Challenging the challenge: a randomized controlled trial evaluating the inflammatory response and pain perception of healthy volunteers after single-dose LPS administration, as a potential model for inflammatory pain in early-phase drug development

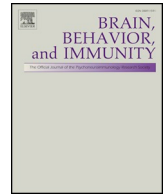Hijma, H.J.; Moss, L.M.; Gal, P.; Ziagkos, D.; Kam, M.L. de; Moerland, M.; Groeneveld, G.J.

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Challenging the challenge: A randomized controlled trial evaluating the inflammatory response and pain perception of healthy volunteers after single-dose LPS administration, as a potential model for inflammatory pain in early-phase drug development

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ABSTRACT

Background and aims: Following an infection, cytokines not only regulate the acute immune response, but also contribute to symptoms such as inflammatory hyperalgesia. We aimed to characterize the acute inflammatory response induced by a human endotoxemia model, and its effect on pain perception using evoked pain tests in two different dose levels. We also attempted to determine whether combining a human endotoxemia challenge with measurement of pain thresholds in healthy subjects could serve as a model to study drug effects on inflammatory pain.

Methods and results: This was a placebo-controlled, randomized, cross-over study in 24 healthy males. Twelve subjects were administered a bolus of 1 ng/kg LPS intravenously, and twelve 2 ng/kg LPS. Before days of placebo/LPS administration, subjects completed a full study day without study drug administration, but with identical pain threshold testing. Blood sampling and evoked pain tests (electrical burst and -stair, heat, pressure, and cold pressor test) were performed pre-dose and at frequent intervals up to 10hr post-dose. Data were analysed with a repeated-measures ANCOVA. For both dose levels, LPS induced an evident acute inflammatory response, but did not significantly affect any of the pain modalities. In a post-hoc analysis, lowering of pain thresholds was observed in the first 3 h after dosing, corresponding with the peak of the acute inflammatory response around 1–3 h post-dose.

Conclusion: Mild acute systemic inflammation, as induced by 1 ng/kg and 2 ng/kg LPS intravenous administration, did not significantly change pain thresholds in this study. The endotoxemia model in combination with evoked pain tests is not suitable to study acute inflammatory hyperalgesia in healthy males.

1. Introduction

The experience of pain, a main symptom in virtually any medical condition, can dramatically decrease a patient's quality of life (Breivik et al., 2008) and has been linked to many pathogenic mechanisms (Dinakar and Stillman, 2016). Tissue injury or (chronic) inflammatory conditions may result in the exaggerated response to certain noxious stimuli, i.e. hyperalgesia – a well-known feeling when affected by an infectious disease, such as the common cold or influenza (Eccles, 2005; Yekkirala et al., 2017) A major underlying mechanism of inflammatory hyperalgesia is the release of various soluble mediators, including bradykinin, sensitization-inducing cytokines (e.g. interleukin -1β, -6 and -8 (IL-1β, IL-6, IL-8) and tumor necrosis factor-alpha (TNF-α)) (Brierley et al., 2010; Paterson et al., 2013; Zhang and An, 2007; Kelley et al., 2003; Follenfant et al., 1989; Poole et al., 1992). While playing a key role in the regulation of the immune response, (Lacy and Stow, 2011) persistent elevation of these cytokines is known to contribute to nerve-inflammation and pathologic pain, and has been linked to diseases such as osteoarthritis, rheumatoid arthritis and inflammatory bowel disease (Zhang and An, 2007; De Jongh et al., 2003; Forrester and Bick-Forrester, 2005).

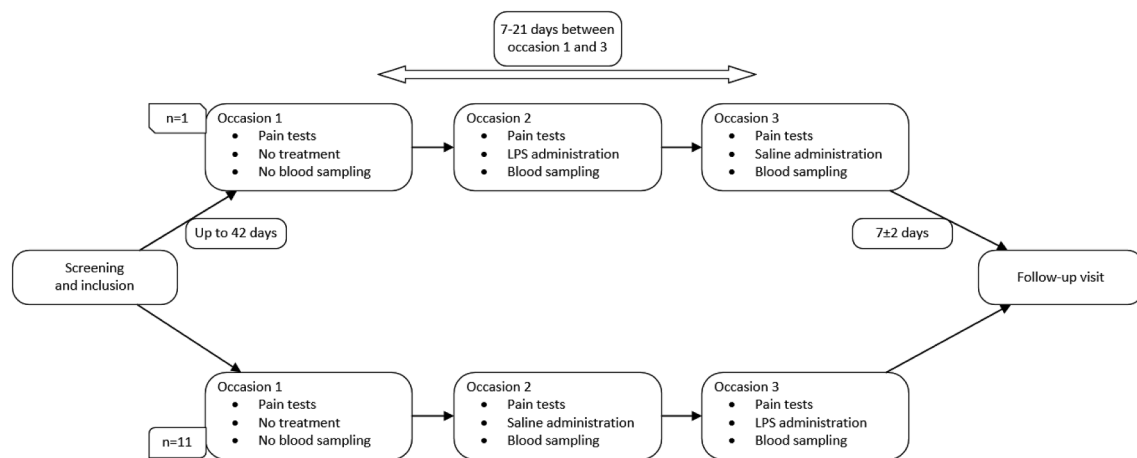Inflammation itself can be divided in the acute phase (processes at

Fig. 1. Flow of study visits per group (n = 12). Screening and inclusion assessments could be performed up to 42 days prior to the first study day (occasion 1). Upon inclusion, subjects were assigned to one of the two treatment arms as indicated (stratified n = 11 and n = 1) and were admitted to the clinical unit three times in total, with a time window of 7–21 days between the first and last date of admittance (i.e. between occasion 1 and occasion 3). Participation was concluded with a safety follow-up visit, 5–9 days after the last dosing performed in occasion 3.

site of inflammation: increase in blood flow, vascular permeability, fluids, leucocytes and inflammatory mediators as listed above) and the chronic phase (recruitment of specific humoral and cellular response, and in cases development of autoimmune conditions) (Feghali and Wright, 1997). To mimic the former, lipopolysaccharide (LPS) may be used to evoke a controlled acute immune response by activation of the Toll-like receptor 4 (TLR4). In rodents, administration of LPS drives an acute immune response, resulting in altered heat and mechanical pain thresholds (Ruiz-Miyazawa et al., 2015; Calil et al., 2014; Naidu et al., 2010; Wilkerson et al., 2016, 2017; Kanaan et al., 1996; Booker et al., 2012). Similarly, in humans, intravenous administration of LPS evokes an acute immune response reflected by increased levels of circulating cytokines, and is dose-dependent (Monnet et al., 2017; van Poelgeest et al., 2018; Dillingh et al., 2014). By combining this human endotoxemia model with evoked pain tests, effects of LPS on multiple pain thresholds have been shown. Following low dose (0.4 and 0.6 ng/kg) LPS administration, significantly reduced pressure- and visceral pain thresholds (Benson et al., 2012; Wegner et al., 2014, 2015; Karshikoff et al., 2015) and altered subjective pain ratings (Benson et al., 2012) were reported between 1 and 3.5 h post-dose. These hyperalgesic effects, although not significant, showed a trend up to 6 h for pressure pain thresholds following a 0.8 ng/kg LPS-dose (Wegner et al., 2014). A high intravenous LPS dose (2 ng/kg) significantly reduced cutaneous (pressure), heat, electrical and cold pressor pain thresholds at 2 h post-dose (de Goeij et al., 2013; Janum et al., 2016). However, in most cases the sample size was small and/or the study design unequipped to compensate for the substantial variability of experimental pain tests (Staahl et al., 2006; Garcia et al., 2004; Diatchenko et al., 2005). Moreover, most previous work on inflammatory hyperalgesia only separately studied the relationship between LPS-dose and pain response, or the time course of the pain response, or the effect of acute inflammation on a few pain modalities, and never all in a controlled and integrated fashion.

The current study was designed to systematically evaluate the effect of an acute systemic inflammatory challenge on pain thresholds in healthy male volunteers, and to validate the combination of experimental endotoxemia with evoked pain tests as a proxy for inflammatory hyperalgesia in early-phase clinical drug studies, if robust effects were to be found. An acute inflammatory response was induced by intravenous administration of LPS. Cytokine and stress hormone responses were frequently monitored over time. In parallel, a validated battery of pain tests, the PainCart, was performed at set times throughout the day. PainCart previously has been validated and used to show the analgesic profile of a wide variety of compound (Loudon

et al., 2018; van Amerongen et al., 2018; Okkerse et al., 2017; Hay et al. (2016)). Two different LPS doses (1 ng/kg and 2 ng/kg bodyweight) were used to evaluate possible dose-dependency. A sample of 12 subjects per dose level (i.e. 24 in total) was chosen to reflect a cohort in a typical phase 1 drug study. We hypothesized, based on prior research as discussed above, that LPS administered to 12 healthy males, could induce robust inflammatory hyperalgesia in an adequately controlled setting. If so, the endotoxemia model combined with evoked pain tests would be of use as a model in early-phase drug testing.

2. Methods

The study was conducted at the Centre For Human Drug Research (CHDR), according to the Declaration of Helsinki of 1975, its amendments and the Guideline for Good Clinical Practice. The study dossier and protocol received Medical Ethics Committee approval prior to initiation of the clinical phase (Medical Ethics Committee: Stichting Beoordeling Ethiek Biomedisch Onderzoek, Assen, The Netherlands). The study was registered under ToetsingOnline number NL65264.056.18 and under ISRCTN number 13923422.

2.1. Study design

This was a double-blind, cross-over, placebo-controlled study in healthy male volunteers receiving a single intravenous dose of LPS or placebo (see for schematic overview Fig. 1). Male subjects aged 18–55, inclusive, were medically screened for general fitness, previous exposure to LPS, and for medical conditions which could create risk for the subject or bias study results (e.g. history of sepsis, cardiovascular disease, acute or chronic pain conditions, previous syncope or malignancies). Medication use (both prescription and over the counter) was prohibited. All participants provided written informed consent prior to any study assessments, and their privacy rights were observed throughout. Being a study exploratory of nature, only men were included in the study.

