No effect of 25 mg oxazepam on physiological and self-rated responses to pain in others: a randomized controlled experiment

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Abstract

Background: Benzodiazepines have been proposed to inhibit empathic responding to others' pain.
Aims: We aimed to investigate the effect of 25 mg oxazepam on self-rated experience, skin conductance, heart rate, and superciliary corrugator muscle activity when observing another person in pain.
Methods: In a double-blind randomized controlled experiment, participants were given 25 mg oxazepam or placebo and then pain stimulated with electrical shocks, alternately with another person whom they thought was a fellow experimental participant.
Results: Oxazepam did not cause increased responses to others' pain, compared to participants' own pain, on any of the measures used. Oxazepam did cause increased ratings of unpleasantness and increased skin conductance responses across conditions. Empathic responses were predicted by self-rated empathy using the interpersonal reactivity index and inversely predicted by self-rated alexithymia using the Toronto Alexithymia Scale-20.
Conclusions: Oxazepam did not inhibit empathic responding in this experiment. Oxazepam did cause a general increase in self-rated unpleasantness and skin conductance responses.

Introduction

Empathy for pain has been investigated using functional brain imaging for more than a decade [1]. A consistent finding is that observation of pain in others is associated with activation in the anterior insula and anterior/middle cingulate cortex [1, 2]. This result is consistent with simulation theory, according to which others' emotional states are understood through a representation in brain networks overlapping with those that represent one's own internal states [3–5]. Such representation of others' emotions has been proposed as a major contributor to prosocial behavior [6, 7].

Benzodiazepines are used clinically for their anxiolytic, sedative, and myorelaxant properties. In forensic psychiatric case series [8–10], Dåderman et al. have reported instrumental use of benzodiazepines, particularly flunitrazepam, to facilitate violent criminal behavior, raising concerns that these drugs may inhibit empathic responses. These findings are consistent with earlier reports of paradoxical reactions with increased agitation and aggressiveness following benzodiazepine use [11–14]. Similarly, midazolam,

triazolam, and flunitrazepam have been found to increase agressive behavior in male rats [15, 16]. Since benzodiazepines act by potentiating $GABA_A$ receptor signalling, this implicates GABA as a potential regulatory neurotransmitter for prosocial/antisocial behavior.

$GABA_A$ receptors are pentameric ligand-gated ion channels composed of α, β, and γ subunits. The GABA binding site is located at the interface of α and β subunits, while the allosteric benzodiazepine binding site is located homologously at the interface between α and γ subunits. In humans, six types of the α subunit have been discovered, which are variably expressed in different brain areas and to which different benzodiazepines bind with varying affinity. Anxiolytic effects of benzodiazepines are thought to be mediated mainly by α-2 subunit containing $GABA_A$ receptors [17], which are strongly expressed in the amygdala [18]. Sedative and anticonvulsant effects are thought to be mediated mainly by α-1 subunit containing $GABA_A$ receptors, which are expressed widely in the cerebral cortex [17–20].

Since the empathic representation of others' emotional states is likely to inhibit aggressive and antisocial behavior, and since benzodiazepines have been reportedly used to facilitate aggressive and violent behavior, we hypothesised that benzodiazepines would inhibit empathic responding. Therefore, we investigated the effect of 25 mg oxazepam, a commonly prescribed benzodiazepine, on empathic responding, using subjective and physiological measures.

Materials and Methods

Study design

The study was a double-blind randomized controlled experiment performed in two waves. We investigated the effect of oxazepam on three different emotional processes: empathy for pain, emotional mimicry, and emotion regulation by reappraisal. This paper describes the experiment on empathy for pain. The other two experiments will be reported elsewhere.

Participants were block-randomized in groups of four according to a list drawn up by a colleague on beforehand. They were randomized both to oxazepam or placebo and to two different orders of stimulus presentation, meant to be counterbalanced. Thus, each group of four would contain four conditions, distributed randomly. However, due to an error in the randomisation procedure in wave 1, stimulus presentation order were instead conflated with treatment groups. In wave 2, where randomisation was conducted as intended, timing of stimulus presentation was revised, and heart rate was added as an outcome measure (see below). The study was approved by the regional ethical review board of Stockholm (no. 2009/1128-31/3).

Participants

Healthy male volunteers were recruited by advertisement on university campuses in Stockholm, Sweden, and using a website (`www.studentkaninen.se`). Participants were required to be right-handed, male, 18-45 years of age, to have no history of neurological or psychiatric disease including substance abuse, to speak and understand Swedish fluently, and not to be habitual consumers of nicotine. Furthermore, students of psychology, behavioural sciences, and medicine (past the 3rd semester) were not included, because we thought they might be more likely to try to uncover the role of the confederate, and because training in medicine likely causes a more detached attitude towards images of injured and sick people, which were used in the reappraisal experiment. We recruited only male participants because the earlier work on crinimal

offenders as well as experimental animals was restricted to males (see introduction), and a study investigating sex differences in brain mechanisms showed that males have a greater capacity for down-regulating empathic responses [6]. We aimed for a sample size of $n = 40$ for each wave, with 20 participants in each group, based on pragmatic considerations. Participants were paid 500 SEK (approx. 50 Euro or 60 USD), subject to tax.

Procedures

Screening, instructions, and intervention On arrival, participants were allowed to acquaint themselves for a few minutes with a confederate who was introduced as another experimental participant, but who was in reality a fellow investigator (ST), through a short scripted interaction.

Participants completed a brief medical screening form to verify that they fulfilled inclusion criteria. They were given written and oral information about the experiment and gave written informed consent. Next, they were given either a tablet of 25 mg oxazepam or a placebo pill, for which we used non-prescription vitamin D3 supplement pills of similar size and shape. Tablets were in pre-prepared sealed envelopes and both the investigators and the participants were blind to the treatment condition. We chose to use oxazepam because it has a favourable side-effect profile and relatively weak sedative effects compared to other benzodiazepines. We used a dose of 25 mg hoping that it would not have so strong subjective effects as to break blinding. Following oral administration, oxazepam reaches its maximal plasma concentration after about 2 hours, and maximum brain concentrations about half an hour after that [21–23]. Elimination occurs through glucuronidation yielding no active metabolites, with a half-life of 5-15 hours [21, 22]. Participants were instructed not to drive until the next day, in order to reduce risks from sedative effects in traffic.

