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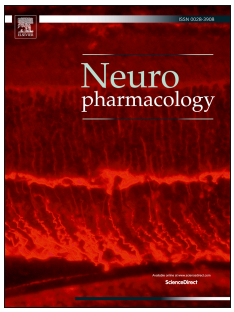

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Pharmacological modulation of colorectal distension evoked potentials in conscious rats

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Abstract

Background: Cerebral evoked potentials (CEP) induced by colorectal distension (CRD) in conscious rats provides a novel method in studies of visceral sensitivity. The aim of this study was to explore the pharmacological effect on CEP of compounds known to reduce the visceromotor response to CRD.

Methods: Epidural electrodes were chronically implanted in eight female Sprague-Dawley rats. Evoked potentials were elicited by colorectal rapid balloon distensions (100 ms, 80 mmHg) and the effect of WIN55 (cannabinoid CB receptor agonist), clonidine (adrenergic α_2 receptor agonist), MPEP (mGluR5 receptor antagonist), pregabalin (ligand of $\alpha_2\delta$ subunits in voltage-gated calcium channels) and baclofen (GABA-B receptor agonist) on amplitudes and latency of CEP were determined.

Results: WIN55 (0.1 µmol kg^{-1}), clonidine (0.05 µmol kg^{-1}), MPEP (10 µmol kg^{-1}) and pregabalin (200 µmol kg^{-1}) caused a significant, $p<0.05$, reduction of the N2 to P2 peak-to-peak amplitude by 23 ±8%, 25 ±8%, 39 ±5%, and 47 ±6% respectively. Baclofen (9 µmol kg^{-1}) induced a prolongation of the N2 peak latency of 18 ±4% but had no significant effect on the amplitudes.

Conclusion: The obtained results suggest that MPEP, WIN55, clonidine, and pregabalin reduce visceral nociceptive input to the brain, whereas the lack of effect of baclofen on CRD evoked CEP amplitudes suggest that the effect on VMR is not due to a direct analgesic effect. Brain responses to colorectal distension provide a useful tool to evaluate pharmacological effects in rats and may serve as a valuable preclinical model for understanding pharmacological mechanisms related to visceral sensitivity.

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List of abbreviations:

CB1:	Cannabinoid receptor 1
CEP:	Cerebral evoked potential
CRD:	Colorectal distension
GABAB:	γ-aminobutyric acid B receptor
IBS:	Irritable bowel syndrome
ICC:	Intra-class correlation coefficients
MPEP:	(2-methyl-6-(phenylethynyl)-pyridine) hydrochloride
VMR:	Visceromotor response
WIN55:	WIN55,212-2 [[(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl-methyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenyl-methanone], mesylate form]

1 Introduction

Colorectal distension (CRD) has been used to study experimental visceral sensitivity in both human and animals (1-6). In human studies, sensory perception is commonly determined by the use of a subjective visual analogue scale (2,7). In contrast, most animal studies use contractions of the abdominal musculature caused by CRD, the so-called visceromotor response (VMR), as a surrogate marker for the evoked sensation (8-10). The VMR is considered a valid indirect marker of visceral sensation in rodents, and the model has been widely used in pharmacological studies assessing potential analgesic effects (5,11). Previous pharmacological studies have shown that the VMR to CRD is reduced by the metabotropic glutamate receptor 5 (mGluR5) antagonist MPEP, the γ-aminobutyric acid B (GABAB) receptor agonist baclofen, the cannabinoid receptor 1 (CB1) agonist WIN55, the α-adrenoceptor agonist clonidine, and the α2-δ ligand pregabalin (8,12-15). However, since the VMR response is abolished in spinalized but not in decerebrated animals, the underlying mechanisms mainly reflect a reflex pathway occurring in the lower brain stem and spinal cord (5,16,17). Therefore, the VMR model does not assess the supraspinal response to visceral stimulation. An alternative method to assess the supraspinal response is to record event-related cortical evoked potentials (CEPs). In humans, CEPs have been recorded in response to mechanical and electrical stimulation of the rectum to objectively study and assess the brain response in comparison to subjective feedback (6,18-20). Rothstein et al. showed a correlation between CEP amplitude and stimulation intensities as well as a correlation to the subjective evaluation (2). We have previously successfully studied CEPs in response to CRD in conscious rats (21). This model showed a correlation between stimulus intensity and amplitude of CEPs and revealed diminished amplitudes in response to CRD when the local

anaesthetic lidocaine was administered rectally, indicating that interfering with peripheral sensory signaling alters supraspinal responses. Furthermore, we recently showed the translational potential of CEPs induced by CRD, by demonstrating that CEPs with similar morphology can be induced in both rats and humans (6).