The study evaluated the effects of two LPS doses: 1 ng/kg and 2 ng/kg, in two separate groups of 12 subjects. The two groups were tested in subsequent order (i.e. first the 1 ng/kg dose group, thereafter the 2 ng/kg dose group). Subjects were randomized to one of two different treatment arms. Per dose level, eleven subjects were allocated to treatment arm A (occasion 1: no treatment; occasion 2: placebo administration; occasion 3: LPS-administration), and one subject was allocated to treatment arm B (occasion 1: no treatment; occasion 2: LPS administration; occasion 3: placebo administration). This disbalanced
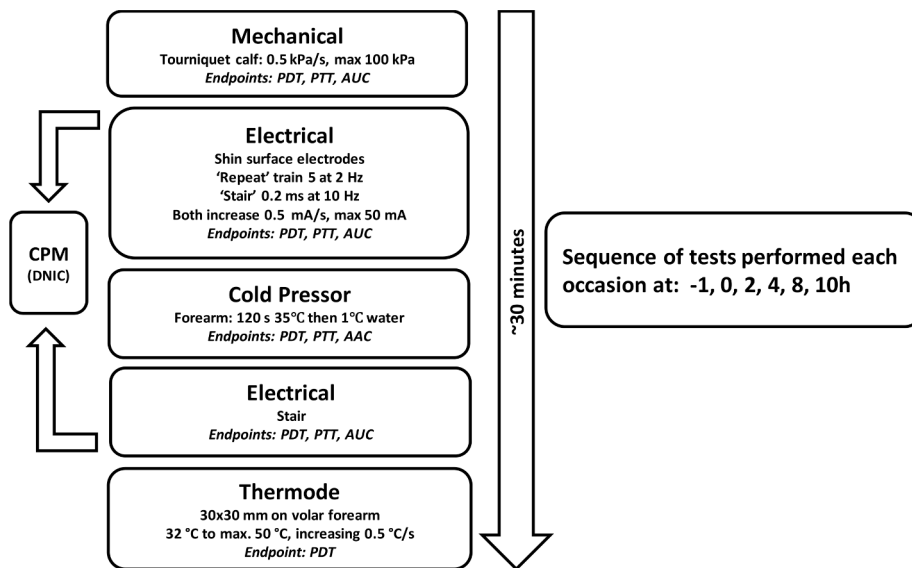
Fig. 2. Sequence and timing of performed pain tests (PainCart) with respective endpoints. CPM: Conditioned Pain Modulation paradigm, DNIC: Diffuse Noxious Inhibitory Control, eVAS: electronic Visual Analogue Scale, LPS: Lipopolysaccharide, PDT: pain detection threshold, PTT: pain tolerance threshold, AAC: area above the eVAS pain curve, AUC: area above/under the eVAS pain curve.

study design was selected since an intravenous LPS challenge cannot be repeatedly performed within one volunteer: LPS induces an innate memory response, regulated at the cellular and epigenetic level, that may last for months (Foster et al., 2007; Motwani et al. (2017); van der Heijden, 2017). Data from a placebo administration day following LPS administration therefore may be biased and is considered to be not reliable for further analysis (see also Section 2.6). By adopting a dis-balanced randomization scheme of 11:1 per dose level, the amount of non-biased data was optimized whilst being able to maintain a double-blind design. Other options, such as a single-blind or open-label design, were not considered valid alternatives due to the high subjectivity and corresponding nocebo effects, which experimental pain models are subject to.

At pre-defined time points throughout the day, blood for quantification of the inflammation and stress markers was sampled and the PainCart test battery, as described below and illustrated in Fig. 2, was performed. Blood was sampled in occasions 2 and 3, PainCart was performed in all three occasions.

2.2. Intravenous LPS challenge

Intravenous LPS challenges were only performed on occasion 2 and 3. Subjects received 1 ng/kg (cohort 1) or 2 ng/kg (cohort 2) *E. Coli*-purified LPS (GMP-grade from Lot#94332B4, List Biological Laboratories Inc. CA, USA), or placebo (0.9% NaCl), administered as a 2-minute infusion. To ensure that subjects would stay adequately hydrated, additionally glucose/saline was infused (2.5% glucose/0.45% sodium chloride) starting 2 h (hours) prior to LPS/placebo administration, until 6 h afterwards.

2.3. Study assessments – blood-based markers

For analysis of various cytokine concentrations, cortisol and C reactive protein (CRP), blood was collected in Natrium Heparin tubes and analyzed using electrochemiluminescence (cytokines analyzed with the Meso Scale Discovery, Rockville, Maryland, USA, with the following Lower limits of quantification (LLOQ): IL-1 receptor antagonist (IL-1ra): 91.6 pg/mL, IL-1β: 0.280 pg/mL, IL-6: 1.49 pg/mL, IL-8: 1.21 pg/mL, IL-10: 0.666 pg/mL, TNF-α: 0.720 pg/mL); cortisol and CRP analyzed using Cobas8000 e602; Roche Diagnostics, with the following LLOQ's: cortisol: 70 nmol/L and CRP: 0.3 mg/L. For the analysis of bradykinin, kallikrein, cortisol and prostaglandin E2 (PGE2), blood was sampled in K2EDTA tubes. Bradykinin, kallikrein and PGE2 were analyzed using ELISA (bradykinin and PGE2: Abcam, Cambridge, UK; bradykinin

LLOQ: 187 pg/mL, and PGE2 LLOQ: 39.1 pg/mL, kallikrein: R&D Systems, Abingdom, UK with LLOQ: 46.9 pg/mL).

2.4. Study assessments – pain tests

On each occasion, nociceptive (pain) detection and tolerance thresholds were measured repeatedly using a fixed sequence battery of pain tests before (at −1h, and 0 h) and after (2, 4, 8 and 10 h)_ LPS administration. (Fig. 2) Tests were performed as described earlier (Loudon et al., 2018; Okkerse et al., 2017) using the following sequence: pressure, electrical burst, electrical stair (1), cold pressor, electrical stair (2), and heat pain test. A training session was part of the screening procedures to reduce any possible learning effects, as well as to exclude any subjects indicating intolerable to pain tests, or achieving tolerance at more than 80% of the maximum input intensity for the cold pressor-, electrical-, or pressure pain test. Assessments were performed with the subject sitting comfortably in a chair, leg raised, in a quiet room that was fitted with ambient lighting. Each subject was assigned to a separate room to minimize any distraction.

2.4.1. Heat pain assessment

To determine primary hyperalgesia to heat, thermal pain detection thresholds (PDTs) were measured with a thermode (Medoc QSense, Israel, contact area: 30 mm × 30 mm), that was placed on the subject's volar forearm. After start of the test, the thermode gradually increased in temperature from 32 °C with 0.5 °C/s, until the subject perceived the stimulus as painful (PDT), or if a temperature of 50 °C was reached. The subject indicated his PDT by pushing the button on the hand-held feedback control. The average of a triplicate measurement was used for further analysis.

2.4.2. Pressure pain assessment

An 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the subject's gastrocnemius muscle. The tourniquet was controlled by an electro-pneumatic regulator (ITV1030-31F2N3-Q, SMC Corporation, Tokyo, Japan), Power1401mkII analogue–to-digital converter and Spike2 software (CED, Cambridge, UK). During the test, the subject indicated his pain intensity using an electronic Visual Analogue Scale (eVAS)-slider, with 0 and 100 defined as "no pain" and "worst pain tolerable", respectively. eVAS > 0 was used as PDT. The pressure evoked by this cuff constantly increased with a rate of 0.5 kPa/s until the subject indicated his Pain Tolerance Threshold (PTT – eVAS to 100), or if 100 kPa was reached.

2.4.3. Cold pressor pain assessment

The subject placed his non-dominant hand into a water bath (minimal depth of 200 mm) at 35 ± 0.5 °C, for 2 min. After 1.45 min, a blood pressure cuff that was placed on the upper arm, was inflated to 20 mmHg below resting diastolic pressure, to limit warm blood returning to the non-dominant hand. After 2 min, the subject changed his hand from the first water bath directly into a similar sized water bath, with a temperature of 1.0 °C. Using the eVAS slider, the subject was instructed to indicate his PDT, the increase in pain intensity and PTT. When the time limit of 120 s, or PTT (eVAS-slider to 100) was reached, the subject removed his hand from the water bath. Simultaneously, the blood pressure cuff was deflated. The time (in seconds) the subject needed to reach PDT, and to reach PTT (or the time limit of 120 s) was used for analysis.

2.4.4. Electrical stimulation assessment (electrical burst and stair)

On clean skin overlying the left tibial bone near the caudal end of the patella, two electrodes (Ag-AgCl) were placed. For the stair test, sole stimuli (10 Hz tetanic pulse with a duration of 0.2 ms) were administered by a constant current stimulator. Current intensity increased from 0 mA to a maximum of 50 mA, in steps of 0.5 mA/s. For the burst test, each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated five times with a frequency of 2 Hz at the same current intensity with a random interval of 3 to 8 s between the repetitions. Current intensity increased identical to the stair test. For both tests, PDT was determined as eVAS > 0; PTT as eVAS = 100 or if 50 mA was reached.

2.4.5. Conditioned pain modulation (CPM)

A possible drug effect on the centrally acting descending inhibitory control pathway, was measured using the conditioned pain modulation (CPM) response, which was quantified by calculating the difference of pain detection and pain tolerance threshold of the electrical stair pain test directly after the cold pressor pain test, minus the electrical stair pain detection and tolerance thresholds prior to the cold pressor pain test (Hay et al., 2016).

2.5. Measures for safety monitoring

The day before each test day (i.e. Day -1 for each occasion) subjects were confined to the clinic and eligibility confirmed by an abbreviated screening of medical history, vital signs and safety laboratory results. During study days, subjects were monitored for overall well-being, as well as any possible adverse events, by clinical staff. Vital signs including temperature were measured at 4 h and whenever deemed necessary. Subjects reported back to clinic 7 ± 2 days after last dosing for a safety follow-up visit.