Immediately after administration of drug or placebo, participants completed a reaction time task, titration of pain thresholds, and several rating scales. These baseline measures were recorded immediately after drug administration rather than before in order to further the efficient use of time, based on the assumption that effects of oxazepam would only appear later (at least 20 minutes after ingestion).

Reaction time test The purpose of the reaction time test was to measure vigilance, in order to gain an independent measure of the effect of oxazepam. The test was administered on a desktop personal computer using the Presentation software (Neurobehavioural Systems, Berkeley, California, USA). At intervals randomized between 2 and 10 seconds, a 200 x 200 pixel white square was shown at a random location on the screen for 1 s. Participants were instructed to press the space bar as fast as possible when the square appeared. There were 40 events, for an average length of 4 minutes for the whole test. Responses slower than 1 s were considered lapses and responses faster that 100 ms would have been considered false starts, had there been any. The outcome of interest was response time, as the test was too short to be sensitive for lapses. Response times were inverse-transformed to better approximate a normal distribution, which is a well-established practice for vigilance tests [24]. For reporting results, model estimates were transformed back to the original scale. Stimulus presentation code is available at [25].

Pain stimulation We used a custom-built concentric stimulation electrode designed to be MRI compatible, as we wanted to be able to use the experimental paradigm during functional magnetic resonance imaging in the future. The electrode consisted of a non-ferromagnetic conducting element of approx. 4 mm ⌀, insulated by a plastic ring

of about 3 mm, surrounded by another conducting element of approx. 1 mm, insulated on the outside by another layer of plastic. We placed the electrode on the volar forearm in order to avoid muscle contractions. Spectra 360® contact gel (GEL104, Biopac Systems, Inc., Goleta, California, USA) was used. The electrode was connected to a Biopac recording system with an STM200 stimulation unit (Biopac Systems, Inc.). Shocks lasted for 200 ms. In order to achieve comparable pain intensities, pain thresholds were titrated individually for each participant using a visual analog scale (VAS). For each participant we identified VAS 10 (perceptible but not painful) and VAS 80 (as painful as they considered to be bearable for the experiment). Titration was repeated at the end of the experiment to verify that pain perception as such had not been inhibited by oxazepam.

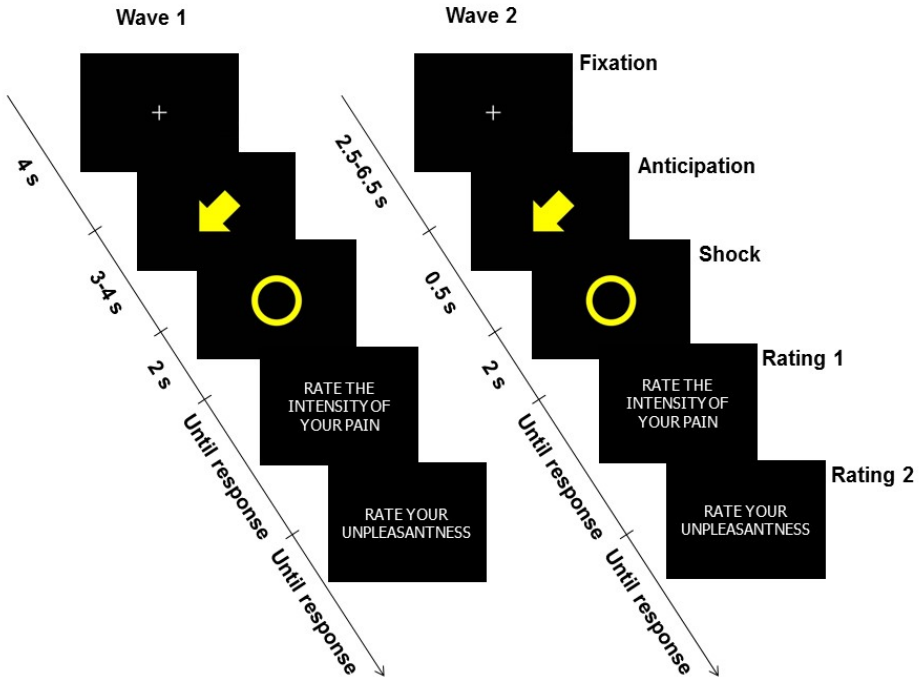
Experimental paradigm The experiment on empathy for pain is adapted from Singer et al. [1]. Participants were seated in front of a table with a computer monitor, and asked to lay their right arm, on which we had placed the stimulus electrode, on the table. The confederate was seated next to the participant with her arm on the table. A screen was placed on the floor between the participant and the confererate so they could see each other's extended arms only. This setting was chosen so as to approximate an experimental situation that could be set up in an MRI scanner.

There were a total of 40 shock events and 40 "null" events. For every shock event, a cue was shown on the computer monitor, in the form of an arrow pointing at either the participant or the confederate. Low intensity shocks were cued by a solid-color arrow, and high-intensity shocks by a striped arrow. At the same time as the shock, a circle was shown on the screen, colour-coded in the same manner as the arrows. Timing is described in figure 1. In wave 2, we shortened the anticipation time in order to better be able to study the effects of the shock itself, rather than effects due to prolonged anticipation (figure 1). Stimulus presentation code and materials are available at [25].

