A crossover study evaluating the sex-dependent and sensitizing effects of sleep deprivation using a nociceptive test battery in healthy subjects

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Aim: We assessed whether total sleep deprivation (TSD) in combination with pain tests yields a reliable method to assess altered pain thresholds, which subsequently may be used to investigate (novel) analgesics in healthy subjects.

Methods: This was a two-part randomized crossover study in 24 healthy men and 24 women. Subjects were randomized 1:1 to first complete a day of nonsleep-deprived nociceptive threshold testing, followed directly by a TSD night and morning of sleep-deprived testing, or first complete the TSD night and morning sleep-deprived testing, returning 7 days later for a day of nonsleep-deprived testing. A validated pain test battery (heat, pressure, electrical burst and stair, cold pressor pain test and conditioned pain modulation [CPM] paradigm) and sleep questionnaires were performed.

Results: Subjects were significantly sleepier after TSD as measured using sleepiness questionnaires. Cold pressor pain tolerance (PTT, estimate of difference [ED] \textsuperscript{-}10.8\%, 95\% CI \textsuperscript{-}17.5 to \textsuperscript{-}3.6\%), CPM PTT (ED \textsuperscript{-}0.69 mA, 95\% CI \textsuperscript{-}1.36 to \textsuperscript{-}0.03 mA), pressure PTT (ED \textsuperscript{-}11.2\%, 95\% CI \textsuperscript{-}17.5\% to \textsuperscript{-}4.3\%) and heat pain detection thresholds (ED \textsuperscript{-}0.74 \textdegree C, 95\% CI \textsuperscript{-}1.34 to \textsuperscript{-}0.14 \textdegree C) were significantly decreased after TSD compared to the baseline morning assessment in the combined analysis (men + women). Heat hyperalgesia was primarily driven by an effect of TSD in men, whereas cold and pressure hyperalgesia was primarily driven by the effects of TSD observed in women.

Conclusions: TSD induced sex-dependent hyperalgesia on cold, heat and pressure pain, and CPM response. These results suggest that the TSD model may be suitable to evaluate (novel) analgesics in early-phase drug studies.

KEYWORDS
drug development, pain, randomized controlled trial
1 | INTRODUCTION

Sleep disturbance is a highly prevalent symptom in chronic pain patients as over 50% also report having impaired sleep. Studies in primary insomnia patients or subjects deprived of sleep reported the development of spontaneous pain and increased sensitivity to (experimentally) evoked pain, therefore proper sleep is necessary to maintain the homeostasis of pain-regulatory processes.

The use of pain models in early-phase pharmacological studies may help to reduce decision-making risks during the translational process from preclinical models to patients with pain, and to determine the biological activity of the studied drug. Most models used to evaluate the effects of (novel) analgesics in healthy subjects are evoked pain tasks eliciting nociceptive pain, eg, pressure or heat application to or on extremities, where there are limited to no disturbances in central pain processing. Such models are less suitable to fully assess the analgesic potential of (novel) neuropathic pain treatments that primarily act in the central nervous system (CNS) and target centrally induced lowering of pain thresholds.

Easily adoptable models that assess the effects on central pain processing are mainly based on peripheral input (eg, secondary hyperalgesia induced in the capsaicin or ultraviolet-B [UVB] hyperalgesia models rely on peripheral activation), which does not apply to sensitization caused by sleep deprivation. In addition, models that evaluate central effects often have limitations that prevent repetitive use in studies with a crossover design (which in pharmacological pain studies is preferred to limit the required sample size through increased statistical power compared to a parallel design). Reasons precluding repetitive use of other central-acting models may be ethical or practical. A high subject burden due to the unpleasantness of the test procedure is one example of the former (eg, intradermal capsaicin injection or high/low-frequency stimulation) and long-term adverse effects are another (eg, the prevalence of post-inflammatory hyperpigmentation with the freeze injury and ultraviolet-B hyperalgesia models). Other models, for example secondary hyperalgesia/allodynia induced by topical capsaicin, appear to be only limitedly sensitive to pharmacological interventions or only limitedly reproducible.

