mood,
415 typically lasts only for short periods of time after laboratory stress induction, orally administering
416 the drug and waiting for it to reach peak concentration after the TSST would not have been feasible.
417 For these reasons we administered the compound prior to stress exposure. However, the resulting
418 suppression of the HPA axis activity before the beginning of the stress induction may have led to
419 the observed difference in the mood response. Accordingly, the raising of cortisol levels, via
420 cortisol administration, prior to stress induction has been shown to have a protective role in
421 women, lowering the negative subjective reaction to stress [51].

422 *Morphine-induced increased aversive reaction to stress is associated with enhanced social*
423 *motivation*

424 Regarding the social reward task, the observed effects are consistent with previous models [3,4]
425 suggesting that, under distress conditions, individuals seek physical social contact to down-
426 regulate the negative state and re-store comfort. Accordingly, we showed that the morphine-
427 induced increased negative reaction to the TSST was accompanied by enhanced social contact
428 seeking. Specifically, we observed greater subjective wanting of the most pleasurable social
429 reward (CT-optimal touch) following morphine administration, compared to placebo. Physical
430 social contact, such as grooming in animals and caressing in humans, has a soothing function and
431 is considered a powerful means to buffer distress and re-store comfort [55]. Interpersonal touch,
432 and particularly slow CT-optimal touch in humans, has been shown to reduce the physiological
433 signs of distress [e.g., 56,57] and the psychological consequences of aversive social situations,
434 such as social rejection [58], representing therefore an especially appetitive stimulus in such
435 situations. However, while previous models predict that the relief of negative affect through social
436 contact is mediated by μ-opioids [3,4], due to the employed design and the opposite effects of the

437 drug on stress responses compared to previous animal research, the role of the MOR system in the
438 observed enhancement of contact seeking remains to be clarified.
439 The results are also consistent with existent evidence on the effects of stress and negative affect
440 on reward processing. Previous research has indeed shown that stress exposure, and the consequent
441 increase of negative affect, boosts the incentive value of appetitive stimuli, such as food, money,
442 or drug cues. This results in a selective increased motivation to obtain the reward, despite an absent
443 increase in the pleasure experienced once it is consumed [e.g., 17,59,60]. Notably, the results
444 reported here replicate previous findings from our group indicating that, as already shown for non-
445 social rewards, psychosocial stress increases wanting, but not liking, of rewards of social nature,
446 such as interpersonal touch [16]. As in Massaccesi et al. 2021, the increased negative mood was
447 accompanied by an increase in the subjective desire of receiving pleasurable social touch, but not
448 in the force exerted to obtain it. While implicit wanting can occur without a conscious experience
449 and mostly depends on dopaminergic mechanisms, explicit (subjective) wanting, or cognitive
450 desire, requires awareness and is strongly linked to the individual's previous liking experiences
451 with and memories of the rewarding stimulus [61]. The cognitive desire of the stimulus is therefore
452 driven by the pleasure that individuals expect to receive once the reward is consumed, based on
453 these memories. Accordingly, a recent study on smokers showed that self-reported wanting and
454 anticipated liking in response to smoking-related cues were strongly correlated and increased in
455 heavy smokers, while this was not the case for consummatory liking, i.e. the pleasure reported
456 after smoking a cigarette [62].
457 As expected, the effect of enhanced wanting for the social reward was selective for the most
458 pleasurable touch (high reward level), characterized by slow stroking velocity (CT-optimal touch).
459 This is different from our previous work [16], were a general increase of wanting, independent of

460 reward magnitude, was observed following stress. The potentiation of wanting for the most
461 pleasurable touch observed here may be due to a specific influence of the enhanced MOR system
462 activity. Indeed, previous research has shown that pharmacological challenges of the MOR system
463 have strongest effects on rewards of greatest magnitude [e.g., 6,18]. Therefore, it can be
464 hypothesized that while stress alone may result in increased motivation for all available rewarding
465 stimuli, an additional potentiation of motivation for the most valuable stimulus is seen for the best
466 reward due to MOR stimulation. Alternatively, a reason for the partially different result pattern
467 might be that, differently from the current study, in Massaccesi et al. 2021 participants were
468 allowed to individually rank the three types of touch as high, low and very low. The high reward
469 was therefore not necessarily the CT-optimal touch. This could have masked a possible impact of
470 the type of touch. While our previous work included different state manipulations, this study
471 focused on the effects of stress. Since slow CT-optimal touch is more likely to convey affiliative
472 intentions such as social support compared to faster touch [63], here the slow CT-optimal touch
473 was kept as fixed high reward to avoid possible confounding effects related to the speed of
474 stroking.

475 *Study limitations*

476 Some limitations of the study should be considered. First, while the use of a within-subject design
477 is usually preferable in pharmacology, in this study we employed a between-subjects design. The
478 choice was mainly motivated by the fact that repeated exposure to the TSST, especially with a
479 short time interval, can lead to habituation of the stress response as well as other repetition effects,
480 resulting in low test re-test reliability [e.g., 64,65] and thus outweighing by far the possibly higher
481 statistical power a within-subject design might have had in principle. Second, despite our efforts
482 in enhancing the social properties of the administered touch (e.g., skin-to-skin administration), the

483 absence of a social relationship between the toucher and the participant, as well as other
484 methodological aspects (e.g., use of a curtain separating participant and toucher), might have
485 affected our results. Nevertheless, in humans an involvement of the MOR system in bond
486 formation, rather than just maintenance, has also been observed [12]. Third, the current study
487 tested a sample of healthy female participants, preventing a generalization to male individuals.
488 Finally, it is unlikely that a complex behavior such as social motivation can be fully explained by
489 the activity of a single neurochemical system only. For instance, the neuropeptides oxytocin and
490 vasopressin are well-known for their crucial role in bonding and reproductive functions [66]. Stress
491 exposure induces a potent activation of the dopaminergic system [67,68], and it has been shown
492 that MOR stimulation disinhibits dopaminergic neurons in the ventral tegmental area and increases
493 dopamine release in the nucleus accumbens [69]. To investigate how these systems interact in
494 driving social contact seeking during aversive states seems therefore necessary to understand the
495 neurobiology of social motivation.

496 *Conclusions*

497 To conclude, our findings show that enhancing the MOR system activity before psychosocial stress
498 exposure increases, rather than reduces, the aversive reaction to stress, leading to an increased
499 subjective motivation for the attainment of highly rewarding social contact. Specifically, we
500 showed that morphine administration blunted cortisol reactivity to stress and increased the
501 negativity of the adverse experience, in line with a mood-buffering effect of cortisol. Further, the
502 results indicate that this enhanced morphine-induced stress response was followed by increased
503 desire to attain social rewards.

504 Overall, the results significantly extend previous human evidence on the modulation of the
505 physiological and subjective responses to social stress by the MOR system and indicate a state-

506 dependent regulation of social motivation. A better understanding of the effects of opioids on
507 mood, wellbeing and social behaviors via opioid manipulations in healthy, opioid-naïve
508 individuals may have important implications as drugs that act on the opioid system are widely
509 consumed in both medical and non-medical contexts. To clarify the role of the MOR system in
510 modulating contact seeking behaviors under distress, future studies should investigate the effects
511 of MOR agonists and antagonists administered after stress induction (for instance via intravenous
512 administration), as well as the interaction with other neurochemical systems, such as dopamine.

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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.

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