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Reducing Social Stress Elicits Empathy for Pain in Mouse and Human Strangers

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Running Title: Stress and emotional contagion in mice and humans

Summary

Background: Empathy is a multidimensional construct; at its core is the phenomenon of emotional contagion (state matching). Empathy for another's physical pain is well known in humans, and emotional contagion of pain has been demonstrated in mice. In both species, empathy is stronger between familiars. Using a translational approach, we investigated the effect of stress on emotional contagion of pain in familiar and stranger dyads of both species.

Results: Mice and undergraduates were tested for sensitivity to noxious stimulation alone and/or together (dyads). In familiar—but not stranger—pairs, dyadic testing was associated with increased pain behaviors or ratings (i.e., hyperalgesia) compared to isolated testing. Pharmacological blockade of glucocorticoid synthesis or glucocorticoid/mineralocorticoid receptors revealed the hyperalgesia in mice and human stranger dyads, despite having no effect (or even an opposite effect) on pain sensitivity *per se*, as did a shared gaming experience (the video game Rock Band®) in human strangers.

Conclusions: We show here that blockade of glucocorticoid synthesis or receptors for adrenal stress hormones elicits the expression of emotional contagion of pain in strangers of both species. Our results demonstrate that emotional contagion is prevented, in an evolutionarily conserved manner, by the stress of a social interaction with an unfamiliar conspecific, and can be evoked by blocking the endocrine stress response.

Introduction

Empathy—the ability to understand another's emotions and intentions, and to respond appropriately—is a basic feature of social experience [1], and is vital to interpersonal well-being and to societal functioning more generally as it underlies a variety of prosocial, altruistic behaviors [2]. Conversely, the absence of empathy is a defining feature of disorders marked by deficits in social functioning and anti-social behavior like psychopathy [3]. Empathy—an affective state more appropriate to the situation of another compared to one's own [4]—is a multidimensional construct with emotional contagion as a basic, lower-level form [5]; higher "levels" of empathy include helping or pro-social behavior. We previously demonstrated that laboratory mice are capable of emotional contagion [6]. The primary finding was that mice of both sexes tested for sensitivity to noxious stimuli in the presence of a cagemate also in pain, but not a stranger also in pain, displayed significantly higher levels of pain behavior (i.e., hyperalgesia) than mice tested in isolation. We also found that in tests in which only one mouse was in pain, the presence of an unaffected stranger male mouse produced hypoalgesia in the affected male subject [6]; we have subsequently shown that this is a social form of stress-induced analgesia, dependent on intact testosterone levels in both mice [7]. Stress levels—as measured by numbers of fecal boli [6], plasma corticosterone (the species-typical glucocorticoid) [7] and expression of the corticotrophin releasing hormone gene in stress-relevant brain loci (manuscript in preparation)— in stranger dyads are higher than in cagemate dyads or isolated mice, suggesting that stress might be responsible for the absence of empathy for the pain of strangers. The current study was designed to investigate the relationship between stress and pain empathy in both mice and humans, using as similar a protocol for both species as possible.

Results

Stress Affects Emotional Contagion of Pain Bi-Directionally in Mice

Here we tested male mice for sensitivity to noxious stimuli on the abdominal constriction test either in isolation or in dyads in which both mice were injected with 0.9% acetic acid ("both in pain" or BP dyad condition; Figure 1A). Male mice tested in stranger dyads in which only one mouse received the noxious stimulus (and the other a vehicle injection; "one in pain" or OP dyad condition) displayed naloxone-reversible (i.e., opioid-mediated) stress-induced analgesia compared to isolated mice (Figure S1), as we have previously observed [6, 7], and were thus not appropriate as a control group. Pretreatment with the glucocorticoid synthesis inhibitor, metyrapone (50 mg/kg), produced no effect on pain behavior in the isolated condition (t_{30} = 0.6, p=0.53) (Figure 1A). Significant main effects of social context (Isolated/Cagemate/Stranger: $F_{2,102}$ = 10.8, p<0.001) and drug ($F_{1,102}$ = 10.7, p<0.01), and a significant interaction ($F_{2,102}$ = 3.3, p<0.05) were obtained. In vehicle-treated mice, pain behavior in the dyadic condition increased significantly in cagemates (p<0.05) but not strangers (p=0.73), replicating our previous findings [6]. However, pretreatment with metyrapone significantly increased pain behavior in stranger dyads as well as cagemate dyads (both p<0.05) (Figure 1A). That is, metyrapone appeared to evoke, or allow, an empathic response (emotional contagion) between strangers normally observed only between cagemates. A higher dose of metyrapone (75 mg/kg) also elicited emotional contagion in a separate group of stranger dyads, while producing frank analgesia in isolated mice (Figure S2). The effect of metyrapone was not mediated by opioid receptors (Figure S3), nor were these data confounded by freezing behavior or aggression, which did not differ between conditions (data not shown).