Skin conductance Skin conductance responses were measured using two 6 mm ⌀Ag/AgCl finger electrodes (TSD203, Biopac Systems, Inc.) with isotonic 0.05 M NaCl electrode paste (GEL101, Biopac Systems, Inc.), connected to a GSR100C amplifier (Biopac Systems, Inc.) with the following acquisitions settings: 5 μ℧/V, 1 Hz low-pass filter, and direct current. To remove non-physiological noise, data were further filtered in the Acqknowledge software using a low pass filter with a 1 Hz cutoff and 4000 coefficients and converted from direct to alternating currrent using an 0.05 Hz high pass filter. Responses were identified manually after each stimulus by inspection of the curve in the interval from cue onset to 2 seconds after shock onset. It was not possible to differentiate responses to the cue and responses to the shock, and the greatest response in the interval was recorded. A response was defined as a wave starting from a slope of 0, unless the baseline was trending upwards, in which case the point with the lowest slope (derivative) was used as baseline. Amplitude was defined as the height of the peak, which was allowed to be anywhere within 6 seconds from onset, in μSiemens. If no peak appeared within 6 seconds, the response was excluded from analysis. Data were square root transformed before statistical analysis, in order to better approximate a normal distribution.

Electromyography (EMG) EMG was measured over the superciliary corrugator muscles following established guidelines [26]. As described above, we also performed an experiment on emotional contagion, and for that reason we collected data from the major zygomatic muscle as well. In the experiment on empathy for pain, only superciliary corrugator EMG was analysed, since it represents a negatively valenced emotional expression. 4 mm ⌀Ag-AgCl electrodes (EL254S, Biopac Systems, Inc.) were

Figure 1. Stimulus sequence for the empathy for pain experiment. In wave 2, timing was optimised to reduce uncertainty about the contribution of anticipation to observed responses, and to improve jittering so the experiment could later be converted into an fMRI experiment with minimal changes. Shown here are stimuli for a low-intensity shock to the participant. In half of the trials, the fixation cross was followed instead by a rest event of 5.5 s. In wave 2, fixation crosses after rest events were jittered not between 2.5 and 6.5 seconds but between 1 and 5 seconds, to save time. The rating questions were presented in Swedish, but are shown here translated to English.



used with a contact gel (GEL100, Biopac Systems, Inc.). Electrodes were connected to EMG100C amplifiers (Biopac Systems, Inc.) with the following acquisition settings: gain 500, low-pass filter 500 Hz, notch filter off, and high-pass filter 10 Hz. Sampling was at 1000 Hz. The signal was further filtered in the Acqknowledge software using a band pass filter of 30 to 300 Hz to remove signal not due to muscle activity. A band stop filter at 49 to 51 Hz was used to filter out line noise. Average rectified EMG signal was determined. Recordings were downsampled to 100 Hz in order to decrease file size, and data were exported as text files. Before analyses, recordings were further downsampled to 10 Hz using a loess curve in R. Responses were averaged over a time window of 2 seconds (see figure 6A-D) and log-transformed before statistical analysis, in order to better approximate a normal distribution.

Heart rate We recorded heart rate in wave 2 only. A 3-lead EKG was acquired by placing disposable Ag/AgCl electrodes (EL503, Biopac Systems, Inc.) on the right side of the neck, on the left upper arm, and on the left ankle (ground reference). ECG100 amplifiers (Biopac Systems, Inc.) were used with the following settings: Gain 2000, Mode R wave, 35HzLPN on, high-pass filter 0.5 Hz. Sampling was at 1000 Hz. Recordings were downsampled to 100 Hz in order to decrease file size, and data were

exported from the Acqknowledge software as text files. Of the 39 participants from whom EKG was recorded, 1 was excluded due to electrode disattachment and 2 were excluded due to frequent extrasystoles. Heart rate was derived from raw curves by a peak finding algorithm in R. Estimated heart rate of <40 or >200 beats per minutes was rejected (0.2% of data). For each event, heart rate was normalised to the 2 seconds preceding stimulus onset and averaged over a time window from 2.5 to 4 seconds from stimulus onset.

Rating scales

Interpersonal Reactivity Index (IRI) The IRI has four subscales which measure different dimensions of trait empathy: empathic concern (EC), perspective taking (PT), personal distress (PD), and fantasy (FS) [27, 28]. The IRI has been validated in Swedish [29], although the four-factor structure could not be replicated. Instead, EC formed one factor and PT, PD, and FS together formed another factor. Two participants were excluded on this measure because they had a large and nonrandom number of missing items (due to failing to turn over the page). One additional item response was missing, and it was imputed based on the mean of the subscale.

Toronto Alexithymia Scale-20 (TAS-20) The TAS-20 measures alexithymia, a construct thought to represent difficulties in identifying and describing one's own emotions. It has three subscales: Difficulty Identifying Feelings, Difficulty Describing Feelings, and Externally-Oriented Thinking [30]. We analysed only total scores. The scale has been validated in Swedish [31]. Four participants were excluded on this measure for failing to respond to a large number of the items. One additional item response was missing, and it was imputed based on the mean of the subscale.

State-Trait Anxiety Inventory (STAI) The STAI has a state and a trait subscale [32]. We used a non-validated Swedish translation with which we have considerable experience, and which can be found at [25]. The state subscale (S) was administered before the experiment, and then again at the end of the experiment. For the trait subscale (T), 4 participants each missed 1 item. These data were imputed using the average of the remaining items, rounded to the nearest integer. For the state subscale, two participants were not administered the scale the second time. One participant gave three illegible responses and three participants each missed one item. Imputation was performed using the average of the remaining items.

Psychopathy Personality Inventory-Revised (PPI-R) The PPI-R measures psychopathic traits [33]. Validation of the Swedish version of the PPI-R is ongoing, based partly on the data collected in this study (Sörman et al, submitted). Therefore, PPI-R results are not reported in this paper.

Analyses and data

Data and analysis code for this paper are openly available at [34]. In order to preserve anonymity, participants' age and educational background have been omitted from the published dataset. All analyses were made with R [35], using the packages **RCurl** [36] to read data from GitHub, **quantmod** [37] to find EKG R wave peaks, **nlme** [38] to build mixed-effects models, **effects** [39] to get confidence intervals on estimates, and **RColorBrewer** [40] for graphing. Mixed-effects models have been used throughout unless otherwise indicated. For reference, full output tables of regression models for

main outcomes are also published in the Github repository, for both waves together and separately.

