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Activation of the descending pain modulatory system using cuff pressure algometry: Back translation from man to rat

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Abstract

Background: Diffuse noxious inhibitory controls (DNIC) as measured in rat and conditioned pain modulation (CPM), the supposed psychophysical paradigm of DNIC measured in humans, are unique manifestations of an endogenous descending modulatory pathway that is activated by the application of a noxious conditioning stimulus. The predictive value of the human CPM processing is crucial when deliberating the translational worth of the two phenomena.

Methods: For CPM or DNIC measurement, test and conditioning stimuli were delivered using a computer-controlled cuff algometry system or manual inflation of neonate blood pressure cuffs, respectively. In humans ($n = 20$), cuff pain intensity (for pain detection and pain tolerance thresholds) was measured using an electronic visual analogue scale. In isoflurane-anaesthetized naïve rats, nociception was measured by recording deep dorsal horn wide dynamic range (WDR) neuronal firing rates ($n = 7$) using in vivo electrophysiology.

Results: A painful cuff-pressure conditioning stimulus on the leg increased pain detection and pain tolerance thresholds recorded by cuff stimulation on the contralateral leg in humans by $32\% \pm 3\%$ and $24\% \pm 2\%$ (mean $\pm$ *SEM*) of baseline responses, respectively ($p < .001$). This finding was back-translated by revealing that a comparable cuff-pressure conditioning stimulus (40 kPa) on the hind paw inhibited the responses of WDR neurons to noxious contralateral cuff test stimulation to $42\% \pm 9\%$ of the baseline neuronal response ($p = .003$).

Conclusions: These data substantiate that the noxious cuff pressure paradigm activates the descending pain modulatory system in rodent (DNIC) and man (CPM), respectively. Future back and forward translational studies using cuff pressure algometry may reveal novel mechanisms in varied chronic pain states.

Significance: This study provides novel evidence that a comparable noxious cuff pressure paradigm activates a unique form of endogenous inhibitory control in healthy rat and man. This has important implications for the forward translation of bench and experimental pain research findings to the clinical domain. If translatable

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mechanisms underlying dysfunctional endogenous inhibitory descending pathway expression (previously evidenced in painful states in rat and man) were revealed using cuff pressure algometry, the identification of new analgesic targets could be expedited.

1 | INTRODUCTION

Descending brainstem control pathways regulate spinal nociceptive processing and likely impact the initiation, propagation and maintenance of particular chronic pain states. Diffuse noxious inhibitory controls (DNIC) represent a type of descending inhibitory pathway that projects to the dorsal horn of the spinal cord to modulate pain processing via inhibition of wide dynamic range (WDR) neuronal activity, as evidenced in rodents (Bouhassira, Villanueva, Bing, & le Bars, 1992). This phenomenon is also observed in humans (Le Bars, Villanueva, Bouhassira, & Willer, 1992; Roby-Brami, Bussel, Willer, & Le Bars, 1987; Willer, Le Bars, & De Broucker, 1990) and modalities previously used for testing the efficiency of the DNIC system in a translational setting include electrical, thermal, mechanical and chemical stimuli.

Nowadays the human counterpart of DNIC is referred to as conditioned pain modulation (CPM) and CPM paradigms represent psychophysical protocols that are used to assess reduced pain sensitivity due to noxious conditioning (Yarnitsky, 2010). CPM is dysfunctional in chronic pain patients (Lewis, Heales, Rice, Rome, & McNair, 2012; Yarnitsky, Granot, Nahman-Averbuch, Khamaisi, & Granovsky, 2012) and underlying noradrenergic mechanisms explain the beneficial use of pharmacotherapies that target top-down monoaminergic signalling (Ossipov, Morimura, & Porreca, 2014). Specifically, both tapentadol (μ-opioid receptor agonist and noradrenaline reuptake inhibitor (NRI)) and duloxetine (serotonin NRI) reinstate dysfunctional CPM in certain chronic pain patients (Niesters et al., 2014; Yarnitsky et al., 2012). This back-translates to rodents where translational relevance is suggested by studies that highlight a role for α2 adrenoceptor-mediated mechanisms in the normal expression of DNIC, as well as in restoration of DNIC expression in rodent models of chronicity (Bannister, Patel, Goncalves, Townson, & Dickenson, 2015).

Recording DNIC and CPM at the bench and bedside, respectively, offers preclinical and clinical researchers a surrogate measure of top-down processing functionality. CPM modulation by forebrain mechanisms is likely (Nir, Yarnitsky, Honigman, & Granot, 2012), and, even when recorded in anaesthetized rodents, the functional expression of DNIC is influenced by subcortical brain regions associated with emotional processing (Phelps, Navratilova, Dickenson, Porreca, & Bannister, 2019).