If stress reduction can elicit emotional contagion in strangers, the induction of stress might be expected to abolish the effect in cagemates. Cagemates simultaneously experiencing a 15-min restraint immediately prior to dyadic pain testing still exhibited hyperalgesia ($F_{2,33} = 4.7$, $p<0.05$; $p<0.05$ compared to isolated testing); however, after a 30-min restraint producing significant stress-induced analgesia ($p<0.01$ compared to no restraint), mice in the cagemates–BP dyad condition displayed no trace of increased pain behavior (i.e., emotional contagion) ($F_{2,33} = 0.2$, $p=0.76$) (Figure 1B).

Glucocorticoids act through two different receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). To determine the receptor(s) responsible for the metyrapone effect, we pretreated mice in a separate experiment (stranger dyads only) with the GR antagonist, mifepristone (RU 486; 10 mg/kg), the MR antagonist, RU 26752 (5 mg/kg), or a combination of the two drugs. None of these pretreatments affected pain behavior in mice tested in isolation ($F_{3,50} = 0.4$, $p=0.77$). However, in the strangers–BP condition the combination of mifepristone and RU 26752 significantly increased pain behavior (social context x drug: $F_{3,110} = 3.2$, $p<0.05$) (Figure 1C), recapitulating the empathy-evoking effect of metyrapone. Mifepristone alone was also ineffective at a higher dose of 20 mg/kg (data not shown). Taken together, these findings demonstrate that corticosterone acting at both GRs and MRs prevents empathy for pain in stranger mice.

Humans Also Show Emotional Contagion of Pain

Although empathy for pain in humans is well studied [8], the emotional contagion effects we observe in mice have never been directly investigated using noxious stimuli applied to simultaneously tested human dyads. We recruited university undergraduates, some of whom

were instructed to bring a same-sex friend with them to the experiment, whereas others were paired with a same-sex participant, unknown to them, also scheduled to be tested the same day. Before any pain testing occurred participants completed state mood measure (SMM) and pain catastrophizing scale (PCS) questionnaires (Figure 2A). Participants were then tested for sensitivity to noxious stimuli on the cold pressor test twice, in counterbalanced order, once alone and once silently facing a partner who was simply observing (OP dyad condition) or was also being tested (BP dyad condition). Other than during dyadic pain testing both participants were separated for the entire study. Dyadic testing was justified to participants with a ruse suggesting that it was a last-minute modification of the protocol necessitated by time pressures. After a 30-s immersion of the non-dominant hand in 4 °C water, participants were asked to rate pain intensity and unpleasantness on a 10-cm visual analog scale; these ratings were not visible to dyad partners. After all testing was completed, each participant completed questionnaires of dispositional empathy (Interpersonal Reactivity Index; IRI) and feelings of friendship toward the other person tested. See Table S1 for demographic and questionnaire item effects in the isolated condition. Ratings of stimulus intensity and unpleasantness did not differ between groups when tested in isolation (all $p>0.05$) in this and all subsequent experiments. However, as shown in Figure 2B, ratings of noxious stimulus intensity were significantly higher in friends–BP dyads compared to isolated testing (one-sample *t*-test: $t_{16} = 3.4$, $p=0.004$, Bonferroni-corrected $p=0.02$); by contrast, ratings were not altered in strangers–BP dyads or in either OP dyad compared to isolated testing (all $p>0.05$). The size of the increase was comparable in both male–male and female–female dyads (condition x sex interaction: $F_{1,39} = 0.3$, $p=0.59$) in this and all subsequent experiments. These findings are highly analogous overall to those observed in the mouse studies, except that no hypoalgesia was observed in human male OP dyads, likely because

the fear of aggression was minimal in the context of a university laboratory study. In this and all subsequent experiments no statistically significant main or interaction effects of gender or order-of-testing were observed, and similar results were obtained using unpleasantness ratings instead of intensity ratings (Figure S4). Correlations between questionnaire items and isolated–dyad rating change scores were investigated; the only such correlations achieving statistical significance (uncorrected) were negative correlations between self-reported anxiety and pain intensity rating change in strangers (r = -0.44, p<0.05) (Figure 2C) but not friends (r = 0.16, p=0.54) (Figure 2D), suggestive of stress-induced analgesia in the strangers only.

