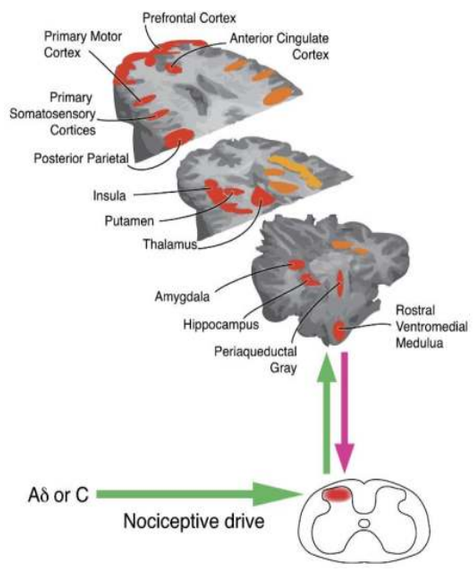
Preclinical and clinical measurements of descending controls: a translational study

Tatum Cummins, Mateusz Kucharczyk, Stephen B. McMahon and Kirsty Bannister
Wolfson Centre for Age-Related Diseases, King's College London, UK

Poster: P146

INTRODUCTION

- The conditioned pain modulation (CPM) paradigm is a measurement of the 'pain inhibits pain' phenomenon
- In CPM, a distally, heterotopically placed noxious conditioning stimulus modulates pain perception of a noxious test stimulus
- CPM is the supposed clinical paradigm of diffuse noxious inhibitory controls (DNIC) as measured in rodents
- How translatable CPM is to DNIC is unclear, but there is some evidence of shared circuitry
- Following a reliability study in healthy humans using computerised-cuff algometry as both test and conditioning stimuli, we back-translated this for an electrophysiological study in naïve rats



Tracey I & Mantyh PW, 2007, *Neuron*

- Fixed cuff pressure conditioning significantly inhibits deep dorsal horn wide dynamic rage (WDR) neurons to von Frey test stimuli comparable to noxious ear pinch
- Here, we show that cuff algometry reliably activates the descending pain modulatory system in both man and rat, thus validating this technique for back and forward translational use

EXPERIMENTAL PROTOCOLS

Experiment 1

- **Computerised cuff-pressure algometry** was used in a test-retest reliability study with twenty healthy male and female subjects aged between 22 and 52 (31.05 ± 7 years) – the test/conditioning-cuffs were placed around the gastrocnemius muscle of each leg
- The test stimulus was a ramped increase in pressure (1 kPa/s) to a possible max. of 100 kPa, with subjects rating first pain (PDT) and pain tolerance threshold (PTT) using an electronic VAS scale - conditioning pressure was individually calibrated to 70% PTT
- CPM effect = absolute difference in PDT and PTT between baseline and conditioning. The subjects' mean conditioning pressure was 42.72 kPa which informed the conditioning pressure in the equivalent DNIC study below

Experiment 2 a&b

- Male Sprague-Dawley rats (250-300 g) were used for electrophysiological experiments - All the neurons recorded from were determined to be WDR
- **Von Frey test stimulus with cuff conditioning**: a cuff conditioning pressure of 40 kPa (as determined in Experiment 1) was applied to the gastrocnemius leg muscle for 5 s prior to von Frey stimuli being applied to the contralateral paw – conditioning pressure was constant during test stimulus application, delivered using an adapted neo-natal cuff
- **Cuff test stimulus with cuff conditioning**: the test stimulus cuff was placed around the contralateral paw (pressure ramp, 1.3 kPa increments every 1 s, in the range of 0-40 kPa) at baseline and during parallel conditioning as before