Conceivably, the anatomy of the CRD-evoked VMR (spinal/lower brain stem) may differ from pathways involved in CRD-evoked CEPs (supraspinal). The aim of the current study was to explore the effect of selected pharmacological agents, that have previously been shown to reduce the VMR to CRD, on the CRD-evoked CEPs in the novel model.

2 Material and Methods

2.1 Animals:

Eight female Sprague-Dawley rats (Harlan Laboratories, Venray, The Netherlands), weighing 250-300 g were used in the study. The rats were allowed to acclimatize in the animal facility for at least one week after arrival and were group-housed in an enriched environment with free access to food (Standard pellets, R3, Lactamin, Kimstad, Sweden) and water on a 12:12 h light-dark cycle. The estrous stage of the rats was not accounted for in the current study.

All experiments were approved by the local animal ethics review committee in Gothenburg, Sweden (17-12-2008, 403-2008), and conducted at AstraZeneca, Mölndal, Sweden. All animal experiments comply with the ARRIVE guidelines and are carried out in accordance EU Directive 2010/63/EU for animal experiments.

2.2 Surgical preparation:

The surgical approach to implant the recording electrodes has previously been described by our group (6, 21). Implantation of skull electrodes and positioning of an abdominal connector was done during anesthesia. From the abdominal connector silicon-coated spiral wires were tunneled subcutaneously across the thorax to the incision on top of the head. Three electrodes for monopolar recordings were placed on the right side of the skull (Figure 1 A). The positions were selected based on experience from a previous study (21).

The rats were housed individually in cages for up to 14 days prior to any experimental procedures. The rats were then group-housed and were included in studies for up to three months.

2.3 Experimental preparations

The experimental preparation has previously described by our group (21). Rats were habituated to Bollmann cages, 30 min per day for 3 consecutive days prior to experiments, in order to reduce motion artifacts due to restraint stress. In case of intravenous administration, a catheter was inserted in the tail vein. A 3 cm polyethylene balloon with a maximal diameter of 10 mm secured to a connecting catheter, was inserted in the rectum, with the distal end of the balloon 3 cm cranial to the anal verge. Animal preparations were performed during light isoflurane anesthesia and rats were allowed to recover from sedation in the Bollmann cages for at least 15 min before the start of experiments.

2.4 Colorectal distension

Colorectal distension was applied by 100 ms long pressure pulses at 80 mmHg with a random inter-stimulus interval of 5±2 seconds. Five periods of stimulation (S1 to S5) were recorded for each experiment (Figure 1 B). Each period lasted four minutes separated by a resting period of 6 minutes, with the exception of the resting period between S3 and S4 which lasted 10 minutes due to administration of either compound or vehicle. The first two periods of stimulation (S1 and S2) served as training sessions to familiarize the rats to the nature of the stimulation in order to obtain a stable response and these recordings were not included in the analysis. The third stimulation period (S3) served as baseline. Forty-eight stimulations were on average recorded for each stimulation period and evoked potentials were obtained as the average cerebral response during each stimulation period.

********** Figure 1 **********

2.5 Compounds

MPEP (2-methyl-6-(phenylethynyl)-pyridine) hydrochloride (AstraZeneca, Mölndal, Sweden) R(+)-baclofen (Sigma-RBI, Natick, MA, USA), clonidine [N-(2,6-dichlorophenyl)-4,5-dihydro-1H-imidazol-2-amine] (Sigma-Aldrich, St. Louis, MO, USA) and pregabalin ((S)-(+)-3-(aminomethyl)-5-methylhexanoic acid) (AstraZeneca, Mölndal, Sweden) were dissolved in 0.9 % saline solution at the appropriate concentrations. WIN55,212-2 [[(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl-methyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenyl-methanone], mesylate form] (WIN55) (Tocris Biosciences, Bristol, UK) was dissolved in 5% ethanol:5% Solutol HS15:90% saline.