Metyrapone Pretreatment Enables Emotional Contagion in Human Strangers

Based on findings in the mouse, we repeated the experiment described above using stranger dyads only, but with all participants pre-treated with either 750 mg oral metyrapone or placebo 60 min before the first pain test. The experimental procedure was identical to that described above except that saliva samples were obtained before and after pain testing, and subjects were covertly videotaped for later analysis (Figure 3A). As shown in Figure 3B, strangers given metyrapone but not placebo displayed significantly increased stimulus intensity compared to isolated testing (t_{18} = 3.6, p=0.002, Bonferroni-corrected p=0.008); ratings were not altered in placebo–BP dyads or in either OP dyad (all p>0.05). This increased sensitivity in metyrapone–BP dyads occurred despite the fact that this dose of metyrapone produced a hypoalgesic effect in participants when tested alone (t_{61} = 2.4, p<0.05) (Figure 3C). Thus, as in mice, blocking the endocrine stress response elicited empathy for pain in strangers. Analysis of saliva samples confirmed the reduction of cortisol levels by metyrapone compared to placebo (t_{35} = 2.8, p<0.01) (Figure 3D). Videotape analysis of pain testing by coders blinded to drug condition revealed that

in metyrapone- but not placebo-treated subjects there was a significantly greater number of painful facial expressions and post-testing pain behaviors (hand touching and holding) in the BP dyadic versus isolated condition (t_{25} = 2.2, 2.1 and 2.2, respectively; all p<0.05) (Figure 3E), providing further behavioral evidence of the effect of stress reduction on empathy for pain.

A Shared Social Experience Enables Emotional Contagion in Human Strangers

It is likely that in both mice and humans the stressor preventing the emergence of emotional contagion is related to the forced social interaction between strangers in a novel environment, because all other test-related stressors in both species are equivalent across condition. We thus reasoned that we might be able to diminish this social stress directly in humans, by having strangers engage in a social bonding activity immediately prior to dyadic pain testing. To this end, we repeated the original experiment but added a brief, between-subject social or non-social pleasurable experience whereby participants (all strangers) "played" four well-known songs by The Beatles on the video game Rock Band® (Figure 4A). Half of the participants played the game alone, whereas half played together in a cooperative game mode in which the players' game score is based on joint performance. As shown in Figure 4B, only those who played together demonstrated the empathy effect of increased stimulus intensity ratings in BP-dyadic versus isolated testing (t_{15} = 3.3, p=0.005). Moreover, the size of the effect correlated significantly with self-report indices of trust/comfort with the stranger (r=0.55, p<0.05) (Figure 4C). The cooperative gaming experience also decreased plasma cortisol levels similarly to metyrapone (t_{36} = 2.4, p<0.05, compared to playing alone) (Figure 4D). Finally, significant changes in the Inclusion of Other in the Self (IOS) scale of interpersonal closeness (t_{37} = 2.8,

$p<0.01$) and the amount of money offered to the stranger in the Dictator Game ($t_{37} = 2.0$; $p<0.05$) demonstrated that playing the video game together increased affiliation (Figure 4E,F).

Discussion

Here, we replicate our previous findings that emotional contagion of pain occurs between familiar mice but not strangers, and extend those findings to humans. The translation between species was surprisingly direct, with effects of similar magnitude demonstrated using similar sample sizes. That a form of empathy would be present only in cagemates and friends is directly predicted by the perception-action model of empathy [5], which posits that empathy increases with both familiarity (subject's previous experience with object) and similarity (perceived overlap between subject and object). Perhaps the most relevant supporting data are the recent observations that contagious yawning is positively correlated with social familiarity in bonobos [9], baboons [10] and humans [11]. It was also shown recently that cortical activation patterns of pain-related (electric shock) threat to the participant correlated with patterns associated with the same threat to an opposite-gender friend, but not to a stranger [12].

The present findings are unlikely to be due to social support or buffering (i.e., the mere presence of a friend) [13], since this would be expected to operate as well or better in the OP dyads, where in fact no effects on pain were observed. Social buffering of experimental pain in humans has been demonstrated [14, 15], but is highly context-dependent [16], and solicitous partners have been shown to worsen pain [17-19]. We are unaware of any existing studies similar to our BP dyads, in which two human participants are tested for pain sensitivity

simultaneously. Most existing studies of pain empathy in humans have involved visual stimuli (pictures, video, or arbitrary cues) presented to a single participant [20-25].