Within CPM protocols test stimuli may be applied sequentially or in parallel to a noxious conditioning stimulus (Baron et al., 2017; Yarnitsky et al., 2012). The use of cuff pressure algometry with a cuff-pressure conditioning stimulus is emerging as a tool to assess CPM with moderate to high reliability (Graven-Nielsen, Izumi, Petersen, & Arendt-Nielsen, 2017; Graven-Nielsen, Vaegter, Finocchietti, Handberg, & Arendt-Nielsen, 2015). In this study, our novel approach evaluated the validity of the CPM paradigm using cuff pressure algometry as a tool for testing the efficiency of the DNIC system in humans. Specifically, the characteristics of the conditioning stimulus evoking a CPM response in humans were used in a backward translational study; can identical pressure conditioning inhibit spinal dorsal horn WDR neuronal responses to a pressure cuff test stimulus in naïve rats? It was hypothesized that a comparable nociceptive cuff-conditioning stimulus would decrease the pain sensitivity, and WDR neuronal firing, to cuff test-stimuli in humans and rats, respectively. Is it possible to link DNIC research data collected in rat with CPM research data collected in man when using cuff pressure algometry as a tool to evoke descending pain modulatory systems?

2 | MATERIALS AND METHODS

2.1 | Subjects for the CPM study

Twenty healthy subjects aged between 22 and 54 years (30 ± 1.6 mean $\pm$ standard error of the mean, 12 women, 8 men) participated in the study. Subjects were recruited within the Wolfson CARD, King's College London. Inclusion criteria specified that subjects should be free of pain and pain medication on the day of testing. Exclusion criteria included acute or chronic pain conditions, neurological disorders and musculoskeletal or inflammatory conditions. Participants were requested to not take any non-steroidal anti-inflammatory drugs (NSAIDs) in the 24-hr period prior to testing, and to avoid excessively strenuous exercise of the legs. All participants provided informed consent prior to testing. The study was approved by King's College London Research Ethics Committee (HR-15/16-2058) and performed according to the Helsinki Declaration.

2.2 | Experimental design of the CPM study

Cuff-pressure pain sensitivity and CPM were assessed using a computer-controlled cuff algometry system (Nocitech,

Aalborg University, Denmark), with pressure delivered via two 10-cm wide tourniquet cuffs (VBM Medizintechnik GmbH, Sulz am Neckar, Germany). Cuff pain intensity was measured using an electronic visual analogue scale (VAS). The VAS was anchored with 'no pain' as 0-cm and 'worst pain imaginable' as 10-cm. The subject lay supine at a slight incline on a medical bench with a pillow placed under the head and under the knees, and the cuffs were placed at the widest point of the lower leg around the gastrocnemius muscle. The test stimuli were ramped increases in pressure (1 kPa/s) to a possible maximum of 100 kPa. The VAS was used by the subjects to provide continuous ratings of their pain intensity during cuff inflation from their first pain (PDT) and was operated by sliding a bar along a 10 cm scale anchored at 'no pain' and 'max pain'. When the VAS reached 1 cm, the corresponding cuff pressure was taken as the subject's PDT (Graven-Nielsen et al., 2015). Once the pressure reached the subject's tolerance limit, they pushed the stop button on the VAS device and this cuff pressure was taken as their pain tolerance threshold (PTT) and cuff deflation occurred immediately. Moreover, the VAS score at PTT was extracted and defined as the pressure tolerance level. For subjects who reached the maximum pressure of 100 kPa, this was taken as their PTT (Hoegh, Petersen, & Graven-Nielsen, 2018). Cuff algometry parameters were assessed by the same experimenter (TMC). Each subject was familiarized with the cuff algometry device during a 'training' phase at the start of the session, with the subject seated and the cuff placed around the arm bicep. Subjects were trained on how to use the electronic VAS and how to stop cuff inflation. CPM was assessed using cuff algometry, with ramped test-stimuli (as above to assess PDT and PTT) applied to the dominant leg and a conditioning stimulus of tonic cuff pressure applied to the non-dominant leg. Baseline measures (PDT and PTT) were recorded at the dominant leg followed by a 10-min rest. Next, a test-stimulus ramp was given on the non-dominant leg to determine the PTT value used to calibrate the conditioning pressure and was followed by a 5-min rest. Lastly, CPM was measured using tonic cuff pressure stimulation at 70% PTT applied to the non-dominant leg and maintained until the end of the last test-stimulus. Subjects were asked to focus their attention on the sensation of the dominant leg and rate their PDT and PTT as before. The conditioning cuff stimulation, delivering the tonic cuff pressure, is inflated 3s before the test-stimulus cuff inflation begins. Both cuffs were deflated once subjects reached their PTT on the dominant leg. PDT and PTT values were extracted for each test-stimulus. If the PTT was not reached before the 100 kPa safety limit it was conservatively estimated as 100 kPa for further analysis. The CPM-effect was calculated as the conditioned PDT or PPT value minus the similar measures taken at baseline. The participants were not informed of the experimental hypothesis nor the study that their test data would be used

for. Participants were not told what was expected from them further than the instruction to begin moving the electronic VAS upon pain detection and to push the terminating button upon reaching their pain tolerance limit.