MPEP (3, 6 and 10 µmol kg^{-1}), baclofen (9 µmol kg^{-1}) and WIN55 (0.1 µmol kg^{-1}) were administered as intravenous bolus injections (1 mL kg^{-1}) immediately after the third stimulation period. Clonidine (0.05 µmol kg^{-1}) and pregabalin (200 µmol kg^{-1}) were administered as an oral gavage (5 mL kg^{-1}) 15 min prior to the first stimulation. Isotonic saline was used as vehicle control in all cases. Not all rats received all compounds, but each compound was given to six rats and each rat received vehicle as well as multiple compounds on different occasions. There was a wash out period of at least one week between administration of the different compounds. The sequence of drug administration was in the same order for all rats. The different drugs were in the following order MPEP, Baclofen, Clonidine, Pregabalin and WIN55.

2.6 Data collection and analysis

The EEG was recorded by use of an in-house built amplifier (gain 10000, bandwidth 0.3 Hz – 1 kHz) at a sampling frequency of 2000 Hz. The recordings were obtained in a dimmed room, and all unnecessary electrical equipment was turned off to avoid 50 Hz contamination of the signals. The EEG signals were filtered with a 0.3 - 200 Hz band pass filter. The signals were stored for further analysis using in-house developed software for EEG analysis.

2.7 Cerebral evoked potentials

The CEP waveforms consisted of a number of negative (N) and positive (P) peaks numbered in order of occurrence (see figure 2). In order to evaluate pharmacological effects, the most prominent and consistent peaks (N2 and P2) were used for analysis of latency and amplitude. Maximal amplitudes were recorded at the electrode 1.5 mm posterior of bregma and 1.5 mm lateral to the midline, and hence recordings from this electrode were used for further analysis. Each peak was manually identified and the amplitude and latency were determined. The latency (ms) of the cortical responses was measured at the peak of the distinct negative and positive peaks. The cortical amplitude was measured for the N2 and P2 peaks and the N2-P2 peak-to-peak amplitude. EEG signals were analyzed in the interval 0-400 ms after stimulation. CEPs were generated from averaging EEG signals recorded in each session, and off-line analysis of the averaged CEPs was done using customized software.
Drug administration was not blinded during the experiments, but data analysis was performed by another person, who was blinded with regard to the drug administered.

2.8 Evaluation of compound efficacy

The effects of intravenously administrated drugs were determined as the difference in response (latency and amplitude) between baseline (S3) and stimulation period 5 (S5). Compound and vehicle induced changes in brain responses were compared to test pharmacological efficacy. Effects of orally administered drugs were determined by comparing the response during stimulation period 3 (S3), with baseline (S3) recordings from vehicle administration, since no baseline recordings before oral administration were done.

2.9 Statistical analysis

All data are represented as mean ± standard error of mean (SEM). Repeated measurement ANOVA was used to assess the effect of the different drugs using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). P-values below 0.05 were considered as statistically significant. To evaluate the reproducibility intra-class correlation coefficients (ICC) were calculated. ICC-values describe the variation within the individual rat in response to repeated stimulations compared to the variation between rats. Previously acceptable level of ICC has been set to ≥0.6 (22-25).

3 Results

Cerebral evoked potentials to mechanical distension at 80 mmHg were successfully recorded in all eight rats, and no adverse reactions were observed. The CEPs had a pattern consisting of three main peaks labeled P1, N2 and P2 (Figure 2). As the N2-P2 complex was the most reproducible response across all animals, it was used to evaluate compound efficacy in the present study.

********** Figure 2 **********

********** Table I **********

Cerebral evoked potentials

Administration of vehicle caused no significant differences in neither latency nor amplitude (all p-values >0.2, Table I and Figure 2). Both latencies and amplitudes had high ICC values, indicating that each rat produce a similar CEP in response to mechanical distension at 80 mmHg before and after administration of vehicle (all ICC values ≥0.97).

********** Figure 3 **********

MPEP caused a dose related reduction of the N2-P2 amplitude (Figure 3). The doses of 3, 6 and 10 µmol kg^{-1} MPEP resulted in statistically significant reductions of 11%, 19% and 39%, respectively (p=0.01, p=0.02, and p=0.002, respectively, see Table II). The largest effect of MPEP was on the P2 component. The latency was not significantly affected by any of the MPEP doses.