Although the neuroanatomical basis of empathy for pain is now well characterized [26], the neurophysiology and neurochemistry of empathy remains obscure. The present study suggests for the first time that the hypothalamo-pituitary-adrenal stress axis is an important modulatory system. The effects of stress on emotional contagion appear to be mediated by both high affinity MRs, known to mediate the effects of basal glucocorticoids, and the lower affinity GRs, which predominantly mediate signaling by stress-induced increases in glucocorticoid levels [27]. In fact, MRs exhibit such a high affinity for glucocorticoids that most of these receptors are constantly occupied, even during periods of low basal release [28]. Thus, it was surprising that an acute stress-related phenomenon would involve MRs, since GR activation would presumably be sufficient. Both MRs and GRs are typically regarded as intracellular receptors; emerging evidence indicates that MRs can also be positioned on the membrane of limbic region neurons, where they possess GR-like corticosterone affinity and drive fast signaling cascades [29]. Membrane-bound MRs have been shown to be relevant for acute stress-related effects on memory [30] and aggression [31].

It has been reported that intranasal oxytocin (and oxytocin receptor gene variants) affect both stress and various components of empathy in humans [32-35], albeit in a complex manner [36]. Indeed, we attempted to replicate the human metyrapone experiment using intranasal (24 IU) oxytocin, and found no significant effects of oxytocin on pain contagion, nor did oxytocin in our hands produce any reduction in cortisol levels (data not shown).

Since our previous demonstration of emotional contagion in mice [6], a number of studies have been published suggestive of empathy-related abilities in laboratory rodents and other

non-primate species, including contagious yawn [37, 38], observational fear learning (via affect matching) [39-41], and prosocial (helping) behavior [42, 43]. Several excellent reviews of this literature have recently been published [44-46]. The capability of rodents to display helping behavior is controversial [47, 48], however. Of direct interest are the observations by Ben-Ami Bartal and colleagues (Society for Neuroscience Annual Meeting, 2012) that emotional contagion is required for helping behavior in rats (i.e., freeing a trapped and distressed conspecific), and that rats with lower corticosterone levels are more likely to engage in this behavior. The current demonstration that emotional contagion is perfectly translatable from mice to humans provides an excellent opportunity to exploit mouse genetics and physiology to better understand underlying mechanisms of the phenomenon, on which higher forms of empathy are dependent.

We are unaware of any prior demonstration or speculation that stress can directly affect emotional contagion, although it has been shown that: 1) stress itself can be directly contagious [49], displaying physiological resonance between individuals; 2) that empathy for negative emotions, including stress, can be stressful to the empathizer [49, 50]; and, 3) that contagious laughing and yawning are impaired in individuals with post-traumatic stress disorder [51]. The present demonstration that stress can impair emotional contagion in familiar mice, and that stress reduction can enable it in stranger mice and humans, raises the tantalizing possibility that higher forms of empathy are similarly controlled. If so, simple and readily achievable strategies for increasing empathic behavior among strangers in specific contexts are suggested, as are strategies for increasing empathy in the context of chronically stressed relationships. However, recent experiments have demonstrated that social stress can increase prosocial behaviors in humans, such as trust and sharing [52], especially in situations when immediate helpful

responses are available to an observer [53]. The apparently contradictory direction of the effects observed here may reflect differences between emotional contagion and prosocial behavior, differences between empathy for pain and for stress itself, or because in our paradigm no overt helping behaviors were available to either participant.

Experimental Procedures

Animal Subjects

All behavioral experiments were performed on naive, male CD-1® (ICR:Crl) mice (6–12 weeks of age) that were bred in-house from breeders obtained from Charles River (Boucherville, QC). Male mice only were used because only male mice display both phenomena under study (i.e., hyperalgesia in cagemate dyads with both in pain, analgesia in stranger dyads with one in pain), and because we considered the demonstration of empathy in stranger male dyads to be a more challenging test of our hypothesis. All mice were housed 2–4 per cage in standard shoebox cages, maintained in a temperature-controlled (20 ± 1°C) environment (14:10 h light/dark cycle, with lights on at 07:00 h), and fed (Harlan Teklad 8604) and watered *ad libitum*. All animal studies were approved by a local animal care and use committee and were consistent with national guidelines.