2.3 | DNIC study design: Animals

Male Sprague–Dawley rats (Charles River, UK) were used for electrophysiological experiments. Animals were group housed on a 12 h:12 h light–dark cycle. Food and water were available ad libitum. All procedures described were approved by the Home Office and adhered to the Animals (Scientific Procedures) Act 1986. Every effort was made to reduce animal suffering and the number of animals used in accordance with the IASP ethic guidelines (Zimmermann, 1983).

2.4 | DNIC study design: Electrophysiology

In vivo electrophysiology experiments were conducted on naive rats (weight 250 g ± 10%) as previously described (Urch & Dickenson, 2003). Briefly, animals were anaesthetized and maintained for the duration of the experiment with isoflurane (1.5%) delivered in a gaseous mix of N_2O (66%) and O_2 (33%) via trachea tube. Core body temperature was monitored and maintained at 37°C by a heating blanket with rectal probe. A laminectomy was performed to expose the L4-5 segments of the spinal cord. Extracellular recordings were made from deep dorsal horn neurons (lamina V-VI) using parylene-coated tungsten electrodes (125 μm diameter, 2 MΩ impedance, A-M systems, USA). All neurons recorded were WDR and responded to low- and high-intensity natural stimuli in a graded manner with coding of increasing intensity. In keeping with the 3Rs, where possible, more than one WDR neuron was recorded per animal (for this study 7 WDR neuronal recordings were made from 5 rats). Neuronal firing was captured, amplified (30–40 k times), band-pass filtered and digitalized at 20 kHz sampling rate by a CED 1,401 interface coupled to a Pentium computer with Spike 2 software (Cambridge Electronic Design; rate functions). The experimenter was not blinded to test or conditioning stimulus type; however, the endpoint used in the animal study is an objective measure free from subjective bias (i.e. total evoked spikes to a given stimulus). All electrophysiology procedures commenced approximately 2 hr into the light cycle and lasted around 2–4 hr in total; upon isolating a single wide dynamic range spinal neuron 3 consecutive stable single-unit recording trials were performed (<10% variation for von Frey-evoked neuronal responses) in order to collect baseline responses before application of a conditioning stimulus. Following data collection, the rat was overdosed on isoflurane (5%) and, upon cessation of heartbeat, cervical dislocation was performed.

2.5 | DNIC study design: conditioning of cuff test stimuli

This paradigm was developed to mimic the human cuff algometry design. The setup consisted of two identical neonatal pressure cuffs (E-Medical Medical Supplies Neonate Disposable BP Cuff, size #2, individually connected to a manometer and air pump—air-filled 60 ml syringe—for manual inflation). For the test stimulus, the first 'test cuff' overlaid the WDR neuronal receptive field (hind paw). The pressure in the test cuff was monitored with the electronic pressure amplifier (Neurolog systems), connected via CED1401 to the PC and displayed in Spike2 software along the neuronal recording (Cambridge Electronic Design). The number of action potentials fired by the WDR neuron upon incremental pressure increases of the 'test cuff' (pressure ramp, 1.3 kPa/s, in the range of 0–40 kPa) was recorded. The procedure was repeated in the presence of noxious cuff pressure; the 'conditioning cuff' was applied to the contralateral calf and was inflated 5 s before the incremental increase in pressure of 'test cuff'. Based on the human study a cuff conditioning intensity provoking CPM was extracted and a comparable value used in the animal study. The cuff pressure conditioning intensity was deemed to be sufficiently noxious based on prior demonstration of the recruitment of small diameter afferents using cuff pressure as low as 14 kPa (Kucharczyk et al., 2020). The ipsilateral ('dominant leg') receptive field was chosen as the same side from which the WDR neuron was being recorded.