Sleep deprivation in combination with evoked pain tests may be a suitable alternative to study the effects of centrally acting analgesic drugs. By depriving healthy subjects of sleep, central pain pathways are affected: disturbed sleep significantly decreased pain tolerance to peripheral mechanical, heat and cold stimuli. Sleep deprivation-induced alterations in the endogenous pain inhibition pathway have also been reported, for example the impaired conditioned pain modulation (CPM) response, a centrally acting mechanism. Use of sleep deprivation as a tool to evaluate (investigational) analgesics, however, is precluded by discrepant findings. Effects on thermal pain tolerance, for example, differed between those reported by Onen et al (no significant effect) those reported by Kundermann et al (significantly lower heat and cold pain thresholds) and those reported by Eichhorn et al (heat pain solely decreased in women, cold hyperalgesia was induced sex-independently). Given this discrepancy, and to accurately assess a drug’s potential analgesic effect, it is necessary that the sleep-deprivation model is first validated without intervention prior to further use in studies with (investigational) analgesics(s).

Here, we investigated the sex-dependent effects of sleep deprivation on pain responses in healthy subjects using a comprehensive and validated evoked pain test battery.

2 | METHODS

2.1 | General considerations

This study was performed at the Centre for Human Drug Research, Leiden, the Netherlands in accordance with the Declaration of Helsinki of 1975, as revised in 2013. Approval of the Medical Ethics Committee Stichting Beoordeling Ethiek Biomedisch Onderzoek, Assen, the Netherlands, was obtained prior to study start. The results reported here are part of a larger study that also answered distinctly different objectives: to determine the effects of sleep deprivation on driving performance and to determine the validity of an intra-epidermal stimulation method. Those results will be or are reported elsewhere. The trial was registered in the Netherlands Trial Register (NTR number NL7517).
2.2 Study subjects and design

Twenty-four men and 24 women were enrolled. All subjects provided consent before any study procedures took place. Interested subjects were medically screened and enrolled if they were men (part A) or women (part B), 18-35 years of age (inclusive), and excluded if they had sleep disturbances, irregular sleeping patterns (eg, night shifts) or went through a change in time zone(s) 7 days prior to the first test day. Regular smokers (>10 cigarettes or equivalent per day) were excluded from participation, as were those that smoked 24 hours before each visit, used (illicit) drugs, consumed alcohol within 24 hours prior to each visit, consumed >8 units of (methyl-)xanthine-holding products per day or consumed these 4 hours before each visit. Subjects were acquainted with all tests during a training session (section 2.4) that was part of the screening procedure. Subjects who did not understand the instructions, indicated intolerance during the pain test training or achieved tolerance at >80% of the maximum input intensity for the cold, pressure and/or electrical pain task(s) were excluded. This tolerance threshold of 80% was included to ensure that both an increase and decrease in pain response could be determined during the test visits.

The menstrual cycle may influence (evoked) pain perception. To minimize a possible influence on test results, women (part B) were required to use a reliable hormonal contraception method at least 30 days before the first study day until the end of the study. In addition, one of the following was required for participation: (i) use of the contraceptive pill continuously (no stop week) throughout the study; (ii) use of the contraceptive pill with a planned stop week, in which the study days were > 2 days after restart of contraceptive pill use; (iii) in case of other hormonal contraceptives, study days were > 2 days after end of withdrawal bleeding.

Subjects in both parts (A and B) were randomized in a 1:1 ratio (Figure 1). Per part, 12 subjects first completed a visit in which two baseline measurement rounds were performed, one in the morning (MORN) and one in the afternoon (AFT). This was followed directly by a night of total sleep deprivation (TSD) and one measurement round post-sleep deprivation (Figure 1, arm 2). The other 12 subjects first completed the TSD night and subsequent measurement round, after which they went home to revert to their normal sleep pattern. At least 7 days later, these subjects reported back to the clinical unit for a second visit during which the two baseline measurement rounds (MORN and AFT) took place (Figure 1, arm 1). This design was identical for both study parts.

Due to the COVID-19 pandemic and related local regulations enforced at the time of part B of the study, subjects in part B, arm 1 were required to report to the clinic at 8.00 instead of 19.00 (see section 2.3) to allow for a day of quarantine and COVID-19 screening, whereas subjects that participated in part B, arm 2 (Figure 1) were required to stay an additional full study day at our unit in quarantine, including COVID-19 screening. The quarantine day in this arm preceded the regular study visits. Part A was completed before the COVID-19 pandemic thus was unaffected.