********** Table II **********

Baclofen (9 µmol kg^{-1}) had no significant effect on the amplitude of any component, but caused a significant increase in latency to N2 (Table II, Figure 3). Latency to N2 was increased by 8.8 ± 2ms, corresponding to 18 ±4% (p=0.01). Latency to P2 was not affected.

The cannabinoid receptor agonist WIN55 (0.1 µmol kg^{-1}) caused a significant reduction of the amplitude. The effect was significant at both N2 and N2-P2, reducing the amplitude by

29% and 23%, respectively (p=0.006 and p=0.046, respectively, see Table II and Figure 3). There was no significant effect on the latencies.

Clonidine (0.05 µmol kg^{-1}) caused a statistical significant reduction of amplitude on the P2 component and the N2-P2 amplitude (p=0.048 and p=0.048, respectively, see Table III, Figure 3). The P2 component was reduced by 29%. The effect on the latency was not significant, but a trend towards a reduction in latency was seen for both N2 and P2 (p=0.07 and p=0.07, respectively).

********** Table III **********

Pregabalin (200 µmol kg^{-1}) significantly reduced both amplitude and latency (Table III, Figure 3). The amplitude of N2 and P2 was reduced approximately by the same magnitude, 48% and 42%, respectively (p=0.01 and p=0.037, respectively). The effect on latency was most pronounced on the P2 component, which was reduced by 30%, whereas the N2 latency was reduced by 18%.

4 Discussion

In the present model we used rapid CRD at 80 mmHg to generate robust and reproducible CEPs. These cerebral responses and how they are affected by pharmacological compounds may provide additional information to the overall response of the animal to a noxious visceral stimulus. The typically used VMR model assesses contraction of the abdominal musculature, which is believed to be mediated through a brainstem loop, and hence the response probably does not include the supraspinal modulation of the evoked sensation (5). Therefore,

comparing efficacy of compounds in the VMR model with the current CEP model is of interest. We have previously demonstrated a close correlation between stimulation pressure (between 20-80 mmHg) and the amplitudes of CEPs (21). In addition, locally administered lidocaine attenuated CEPs in a similar fashion as previously shown for VMRs (10,26). In the current study we extend these findings by investigating the effects of other pharmacological compounds known to reduce the VMR to CRD in conscious rats.

The CRD-induced CEP consisted of a number of negative and positive peaks numbered in order of occurrence in accordance with previous results (21). To obtain a robust model for evaluating pharmacological effects only the two most prominent and consistent peaks were used for analysis of latency and amplitude. It cannot be excluded that more detailed analyses of the full response, including all peaks, may reveal further information.

The mGluR5 receptor antagonist MPEP (3, 6 and 10 umol kg^{-1}) caused a dose-dependent reduction of the N2-P2 amplitude evoked by CRD by up to 39% at the highest dose. Interestingly, these results are strikingly similar with results from the VMR and cardiovascular response models, where a similar dose-dependent reduction was seen (12). Furthermore, it has been shown that MPEP (2.5 mg kg^{-1}, i.p.; approximately 10 µmol kg^{-1}) reduced the number of Fos-positive neurons in the spinal cord in response to i.p. injection of acetic acid in rats which is considered a chemical model of visceral pain (27). The results from the current study contributes to the description of how MPEP affects the transmission of noxious sensations from the viscera to the supraspinal structures. However, from the present and previous results, it cannot be determined, if MPEP has an effect in the periphery, at the spinal, at the supraspinal level, or at multiple levels. Taken together, results from the present and previously studies indicate a potential analgesic effect of MPEP against visceral pain.

The dual CB1/CB2-receptor agonist WIN55 (0.1 µmol kg^{-1}) reduced the N2-P2 peak-to-peak amplitude by 23%. In the VMR model, WIN55 reduced the response by up to 40% and also reduced chemically-induced colonic hypersensitivity in rodents (14,28,29). The effects of WIN55 appear to be mediated via cannabinoid CB_1 receptors (14). By contrast, drabinol, a mixed CB1/CB2 receptor agonist failed to modify visceral perception to rectal distension in healthy volunteers and IBS patients (Klooker et al., 2011). Hence, the effects of cannabinoids on visceral pain have not yet been demonstrated to translate from rodents to man.