Results

Participants

39 participants completed each wave. In addition, 8 participants were pilots, tested as a "run-in" before the experiment began. Pilot participants are not included in any analyses, but their data are published along with the other participants' data. From wave 1, 2 participants were excluded after debriefing because it emerged they had not understood the instructions. 1 participant was excluded due to problems with the recording equipment. A further 3 were excluded because they voiced suspicions about the nature of the confederate at debriefing. From wave 2, 1 participant was excluded because he was found to have a psychiatric diagnosis after the experiment. 4 were excluded due to not reaching VAS 80 and 4 were excluded because they voiced suspicions about the nature of the confederate at debriefing.

Participant characteristics are shown in table 1. In wave 1, the oxazepam group had higher ratings on the IRI-EC. Since the form was completed approx. 20 minutes after drug administration, we had to consider the possibility that ratings were affected by the drug. To exclude this putative explanation, we asked the participants to complete the IRI again by mail after the experiment. 24 out of 35 participants responded (69 %), and the mean change in IRI-EC was -0.02 (SD 0.51). Furthermore, in wave 2, we administered the IRI before drug administration, and then again with items in a scrambled order after drug administration, and found no difference in IRI-EC responses due to oxazepam (-0.04 [-0.28, 0.19], $p = 0.70$). Thus, we conclude that the group difference in IRI-EC responses in wave 1 is more likely explained by chance than by a drug effect.

Table 1. Characteristics of participants Means and standard deviations are given unless otherwise indicated.

	Wave 1		**Wave 2**	
	Placebo	**Oxazepam**	**Placebo**	**Oxazepam**
n	16	17	16	14
Age (median, range)	20.5 (18-28)	21 (18-25)	21.5 (18-44)	23.5 (19-41)
Any tertiary education (n, %)	12 (75%)	14 (82%)	10 (63%)	12 (86%)
IRI-EC	3.23 (0.71)	3.79 (0.51)	3.84 (0.61)	3.79 (0.35)
IRI-PT	3.38 (0.79)	3.70 (0.49)	3.61 (0.36)	3.37 (0.41)
IRI-PD	2.60 (0.59)	2.50 (0.65)	2.55 (0.47)	2.42 (0.59)
IRI-F	2.94 (0.52)	3.28 (0.45)	3.30 (0.71)	3.24 (0.68)
STAI-T	40.9 (8.5)	36.6 (6.1)	39.8 (5.2)	35.1 (6.9)
TAS-20	45.3 (10.8)	39.6 (8.9)	40.4 (9.9)	36.4 (8.7)

Efficacy of intervention

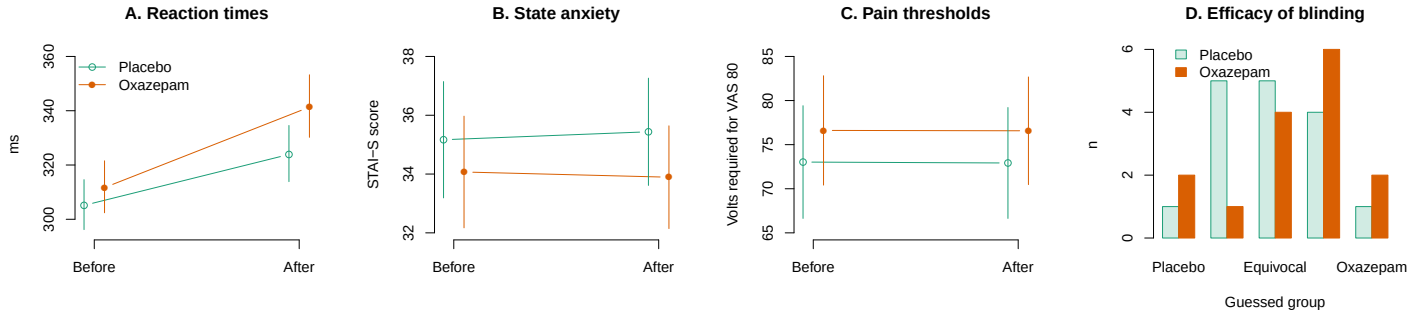
Reaction times Oxazepam caused slower reaction times, seen as an interaction between treatment and first/second test (8.3 ms, [3.3, 13.1], estimates back-transformed from the inverse, $p = 0.001$, figure 2A), confirming biological activity of the drug. Reaction times were slower in the second test (24.5 ms, [21.3, 27.6], $p < 0.0001$, figure 2A)).

State anxiety Oxazepam did not cause decreased state anxiety, although the effect was in the direction of an increase in the placebo group, seen as an interaction between treatment group and first/second test (2.54, [-0.66, 5.74], $p = 0.06$ (one-sided), figure 2B). No change in anxiety from the first to the second test time was seen (-0.97, [-3.19, 1.26], $p = 0.39$), nor any main effect of oxazepam (-2.02, [-7.43, 3.38], $p = 0.46$).

Pain thresholds Oxazepam did not cause increased pain thresholds, seen as an interaction between treatment group and first/second test (-0.31 V, [-4.34, 3.72], $p = 0.88$, figure 2C), confirming the expected lack of analgesic effect. No change in pain thresholds from the first to the second test time was seen (-0.21 V, [-3.03, 2.62], $p = 0.88$), nor any main effect of oxazepam (-3.28, [-13.92, 7.36], $p = 0.54$).

Efficacy of blinding Participants were not able to guess better than chance whether they had received oxazepam or placebo (1.0, [-0.00003, ∞], $p = 0.13$, one-sided Wilcoxon rank sum test, figure 2D), confirming the integrity of the blinding, although the effect was in the direction of detection of true group membership.