2.6. Statistical analysis

Data are presented as mean ± standard deviation (SD). eVAS versus time was used to calculate the Area Above the eVAS pain Curve (AAC; for the cold pressor pain test) or Area Under the eVAS pain Curve (AUC; for the pressure-, electrical burst- and stair pain test and CPM). Parameters were initially analyzed without transformation, but as the data suggested otherwise, log-transformation was applied. Log-transformed parameters were back-transformed after analysis allowing results to be interpreted as percentage change. To establish whether significant treatment effects could be detected, all repeatedly measured parameters were analyzed with a mixed model analysis of covariance (ANCOVA) with treatment, time and treatment by time as fixed factors and subject, subject by treatment and subject by time as random factors and the (average) baseline measurement as covariate. The Kenward-Roger approximation was used to estimate denominator degrees of freedom and model parameters were estimated using the restricted maximum likelihood method. Contrasts were calculated between LPS versus placebo (occasion 3 vs occasion 2, only for data from subjects receiving LPS in occasion 3), LPS versus no treatment (occasion 2 or 3 vs occasion 1 – all subjects) and placebo versus no treatment (occasion 2 or 3 vs occasion 1 – all subjects). For all parameters included in the analysis, contrasts were calculated for a time window ranging from pre-dose up until 10 h post-LPS-administration. A sample size of 12 subjects per cohort was based on previous cross-over studies investigating similar objectives, and on the feasibility of including the proposed model in early-phase pharmacological studies (Benson et al., 2012; Wegner et al., 2015; Karshikoff et al., 2015; Hutchinson et al., 2013).

3. Results

3.1. Baseline characteristics

24 subjects were enrolled and finished the complete study. Apart from these 24 subjects, two subjects did participate in occasion 1, but stopped participation before being dosed in the occasion 2 for personal reasons, and were therefore replaced. Although the protocol allowed for flexibility in when subjects returned to clinic (i.e. 7–21 days between occasion 1 and 3), all subjects but one (due to personal circumstances) reported to the clinic once a week (e.g. every Monday). Mean age was 30.8 ± 9.5 years, mean body mass index was 23.8 ± 2.3 m^2; most subjects (58.3%) were Caucasian. Further baseline characteristics can be found in Table 1. Although there were a few treatment-emergent adverse events observed around the projected E$_{max}$ (around 2 h post-dose, see section 3.2. below), such as chills or short and transient nausea symptoms, this was not reflected in out-of-range vital signs or other clinically significant safety findings.

3.2. Inflammatory response to LPS

All markers were assessed from pre-dose up until 10 h post-LPS administration. For IL-6, IL-8 and TNF-α in both dose levels (1 ng/kg and 2 ng/kg), a time-dependent and significant increase was observed shortly after LPS administration in comparison to placebo. Peak concentrations for TNF-α were observed at 1 h post-dose (peak concentration 1 ng/kg dose: 66.49 pg/mL; 2 ng/kg dose: 249.35 pg/mL); for IL-1b, IL-6, IL-8 and IL-10 peaks were observed at 2 h post-dose (IL-1b 1 ng/kg dose: 0.39 pg/mL; IL-1b 2 ng/kg dose: 1.10 pg/mL; IL-6 1n/kg dose: 65.55 pg/mL; IL-6 2 ng/kg dose: 200.88 pg/mL; IL-8 1 ng/kg dose: 195.96 pg/kg; IL-8 2 ng/kg dose: 515.83 pg/mL; IL-10 1 ng/kg

Table 1
Subject baseline characteristics.

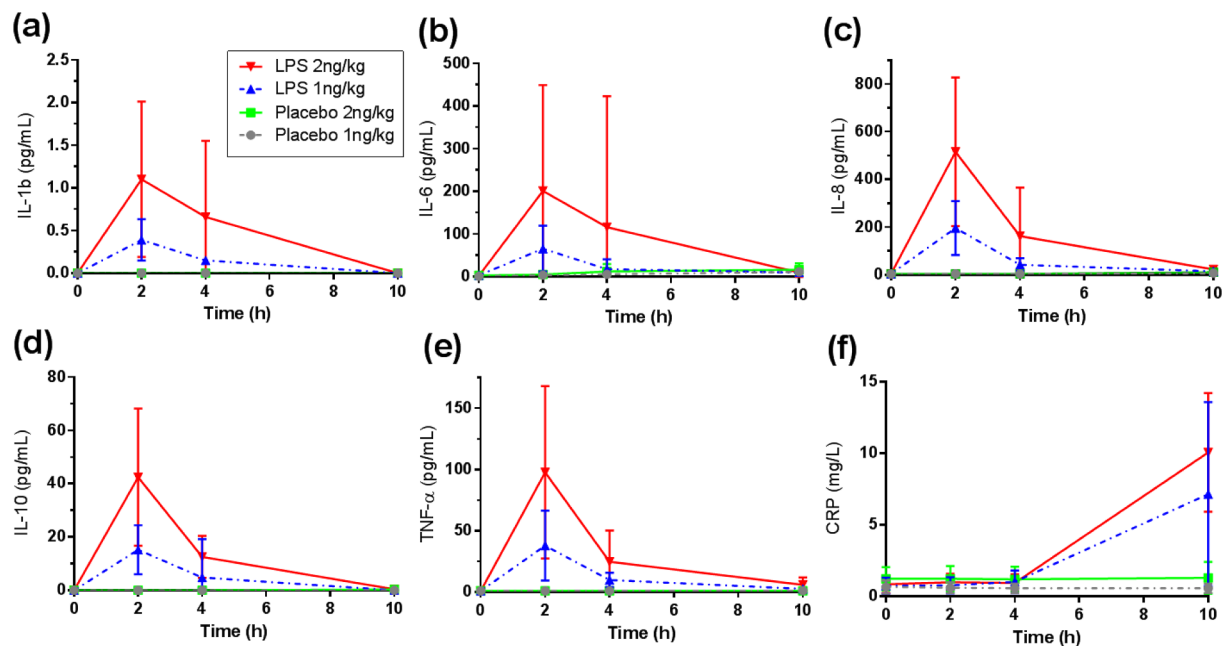| Total subjects	24
Age (years)	
Mean (SD)	30.8 (9.5)
Range	19–52
Gender	
Male	100%
Ethnicity	
Caucasian	58.3%
Mixed	12.5%
Black/African	12.5%
Asian	8.3%
Other	8.3%
Height (cm)	
Mean (SD)	179 (6.9)
Range	168.5–190.4
Weight (kg)	
Mean (SD)	76.4 (9.7)
Range	58.2–94.5
BMI (kg/m^2)	
Mean (SD)	23.8 (2.3)
Range	20.1–27.9

BMI: Body Mass Index

Fig. 3. Cytokine concentrations after LPS or placebo administration, measured pre-dose (0 h) up until 10 h (hours) post-dose. Vertical lines represent standard deviations. a: IL-1b concentrations (pg/mL), b: IL-6 concentrations (pg/mL), c: IL-8 concentrations (pg/mL), d: IL-10 concentrations (pg/mL), e: TNF-α concentrations (pg/mL), f: CRP concentrations (mg/mL). CRP: C Reactive protein, IL: interleukin, pg/mL: LPS: Lipopolysaccharide, mg or pg/mL: microgram or picogram/milliliter, respectively.

dose: 15.25 pg/mL; IL-10 2 ng/kg: 42.41 pg/mL). After peaking, concentrations of all these markers rapidly decreased, and approached baseline values again at 10 h post-dose (Fig. 3-a, -b, -c and -d). For IL-1b and IL-10, no statistical testing could be performed given most results from the placebo occasion were below LLOQ, as expected.

IL-1ra concentrations increased shortly after LPS exposure compared to placebo, yet also remained vastly elevated: between 2 and 10 h post-dose > 57% of the samples at the 1 ng/kg dose level, and > 93% of the samples at the 2 ng/kg dose level were above the upper limit of quantification of 2930 pg/mL. CRP concentrations showed a delayed response to LPS, by increasing from 4 h onwards without a tendency to decrease at our last measured time point (at 10 h post-dose; concentration 1 ng/kg dose: 7.15 mg/mL; 2 ng/kg dose: 10.07 mg/mL) (Fig. 3-f).

3.3. Measures for inflammatory hyperalgesia

3.3.1. Primary analysis

Overall, LPS administration did not significantly alter pain thresholds over time, i.e. PDT, PTT and/or AUC endpoints from pre-dose up until 10 h post-dose, as shown in Table 2 and Fig. 4. Baseline values, summary graphs and the statistical table may be found in Appendix A, B and C.1, respectively. For both dose levels, LPS decreased heat pain PDT and electrical stair PTT between 1 and 2 h post-administration (Fig. 4-a, b, g and h), although the contrast with the no treatment- or placebo occasion was not significant when analyzed for the full time profile (0–10 h post-dose, Table 2). Lowering of pain thresholds around 2 h post-administration were also observed for pressure pain PTT and electrical burst PTT after 2 ng/kg LPS administration (Fig. 4-f and j).

A significant effect of 2 ng/kg LPS was observed for the CPM AUC endpoint (versus placebo; ED: −71.04%, 95% CI: −139.76 − −2.33%). Fig. 4-k and l show that CPM, although unaffected by LPS at 1 ng/kg, is decreased (i.e. lower endogenous inhibition) around 2 h post-administration. Thereafter, CPM rapidly returns around baseline at 4 h, before decreasing until last measured time point at 10 h post-administration.

Cold pressor PTT and AAC, electrical burst PDT and pressure pain PDT were significantly reduced by 1 ng/kg LPS compared to placebo

(cold pressor PTT: ED: −15.8%, 95% CI: −25.7 − −4.7% and AAC: ED: −14.9%, 95% CI: −27.2 − −0.6%, electrical burst pain test PDT ED: −15.8%, 95% CI: −28.3 − −1.1% and pressure pain test PDT (ED: 23.7%, 95% CI: 3.6 − 47.7%). All these PainCart modalities simultaneously showed an LPS effect versus no treatment (pressure pain test PDT: ED: 41.3%, 95% CI: 18.9 − 68.0%) or placebo effect versus no treatment (cold pressor test PTT: ED: 18.9%, 95% CI: 6.1 − 33.3%; cold pressor test AAC: ED: 16.5%, 95% CI: 1.3 − 33.9%; electrical burst pain test PDT: ED: 30.1%, 95% CI: 12.9 − 50.0%) (Table 2).