2.6 | Statistical analysis

Statistical analyses were performed using SPSS (version 24; IBM Corp, 2016). One-way repeated measures analysis of variance (ANOVA) was conducted for human studies (within-subject factor time: baseline, conditioning) for both PDT and PTT to evaluate significant contrasts. For animal studies the statistical difference in neuronal response to cuff pressure algometry was determined using a two-tailed paired Student's t-test. There were no missing animals from analysis and no outliers were excluded from the analysis. Minimum group sizes were determined by a priori calculations using the following assumptions ($\alpha = 0.05$, 1-$\beta = 0.8$, $\varepsilon = 1$, effect size range $d = 0.5$–0.8). Effect sizes of conditioning stimulus on mechanical evoked responses were determined from historical datasets; the typical means and variation in neuronal sample sizes ranging from 5 to 10 were comparable to this study. The animal experimental group contained five animals (and 7 single unit recorded cells) to ensure statistical robustness while adhering to the '3 R's' (refine, reduce, replace—https://www.nc3rs.org.uk/the-3rs). The primary outcome measure was a change in the neuronal response. Results are presented as mean $\pm$ standard error of the mean (SEM) in-text and figures unless described otherwise. Significance accepted at $p < .05$.

TABLE 1 Mean cuff algometry parameters (group level)

	Mean $\pm$ *SEM*
Pressure detection threshold (kPa)	
Baseline	33.5 ± 2.4
Conditioned	44.3 ± 3.2
CPM effect	10.8 ± 2.2
Pressure tolerance threshold (kPa)	
Baseline	61.3 ± 3.0
Conditioned	75.9 ± 3.7
CPM effect	14.6 ± 1.5
Pressure tolerance level (VAS)	
Baseline	6.4 ± 0.4
Conditioned	6.6 ± 0.4

Note: Mean ($\pm SEM$, $n = 20$) cuff pressure pain detection threshold (PDT), pain tolerance threshold (PTT) and visual analogue scores (VAS) of the pain intensity at PTT ('pressure tolerance level') assessed at the dominant leg at baseline and during measurement of conditioned responses. There was a significant group-level increase in PDT and PTT, respectively (*$p < .001$).

3 | RESULTS

3.1 | Cuff test stimuli for baseline and conditioned responses

Three subjects reached 100 kPa (safety limit) when conditioned but none did at baseline. The mean conditioning intensity was 70% PTT (41.6 ± 3 kPa) as assessed at the non-dominant leg. At the group-level, there was an increase in PDT (Table 1; one-way RM-ANOVA; $p < .001$, $F_{(1, 19)} = 23.92$) and PTT (one-way RM-ANOVA; $p < .001$, $F_{(1, 19)} = 99.03$) as assessed at the dominant leg when rated in the presence of a conditioning stimulus (noxious cuff pressure). The difference in PDT and PTT (the 'CPM effect') is visualized in representative raw-trace graphs of eight individual subjects (Figure 1a-h). A shift in time for reporting of PDT and PTT is illustrated when conditioned and quantification of the representative traces is shown in Table 2.

3.2 | Diffuse noxious inhibitory controls are induced using noxious cuff pressure in naïve rats