Figure 2. Efficacy of intervention. A: Reaction times increased from before the experiment to after, and more so in the oxazepam group, confirming that the administered drug had a biological effect. Estimates were back-transformed from the inverse for plotting. B: Oxazepam did not decrease state anxiety. C: Oxazepam did not affect participants' pain thresholds. D: Participants in wave 2 guessed after the experiment which treatment group they were in, using a 5-level Likert-type scale to indicate whether they were sure they were in the placebo group, probably in the placebo group, equivocal, probably in the oxazepam group, or sure they were in the oxazepam group. Labels are omitted for the "probably placebo" and "probably oxazepam" responses.



Oxazepam and empathy

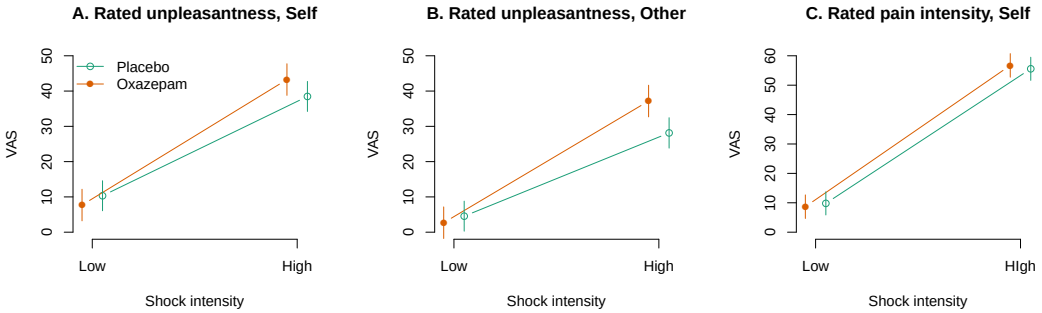
Rated unpleasantness Shocks to other were rated less unpleasant than shocks to self (-5.8, [-7.9, -3.7], $p < 0.0001$, figure 3A, B). Shocks of high intensity were rated more unpleasant than shocks of low intensity (28.1, [26.0, 30.3], $p < 0.0001$, figure 3A, B). There was no main effect of oxazepam on rated unpleasantness (-2.6, [-9.0, 3.7], $p = 0.41$). Shock intensity and self/other condition interacted such that high intensity stimuli were rated less unpleasant in the other condition (-4.6, [-7.7, -1.4], $p = 0.004$, figure 3A, B).

The effect of oxazepam on empathic responding was assessed as a 3-way interaction between treatment, shock intensity, and self/other condition. We had hypothesised that

oxazepam would cause lower rated unpleasantness specifically in the other high condition, but this effect was not seen (3.5, [-0.9, 7.9], $p = 0.12$, figure 3A, B).

Rated intensity Rated pain intensity was not affected by oxazepam (-1.1, [-6.9, 4.6], $p = 0.70$, figure 3C). As expected, rated pain intensity was higher to high shock intensity (46.8, [44.0, 47.5], $p < 0.0001$, figure 3C. Oxazepam did not interact with shock intensity (2.3, [-0.2, 4.8], $p = 0.07$, figure 3C).

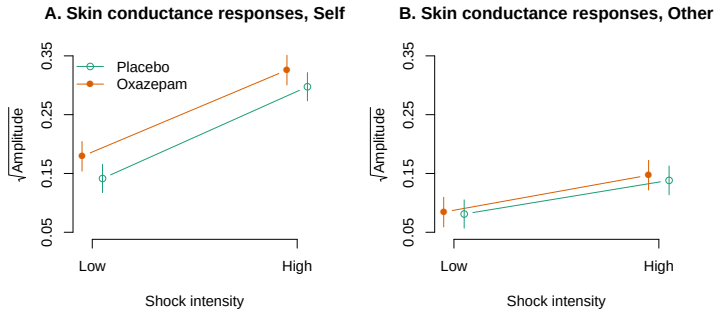
Figure 3. Ratings.



Skin conductance There were main effects of other vs self condition (-0.06, [-0.08, -0.05], $p < 0.0001$) and of high vs low shock intensity (0.16, [0.14, 0.17], $p < 0.0001$), and a 2-way interaction (-0.10, [-0.12, -0.08], $p < 0.0001$, figure 4A, B), such that skin conductance responses were highest in response to high-intensity shocks and to self. Oxazepam had a main effect on skin conductance (0.04, [0.02, 0.07], $p = 0.04$, figure 4A, B).

The effect of oxazepam on empathic responding was assessed as a 3-way interaction between treatment, shock intensity, and self/other condition. We had hypothesised that oxazepam would cause lower skin conductance responses specifically in the other high condition, but this effect was not seen (0.02, [-0.02, 0.05], $p = 0.35$, figure 4A, B).

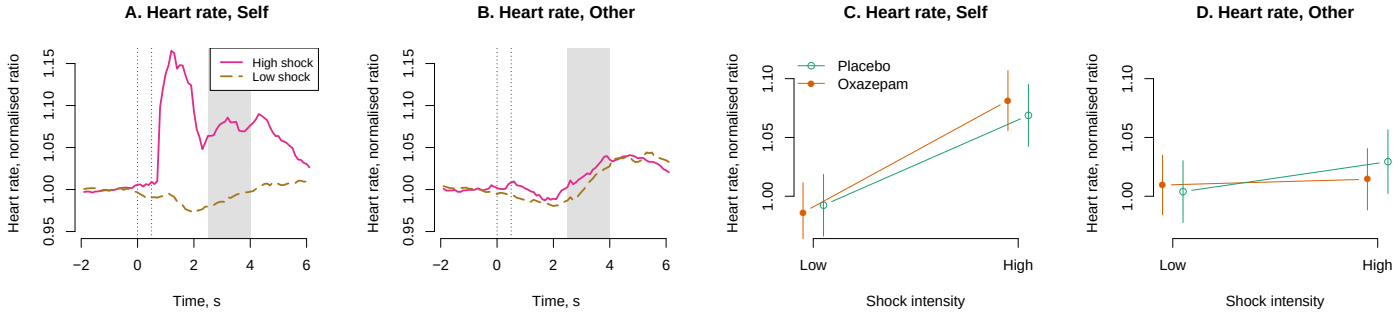
Figure 4. Skin conductance responses.