3.3.2. Post-hoc analysis of PainCart results (pre-dose up until 6 h post-dose)

As with the primary analysis, LPS administration did not significantly alter pain thresholds over time, i.e. PDT, PTT and/or AUC endpoints from pre-dose up until 6 h post-dose, as shown in Table 3 (statistical table in Appendix C.2). No dose-dependency was observed.

In the 1 ng/kg cohort, no significant effect of LPS versus placebo were found. Effects of LPS versus no treatment were found for the pressure PDT (ED: 43.2%, 95% CI: 17.5 − 74.5%); and CPM PDT (ED 1.99%, 95% CI: 0.46 − 3.51%). Placebo differed significantly from no treatment for cold pressor PTT and AAC (PTT: ED: 22.8%, 95% CI: 9.0 − 38.5%; AAC: ED: 18.8%, 95% CI: 2.7 − 37.4%) and for pressure pain PDT (ED: 18.1%, 95% CI: 8.0 − 60.6%).

In the 2 ng/kg cohort, significant effect of LPS versus placebo, and LPS versus no treatment were only found for the electrical stair PTT and AUC endpoints (versus placebo: PTT: ED: −9.5%, 95% CI: −17.0 − −1.2%; AUC: ED: 197.68%, 95% CI: 31.80 − 361.55%; versus no treatment: PTT: ED: −9.6%, 95% CI: −16.9 − −1.6%; AUC: ED: 157.08%, 95% CI: 1.81 − 312.35%). No effects were observed in the placebo versus no treatment contrast.

3.4. Stress hormone response

Cortisol levels significantly increased after both 1 ng/kg and 2 ng/kg LPS administration, peaking at 3 h post-dose and gradually returning to baseline afterwards (Fig. 5-a). No time-dependent fluctuations were observed in the placebo groups. LPS administration did not substantially alter bradykinin or PGE2 levels (Fig. 5-b,c). As half of the

Table 2

PainCart evoked pain model results; pre-dose up until 10 h post-dose.

Modality	Contrast for 1 ng/kg LPS dose			Contrast for 2 ng/kg LPS dose		
	LPS vs placebo	LPS vs none	placebo vs none	LPS vs placebo	LPS vs none	placebo vs none
Heat						
PDT	−0.4 (p = 0.486) (−1.03–0.51)	−0.13 (p = 0.721) (−0.87–0.62)	0.11 (p = 0.757) (−0.65–0.88)	0.18 (p = 0.545) (−0.44–0.81)	−0.22 (p = 0.441) (0.80–0.36)	0.30 (p = 0.286) (−0.88–0.27)
Cold pressor						
PDT	3.5 (p = 0.750) (−17.2–29.5)	29.1 (p = 0.057) (−0.8–68.1)	26.9 (p = 0.075) (−2.6–65.3)	20.5 (p = 0.232) (−12.3–65.7)	13.8 (p = 0.305) (−35.9–15.8)	**28.3 (p = 0.0292)** (−46.6–3.7)
PTT	**−15.8 (p = 0.010)** (−25.7–4.7)	0.8 (p = 0.8873) (−10.1–13.0)	**18.9 (p = 0.005)** (6.1–33.3)	0.2 (p = 0.976) (−14.6–17.7)	2.4 (p = 0.766) (−13.3–21.0)	3.7 (p = 0.657) (−12.2–22.4)
AAC	**−14.9 (p = 0.044)** (−27.2–0.6)	0.2 (p = 0.979) (−12.9–15.2)	**16.5 (p = 0.034)** (1.3–33.9)	3.8 (p = 0.657) (−12.8–23.6)	1.1 (p = 0.892) (14.2–19.2)	1.3 (p = 0.865) (−16.2–16.1)
Electrical stair						
PDT	−7.2 (p = 0.498) (−26.2–16.7)	−3.3 (p = 0.747) (−22.2–20.1)	5.4 (p = 0.710) (−14.8–30.4)	11.3 (p = 0.399) (−33.7–18.8)	−8.9 (p = 0.463) (−29.8–18.1)	2.0 (p = 0.874) (−21.3–32.2)
PTT	−5.0 (p = 0.252) (−13.3–4.1)	−2.7 (p = 0.503) (−10.4–5.8)	2.5 (p = 0.545) (−5.7–11.3)	−7.4 (p = 0.059) (−14.6–0.3)	−6.0 (p = 0.118) (−13.1–1.7)	2.3 (p = 0.552) (−5.4–10.6)
AUC	50.0 (p = 0.717) (−239.19–339.19)	134.19 (p = 0.293) (−127–395.39)	89.28 (p = 0.476) (−169.69–348.26)	123.47 (p = 0.119) (−35.09–282.04)	79.47 (p = 0.279) (−69.29–228.23)	−47.69 (p = 0.507) (−195.07–99.70)
Electrical burst						
PDT	−15.8 (p = 0.037) (−28.3–1.1)	12.2 (p = 0.124) (−3.4–30.3)	**30.1 (p = 0.001)** (12.9–50.0)	0.8 (p = 0.960) (−26.9–38.9)	11.1 (p = 0.474) (−17.6–49.8)	12.1 (p = 0.436) (−16.8–51.0)
PTT	3.7 (p = 0.568) (−9.1–18.1)	1.5 (p = 0.794) (−9.7–14.0)	−2.6 (p = 0.650) (−13.4–9.6)	−9.6 (p = 0.165) (−21.8–4.6)	−4.4 (p = 0.495) (16.5–9.4)	6.9 (p = 0.315) (−6.6–22.5)
AUC	−4.29 (p = 0.920) (−92.90–84.33)	−11.98 (p = 0.772) (−96.71–72.76)	−19.92 (p = 0.620) (−102.07–62.23)	39.59 (p = 0.325) (−42.61–121.80)	−0.06 (p = 0.999) (−76.12–76.0)	−44.84 (p = 0.233) (−120.92–31.24)
Pressure						
PDT	**23.7 (p = 0.022)** (3.6–47.7)	**41.3 (p < 0.001)** (18.9–68.0)	18.1 (p = 0.060) (−0.8–40.5)	6.2 (p = 0.419) (−8.8–23.6)	3.0 (p = 0.675) (−10.9–19.1)	−2.2 (p = 0.752) (−15.3–12.9)
PTT	5.5 (p = 0.522) (−10.5–24.2)	5.1 (p = 0.526) (−10.5–23.3)	1.9 (p = 0.811) (−13.2–19.6)	−2.6 (p = 0.622) (−12.7–8.7)	−10.8 (p = 0.058) (−20.8–0.4)	−9.1 (p = 0.116) (−19.5–2.6)
AUC	123.79 (p = 0.987) (−221.28–468.86)	38.09 (p = 0.862) (−324.06–400.24)	−6.63 (p = 0.991) (−365.29–352.02)	248.34 (p = 0.194) (−137.84–634.52)	299.26 (p = 0.296) (−283.15–881.68)	151.13 (p = 0.593) (−429.42–731.67)
CPM						
PDT	0.46 (p = 0.421) (−0.71–1.63)	1.06 (p = 0.070) (−0.1–2.21)	0.91 (p = 0.101) (−0.19–2.02)	0.30 (p = 0.556) (−0.74–1.34)	0.70 (p = 0.159) (−0.29–1.69)	0.58 (p = 0.220) (−0.38–1.54)
PTT	0.49 (p = 0.233) (−0.35–1.34)	−0.23 (p = 0.568) (−1.09–0.62)	0.72 (p = 0.085) (−1.54–0.11)	0.08 (p = 0.956) (−0.37–0.53)	0.33 (p = 0.347) (−0.80–0.13)	−0.32 (p = 0.696) (−0.78–0.14)
AUC	−72.57 (p = 0.185) (−183.38–38.25)	30.43 (p = 0.575) (−141.70–80.85)	26.55 (p = 0.611) (−80.62–133.73)	**−71.04 (p = 0.043)** (−139.76–2.33)	−47.78 (p = 0.171) (−117.69–22.12)	5.13 (p = 0.881) (−64.91–75.16)

Numbers represent estimates of the difference (in %), next to the p-value which is displayed in italic. Lower and upper limit (in %) of 95% confidence interval are shown between parentheses. AAC: area above the eVAS pain curve, AUC: area under the eVAS pain curve, CPM: conditioned pain modulation paradigm, eVAS: electronic Visual Analogue Scale, LPS: Lipopolysaccharide, PDT: pain detection threshold, PTT: pain tolerance threshold. Estimates > 0 favor the first mentioned condition (i.e. LPS in LPS vs placebo contrast), estimates < 0 favor the second condition (i.e. placebo in LPS vs placebo contrast).

results were below LLOQ, kallikrein concentrations were not interpretable.

4. Discussion

The goal of this study was to evaluate the effect and dose-dependency of an acute LPS-driven inflammatory response on pain perception using evoked pain tests. We did not observe significant pain threshold lowering in 12 healthy male subjects per dose level in a highly controlled setting – correcting for treatment-, placebo-, and long-term carry-over-effects, when analyzed over a 10 h period.