The number of action potentials fired by WDR neurons upon incremental pressure increase in the 'test cuff' (pressure ramp, 1.3 kPa/s, in the range of 0–40 kPa) was recorded. DNIC were induced by application of continuous noxious cuff pressure (40 kPa, corresponding closely to the human group-level mean conditioning pressure) on the calf ipsilateral to the WDR neuron being recorded. A pronounced reduction in cuff pressure ramp-evoked WDR
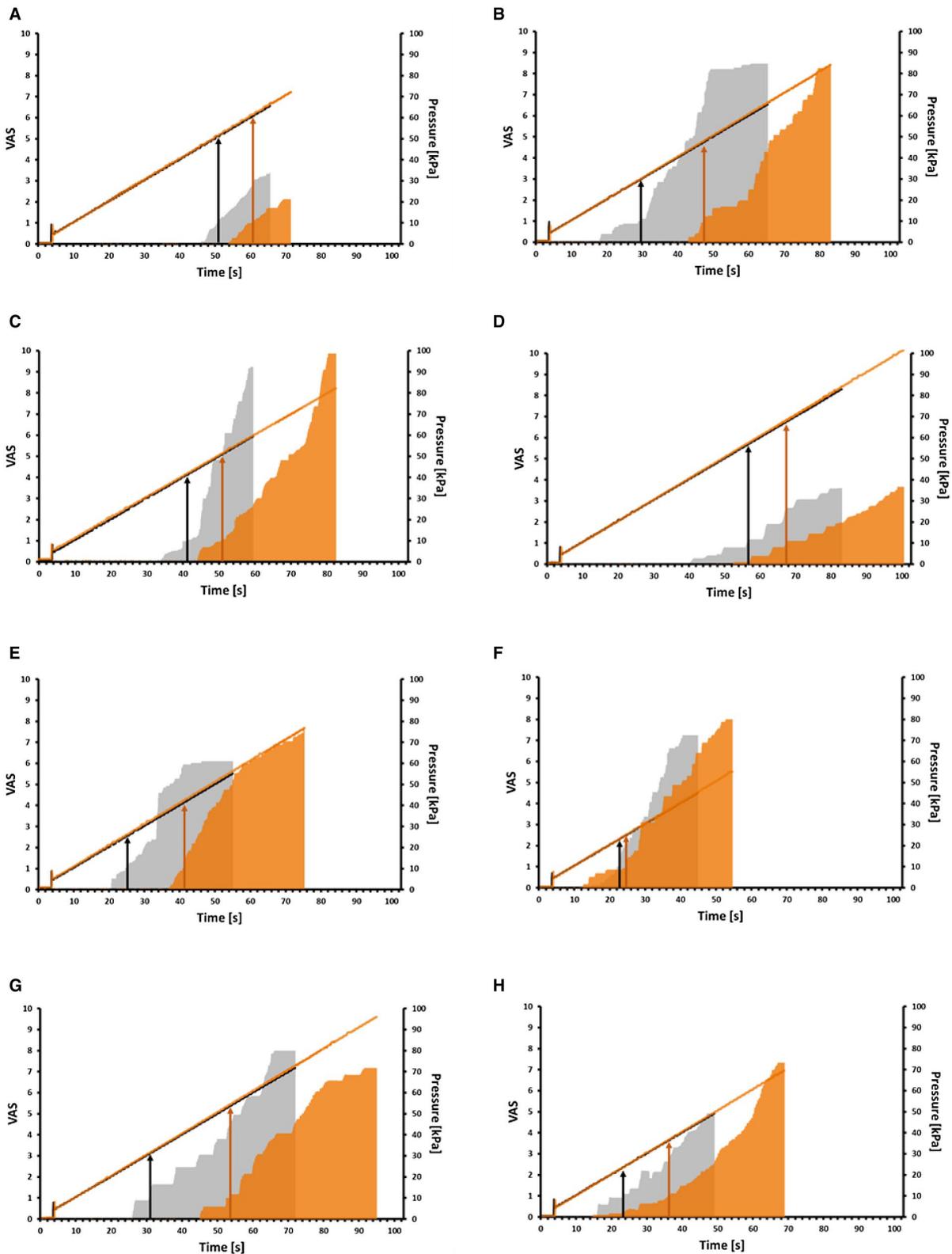
FIGURE 1 Raw pressure-time/VAS traces of eight subjects. A selection of representative raw traces are shown to illustrate the shift in time in subjective reporting of pain detection threshold (PDT), and the increase in intensity for pain tolerance threshold (PTT) during constant cuff conditioning (orange, conditioned response) compared with baseline (grey). The grey and orange arrows indicate the visual analogue scale (VAS) rating at 1 cm at baseline and during the conditioned response, respectively. Moreover conditioned responses (orange) compared with baseline (grey) VAS ratings in 50% of subjects are illustrated to comparable cuff test pressure intensities (see summary Table 2)

TABLE 2 Summary of raw pressure traces (shown in Figure 1)

	Baseline			Conditioned			% Change		
Figure 1	PDT(kPa)	PTT(kPa)	VAS(cm)	PDT(kPa)	PTT(kPa)	VAS(cm)	PDT(kPa)	PTT(kPa)	VAS(cm)
A	51.3	65.6	3.4	60.6	70.9	2.1	18.3	8.1	−38.1
B	29.7	65.3	8.5	47.5	82.8	8.2	59.8	26.8	−3.4
C	41.2	59.4	9.2	51.0	80.2	9.7	23.6	35.1	4.4
D	56.4	83.0	3.6	67.5	100.4	3.6	19.5	21.0	−0.3
E	25.1	55.3	6.1	41.2	75.3	7.6	64.1	36.3	23.6
F	22.5	45.1	7.2	24.7	54.3	7.9	9.7	20.4	8.7
G	30.8	71.7	8.0	53.5	94.4	7.0	73.7	31.7	−11.9
H	23.8	49.2	4.9	36.1	68.6	7.2	51.7	39.5	46.6