Heart rate There was a main effect of high vs low shock intensity (0.076, [0.049, 0.104], $p < 0.0001$), but not of other vs self condition (0.012, [-0.016, 0.039], $p = 0.41$), and a 2-way interaction (-0.051, [-0.091, -0.011], $p = 0.01$, figure 5A, B), such that heart rate responses were highest in response to high-intensity shocks and to self. Oxazepam did not have a main effect on heart rate (-0.006, [-0.044, 0.032], $p = 0.74$, figure 5A, B).

The effect of oxazepam on empathic responding was assessed as a 3-way interaction between treatment, shock intensity, and self/other condition. We had hypothesised that oxazepam would cause lower heart rate responses specifically in the other high condition, but this effect was not seen (-0.039, [-0.093, 0.014], $p = 0.15$, figure 5A, B).

Figure 5. Heart rate. A and B: The first dotted vertical line shows the onset of the stimulus cue. The second dotted vertical line shows the onset of the shock and the shock cue. The gray area shows the time window for which signal was averaged. In the self high condition, there was a biphasic response with a large peristimulus peak. We did not include this peak in the time window for further analysis, since it may partly represent non-cardiac signal sources, i.e. the electrical pain stimulus and associated muscle activity.



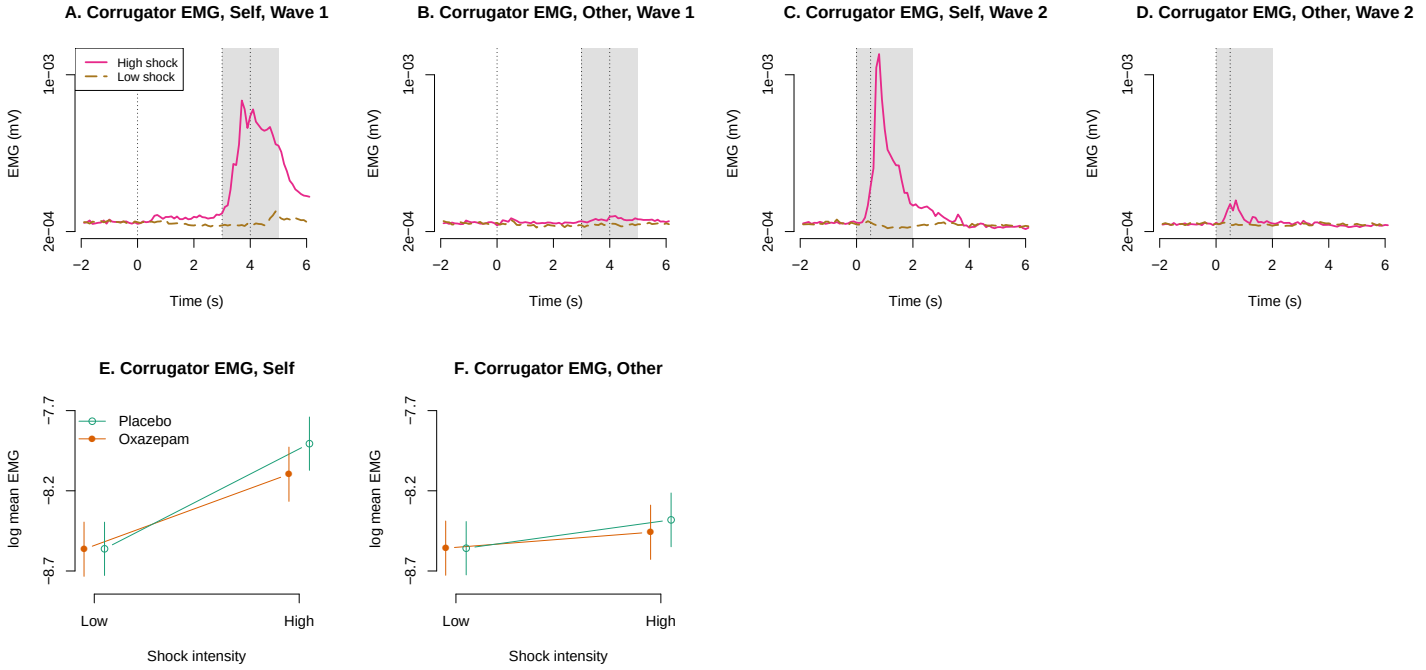
Superciliary corrugator activity There was a main effect of high vs low shock intensity (0.66, [-0.39, -0.29], $p < 0.0001$) but not of other vs self condition (0.00, [0.26, 0.36], $p = 0.91$), and a 2-way interaction (-0.48, [-0.58, -0.38], $p < 0.0001$, figure 6A, B), such that corrugator EMG responses were highest in response to high-intensity shocks and to self. Oxazepam did not have a main effect on EMG responses (-0.00, [-0.25, 0.24], $p = 0.98$, figure 6A, B), but it did show a 2-way interaction with shock intensity (-0.19, [-0.28, -0.09], $p = 0.0001$, figure 6A, B), such that responses to shocks of high intensity were lower in the oxazepam group.

The effect of oxazepam on empathic responding was assessed as a 3-way interaction between treatment, shock intensity, and self/other condition. We had hypothesised that oxazepam would cause lower corrugator EMG responses specifically in the other high condition, but this effect was not seen (0.11, [-0.02, 0.22], $p = 0.11$, figure 6C, D).