RESULTS

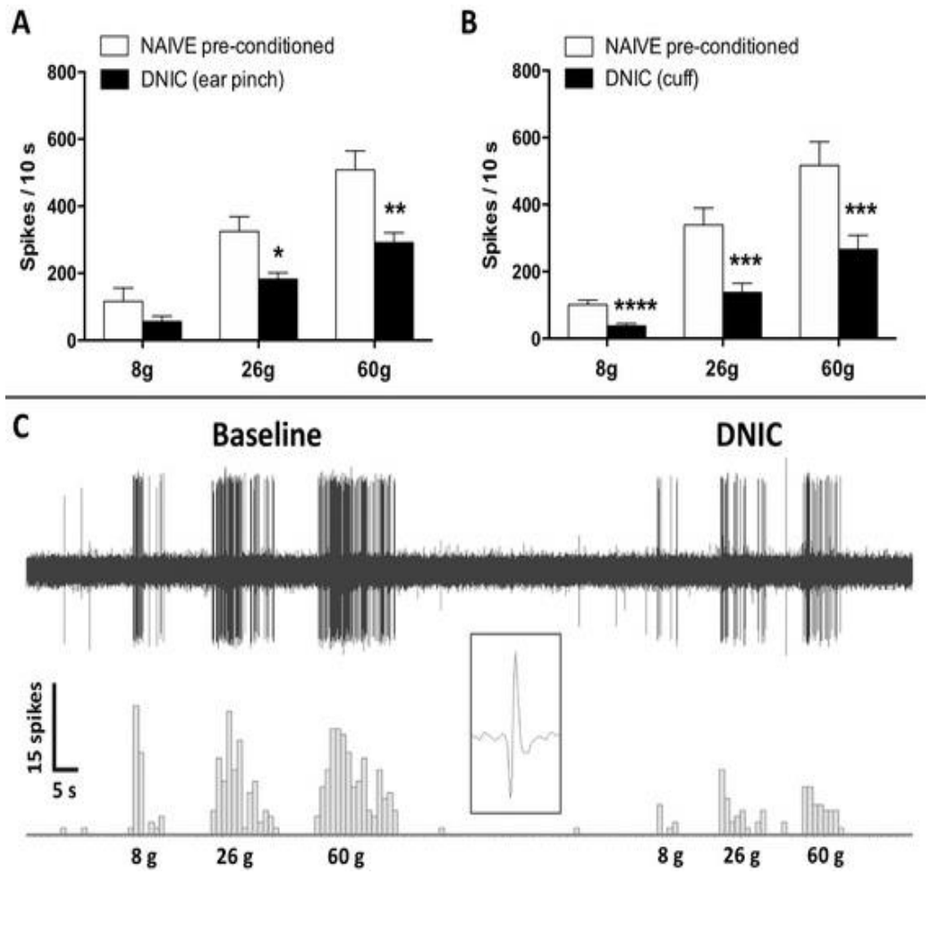


Figure 1. DNIC can be induced by either ear pinch or cuff pressure in naïve rats

In vivo single unit recordings of DDH WDR neurons were performed in naïve rats under light isoflurane anaesthesia. DDH WDR neuronal responses to punctate mechanical stimulation (von Frey; test stimulus) of the receptive field (ipsilateral hind paw) before and after ipsilateral ear pinch (conditioning stimulus) are shown **(A)**. The magnitude of inhibition of mechanically-evoked (von Frey) DDH WDR neuronal responses following parallel cuff pressure conditioning to the contralateral calf in naïve rats is shown **(B)**. Representative action potentials fired after von Frey stimulation to the ipsilateral paw at baseline and after (DNIC) parallel cuff stimulation applied to the contralateral calf are shown **(C)**. All data represent the mean ± SEM from naïve rats (n = 6 cells from 6 rats in **(A)**, and n = 10 cells from 7 rats in **(B)**).

2-way RM-ANOVA with Bonferroni post-hoc: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 vs. corresponding baseline.

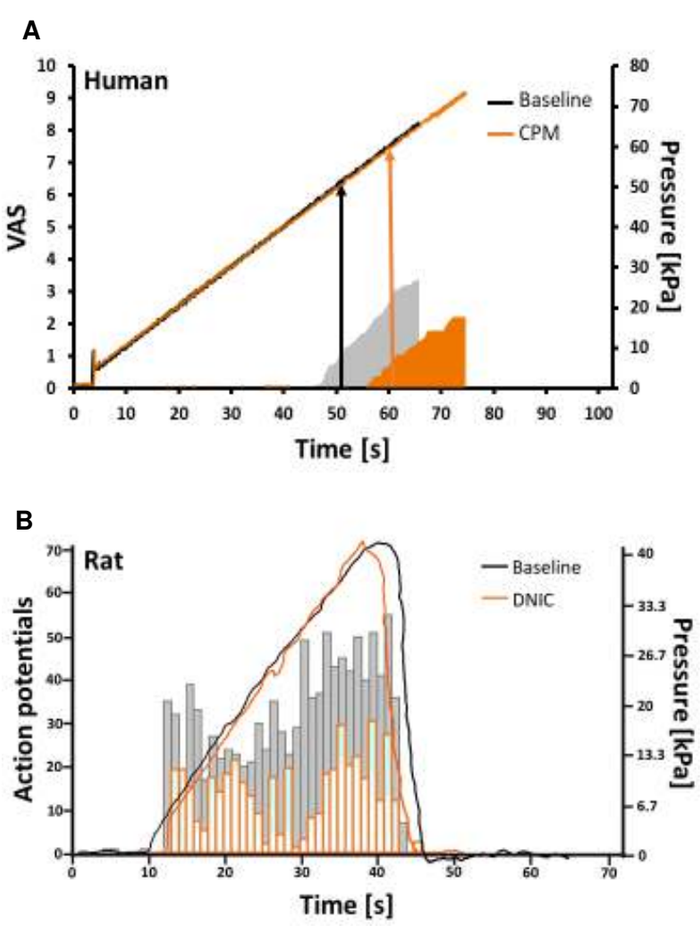


Figure 2. Illustration of 'CPM effect' in human and DNIC activation in rat during cuff-cuff stimulation