Note: In the representative subjects an average increase of 40.1% was seen for PDT, 27.3% for PTT and 3.7% for VAS rating. Only 50% of these arbitrarily selected subjects showed an increase in VAS rating when conditioned despite all showing increases for the other measures.
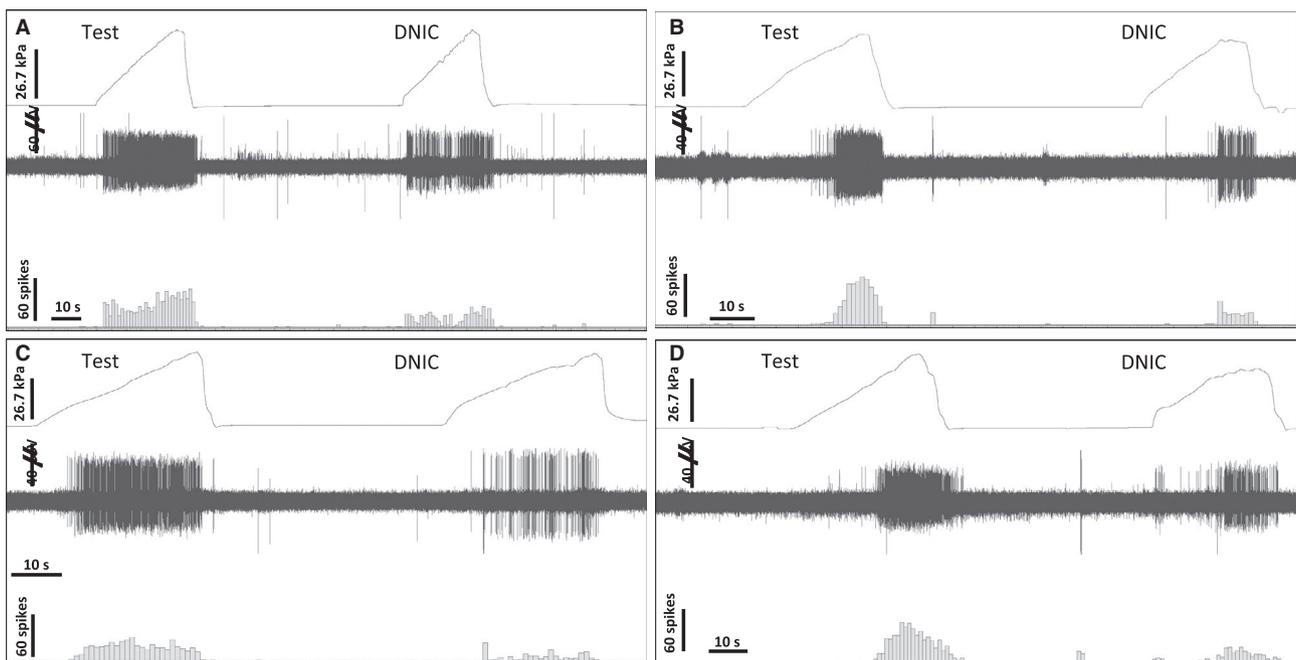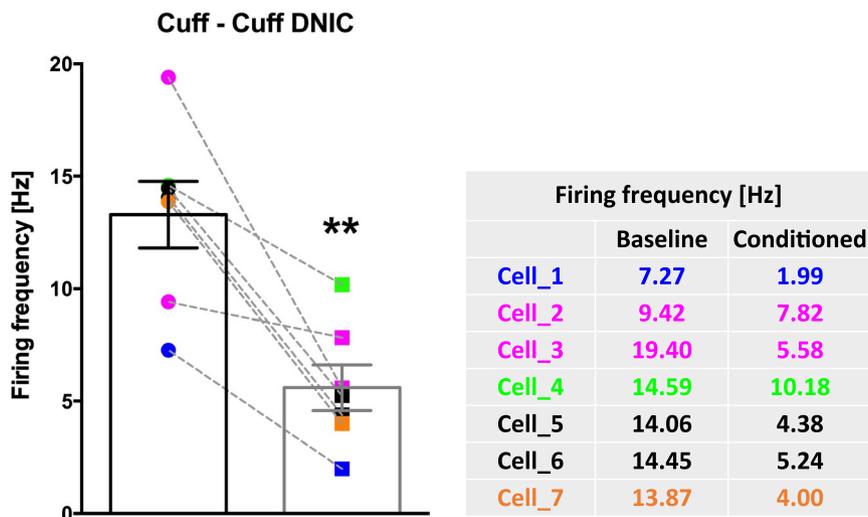