Predictors for responsiveness to stimuli

Personality measures were assessed as predictors of general responding to stimuli as well as empathic responding, i.e. responding specifically in the other high condition. We hypothesised that IRI-EC would predict empathic responses. Associations between PPI-R and empathic responding will be reported elsewhere (Sörman et al, submitted). Predictors for responding in the empathy condition (high instensity stimulus to the other person) are shown in (figure 7). IRI subscales predicted increased empathic responding on ratings, skin conductance, and EMG, but not heart rate. Conversely, TAS-20 predicted lesser empathic responses on ratings, skin conductance, and EMG, but not heart rate. None of the personality measures predicted responding across conditions S1 Predictors of responding across conditions. Besides the rating scales, we also investigated rated likability of the confederate, and it did not predict empathic

Figure 6. Superciliary corrugator activity. Since stimulus timing differed between waves 1 and 2, different windows were used for inclusion of data into statistical modelling. A and B: The first dotted vertical line shows onset of the stimulus cue. The second and third dotted vertical line bound the interval in which the shock and the shock cue appeared. The gray area shows the time window for which signal was averaged. C and D: The first dotted vertical line shows the onset of the stimulus cue. The second vertical line shows when the shock and the shock cue appeared. The gray area shows the time window for which signal was averaged. In A-D, the solid data line shows high shock intensity and the dashed data line shows low shock intensity.



responses on unpleasantness (2.24 [-0.16, 4.64], $p = 0.07$), skin conductance responses (0.017, [-0.020, 0.013], $p = 0.11$), corrugator EMG (-0.065, [-0.134, 0.004], $p = 0.07$), nor heart rate (0.003, [-0.017, 0.023], $p = 0.80$).

Adverse events

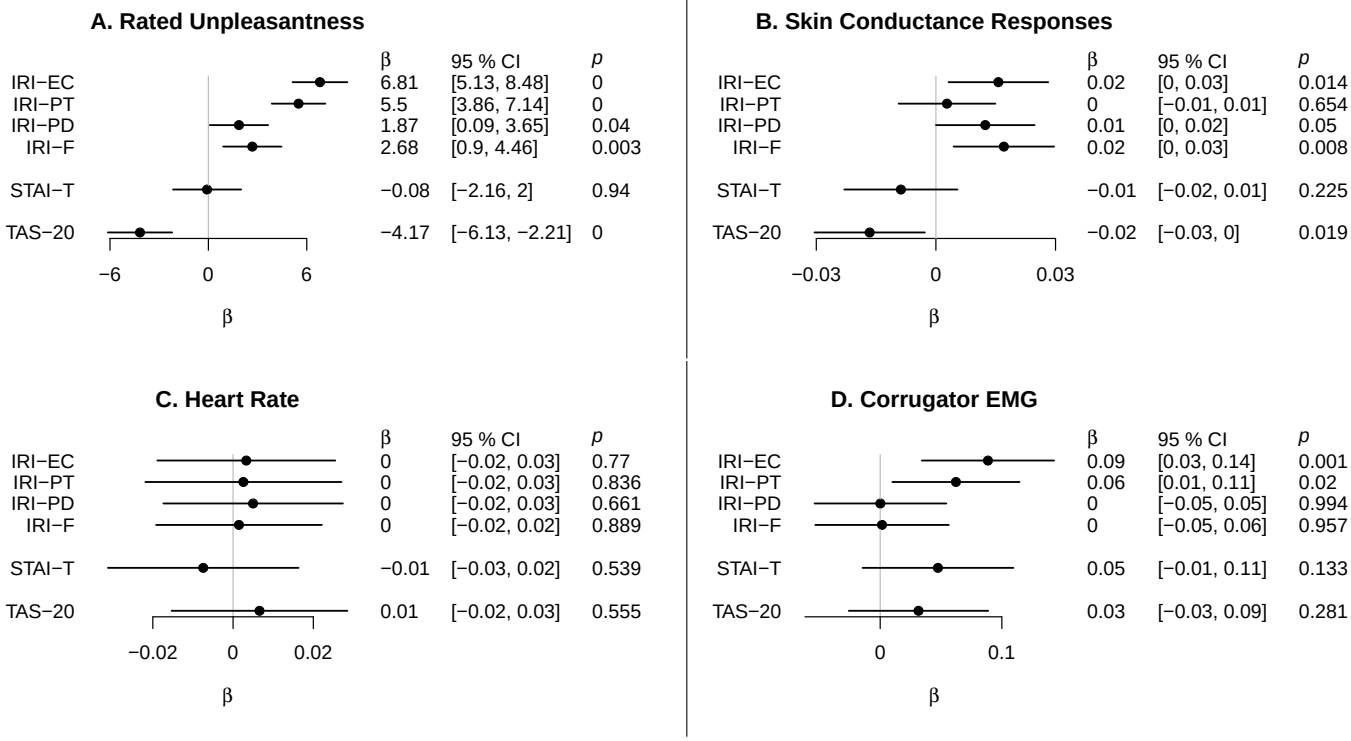
The shock electrode caused minor dermal injuries measuring up to approx. 1 mm at greatest diameter to 11 participants. Use of this electrode has been discontinued. Of the 39 participants from whom we recorded EKG, 2 were found to have irregular heart rhythm and were recommended to consult a physician.

Discussion

Conclusions

25 mg oxazepam did not inhibit empathic responses to others' pain. Oxazepam showed an expected effect on reaction times, confirming that the drug was biologically active. The experimental paradigm caused responses to others' pain on all investigated outcomes, confirming that the experimental paradigm worked. While subjective ratings

Figure 7. Predictors of empathic responding



were likely affected by demand effects, i.e. participants rating in a manner they believe to be expected of them, physiological measures were probably not much affected by such biases, since the participants were not well aware of the nature of the recordings.

Oxazepam caused increased ratings of unpleasantness across stimulus conditions. This would seem to be at odds with the anxiolytic effects for which oxazepam is used in the clinic. One explanation could be that oxazepam caused increased sleepiness, which is known to cause worse ratings of subjective experience [41]. While oxazepam is not mainly prescribed for its hypnotic properties, our reaction time results showed that participants in the oxazepam group did show a decrease in psychomotor vigilance, consistent with this interpretation.