The underlying mechanism of inflammatory hyperalgesia is through the activation of the primary afferent nociceptors following increased release of sensitization-inducing mediators such as TNF-α, IL-1β, and bradykinin: molecules which are known to induce thermal and mechanical hyperalgesia (Paterson et al., 2013; Wu et al., 2010; Eidson and Murphy, 2013). Increased expression of these mediators is a downstream effect of the enhanced production of PGE2, which in turn is caused by activation of cyclooxygenase-1 or -2 by a pro-inflammatory stimulus (Agard et al., 2013; Fujikawa et al., 2012). LPS drives this response by activation of the TLR4 that, apart from being located on

inflammatory cells, is also found on the dorsal root ganglia, dorsal root horn, Schwann cells and neuraxial glia. Based on these physiological mechanisms, and literature showing a link between LPS-induced acute inflammation and reduction in pain thresholds in both rodents and humans, the current study was performed. Adding to available reports, we have set-up our trial to study the dose-effect relationship between LPS and pain perception using comprehensive battery of evoked pain tests, and have evaluated these over-time. Here, LPS indeed induced a clear acute inflammatory response at both dose levels (1 ng/kg and 2 ng/kg), but this did not translate to significant effects on pain thresholds. A few isolated significant contrasts were observed, but no evident dose-dependent effects were found over the full-time course (pre-dose up to 10 h post-dose). However, when looking at the profiles for both doses in more detail – using a post-hoc analysis to assess pain thresholds from pre-dose to 6 h post-dose –, significant effects were reported for the cold pressor- and electrical stair pain test. Hyperalgesic effects were most pronounced two hours after dosing, and seem to correspond with the acute inflammatory response peak.

Previous human endotoxemia studies evaluating pain perception are inconclusive on potential sex-related differences (Wegner et al., 2015; Karshikoff et al., 2015). To exclude for a potential effect of
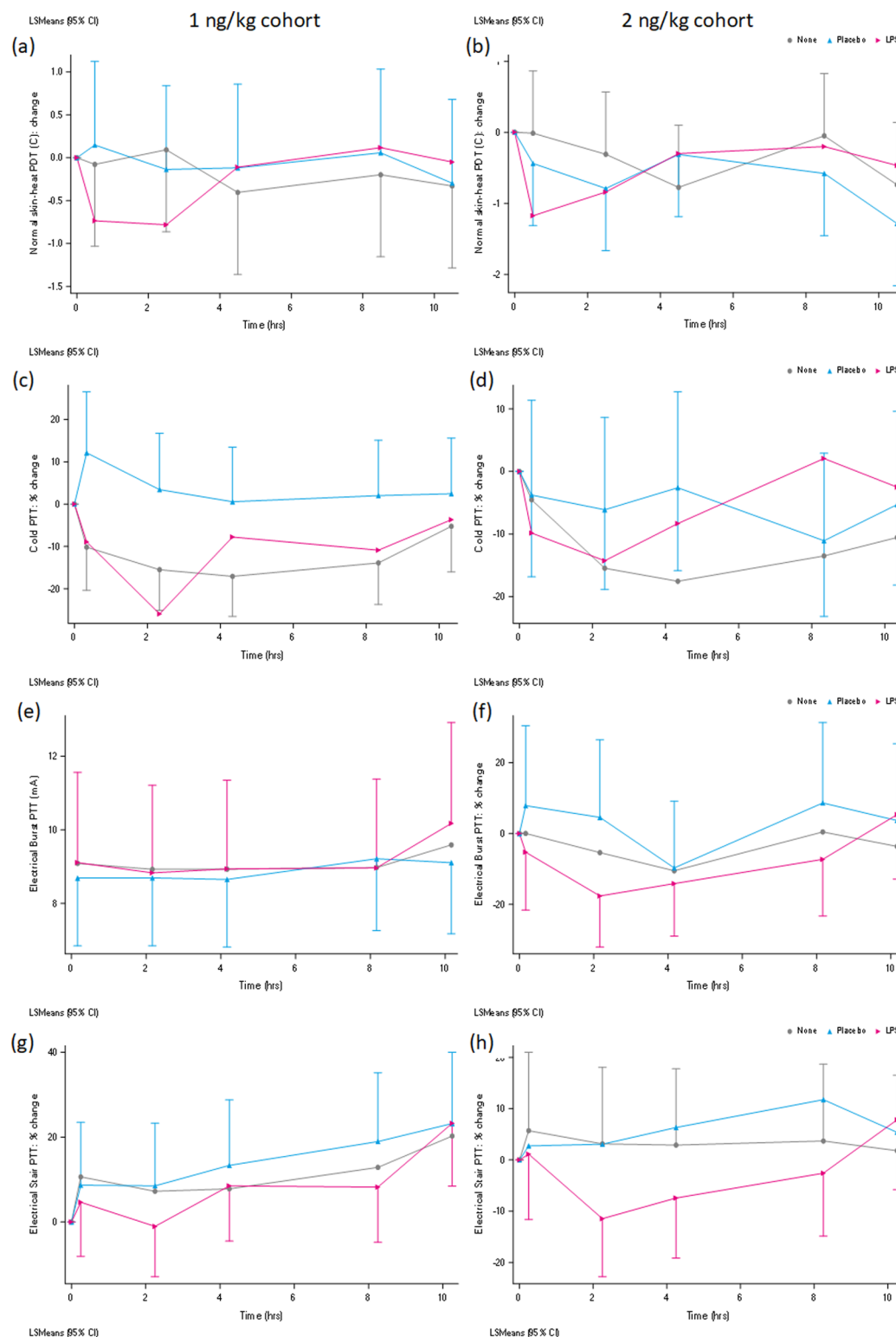
Fig. 4. Selection of PainCart test results. Graphs in the left column represent subjects that received 1 ng/kg LPS, right column for subjects that received 2 ng/kg. Data represented as change from baseline in percentages (%), in which baseline has been defined as the pre-dose measurement of that occasion. Values on y-axis represent the least square means change of the 95% confidence interval, time is described in hours on the x-axis. a and b: heat pain PDT; c and d: cold pressor PTT; e and f: electrical bust PTT, g and h: electrical stair PTT; i and j: pressure pain PTT, k and l: CPM PTT. CPM: conditioned pain modulation, PDT: pain detection threshold, PTT: pain tolerance threshold.

gender, we limited our study to men. Although the selected LPS dose for our study was low (1 ng/kg and 2 ng/kg), approximately 50% of the subjects reported effects as feeling cold and/or sick. These clinical symptoms, though inevitably related to LPS exposure, may have

interfered with (heat) pain testing and treatment blinding. We mitigated bias as much as possible by use of a double-blind design, allocation of subjects to separate testing rooms during study days, and standardized sequence and timing of pain tests. The electrical stair PTT

Fig. 4. (*continued*)

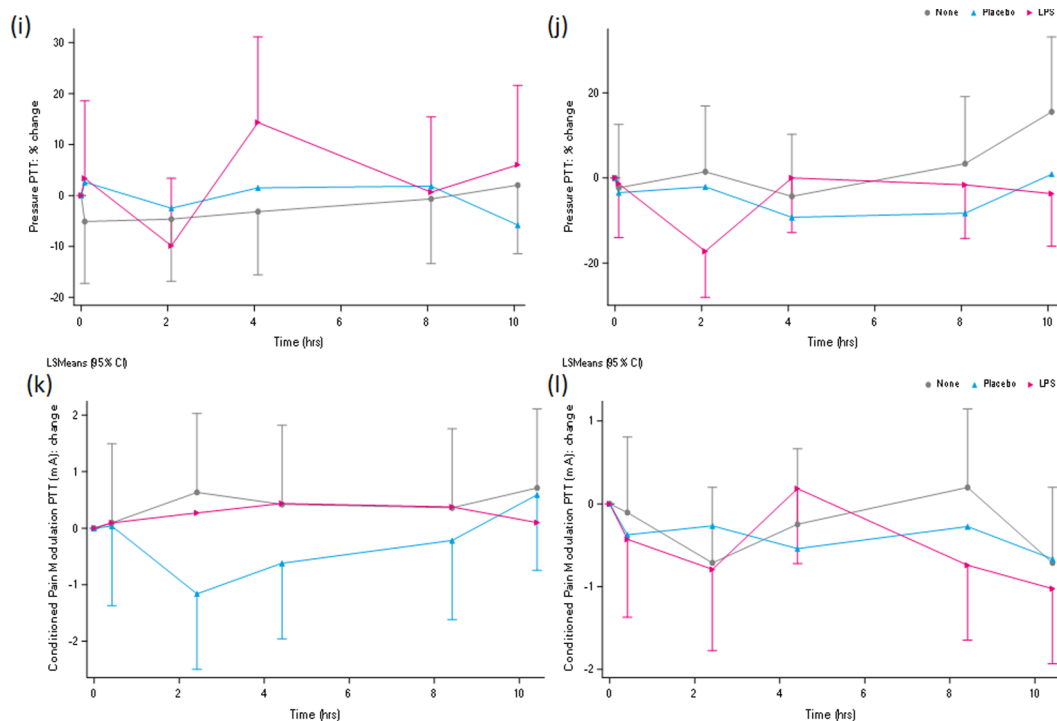immediately after the cold pressor test was used to quantify the conditioned pain modulation (CPM) response. Heat PDT's were quantified after the post-cold pressor electrical stair (see Fig. 2) and may therefore possibly have been influenced through an ongoing CPM response (Fig. 2). However, the possible bias – if at all present – will have been limited, as CPM is typically only short-lived (Arendt-Nielsen et al., 2008; Tuveson et al., 2006; Campbell et al., 2008; Kakigi, 1994; Yarnitsky et al., 2015) and because effects of LPS on pain thresholds were determined in a controlled fashion in which heat PDTs were always determined in the same order; they will therefore have been equally affected during each cross-over occasion. Our study included a sample size of 12 subjects per cohort. Using a Minimal Detectable Effect Size (MDES) calculation and results from the 12 subjects in the 2 ng/kg cohort, for heat PDT with a power of 80%, we could have detected a mean difference of 0.87 °C assuming a SD of differences of 0.98 °C; and for pressure PTT a mean difference of 16.3 kPa assuming a SD of differences on a 0.17 log scale. A size of 12 subjects per group was chosen to reflect a typical phase I drug study cohort, aligning with our study objective to validate the model for use in such a study.