Acetic Acid Test

Mice were assessed for nociceptive sensitivity using the acetic acid "writhing" test, and previously used by our lab to demonstrate that mice have the capacity for emotional contagion [6]. All testing occurred near mid-photoperiod (10:00–16:00 h). Drug or vehicle was administered, and then mice were placed on a glass surface within transparent Plexiglas cylinders (30 cm high x 15cm diameter) and allowed to habituate to the cylinder for 30 min. Mice were briefly removed, and 0.9% acetic acid (in physiological saline) was injected i.p. (10 ml/kg). In the OP condition (see below), one mouse in the dyad, chosen randomly, received physiological saline instead of acetic acid. Mice were placed back in their cylinders and observed continuously for 30 min. Stereotypical abdominal constrictions (lengthwise constrictions of the torso with a

concomitant concave arching of the back) were counted over this period, sampling for 5 s every 20 s for a total of 90 observations. We have found sampling in this manner to feature high accuracy and higher interrater reliability than counting constrictions [6]. Four mice were observed and scored per run. In all cases, the scorer was blinded to drug condition and cagemate/stranger status (see below), although for obvious reasons blinding to acetic acid injection was not possible.

Social Conditions

Mice were tested either in isolation (one mouse per cylinder) or in dyads (two mice per cylinder). In the OP (*o*ne in *p*ain) dyad condition, one mouse was injected with acetic acid and one was injected with saline within 20 s of each other; in the BP (*b*oth in *p*ain) dyad condition, both mice were injected with acetic acid within 20 s of each other. Since writhing behavior can only be observed in mice injected with a noxious stimulus, writhing data were collected from both mice in BP dyads and only the acetic acid-injected mouse in OP dyads. Mice forming dyads were either drawn from the same cage (non-sibling "cagemates", having lived together since weaning) or from different cages ("strangers").

Drugs

Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone), mifepristone (11β-(4-dimethyl-amino)-phenyl-17β-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one; RU 486) and RU 26752 (17α-Hydroxy-3-oxo-7α-propylpregn-4-ene-21-carboxylic acid γ-lactone) were purchased from Sigma Aldrich (St. Louis, MO). Metyrapone was dissolved in saline and injected, 30 min prior to acetic acid injection, subcutaneously at a volume of 10 ml/kg.

Mifepristone and RU 26752 were prepared in a suspension of Tween 80 (2%, vol./vol) and methylcellulose (0.5%, weight/vol.) and administered, either individually or together, 30 min prior to acetic acid injection, per oral at a volume of 10 ml/kg. In OP conditions, the same mouse receiving the acetic acid received the drugs listed above. In BP conditions, both mice received the drugs.

Restraint Stress

Mice were restrained in a 50-ml conical tube, with air holes, for either 15 or 30 min. They were injected with acetic acid immediately after being removed from the tube.

Human Participants

McGill University undergraduate students of both sexes (age range 18–35) were recruited by responding to an online advertisement that offered a monetary reward (between CAD$10–$20, depending on the experiment) for participating in a study examining sensory perception to cold water. Participants were pre-screened and excluded if they were currently taking prescription drugs, over-the-counter drugs (e.g., antihistamines, cold or flu remedies, sleeping aids), analgesics (aspirin, Tylenol, Advil, or any other pain reliever), or recreational drugs (e.g., marijuana). Further exclusion criteria included circulation or blood pressure problems, Reynaud's disease, diabetes, epilepsy, asthma and cardiac disease. All human studies were approved by a local Research Ethics Board and were consistent with national guidelines.

Cold Pressor Test

Pain ratings were obtained twice for each subject, using the cold pressor test. Each time, participants rated both the intensity and unpleasantness of pain on a 10-cm visual analog scale labeled at each end as "No Pain" and "Worst Pain Imaginable" (for intensity) and "Not at all Unpleasant" and "Most Unpleasant Pain Imaginable" (for unpleasantness). Prior to their first test, we explained to each participant the basic principle of the distinction between sensory-discriminative and motivational-affective components of pain: that *intensity* refers to the amount of pain and *unpleasantness* refers to how upsetting or emotionally salient the pain was. Each cold pressor trial required participants to immerse their non-dominant hand in water maintained at 4 ºC with a cooling probe (Thermo Fischer Scientific, Hudson, NH) and water circulator (Thermo Fischer Scientific) for 30 s. The water immersion time was set at 30 s because: 1) in pilot testing virtually all participants were able to submerse their hand for this length of time, and we did not want the withdrawal response of one participant to influence the withdrawal response of the other participant; and, 2) hyperalgesia was more likely to be detected using modest pain intensities.