The present negative result is similar to the finding by Olofsson et al. that 20 mg oxazepam did not influence event-related potentials in response to emotional image stimuli [42]. On the other hand, Siepmann et al. found that 0.5 mg lorazepam caused decreased skin conductance responses to aversive stimuli in humans, however with no significant effects on pupil dilation, vigilance, nor mood [43].

Recently, Wang et al. [44] showed, using magnetic resonance spectroscopy, that higher levels of GABA in the anterior insula, a key region for empathy, predicted higher self-reported trait empathy on the IRI empathic concern and perspective taking subscales. This finding suggests the hypothesis that increased GABA signalling in the anterior insula would cause greater empathic responding, i.e. an effect in the opposite direction from what we hypothesised. Our results do not, however, provide support for a behavioral correlate of the finding by Wang et al.

We found that subscales of the interpersonal reactivity index (IRI) predicted empathic responding, supporting the notion that our experimental paradigm caused participants to experience sharing of the other persons's emotion. We also found that the Toronto Alexithymia Scale-20 (TAS-20) total score predicted less empathic

responding. Previous neuroimaging studies have shown that TAS-20 scores predict both higher [45] and lower [46] responses in anterior insula to viewing others in pain. Both these studies however found that TAS-20 predicted lower behavioral responses to other's pain, as we have found here.

Limitations

One limitation of this study is that we do not know whether the observed lack of effect extends to other benzodiazepines, such as flunitrazepam and chlordiazepoxide, which have been proposed to cause aggressive behavior. Furthermore, the facilitating effect of benzodiazepines on aggression seems to be potentiated by alcohol in real life and in the laboratory [9, 47], whereas we have studied the effect of oxazepam in isolation. Also, we cannot say whether a higher dose of oxazepam would have inhibited empathic responding. Finally, the nature of the participant sample (all-male, largely university students) limits generalisability of results.

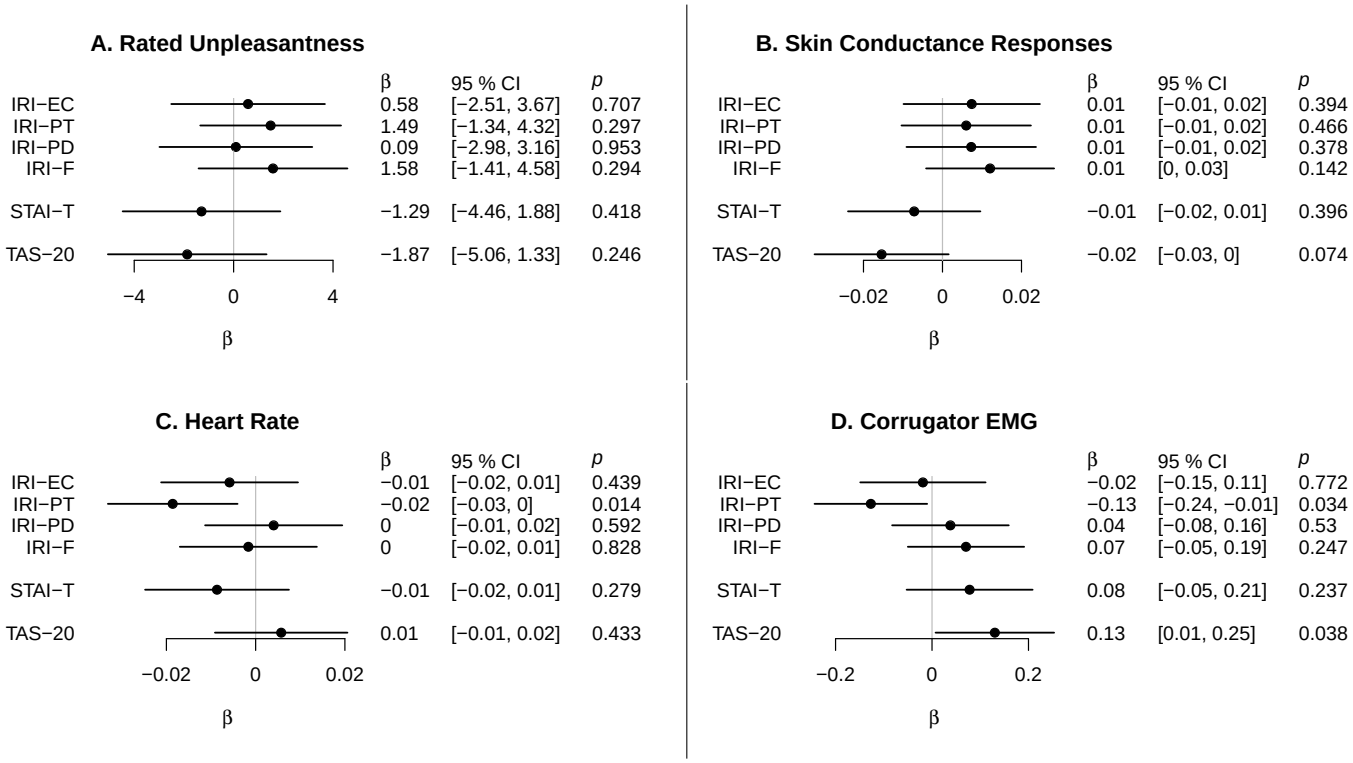
Conclusions

This experiment showed that 25 mg oxazepam did not inhibit empathic responding. This finding does not support future neuroimaging experiments using the intervention and experimental paradigm investigated here.

Supporting Information

S1 Predictors of responding across conditions

None of the rating scales predicted responding across conditions.



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Author contributions

Conceived and designed the experiments: GN, ST, AG, AO, MI, PP. Performed the experiments: GN, ST. Analyzed the data: GN, ST. Interpreted results: GN, ST, AG, AO, MI, PP. Drafted the paper: GN. All authors read and approved the final version of the manuscript.

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