FIGURE 1 Study design. Schematic study design of parts A and B. Due to the COVID-19 pandemic, subjects in part B, arm 1 were required to report to clinic at 8.00 instead of 19.00 to allow for a day of quarantine and COVID-19 screening, whereas subjects that participated in part B, arm 2 were required to stay an additional full study day at our unit in quarantine, including COVID-19 screening (see section 2.2). The quarantine day in this arm preceded the regular study visits. Part A was completed before the COVID-19 pandemic and thus was unaffected. T, moments when test rounds were performed.
2.3 Study intervention: Sleep deprivation

Subjects were deprived of sleep for at least 24 hours at time of the measurements following TSD. The night before TSD, subjects were instructed to go to bed between 22:00 and 23:00, wake up between 07:00 and 08:00 the next day and report to the clinic around 19.00 to start the TSD night. To aid subjects in staying awake for the duration of the TSD, a personal activities schedule was created at the start of the visit together with the subject (eg, playing [video] games, splashing cold water on the face, light exercise) to provide structure during the night. Subjects were allowed to deviate from this schedule if, for example, an activity was found to be effective to promote wakefulness and prevent sleepiness. During the TSD night (19:00-07:00), study staff closely monitored the subject and reinforced motivation to ensure compliance and that subjects did not take any naps. Caffeine use was not allowed during TSD.

2.4 Study procedures: Evoked pain tasks

Pain detection thresholds (PDT) and pain tolerance thresholds (PTT) were evaluated using a multimodal and fixed-sequence pain test battery at prespecified timepoints (each test round is indicated by ‘T’ in Figure 1). All subjects thus completed three rounds of pain tests. Details of the procedures have been described extensively elsewhere and therefore are only briefly repeated here.\(^24,25\) Measurements were performed in the following sequence: heat pain task, pressure pain task, electrical pain task – repeated stimulus (‘burst’), electrical pain task – single stimulus (‘stair’) (pre-cold pressor, #1), cold pressor pain task, electrical pain task – single stimulus (‘stair’) (post-cold pressor, #2), ending with the intra-epidermal stimulation test (results reported elsewhere).\(^20\) Pain intensity for all tests except the heat pain test was captured using an electronic visual analogue scale (eVAS) slider: 0, “no pain” to 100, “worst pain tolerable”. PDT was defined as eVAS > 0 and PTT as eVAS = 100.

Heat PDTs were measured with a thermode (contact area 30 × 30 mm; QSense, Medoc, Israel) placed on the volar forearm that gradually increased from 32 °C at 0.5 °C/s. The test continued until the subject indicated his/her PDT by pushing a button on a hand-held feedback control or when the safety cut-off of 50 °C was reached. The average of three measurements was used for analysis.

For the pressure pain test, an 11-cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle. Pressure was computer-controlled with an electro-pneumatic regulator (ITV1030-31F2N3-Q, SMC Corporation, Tokyo, Japan). Power1401mkl1 analogue-to-digital converter and Spike2 software (CED, Cambridge, UK). Pressure constantly increased at 0.5 kPa/s until PTT or the safety cut-off of 100 kPa was reached.

At start of the cold pressor test, the nondominant hand was placed in a bath (minimal depth of 200 mm) filled with circulating warm water (35 ± 0.5 °C) for 2 minutes. After 1 minute and 45 seconds, a blood pressure cuff was wrapped around the nondominant upper arm and inflated to 20 mmHg below resting diastolic pressure, limiting warm blood to return to the hand. At 2 minutes, the hand was moved from the warm bath to a similar bath filled with circulating cold water (1.0 °C). Using the eVAS slider, PDT and PTT were recorded in seconds. The hand was immediately removed from the cold water when PTT was reported or when the time limit of 120 s was reached, at which point the cuff would also deflate and the hand was removed from the water bath.

Two types of electrical pain paradigms were included (single stimulus [‘stair’] and repeated stimulus [‘burst’]) by placing two electrodes (Ag-AgCl) on cleaned skin overlying the left tibial bone. The ‘stair’ test directly stimulates the nerve and bypasses nociceptors,\(^26\) whereas the ‘burst’ paradigm serves as proxy for temporal summation/wind-up.\(^27\) With the stair test, single stimuli (10 Hz tetanic pulse, 120 s) was used. In the ‘burst’ paradigm, tetanic pain was delivered to the skin following the single stimulus (10 Hz tetanic pulse, 50 s).