Coding of Pain Behaviors

In one experiment, face and body gestures of participants were recorded by video during the cold pressor task. We used a coding system modeled after previous work [54]. Briefly, the current coding system was comprised of the following categories and criteria: 1) *Action involving the immersed arm*. This category was only coded for the period following the removal of the arm from the cold pressor. Positive pain behaviors included abnormally rigid or stiff movements, bracing, holding, shaking, and rubbing of the hand or arm. 2) *Action not involving the immersed*

arm. Characteristically this category included behaviors such as rocking, arching of the neck, bending, or bouncing. 3) *Action involving the face*. This category included actions such as grimacing, narrowed eyes, furrowed brow, tightened lips, and pulling back or clenching of the corners of the mouth. 4) *Vocalizations*. Criteria for this category included verbal and para-verbal expressions such as gasping, sighing, and grunting. Two trained coders, blind to the experimental hypotheses, assessed the acquired footage for instances of pain behavior. The coders provided a frequency count for the behaviors observed and recorded their duration (in s) in the 30-s period following the immersion.

Salivary Cortisol

Salivary cortisol samples were collected using a Sarstedt salivette device (Sarstedt, Germany) and stored at −80 °C until assayed. Samples were thawed and spun at 2000 rpm at 4 °C for 2 min, and cortisol concentrations were determined using Salimetrics high sensitivity salivary assay kits (State College, PA, Kit#1305503). Samples and standards were assayed undiluted in duplicate according to the manufacturer's protocol. Single absorbance readings for samples and standards were obtained at 450 nm (BioTekELx800 plate reader), and these values were used for calculation of cortisol levels (ng/ml) based on a linear regression of the standard curve using a log-logit transformation.

Friends vs. Strangers Experiment

Participants were first randomly assigned using block randomization to either the friend or stranger condition. Those assigned to the friend condition were contacted and asked to bring a same-sex friend with them on the day of the experiment under the pretense that this would

rapidly facilitate data collection. Those who were unwilling or unable to do so were excluded from the study. Participants in the stranger condition were randomly paired with another same-sex participant who could attend the same testing session.

When participants arrived to the lab, we obtained informed consent from each in a waiting room separate from the testing room. Each participant then filled out a State Mood Measure (SMM) questionnaire (12 adjectives taken from the Profile of Mood States) [55] to assess their mood at the time of the experiment, and the Pain Catastrophizing Scale (PCS), which assesses the degree and magnitude of negative pain-related thoughts [55]. We next tested one of the participants, picked at random by flipping a coin, for pain in a separate room, while the other participant waited with an assistant. After this first test (isolated trial), we told both participants that due to time constraints one of their pain tests would be done together (dyad trial). Our real reason for doing this was to determine whether their pain ratings changed when tested together. During the dyad trial, we instructed participants to look straight ahead (thus, directly at the other participant) and to remain silent; we did not tell them explicitly to either engage or avoid the other's gaze. Following the dyad trial, the second participant completed their isolated trial. Conducting the experiment in this manner effectively counterbalanced isolated and dyad trials within-subject. To control for changes in sensitivity across trials, subjects were always given at least 5 min between trials for their hands to recover. We also counterbalanced the "together" condition throughout the experiment between-subject, with a mix of friends and strangers participating on each day. Finally, human OP and BP conditions were counterbalanced between-subjects in the dyad trial, with both participants immersing one of their hands (BP) or only one participant immersing his or her hand while the other simply observed (OP).

Following the cold pressor testing sessions, each participant was taken to a separate room and completed a questionnaire (Table S2) specifically constructed to assess their relationship with and feelings toward the other person participating in the experiment with them. Specifically, questions assessed feelings of affiliation and the degree of perceived friendship between the participants, and thus could be given to participants in both the friends and stranger conditions. Following the friendship questionnaire, we assessed participants' level of Empathic Concern and Perspective Taking, which are sub-scales of the Interpersonal Reactivity Index (IRI) [56]. These sub-scales provided a measure of trait empathic tendencies. After the experiment was over, subjects were debriefed in separate rooms. See Figure 2A for a detailed timeline.