Others have previously reported significant effects of intravenous LPS administration on nociception and pain tolerance thresholds, which contrasts with our findings. For example, de Goeij et al. showed that the inflammatory response following a 2 ng/kg intravenous LPS challenge significantly influenced the thermal, pressure and cold pressor pain test 2 h after the challenge (de Goeij et al., 2013). It is important to note, however, that this 27 subject-study was performed in a non-cross-over fashion. In another study, Janum et al. demonstrated the effects of 2 ng/kg LPS on thermal pain and mechanical pain at 2 h post-dose, and on mechanical pain up to 6 h post-dose (Janum et al., 2016). While noting that LPS-induced hyperalgesia was majorly reported for mechanical pain assessed with a methodology different from ours (i.e. a handheld algometer to measure pain sensitivity with, versus a tourniquet cuff to measure pain sensitivity (PDT) and tolerance (PTT) with) and so possibly contributes to the discrepancy between study outcomes, the current study has several key advantages over both de Goeij and Janum s work. First, this study was designed to control for the substantial inter- and intra-subject variability of evoked pain tests, which is key for clinical pain research (Staahl et al., 2006; Garcia et al., 2004;

Diatchenko et al., 2005). This contrasts with earlier studies that did not use a cross-over study design (Wegner et al., 2014; Karshikoff et al., 2015; de Goeij et al., 2013). Moreover, as described in the methods sections, LPS has a long-term effect on the innate immune response, a factor that has not been taken into account in the other experimental pain studies of Wegner et al., de Goeij et al. and Karshikoff et al., (van Lier et al., 2019; Wegner et al., 2014, 2015; Karshikoff et al., 2015; de Goeij et al., 2013) yet has been mitigated in the current study. Finally, the majority of previous studies assessed LPS-induced hyperalgesia solely at one time point, (Benson et al., 2012; Wegner et al., 2015; Karshikoff et al., 2015; de Goeij et al., 2013) or at a maximum of three time points, (Wegner et al., 2014) and were performed around the E_{max} of LPS (2–3 h post-dose). Although results of Wegner et al. hint that effects are subtle after 3 h and non-significant at 6 h post-dose, the exact temporal relationship between the inflammatory response and hyperalgesia is not known. Only assessing specifically around the E_{max} is therefore a suboptimal experimental approach. In the current study, extensive time courses (pre-dose up to 10 h) were generated for cytokine and pain responses, allowing the full integration of both, and showing that the hyperalgesic effects of LPS are more subtle and time constrained (only briefly around 2 h post-dose) than assumed thus far.

Nonetheless, preclinical work also reported positive results that are discrepant from ours (Ruiz-Miyazawa et al., 2015; Calil et al., 2014; Naidu et al., 2010; Wilkerson et al., 2016, 2017; Kanaan et al., 1996; Booker et al., 2012). The reason for this may be two-fold. Apart from translatability issues, i.e. the fact that pre-clinical models often cannot be confirmed in other animal- or clinical models due to substantial inter-species variability, (Wendler and Wehling, 2010; Clark, 2016) the LPS dose given to mice plausibly induced a more severe acute inflammatory response – yielding more pronounced clinical symptoms and therefore potential effects on pain thresholds –, when compared to the dose we administered to humans. We consciously did not exceed an LPS dose of 2 ng/kg knowing that notable flu-like symptoms would hamper execution of the (pain) tests, and that high-dose LPS administration may result in severe side effects such as (fatal) cardiac issues, sepsis and renal and/or kidney injury (Nozaki et al. (2017); Suffredini et al., 1989; Virzi et al., 2017). LPS doses, such as the ones we used, that induce a significant cytokine response with a mild adverse effect profile

Table 3
PainCart evoked pain model results; pre−dose up until 6 h post-dose.

	Contrast for 1 ng/kg LPS dose			Contrast for 2 ng/kg LPS dose		
Modality	LPS vs placebo	LPS vs none	placebo vs none	LPS vs placebo	LPS vs none	placebo vs none
Heat						
PDT	−0.52 (p = 0.188)	−0.41 (p = 0.273)	0.09 (p = 0.805)	−0.16 (p = 0.638)	−0.41 (p = 0.217)	0.15 (p = 0.643)
	(−1.304–0.274)	(−0.18–0.35)	(−0.69–0.88)	(−0.87–0.54)	(−1.06–0.25)	(−0.79–0.50)
Cold pressor						
PDT	4.4 (p = 0.739)	29.4 (p = 0.092)	26.5 (p = 0.125)	6.4 (p = 0.728)	−5.8 (p = 0.721)	−13.6 (p = 0.0384)
	(−19.5 – 35.3)	(−4.3–75.1)	(−6.7–71.6)	(−26.2–53.5)	(−33.0 – 32.5)	(−38.5–21.4)
PTT	−19.3 (p = 0.003)*	−0.4 (p = 0.947)	**22.8 (p = 0.002)**	−7.1 (p = 0.34)	2.1 (p = 0.795)	9.8 (p = 0.254)
	(−29.1–8.1)	(−11.7–12.3)	(9.0–38.5)	(−20.6 – 8.6)	(−13.4–20.4)	(−6.9 – 29.3)
AAC	−18.2 (p = 0.019)*	−3.3 (p = 0.743)	**18.8 (p = 0.023)**	−3.4 (p = 0.708)	3.0 (p = 0.736)	6.2 (p = 0.495)
	(−30.3–4.1)	(−15.5–13.0)	(2.7–37.4)	(−20.0–16.7)	(−13.9 – 23.2)	(−11.1–26.8)
Electrical stair						
PDT	−13.6 (p = 0.252)	−7.0 (p = 0.543)	10.1 (p = 0.414)	−23.1 (p = 0.089)	−20.0 (p = 0.108)	3.3 (p = 0.809)
	(−33.1–11.6)	(−26.9 – 18.3)	(−13.2 – 39.7)	(−43.4–4.4)	(−39.2–5.3)	(−21.4–35.7)
PTT	−4.7 (p = 0.337)	−4.2 (p = 0.349)	1.5 (p = 0.748)	**−9.5 (p = 0.027)**	**−9.6 (p = 0.021)**	0.1 (p = 0.977)
	(−13.9–5.5)	(−12.7 – 5.1)	(−7.5–11.3)	(−17.0–1.2)	(−16.9 − 1.6)	(−8.0 – 8.9)
AUC	38.3 (p = 0.787)	157.94 (p = 0.233)	110.30 (p = 0.397)	**197.68 (p = 0.022)**	**157.08 (p = 0.048)**	−32.75 (p = 0.664)
	(−257.12–333.72)	(−110.28–426.16)	(−155.74–376.34)	(31.80–361.55)	(1.81–312.35)	(−186.45–120.95)
Electrical burst						
PDT	−17. (p = 0.064)	1.4 (p = 0.882)	17.9 (p = 0.071)	−1.4 (p = 0.934)	6.7 (p = 0.686)	11.6 (p = 0.493)
	(−31.8–1.2)	(−15.7–22.0)	(−1.4 – 41.1)	(−30.3 – 39.5)	(−22.8–47.5)	(−19.1–54.0)
PTT	3.2 (p = 0.635)	−0.1 (p = 0.991)	−3.7 (p = 0.536)	−13.2 (p = 0.070)	−7.5 (p = 0.274)	6.3 (p = 0.388)
	(−10.1–18.5)	(−11.7–13.1)	(−15.1–9.1)	(−26.6–1.2)	(−19.8 – 6.7)	(−7.9 – 22.8)
AUC	−1.22 (p = 0. 979)	−4.52 (p = 0.920)	−8.74 (p = 0.843)	62.0 (p = 0.155)	26.43 (p = 0.508)	−34.75 (p = 0.384)
	(−97.23–94.79)	(−96.06–87.03)	(−97.91–80.44)	(−25.13–149.13)	(−54.37–107.22)	(−115.4–45.89)
Pressure						
PDT	12.3 (p = 0.259)	**43.2 (p < 0.001)**	**18.1 (p = 0.008)**	6.8 (p = 0.467)	8.5 (p = 0.344)	3.6 (p = 0.676)
	(−8.6–37.9)	(17.5–74.5)	(8.0–60.6)	(−10.9–27.6)	(−8.7–29.1)	(−12.6–22.9)
PTT	3.9 (p = 0.650)	6.7 (p = 0.429)	1.9 (p = 0.550)	−4.0 (p = 0.481)	−4.9 (p = 0.394)	−3.3 (p = 0.567)
	(−12.5–23.4)	(−9.7–26.2)	(−11.1–24.2)	(−14.7–8.0)	(−15.5–7.1)	(−14.1 – 8.9)
AUC	81.74 (p = 0.814)	20.47 (p = 0.950)	−114.24 (p = 0.727)	295.75 (p = 0.152)	41.15 (p = 0.840)	−159.87 (p = 0.433)
	(−221.28–468.86)	(−648.83–689.77)	(−783.11–554.63)	(−115.85–707.35)	(−376.34–458.63)	(−575.95–256.21)
CPM						
PDT	1.22 (p = 0.121)	**1.99 (p = 0.011)**	0.69 (p = 0.363)	0.56 (p = 0.441)	0.95 (p = 0.164)	0.45 (p = 0.489)
	(−0.33 – 2.76)	(0.46 – 3.51)	(−0.91–2.18)	(−0.88 – 2.00)	(−0.40 – 2.31)	(−0.85–1.76)
PTT	0.62 (p = 0.243)	−0.12 (p = 0.835)	−0.96 (p = 0.080)	0.06 (p = 0.862)	0.01 (p = 0.977)	−0.04 (p = 0.911)
	(−0.43–1.67)	(−1.23 – 1.00)	(−2.05–0.12)	(−0.65–0.77)	(−0.66–0.68)	(−0.73–0.65)
AUC	−68.54 (p = 0.275)	−59.83 (p = 0.360)	36.71 (p = 0.565)	−69.33 (p = 0.133)	−62.93 (p = 0.164)	2.36 (p = 0.958)
	(−194.26–57.18)	(−190.42–80.76)	(−90.93–164.34)	(−160.44–21.79)	(−152.21–26.35)	(−85.74–90.47)

Numbers represent estimates of the difference (in %), next to the p-value which is displayed in italic. Lower and upper limit (in %) of 95% confidence interval are shown between parentheses. AAC: area above the eVAS pain curve, AUC: area under the eVAS pain curve, CPM: conditioned pain modulation paradigm, eVAS: electronic Visual Analogue Scale, LPS: Lipopolysaccharide, PDT: pain detection threshold, PTT: pain tolerance threshold. Estimates > 0 favor the first mentioned condition (i.e. LPS in LPS *vs* placebo contrast), estimates < 0 favor the second condition (i.e. placebo in LPS *vs* placebo contrast).

therefore might not be sufficient to evidently alter pain thresholds in humans. The marked cortisol response as shown in Fig. 5-a may be causative for this lack in response. Elevated cortisol levels namely can increase pain thresholds when pain itself is not the "stressor" (Timmers et al., 2018; Butler and Finn, 2009; Hannibal and Bishop, 2014) and so

may have diminished the cytokine-driven hyperalgesia.