### Table 1: Subject characteristics

| Demographic category | Total (n = 50) | Men (n = 26) | Women (n = 24) |
|----------------------|---------------|-------------|---------------|
| Age (years)          | 26.1 (2.6)    | 26 (2.2)    | 25.9 (3)      |
| Weight (kg)          | 70.8 (11.6)   | 77.5 (11.2) | 64.1 (7.5)    |
| Height (cm)          | 176 (10.1)    | 183.1 (8.5) | 168.6 (4.9)   |
| BMI (kg/m²)          | 22.8 (2.4)    | 23 (2.1)    | 22.7 (2.7)    |
| Fitzpatrick skin type, n (%) |
| Type I               | 3 (6%)        | 0           | 3 (12%)       |
| Type II              | 28 (56%)      | 13 (50%)    | 15 (63%)      |
| Type III             | 17 (34%)      | 13 (50%)    | 4 (17%)       |
| Type IV              | 2 (4%)        | 0           | 2 (8%)        |

Note: Table references subjects that completed the study or were replacement subjects for those that could not complete the study and were replaced (see section 2.2). Abbreviations: BMI, body mass index; n, number of subjects; SD, standard deviation.
### TABLE 2 Results of primary evoked pain task endpoints, study parts combined

| Test          | Combined group (all measurements n = 47) | SD vs MORN | SD vs AFT | AFT vs MORN | Men vs women |
|---------------|-----------------------------------------|------------|----------|-------------|--------------|
| **Cold pressor** |                                         |            |          |             |              |
| PDT           | −11.5%, P = .32 (−30.6-12.9%)           | −18.8%, P = .09 (−36.2-3.4%) | 9%, P = .47 (−14.1-38.3%) | 49.4%, P = .03 (4.5-113.6%) |
| PTT           | −10.8%, P = .004 (−17.5 to −3.6%)       | −8.2%, P = .03 (−15 to −0.7%) | −2.9%, P = .45 (−10-4.8%) | 22.9%, P = .24 (−13-73.7%) |
| **El burst**  |                                         |            |          |             |              |
| PDT           | 4.4%, P = .63 (−12.7-24.9%)             | −7.2%, P = .41 (−22.4-11%) | 12.5%, P = .19 (−5.7-34.1%) | −10.2%, P = .47 (−33.1-20.7%) |
| PTT           | −3.2, P = .54 (−12.8-7.5%)              | −7.6%, P = .14 (−16.7-2.7%) | 4.7%, P = .37 (−5.5-16%) | −1.2%, P = .92 (−23.5-27.6%) |
| **El stair**  |                                         |            |          |             |              |
| PDT           | 13.5%, P = .12 (−3.3-33.3%)             | 2.7%, P = .74 (−12.5-20.6%) | 10.5%, P = .21 (−5.7-29.4%) | −8.3%, P = .53 (−30.4-20.9%) |
| PTT           | .9%, P = .84 (−7.6-10.2%)               | −4.6%, P = .27 (−12.7-4.2%) | 5.8%, P = .2 (−29.5-15.4%) | 1.6%, P = .88 (−17.4-24.8%) |
| **CPM**      |                                         |            |          |             |              |
| PDT           | 0.06 mA, P = .87 (−0.7-0.82 mA)         | −0.46 mA, P = .24 (−1.22-0.01 mA) | 0.52 mA, P = .18 (−0.25-1.28 mA) | −0.29 mA, P = .42 (−0.99-0.42 mA) |
| PTT           | −0.69 mA, P = .04 (−1.36 to −0.03 mA)   | −0.49 mA, P = .15 (−1.16-0.18 mA) | −0.2 mA, P = .55 (−0.87-0.47 mA) | 0.37 mA, P = .2 (−0.2-0.95 mA) |
| **Heat**     |                                         |            |          |             |              |
| PDT           | −0.74 °C, P = .02 (−1.34 to −0.14 °C)   | −0.41 °C, P = .18 (−1.01-0.19 °C) | −0.33 °C, P = .27 (−0.92-0.26 °C) | 1.21 °C, P = .1 (−0.26-2.68 °C) |
| **Pressure** |                                         |            |          |             |              |
| PDT           | −6.1%, P = .35 (−17.8-7.2%)             | −4.2%, P = .53 (−16.2-9.6%) | −2%, P = .76 (−14.2-11.8%) | 32.3%, P = .16 (−10.5-95.6%) |
| PTT           | −11.2%, P = .002 (−17.5-4.3%)           | −3.3%, P = .37 (−10.1-4.1%) | −8.1%, P = .02 (−14.6 to −1.2%) | 21.1%, P = .07 (−1.9-49.6%) |