FIGURE 2 Diffuse noxious inhibitory control (DNIC) can be quantified using a cuff-cuff paradigm. In vivo single unit recordings of deep dorsal horn wide dynamic range (WDR) neurons were performed in naïve rats under light isoflurane anaesthesia. Four representative traces of WDR neuronal responses to cuff pressure ramp evoked stimulation of the receptive field (ipsilateral hind paw) for baseline and conditioned responses upon concurrent application of noxious cuff pressure (conditioning stimulus; second cuff stimulation on the contralateral calf) are shown (a-d). Data represent individual WDR neuronal firing from naïve rats ($n = 4$ cells from 3 rats). The time window for frequency measurement equals the stimulation period of individual test. To summarize, the raw WDR neuronal firing rates in the representative traces: an average decrease of 69% was seen for DNIC (where the average test firing frequency for the four cells shown was 15.5 Hz and the average DNIC firing frequency was 4.8 Hz). Relating to the Figure 3 group-level DNIC quantification, trace A is a cell recorded from rat 2, trace B from rat 5, trace c and d from rat 4

neuronal activity (test stimulus) was observed upon DNIC activation with the noxious cuff pressure (conditioning stimulus). Representative raw traces of four WDR neuronal firing rates from four rats highlight the reductions upon application of the conditioning stimulus (Figure 2). At a group level the inhibitory effect was quantified as an inhibition of WDR neuronal response to the noxious cuff pressure (Paired Student's *t* test, $p = .003$; Figure 3).

4 | DISCUSSION

This study provides novel evidence that CPM in humans and DNIC in animals are commonly activated in the presence of a comparable noxious cuff pressure conditioning stimulus, thus inhibiting pain sensitivity to cuff pressure stimulation in a distant, remote body region and reducing the firing rate of extra-segmental wide dynamic range (WDR) dorsal horn neurons when

Cuff - Cuff DNIC

Firing frequency [Hz]		
	Baseline	**Conditioned**
Cell_1	7.27	1.99
Cell_2	9.42	7.82
Cell_3	19.40	5.58
Cell_4	14.59	10.18
Cell_5	14.06	4.38
Cell_6	14.45	5.24
Cell_7	13.87	4.00

FIGURE 3 Group level DNIC quantification. Significantly reduced WDR neuronal firings compared with baseline recordings is illustrated (Paired Student's *t* test: **$p < .01$). Data represent mean ± *SEM* of seven individual WDR neuronal responses from five naïve rats. The five rats are colour coded allowing visualization of cell(s) recorded from: rat 1 (blue, one cell), rat 2 (green, one cell), rat 3 (purple, two cells), rat 4 (black, two cells) and rat 5 (orange, one cell). Individual firing frequencies (baseline and conditioned) are quantified in the adjoining table

conditioned (during cuff pressure stimulation), respectively. It was inferred that WDR neuronal inhibition may be one of the mechanisms involved in CPM assessed by cuff algometry in humans.

4.1 | Backward translation of CPM to DNIC

CPM and DNIC likely represent an endogenous descending modulatory pathway that originates supra-spinally and is modulated by forebrain mechanisms (Nir et al., 2012; Phelps et al., 2019). The ability of the DNIC modulatory pathway to inhibit the responses of spinal WDR neurons to a test stimulus upon presentation of a noxious ear pinch (conditioning stimulus) in anaesthetized rats has been well documented (Bannister, Lockwood, Goncalves, Patel, & Dickenson, 2017; Bannister et al., 2015; Lockwood, Bannister, & Dickenson, 2019), as has a process of endogenous central pain modulation upon application of varied noxious conditioning stimuli in humans (Le Bars et al., 1992; Roby-Brami et al., 1987; Willer et al., 1990). It remains unknown whether or not conditioned pain modulation (CPM) output entirely represents functional expression of the endogenous descending inhibitory DNIC pathway. However, evidence supports CPM as a relevant measure in clinical populations (Kennedy, Kemp, Ridout, Yarnitsky, & Rice, 2016; McPhee & Graven-Nielsen, 2019; Nahman-Averbuch, Nir, Sprecher, & Yarnitsky, 2016).

Sham-operated rats express DNIC in an identical manner to naïve rats, the maintenance of which translates to patient data (Bannister et al., 2015). In addition, the magnitude of inhibitory effect of DNIC in rodent studies is around 35%, similar to the reduction in pain sensitivity documented in human studies of CPM (Yarnitsky et al., 2012). Furthermore,

the translation of DNIC acting on WDR neurons to humans is supported by the fact that spinal neurons code the intensity and spatial features of stimuli under the same anaesthetic conditions in a manner remarkably parallel to human psychophysics (Sikandar, Ronga, Iannetti, & Dickenson, 2013).