Despite thus being suitable for studying inflammatory pain-targeting compounds in rodents, the endotoxemia model cannot be used in humans for the same purpose: only subtle, non-significant effects of LPS on pain perception were observed over time. To evaluate if a more
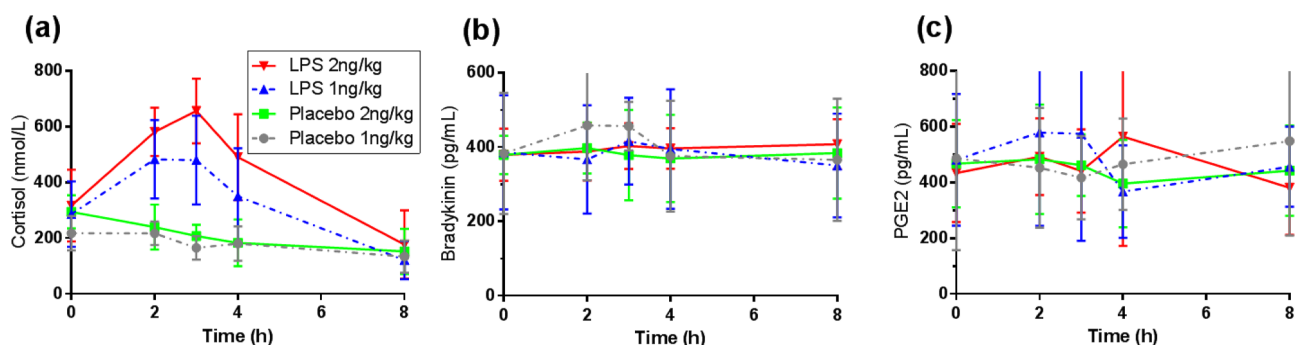

Fig. 5. Stress hormone concentrations after LPS or placebo administration, measured pre-dose (0 h) up until 8 h (hours) post-dose. Vertical lines represent standard deviations. a: cortisol concentrations (nnmol/mL). b: bradykinin concentrations (pg/mL). c: PGE2 concentrations (pg/mL). LPS: Lipopolysaccharide, PGE: prostaglandin E2.

concise time window around the T_{max} of LPS would produce significant hyperalgesic effects, and so confirm positive findings from Wegner et al.'s work with a non-crossover design but similar timeframe (Wegner et al., 2014), we additionally performed a post-hoc analysis (Table 3). Now assessing pain thresholds from pre-dose up until 6 h post-LPS administration, this analysis showed significant effects for LPS *vs* placebo on electrical burst PTT (in 2 ng/kg cohort) and cold pressor PTT (in 1 ng/kg cohort), but still no thermal- or mechanical hyperalgesia was observed. Given that a response on the latter two was expected based on human physiology, as outlined in the beginning of this chapter, we believe that we can conclude that no clear, dose-dependent and reproducible effect of LPS-induced endotoxemia on evoked pain thresholds were observed. The endotoxemia model is therefore not suitable for use in adequately controlled early-phase studies testing analgesics. Continuously infusing LPS as suggested by Kiers et al., (Kiers et al., 2017) as alternative means to create a valid inflammatory hyperalgesia model, is also not a solution. Although the immune response will be extended, and thereby plausibly will induce more pronounced hyperalgesia, Kiers et al. also reported more pronounced and less transient flu-like symptoms, both during and after continuous infusion. In an experimental pain study such adverse effects would make execution of the study unfeasible, as discussed in the previous paragraph. Our conclusion does not relate to the validity of the human endotoxemia model for early-phase drug studies involving different mechanisms of action (i.e. anti-inflammatory), or other scientific settings.

5. Conclusion

Mild acute inflammation, as induced by 1 ng/kg and 2 ng/kg LPS administration, does not significantly change evoked pain thresholds in healthy male subjects. The endotoxemia model in combination with evoked pain tests is therefore not suitable to study drug effects on acute inflammatory hyperalgesia in healthy males.

Funding

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. . Baseline PainCart (i.e. pre-dose) values per modality

	1 ng/kg LPS dose			2 ng/kg LPS dose		
Modality	*LPS*	*Placebo*	No treatment	*LPS*	*Placebo*	No treatment
Heat						
PDT	43.86 °C	44.32 °C	44.38 °C	45.06 °C	45.10 °C	44.84 °C
Cold pressor						
PDT	4.84 s	4.31 s	4.38 s	4.78 s	5.08 s	4.94 s
PTT	19.23	18.87 s	19.40 s	18.56 s	19.34 s	20.00 s
AAC	1303.51 s*%	1205.31 s*%	1199.05 s*%	1206.43 s*%	1210.38 s*%	1381.06 s*%
Electrical stair						
PDT	7.10 mA	6.73 mA	5.27 mA	6.43 mA	6.72 mA	5.58 mA
PTT	22.97 mA	21.51 mA	21.95 mA	17.78 mA	18.78 mA	19.24 mA
AUC	3341.98 mA*%	3395.05 mA*%	3563.98 mA*%	3666.78 mA*%	3612.81 mA*%	3543.85 mA*%
Electrical burst						
PDT	3.02 mA	2.08 mA	1.51 mA	1.79 mA	1.70 mA	1.81 mA
PTT	10.51 mA	10.32 mA	8.53 mA	7.33 mA	7.11 mA	8.44 mA
AUC	4265.32 mA*%	4328.48 mA*%	4470.83 mA*%	4501.16 mA*%	4504.29 mA*%	4436.90 mA*%
Pressure						
PDT	14.51 kPa	14.18 kPa	11.88 kPa	17.29 kPa	16.61 kPa	14.05 kPa
PTT	45.76 kPa	47.48 kPa	43.67 kPa	45.83 kPa	48.61 kPa	44.24 kPa
AUC	6836.51 kPa	6957.73 kPa	7168.09 kPa	6896.49 kPa	6918.77 kPa	6992.39 kPa
CPM						
PDT	−1.083 mA	0.35 mA	0.81 mA	0.66 mA	−0.09 mA	−0.2 mA
PTT	0.86 mA	2.17 mA	2.60 mA	1.24 mA	0.12 mA	2.00 mA
AUC	−26.08 mA*%	−123.88 mA*%	−226.26 mA*%	−44.40 mA*%	−22.53 mA*%	−156.36 mA*%

Data represent mean baseline values for each cohort, 12 subjects per cohort. eVAS: electronic Visual Analogue Scale, AAC: area above the eVAS pain curve, AUC: area under the eVAS pain curve, CPM: conditioned pain modulation paradigm, LPS: Lipopolysaccharide, PDT: pain detection threshold, PTT: pain tolerance threshold. Estimates > 0 favor the first mentioned condition (i.e. LPS in LPS vs placebo contrast), estimates < 0 favor the second condition (i.e. placebo in LPS vs placebo contrast).