Note: Statistical analysis of evoked pain task endpoints for both study parts (A and B) combined. EDs, P values and 95% CIs (in parentheses) are referenced for indicated contrasts. Values are presented either in the unit in which they were measured or in % for tests for which the data were log-transformed. Data in bold and italic denote significant effects (P < .05). EDs < 0 are in favour of first mentioned condition of the contrast (eg, SD in SD vs MORN), >0 in favour of second mentioned condition. Abbreviations: AFT, well-rested afternoon condition; 95% CI, 95% confidence interval; CPM, conditioned pain modulation paradigm; ED, estimate of difference; El stair/burst, electrical stair (single stimulus) and electrical burst (repeated stimulus) pain tests; MORN, well-rested morning condition; n, number of subjects; PDT/PTT, pain detection/tolerance threshold; SD, sleep deprived morning condition.

0.2 ms duration each, intensity increased at 0.5 mA/s) were administered by a constant current stimulator. With the burst paradigm, each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated five times at the same current intensity, at 2 Hz and at a random interval of 3-8 s between repeats. The intensity increased as with the stair test. Tests stopped automatically when PTT or the safety cut-off of 50 mA was reached.

The electrical stair pain task was performed twice per round, once before and once after the cold pressor test, to evaluate the CPM response. The electrical stair pain test post-cold pressor test (used as test stimulus) was performed as soon as possible after completion of the cold pressor test (used in the CPM paradigm as the conditioning stimulus).

#### 2.5 Study procedures: Questionnaires

Subject-reported sleepiness was collected using the nine-point Karolinska Sleepiness Scale (KSS). Early morning behaviour was characterized using the Leeds Sleep Evaluation Questionnaire (LSEQ) questions 8-10, using a 100 mm eVAS (question 8: How did you feel on waking [ranging from tired to alert]; question 9: How do you feel now [ranging from tired to alert]; question 10: How would you describe your balance and co-ordination upon awakening [ranging from clumsy than normal to less clumsy than normal]). Both questionnaires were completed at the start of the well-rested measurements and at the start of the TSD measurements (Figure 1).
2.6 Statistical considerations and analysis

The CPM response was calculated as the difference between the electrical stair PDT or PTT measured pre-cold pressor test and the same parameter post-cold pressor test. As the CPM response is generally short-lived,28 only the results of the electrical stair test within 5 minutes after start of the cold pressor test were used for further analysis to ensure actual CPM effects were evaluated. For CPM PDT and PTT, the data of one subject in part B for the well-rested morning and well-rested afternoon states were excluded. For CPM PDT and PTT in part B, the data for two subjects for the well-rested morning state and for three subjects for the well-rested afternoon state were excluded.

To estimate the differences between groups (sleep deprived/well-rested morning/well-rested afternoon) and sex, and the interaction sex and group, data were analysed with a mixed-model analysis of variance with a fixed factor group, sex and sex by group, and random factor subject. The Kenward-Roger approximation was used to estimate denominator degrees of freedom and model parameters were estimated using the restricted maximum likelihood method. Parameters were initially analysed without transformation. Except for those from the heat and CPM tasks, all the pain test parameters suggested log-transformation was required and therefore applied. Log-transformed parameters were back-transformed after analysis to allow for interpretation as percentage change.