(A). Representative graph of one subject showing delayed reporting of PDT, an increase in PTT and reduced VAS rating during cuff conditioning (orange) compared with baseline (black/grey)* **(B)**. Representative raw trace (grey) shows number of action potentials fired (bars) and the pressure ramp applied (black line) at baseline and during constant cuff conditioning (DNIC) (orange)

*Group level (n = 20) mean absolute difference in PDT was 10.54 ± 1.57 kPa (session I: 9.43 ± 8.30 kPa, *P* <0.001; session II: 11.65 ± 8.93 kPa, *P* <0.001). PTT mean absolute difference between baseline and conditioning was 11.77 ± 3.99 kPa (session I: 14.59 ± 6.58 kPa, *P* <0.001; session II: 8.95 ± 5.57 kPa, *P* <0.001)

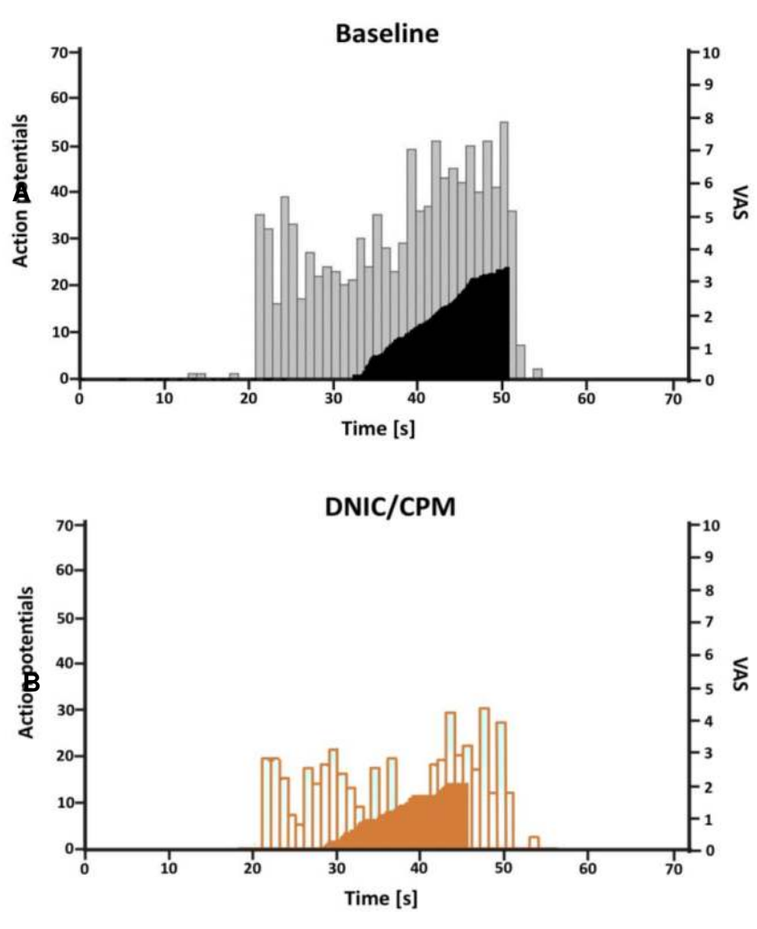


Figure 3. Cuff algometry evokes a comparable measure of the functionality of descending controls in naïve rats and healthy humans

A marked reduction in the ramp-evoked WDR neuronal activity seen during parallel noxious cuff conditioning mirrors results from the human paradigm. **(A)**. Representative human VAS response of one subject at baseline and during conditioning overlaying rat's WDR neuronal responses in the absence **(B)** and presence of cuff conditioning

DDH WDR, deep dorsal horn wide dynamic range; PDT, pain detection threshold; PTT, pain tolerance threshold; VAS, visual analogue scale; ICC, intraclass correlation coefficient

CONCLUSIONS

1. Cuff algometry is a reliable tool for measuring PDT, PTT and VAS in healthy humans
2. DNIC can be induced by noxious ear pinch or cuff pressure in naïve rats
3. DNIC are induced by noxious cuff pressure (conditioning stimulus) in naïve rats upon stimulation with cuff pressure (test stimulus; cuff-cuff paradigm)
4. Human VAS responses can mirror WDR neuronal responses during conditioning

Key references
1. Bannister *et al. Pain*. 2015; 2. Bannister *et al. Eur J Pain.* 2017; 3. Baron *et al. Clin Pract Neurol.* 2006; 4. Graven-Nielsen *et al. Pain.* 2015; 5. Graven-Nielsen *et al. Eur J Pain.* 2017; 6. Ossipov *et al. Curr Opin Support Palliat Care*. 2014; 7. Yarnitsky. *Curr Opin Anaesthesiol*. 2010; 8. Yarnitsky *et al. Pain.* 2012