Appendix B. Summary graphs PainCart results; pre-dose up until 10 h post-dose
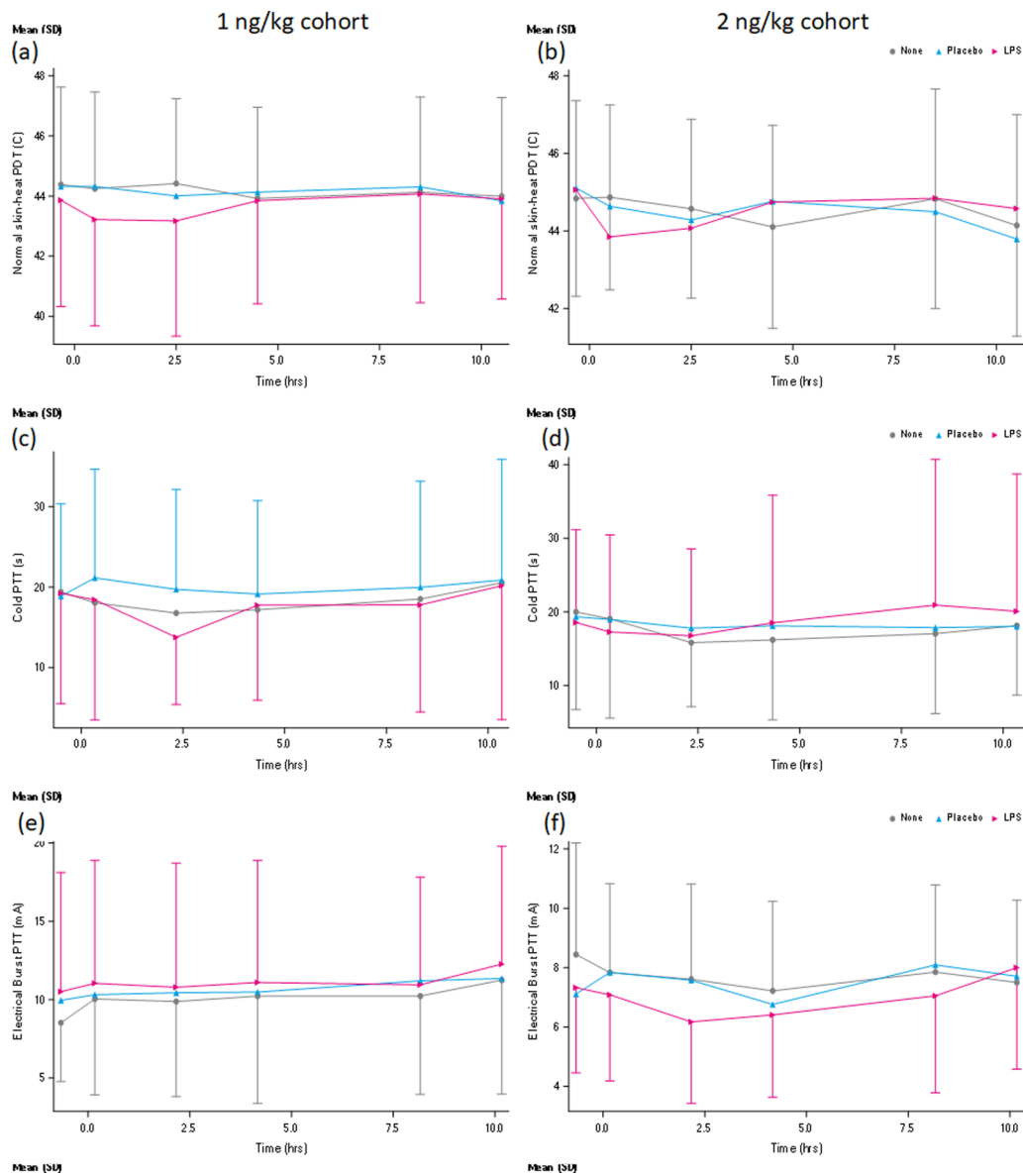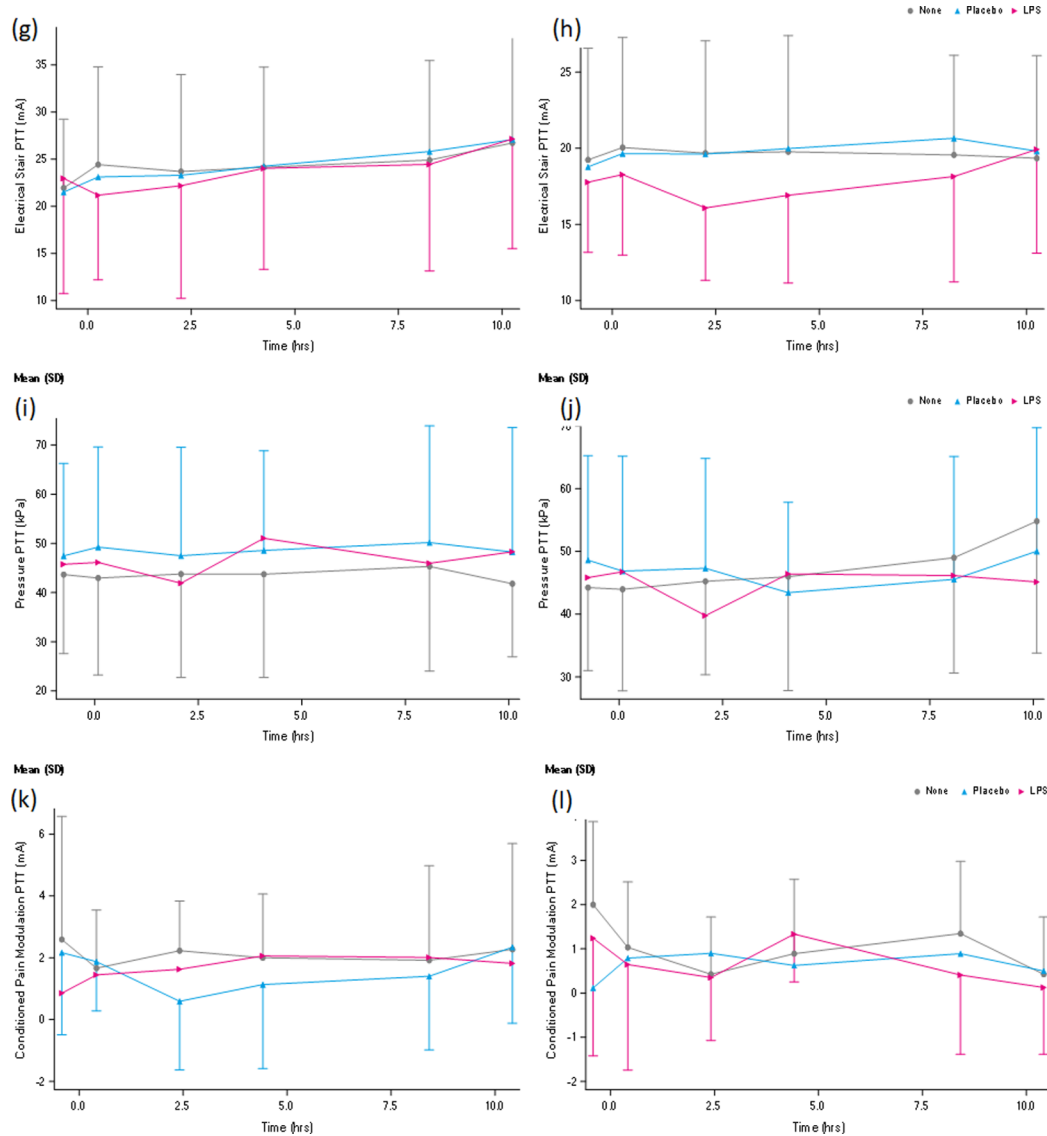
Selection of PainCart test results. Graphs in the left column represent subjects that received 1 ng/kg LPS, right column for subjects that received 2 ng/kg. Values on y-axis represent the averages per treatment, time is described in hours on the x-axis. a and b: heat pain PDT; c and d: cold pressor PTT; e and f: electrical bust PTT; g and h: electrical stair PTT; i and j: pressure pain PTT, k and l: CPM PTT. C: degrees Celsius, CPM: conditioned pain modulation, kPa: kilopascal, mA: milliampère PDT: pain detection threshold, PTT: pain tolerance threshold. s: seconds.

Appendix C. . Statistical tables of PainCart data

Table C.1. Statistical table PainCart data, 1 ng/kg cohort.

		1 ng/kg LPS dose							
	LPS vs placebo			*LPS vs none*			*placebo vs none*		
	DF	t value	*n*	DF	t value	*n*	DF	t value	*n*
Heat									
PDT	16.3	−0.71	11	17.1	−0.36	12	17.3	0.31	12
Cold pressor									
PDT	18.4	0.59	11	19.5	0.34	12	19.8	1.97	12
PTT	15.9	−2.94	11	18.4	0.14	12	18.2	3.18	12
AAC	7.51	−2.42	11	14.7	0.03	12	14.8	2.33	12
Electrical stair									
PDT	16.6	−0.69	11	18.2	−0.33	12	17.9	0.52	12
PTT	15.9	−1.22	11	17.9	−0.74	12	17.7	0.61	12
AUC	14.1	0.37	11	16.7	1.09	12	16.5	0.73	12
Electrical burst									

	DF	t value	n	DF	t value	n	DF	t value	n
PDT	18.8	−2.24	11	20.7	1.6	12	20.4	3.86	12
PTT	15.3	0.58	11	17.8	0.26	12	17.9	−0.46	12
AUC	18.5	−0.1	11	20.6	−0.29	12	20.5	−0.5	12
Pressure									
PDT	18.1	2.52	11	20	4.17	12	20	1.99	12
PTT	16.9	0.69	11	19	0.65	12	19.1	0.24	12
AUC	19.1	0.02	11	21	0.18	12	21	−0.01	12
CPM									
PDT	21.9	0.82	11	23.7	1.9	12	22.2	1.71	12
PTT	14.2	1.32	11	16.4	−0.46	12	15.1	−1.83	12
AUC	17.3	−1.38	11	20.8	−0.57	12	20	0.52	12

AAC: area above the eVAS pain curve, AUC: area under the eVAS pain curve, CPM: conditioned pain modulation paradigm DF: degrees of freedom using the kenward-roger degrees of freedom approximation, n = number of subjects included in the analysis for this contrast, LPS: Lipopolysaccharide, PDT: pain detection threshold, PTT: pain tolerance threshold.

Table C.2. Statistical table PainCart data, 2 ng/kg cohort.

	2 ng/kg LPS dose								
	LPS vs placebo			*LPS vs none*			*placebo vs none*		
	DF	t value	n	DF	t value	n	DF	t value	n
Heat									
PDT	18.6	0.62	11	20.2	−0.79	12	19.6	−1.1	12
Cold pressor									
PDT	19.7	0.6	11	18.1	−0.56	12	18	−1.76	12
PTT	19.4	−0.06	11	20.8	0.82	12	20.6	0.95	12
AAC	19.7	−2.16	11	20.3	0.75	12	19.7	0.42	12
Electrical stair									
PDT	17.3	−0.86	11	21	−0.75	12	20.8	0.16	12
PTT	18.7	−2.01	11	20.7	−1.63	12	20.5	0.6	12
AUC	17.8	1.64	11	20.4	1.11	12	19.9	−0.68	12
Electrical burst									
PDT	19.8	0.05	11	21.4	0.73	12	21.2	0.79	12
PTT	18.6	−1.45	11	21	−0.69	12	21	1.03	12
AUC	17.9	1.01	11	20.1	0	12	20	−1.23	12
Pressure									
PDT	17.4	0.83	11	18.2	0.43	12	17.9	−0.32	12
PTT	18.1	−0.5	11	17.5	−1.45	12	17.7	−1.34	12
AUC	18.5	1.35	11	15.3	0.13	12	15.2	−0.76	12
CPM									
PDT	19.7	0.6	11	18.1	−0.56	12	18	−1.76	12
PTT	19.4	−0.06	11	20.8	0.82	12	20.6	0.95	12
AUC	19.7	−2.16	11	20.3	0.75	12	19.7	0.42	12

3 data points were excluded based on a blind data review (one data point of the cold pressor pain test (one subject at 10 h post-dose) and two data points of the pressure pain test (same subject as for the cold pressor pain test, one data point pre-dose, and one at 0 h). AAC: area above the eVAS pain curve, AUC: area under the eVAS pain curve, CPM: conditioned pain modulation paradigm DF: degrees of freedom using the kenward-roger degrees of freedom approximation, n = number of subjects included in the analysis for this contrast, LPS: Lipopolysaccharide, PDT: pain detection threshold, PTT: pain tolerance threshold.

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