### Table 3: Results of primary evoked pain task endpoints per study part

| Test      | Men SD (n = 24) vs MORN (n = 23) | Men SD (n = 24) vs AFT (n = 23) | Women SD (n = 23) vs MORN (n = 24) | Women SD (n = 23) vs AFT (n = 24) |
|-----------|---------------------------------|---------------------------------|-----------------------------------|-----------------------------------|
| Cold pressor PDT | 4.6%, P = .8 (26-47.8%) | −14.2%, P = .38 (39-20.7%) | 21.8%, P = .24 (12-9.70.4%) | −25.1%, P = .1 (46.8-5.5%) |
| PTT | −2.4%, P = .66 (12-6.9%) | −0.6%, P = .91 (11-11) | −1.8%, P = .73 (11-8.93%) | −18.5%, P = .0003 (26.9 to -9.1) |
| El burst PDT | 22.3%, P = .12 (4.9-57.1%) | 4%, P = .75 (19-33.7) | 17.5%, P = .2 (8.2-50.3%) | −10.8%, P = .38 (30.9-15.1) |
| PTT | 2%, P = .79 (−11.9-18.2%) | −2.4%, P = .75 (15-7.13.1) | 4.5%, P = .54 (−9-5.20.7) | −8.2%, P = .26 (20-9.66) |
| El stair PDT | 19.7%, P = .12 (−4.5-49.9%) | 6.4%, P = .58 (−15-33.3) | 12.4%, P = .3 (−9-40.2) | 7.6%, P = .52 (−14-35.3) |
| PTT | 9.3%, P = .16 (−3.5-23.6%) | −0.6%, P = .93 (−12-1.25) | 9.9%, P = .12 (−2-24) | −6.8%, P = .27 (−17-7.57) |
| CPM PDT | −0.27 mA, P = .61 (−1.32-0.78 mA) | −0.41 mA, P = .43 (−1.46-0.63 mA) | 0.14 mA, P = .78 (−0.9-1.19 mA) | 0.39 mA, P = .48 (−0.7-1.15 mA) |
| PTT | −0.59 mA, P = .2 (−1.51-0.32 mA) | −0.68 mA, P = .14 (−1.59-0.24 mA) | 0.08 mA, P = .86 (−0.83-1 mA) | −0.09 mA, P = .11 (−1.76-0.17 mA) |
| Heat PDT | −1.06 °C, P = .01 (−1.9 to −0.22 °C) | −0.61 °C, P = .15 (−1.45-0.23 °C) | −0.45 °C, P = .28 (−1.27-0.37 °C) | −0.42 °C, P = .34 (−1.27-0.44 °C) |
| Pressure PDT | −6.6%, P = .48 (−22.7-13.0) | −12.2%, P = .18 (−27.6-6.4) | 6.4%, P = .52 (−12-28.7) | −5.7%, P = .54 (−21.7-13.7) |
| PTT | −7.5%, P = .14 (−16.8-2.8) | 0.9%, P = .86 (−9-11.9) | −8.4%, P = .1 (−17-4.17) | −14.7%, P = .003 (−23.1 to −5.3) |

Note: Statistical analysis of evoked pain task endpoints per study part (part A: men; part B: women). EDs, P values and 95% Cls (in parentheses) are referenced for indicated contrasts. Values are presented in the unit in which they were measured or in % for tests for which the data were log-transformed. Data in bold and italic denote significant effects (P < .05). EDs < 0 are in favour of first mentioned condition of the contrast (e.g., SD in SD vs MORN), >0 in favour of second mentioned condition.

Abbreviations: AFT, well-rested afternoon condition; 95% CI, 95% confidence interval; CPM, conditioned pain modulation paradigm; ED, estimate of difference; El stair/burst, electrical stair (single stimulus) and electrical burst (repeated stimulus) pain tests; MORN, well-rested morning condition; n, number of subjects; PDT/PTT, pain detection/tolerance threshold; SD, sleep deprived morning condition.
3 | RESULTS

3.1 | Subject characteristics

See Table 1 for subject characteristics. In part A, 28 men were enrolled of whom 23 completed all study assessments. In treatment arm 1 (Figure 1), one subject had a positive drug test and was replaced; one other withdrew consent and was not replaced. The data obtained from these two subjects from visit 1 could be used for analysis. In treatment arm 2, one subject had a positive drug test and was replaced; his replacement got sick following minor food poisoning and was also replaced. One other withdrew consent following a headache and was not replaced following protocol regulations. The mean age of subjects for which data was used for final analysis was 26 years (SD ± 2.2).

In part B, 24 women were enrolled of whom 23 completed all study assessments. In the second treatment arm, one subject withdrew consent during the TSD night following a headache that did not subside. She was not replaced following protocol regulations. The mean age was 25.9 (SD ± 3.0).

3.2 | Results: Evoked pain tasks

Significant effects of TSD on the total group (ie, men and women combined) were observed, as well as in men only (part A) and in women only (part B) (Tables 2 and 3, Figure 2).

For the cold pressor task, significant effects were noted for the combined and women groups. Cold PTTs were significantly reduced...
after TSD compared to MORN (combined: estimate of difference [ED] $-10.8\%$, 95% confidence interval [95% CI] $-17.5\%$ to $-3.6\%$, $P < .01$; women only: ED $-18.5\%$, 95% CI $-26.9\%$ to $-9.1\%$, $P < .001$). Men reported a significantly higher cold PDT compared to women (ED 49.4%, 95% CI 4.5-113.6%, $P < .05$).