Metyrapone Experiment

In order to assess whether the presence of a stranger, with its concomitant social stress, precluded the expression of hyperalgesia in the dyad condition, we blocked cortisol synthesis by administering metyrapone (750 mg, HPA Pharma, Paris France). Metyrapone or identical placebo pills were administered to participants (only same-sex strangers) in a randomized and double-blind fashion along with a light snack (yogurt) to minimize the nausea that might be caused by metyrapone. Subjects completed the SMM and provided saliva samples before and 90 min after metyrapone/placebo administration, immediately following cessation of all pain testing. Participants were tested twice for pain in either OP or BP conditions as described above for the Friends vs. Strangers experiment, with pain testing starting 60–70 min post-drug. This time frame was chosen because peak plasma concentrations of metyrapone are usually reached 60 min after drug administration. See Figure 3A for a detailed timeline.

Rock Band® Experiment

Because social bonding has been shown to increase empathy and emotional contagion ([see 57], we wanted to determine whether a brief social bonding experience (playing the video game Rock Band®) would elicit empathy for pain in strangers. Participants (only same-sex strangers) were recruited to participate in a study ostensibly examining the effects of video games on sensory perception, and were randomly paired with another person that could attend the same session ("play together" condition). Single ("play alone" condition) participants were also recruited and tested for comparison purposes. After arrival to the lab, participants completed the SMM and PCS questionnaires and were given a brief description/tutorial about the video game, The Beatles™:Rock Band®. In this game players press buttons on a simulated guitar while colored shapes cross the screen; if this is done in synchrony the actual recorded guitar (or bass) notes will play, yielding a strong illusion that one is actually playing the instrument. Participants were told that we were only interested in the effects of this type of video game on sensory perception, and not their success in playing the game. To reduce competitiveness between participants in the "together" condition the video game was played in “band mode”, where scores depend on joint rather than individual performance. All subjects were required to play the same four songs (alternating between the guitar and bass parts), which lasted approximately 15 min including the tutorial/explanation of the game. Following the video game portion one subject was taken (at random) to the cold pressor room, while the other participant waited with an assistant. The remainder of the experiment was completed as described for the Friends vs. Strangers experiment, except that no OP condition was performed. See Figure 4A for a detailed timeline.

Affiliation Tests

To test whether strangers who played Rock Band® together developed affinity for one another, we conducted a series of tests designed to assess affiliation in a separate group of participants who were not tested for pain. Participants first completed the SMM upon entering the lab. All participants then engaged in a Rock Band® video game session as described above, either by themselves (playing alone) or with the other participant (playing together). Participants were then separated if necessary and completed the Inclusion of Other and Self (IOS) Scale of interpersonal closeness (see below), the Dictator Game (see below), the SMM again, the IRI and the friendship questionnaire. Subjects in the playing alone condition completed the tests for affiliation based on the fake biography of a fictitious participant in a separate testing area. Salivary samples were provided at baseline (upon arrival to the lab) and prior to completion of the experiment, at approximately the same time point as in the metyrapone experiment.

The IOS is a widely used single-item pictorial measure of relationship closeness [58]. We used a web-based variant of the original IOS, which allows for output values to be continuously scaled [59]. Participants were instructed to use their mouse to move a "self" figure so that it best represents their relationship with the other participant. The output distance (the number of pixels moved between 0-100) and the overlap area of the two figures were recorded.

For the Dictator Game, each participant was told at the end of the experiment that we had randomly selected one of them to receive an additional $10 in compensation. Each was told, in a separate room, that in fact *they* were the one selected, and that they could decide whether they wanted to share any amount (including $0) of their "extra" money with the other participant. After the subjects specified their monetary donation they were debriefed, and regardless of their response both received the extra $10 compensation.

Statistical Analyses

Data were analyzed (Systat v. 13) using Student's *t*-test or ANOVA followed, where appropriate, by posthoc testing using Tukey's or Dunnett's case-comparison test. A criterion α level of 0.05 was used in all cases. A small number (n=3) of mouse data points were excluded from analysis based on their identification as statistical outliers (Studentized residuals >3). No human data were excluded.

Author Contributions

R.M.S. and J.S.M. conceived of the study. L.J.M., J.A.B., D.J.L. and J.S.M. designed the study. L.J.M., G.H., K.I., S.M., E.L.A., N.N., P.M.S., and W.F.S. performed the study. L.J.M., Z.T., W.F.S., and J.S.M. analyzed the data. L.J.M. and J.S.M. wrote the manuscript, with input from all the authors.

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L.J.M., R.M.S., D.J.L., and J.S.M. conceived and designed the study. L.J.M., G.H., K.I., S.M., E.L.A., N.N., P.M.S., and W.F.S. performed experimental work. Z.T., J.A.B., and W.F.S. designed and oversaw certain experiments. L.J.M. and J.S.M. wrote the paper.