Rodent electrophysiology and human psychophysics outputs have previously been compared when characterizing nociceptive processing, and data to show augmented evoked activity of rat spinal neurons marries with the altered perceptual responses of human subjects to peripheral stimulation in the UVB irradiation model. A correlation in the evoked activity of rat spinal neurons to human pain thresholds was reported and parallel results validated the translational use of both models (O'Neill, Sikandar, McMahon, & Dickenson, 2015). Can DNIC and CPM paradigms measuring spinal neuron activity and pain thresholds, respectively, be utilized to investigate the functionality of descending control systems in health and chronicity? The predictive value of the laboratory DNIC model for human pain processing is a crucial consideration when deliberating the translational worth of the two paradigms. In the first part of this translational study the human pain sensitivity was assessed before and concurrent to application of a conditioning stimulus using the cuff pressure algometry (baseline and conditioned responses, respectively). Decreased pain sensitivity to cuff stimulation was demonstrated during contralateral conditioning stimulation by a cuff inflated to approximately 40 kPa on average, in line with previous findings (Graven-Nielsen et al., 2017). In the second part of this study the present findings were extended to reveal that conditioning noxious cuff pressure (distant noxious cuff pressure, 40 kPa) inhibited WDR neuronal responses to noxious stimulation of the hind paw when using cuff

pressure as the test stimulus. Small diameter primary afferents (presumably nociceptive) were previously activated using this level of cuff pressure stimulation (Kucharczyk et al., 2020). Thus, using the cuff pressure algometry with a fixed cuff-pressure conditioning stimulus paradigm, it was possible to compare rodent DNIC and human CPM functionality. Directly comparable measurable outcomes (using equivalent cuff pressures for the conditioning stimuli in rodent and man) were obtained and we validate the use of the CPM paradigm using cuff pressure algometry as a laboratory tool for testing the efficiency of the DNIC system in humans.

4.2 | Major differences when translating DNIC and CPM

Behavioural paradigms of DNIC have been documented in wakeful animals (Okada-Ogawa, Porreca, & Meng, 2009), but the advantage of electrophysiological rodent studies is that entirely objective measurements of the inhibitory output of the DNIC pathway are made. An important caveat of this study is the fact that CPM is measured in awake subjects although based on a user-independent methodology meaning that the assessment is run completely independently of the experimenter because the cuff algometer is fully computer controlled. In humans, cognitive and higher order brain processes, such as those involved in emotion, directly influence the descending pain modulatory pathways and pain reports are thus subjective; the CPM output is considered to be influenced by the emotional past and present of the test subject (Tracey & Mantyh, 2007). Extracting the net activity in human descending controls from the gross output that is human CPM expression remains challenging, and identifying robust methods for separating the 'noise' generated by cortical and limbic networks will be an important step in further validating the CPM paradigm as a measure of endogenous analgesia. This is directly linked to a further caveat, which relates to the fact that the identical intensity of cuff pressure in use as a conditioning stimulus in rat and man cannot be fully equated because of the underlying neural systems recruited by the stimulus. Nonetheless, the functionality and pharmacology of DNIC/CPM are remarkably similar (Bannister et al., 2015; Niesters et al., 2014), supporting their translational value. Corroborating, drugs that act to reassert a balance between monoaminergic descending excitations and inhibitions not only restore the expression of DNIC but could conceivably be beneficial in the prevention of persistent post-operative pain, where impaired CPM is a predictor of those patients at risk, although more studies are need to support this.

Research to reveal mechanisms underlying dysfunctional pain processing in rodent models of chronicity will allow steps towards the development of a reliable test that can demonstrate activity in descending pathways. Recognizing that DNIC and CPM paradigms assessed by cuff algometry can offer translational value would be a major step forward.

5 | CONCLUSION

This study does not claim that DNIC and CPM responses are the same. However, the WDR neuronal responses to cuff pressure conditioning provide first-step indication of the assumption that the cuff algometry is activating the descending pain modulatory system in man through inhibition of spinal neuronal activity. From this, it might be inferred that the CPM-effect in humans is partly a result of the activation of these same mechanisms. The ability to measure the expression or the absence of a functional descending modulatory pathway could promote personalized pain therapy. Back and forward translational studies between DNIC and CPM in various chronic pain states will inform mechanistic insights and reveal new therapeutic targets, ultimately contributing to the optimization of prescribed analgesic potential.

CONFLICTS OF INTEREST
The authors have no conflicts of interest to declare. Nocitech is partly owned by Aalborg University.

AUTHOR CONTRIBUTIONS
All authors discussed the results, commented on the manuscript and gave final approval of the version to be published. TMC acquired, analysed and interpreted all human data; MMK acquired, analysed and interpreted animal data and contributed to the design of the project; TGN assisted with interpretation of human data and revised critically the manuscript for important intellectual content; KB conceived, designed, acquired, analysed and interpreted data, and drafted and critically revised the manuscript for important intellectual content.

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