The CPM response PTT was significantly decreased in the combined group after TSD compared to MORN (ED $-0.69$ mA/s, 95% CI $-1.36$ to $-0.03$ mA/s, $P < .05$). No significant effects were observed in the men-only or women-only groups.

Heat PDTs were significantly lower in the combined and men-only groups after TSD compared to the MORN (combined: ED $-0.74$ °C, 95% CI $-1.34$ to $-0.14$ °C, $P = .016$; men only: ED $-1.06$ °C, 95% CI $-1.9$ to $-0.22$ °C, $P < .05$).

Pressure PPTs were significantly decreased for the combined and women-only groups when comparing the TSD measurement to the MORN (combined: ED $-11.2\%$, 95% CI $-17.5\%$ to $-4.3\%$, $P < .01$; women only: ED $-14.7\%$, 95% CI $-23.1\%$ to $-5.3\%$, $P < .01$). A significant difference was also noted in the combined group for the AFT versus MORN contrast (ED $-8.1\%$, 95% CI $-14.6\%$ to $-1.2\%$, $P < .05$).

No significant effects were noted for any contrast for the electrical burst or electrical stair pain tests (Tables 2 and 3).

3.3 Results: Questionnaires

Subjects reported being significantly sleepier the morning following TSD compared to MORN, as reported on the KSS (ED 4.3, 95% CI 3.9-4.6, $P < .001$). Men scored significantly higher than women for both measurements combined (ED 0.5, 95% CI 0.2-0.9, $P < .01$). Morning behaviour, as assessed with the LSEQ, was also significantly more tired and unbalanced after TSD compared to behaviour after MORN (ED $-28.23$, 95% CI $-32.8$ to $-23.7$, $P < .001$).

4 DISCUSSION AND CONCLUSION

We evaluated the effects of TSD on pain perception in healthy men and women subjects. Cold pressor pain, the CPM response, heat pain detection and pressure pain thresholds were significantly lowered; effects were sex-dependent.

The intervention to deprive subjects of sleep was successful, as observed from the KSS (sleepiness) and LSEQ (early morning behaviour) questionnaire results. While sleep deprivation significantly reduces attention and vigilance, this likely did not bias results as others have discussed previously. In case of reduced attention, a parallel shift in pain detection and tolerance is expected, plausibly on each pain modality in approximately the same manner. Both men and women could still distinguish pain detection from tolerance as exemplified by significantly altered tolerance to pressure and cold pain (PTT), but not detection of pressure and cold pain (PDT; Table 2). Our data therefore indicate that subjects were sleep deprived yet sufficiently focused to properly conduct the tests.

Preclinical studies indicate that sleep deprivation-induced hyperalgesia is partly caused by inhibition of the endogenous opioid protein synthesis and lowering of mu- and delta-opioid receptor affinity. Other neurotransmitters, such as serotonin (5-HT), appear to play a role in maintaining hyperalgesia. This corroborates clinical evidence showing the importance of opioid and serotonergic supraspinal mechanisms in the descending pain inhibitory pathway, and the impaired CPM response we and others observed following sleep deprivation. The increased sensitivity to the cold pressor test we observed, which aims to activate deep tissue mechanoreceptors, builds on this hypothesis, given that the descending pain pathway, when not impaired, particularly inhibits neural activity residing in deep tissue. The exact pathophysiology underlying the observed sleep deprivation-induced heat and cold hyperalgesia is less clear. Heat-induced hyperalgesia is typically restricted to peripheral sensitization mechanisms. In cold-induced hyperalgesia, both peripheral and central processes are involved but these do not relate to those proposed for sleep deprivation. It is interesting to note that the cold pressor task is commonly employed to study the analgesic effects of opioids in healthy subjects and in patients. Decreased cold pressor thresholds following sleep deprivation may thus support the involvement of a temporarily impaired endogenous opioid system. Further investigation into the underlying mechanisms of sleep deprivation-induced heat and cold hyperalgesia is warranted.