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Figure Legends

Figure 1. Modulation of Emotional Contagion by Stress in Laboratory Mice

(A) Metyrapone (50 mg/kg), which does not affect pain behavior in mice tested in isolation or in cagemate dyads, elicits emotional contagion of pain in stranger BP ("both in pain") dyads otherwise only seen in cagemate BP dyads (n=16–22 mice/social context/drug). OP ("one in pain") dyad data are presented in Figure S1. (B) Thirty min, but not 15 min of restraint stress abolishes emotional contagion of pain in cagemate BP dyads (n=12 mice/condition). Note the duration-dependent pain inhibition (stress-induced analgesia) produced by 15- and 30-min restraint. (C) A combination of the GR antagonist, mifepristone (RU 486; 10 mg/kg) and the MR antagonist, RU 26752 (5 mg/kg) enables emotional contagion of pain in stranger BP dyads, but has no effect on pain behavior in Isolated or OP conditions (n=12–16 mice/social context/drug). All bars represent mean ± SEM percentage of samples positive for abdominal constriction (pain) behavior in a 30-min period following the intraperitoneal injection of 0.9% acetic acid. *p<0.05, ***p<0.001 compared to analogous Isolated group(s) by one-way ANOVA followed by Tukey's posthoc test. ••p<0.01 compared to analogous Vehicle by t-test. °°p<0.01 compared to analogous No Restraint group by one-way ANOVA followed by Tukey's posthoc test. n.t., not tested.

Figure 2. Human Friends, but not Strangers, Demonstrate Emotional Contagion of Pain

(A) Experimental timeline. (B) Emotional contagion of pain was observed in dyads if both participants were familiar and experienced pain together (BP), but not if participants were strangers or if dyadic testing involved only one participant in pain (OP). Bars represent mean ± SEM difference in pain intensity ratings between dyadic and isolated testing; n=10–26

participants/condition. (C,D) Significant correlation between self-reported anxiety and pain intensity difference scores (dyad – isolated) in BP strangers (C), but not in BP friends (D), indicative of stress-induced analgesia in the former. $^{**}p<0.01$ compared to analogous Strangers group by *t*-test.

Figure 3. Metyrapone Elicits Emotional Contagion of Pain in Human Strangers

(A) Experimental timeline. (B) Emotional contagion of pain in stranger BP dyads where both participants have been pretreated with metyrapone. Bars represent mean ± SEM difference in pain intensity ratings between dyadic and isolated testing; n=11–20 participants/condition. (C) Metyrapone (750 mg) produced analgesia *per se*, in participants tested in isolation. Bars represent mean ± SEM pain intensity ratings of all participants during isolated testing. (D) Metyrapone significantly decreased cortisol levels. Bars represent mean ± SEM change in plasma cortisol. (E) Facial expressions and pain-related behaviors of BP dyad participants captured by a video camera for 30 s following removal of the arm. Bars represent mean ± SEM dyad–isolated difference in occurrence of behaviors as a percentage of total observation time. $^{*}p<0.05$, $^{**}p<0.01$ compared to analogous Placebo group by *t*-test.

Figure 4. A Shared Gaming Experience Elicits Emotional Contagion of Pain in Human Strangers

(A) Experimental timeline. (B) Emotional contagion of pain in stranger BP dyads having shared a brief pleasurable social experience (playing the Rock Band video game together); no effect was produced by playing the video game alone. Bars mean ± SEM difference in pain intensity ratings between dyadic and isolated testing; n=15–16 participants/condition. (C) Significant correlation between friendship questionnaire intimacy (FQ-INT) scores and pain intensity

difference scores (dyad – isolated). (D) Playing Rock Band together decreased cortisol levels. Bars represent mean ± SEM change in plasma cortisol. (E,F) Playing Rock Band® together increases two measures of interpersonal affiliation. In the Inclusion of Other in the Self (OIS) scale of interpersonal closeness (E), participants move circles labeled "self" and "other" to overlap each other to the degree most resembling their relationship with the other individual. Bars represent mean ± SEM distance between the center of the circles. In the Dictator Game (F), both participants were told (separately) that they were chosen to receive an extra $10 compensation for the experiment, any portion of which they could donate to the other participant who would otherwise receive nothing. Bars represent mean ± SEM money offered. *$p<0.05$, **$p<0.01$ compared to analogous Play Alone group by *t*-test. n.t., not tested.