Clinical studies evaluating pain thresholds after sleep deprivation reported inconsistent results due to different readouts, different sleep deprivation protocols or different study designs. A comprehensive study using quantitative sensory testing was performed to address this and reported results in line with the results discussed here: heat, cold and pressure thresholds were affected by a night of TSD. A recent follow-up study reported sex-dependent effects of TSD on the CPM response and heat hyperalgesia in women and sex-independent effects on cold and mechanical hyperalgesia. Sex-independent effects of TSD on the cold pressor task were also observed in another study that employed a similar design. Our results confirm that TSD induces heat, pressure and cold hyperalgesia. The significantly lowered threshold of heat pain detection in the combined group is largely driven by the effects induced in men, while the significantly lowered threshold of pressure and cold pain tolerance thresholds are largely driven by the effects induced in women (Table 3 and Figure 2). As the pathophysiology of heat and cold hyperalgesia caused by TSD is yet to be elucidated, it is difficult to dissect why effects on the cold pressor test were seemingly more sex-dependent in our study. The distinct pressure pain protocols used may have played a role in some of the other discrepancies observed. Where Schuh-Hofer et al and Eichhorn et al evaluated more superficial and local pain using an algometer and used this for evaluation of the CPM response, we used a pressure cuff around the leg that also targeted deep tissue nociceptors, which are primarily affected by the descending pain pathway as stated above, and used the electrical stair task as test stimulus for CPM.

Currently available neuropathic pain treatments are only efficacious to a limited extent, resulting in an unmet clinical need.
is great interest in developing drugs for neuropathic pain in general, and in drugs that can treat central sensitization specifically. Here, we showed that TSD lowered pain thresholds and impaired the CPM response, suggesting that TSD alters descending input from the CNS.\textsuperscript{13,15,16} In this study we also observed altered neural activity by increased detection probability of a double-pulse electric intraepidermal stimulus, which further suggests increased facilitation or decreased inhibition (results published elsewhere\textsuperscript{20}). While not strictly related to central sensitization, an imbalance between descending inhibition and ascending facilitation of pain signals is one of the changes found in patients with chronic pain states.\textsuperscript{42,43} Sleep disruption also alters other central pain processes in a sex-dependent manner: an impaired CPM response and increase in temporal summation (a phenomenon often reported in chronic pain disorders) was observed in females.\textsuperscript{13,44} whereas secondary hyperalgesia (a model for neuropathic pain) was induced in males.\textsuperscript{44,45} We discussed in the introduction that, while surrogate models for central sensitization in humans do exist,\textsuperscript{7} they mostly require peripheral input and are of limited use in experimental pain studies with a crossover design. TSD in combination with nociceptive testing may therefore offer an alternative method to evaluate central mechanisms that play a role in chronic pain\textsuperscript{31} and may be suitable to demonstrate and quantify the effects of analgesics aimed at treating central sensitization and neuropathic pain. We intend to confirm this assumption in a future study that will include the same pain test battery and TSD model in which we will administer drugs used to treat neuropathic pain to both males and females. If a sex-dependent drug response is observed in this next study, the results may also aid clinicians in making drug prescription decisions for pain patients suffering from sleep disorders. No opioids will be tested in that study as sleep restriction, a similar yet distinct model to TSD, has been shown to attenuate morphine analgesia.\textsuperscript{46} To the best of the authors’ knowledge, that is the only published study which employed the TSD (or similar) model in the context of analgesic drug testing.\textsuperscript{46} It thus remains to be seen which nociceptive test following TSD will be most sensitive to drug effects.

The results presented here should be read with the following considerations. First, only the short-term effects of TSD over one night were assessed, while pain, including that due to sleep disturbance, is mostly a chronic process. The study results can therefore be used for method development, but are only of limited use for understanding the pathophysiology of sleep deprivation-induced/conditioned pain.\textsuperscript{34} Additionally, the age of the enrolled subjects was relatively young, limiting our conclusions. A young age range was included as this study was part of a larger study in which we also assessed the effects of TSD on driving performance.\textsuperscript{31} Recruiting older subjects for that test was considered unsafe. We did not correct for multiple statistical testing, which may have led to false positives in our results. This was deemed acceptable as the study was exploratory, and is a commonly adopted statistica approach in early-phase exploratory drug studies. In conclusion, TSD induced sex-dependent hyperalgesia on cold, heat and pressure pain, and impaired the CPM response. This confirmed TSD as a method to alter central pain processes. Our data suggest that the model may be used to evaluate (novel) analgesics in experimental pain studies that (partially) target central processes. Investigators should be aware of a sex-dependent response when using the model.

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COMPETING INTERESTS
All authors declared no competing interests for this work.

CONTRIBUTORS
H.H., I.K., R.J.D., R.Z. and G.J.G.: study conception and design. H.H. and I.K.: acquisition of data. E.K.: analysis of the data. H.H.: drafting the article. All authors: interpretation of the data, and critically revision and approval of the manuscript version to be published.

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