The α-adreneceptor agonist clonidine (0.05 µmol kg^{-1}) reduced the N2-P2 peak-to-peak amplitude by 25% in the current study. In the VMR model, clonidine (0.20 µmol kg^{-1}) reduced the abdominal contractions by 35% and also reduced heart rate and blood pressure increases in response to CRD (15). These results are in line with the findings from the current study. A potential analgesic effect of clonidine on visceral pain has also been shown after intrathecal administration in rats (30). Interestingly, and important from a translational point of view, clonidine reduced pain responses to CRD in man and symptoms in patients suffering from irritable bowel syndrome (IBS), thereby implying that the current and previous models may have some predictive value for this pharmacological compound (31-34).

Pregabalin (200 µmol kg^{-1} p.o.) caused a reduction of both latency and amplitude of the CEPs, reducing the N2-P2 peak-to-peak amplitude by 47% and the latency by 30%. Pregabalin has demonstrated efficacy in the VMR model, reducing the response by 27% to 72%, together with a reduction in the CRD induced autonomic responses (8,9,35,36). A possible analgesic effect of pregabalin has also been shown in colonic hyperalgesia, where it reduced the number of Fos-positive neurons in response to CRD (9,10). In healthy humans, pregabalin (200 mg p.o.) caused a 25% reduction of gas and pain sensation ratings in response to colonic balloon distension although the sensation threshold was not affected (37). In

hypersensitive IBS patients, pregabalin increased the pain threshold and appeared to normalize rather than desensitize the perception of rectal distension in hypersensitive patients (38). Thus, results from the current study, together with previous results from CRD-evoked VMRs and autonomic responses, indicate that these models predict the analgesic effect of pregabalin in humans.

The present experiments show that the agents MPEP, WIN55, clonidine and pregabalin have pronounced effects on the N2-P2 peak-to-peak amplitude of the CEPs. As discussed above, these effects are consistent with results from experiments using the VMR model or when monitoring cardiovascular autonomic responses. Thus, the current study re-enforces previous findings suggesting that the compounds are effective in reducing responses to visceral nociceptive stimuli. Reassuringly, the current model detects central efficacy of clonidine and pregabalin, two agents displaying effects on CRD-evoked visceral sensations in man and seem to reduce symptoms in IBS patients.

In contrast to the confirmatory results discussed so far, the results with baclofen in the current study failed to support previous findings. Baclofen (9 μmol kg^{-1}) did not reduce the CEP amplitudes, while previous studies using the VMR model and using similar doses of baclofen have shown increased thresholds to colorectal pressure and a dose-dependent reduction of the abdominal contraction suggesting analgesic effects with baclofen (13). Moreover, the possible analgesic effect is supported by experiments showing that intrathecal administrated baclofen reduced VMR and autonomic responses to CRD (39,40). The lack of effect in the present model does not exclude an analgesic effect of baclofen, as the CEP is not a pain specific measurement (see later in the discussion). The discrepancy between previously and the present study could add information regarding the pharmacological effect of baclofen, with respect to the levels where it exerts the analgesic effect.

It could also be speculated that part of the effect seen in the VMR model is a result of the known muscle relaxing effect of baclofen, rather than an analgesic-like effect. However, in previous experiments colorectal compliance and gross motor coordination were not affected by baclofen at the doses used (13,41).

Although we failed to demonstrate a clear effect of baclofen on CEP amplitudes, baclofen did significantly increase the N2 latency by 18%. A change in latency is difficult to interpret, as it can be affected by several factors. Changes in compliance or synaptic transmission can affect latency, but also amplitude changes of specific components can appear as changes in latency of CEPs (42). From the present data, it was not possible to conclude, why baclofen affected latency or why pregabalin actually shortened latency.

From previous experiments, it has been shown, that CRD at a pressure of 20 mmHg, which is not considered noxious, can elicit a CEP (21). Thus, CRD-induced CEPs are not believed to reflect pain *per se*, but rather a measurement of the visceral sensation. Hence, the pharmacological effects on CRD induced CEP may not necessarily reflect analgesia, but rather a change in the sensation evoked by visceral stimulation.

A potential confounding factor could be the possible pharmacologically induced sedation, which could affect the obtained CEP. Due to the experimental setup, where the rats were placed in a Bollmann cage, evaluation of behavior during the experiment was limited. Effects on behavior have, however, not been reported from previous studies where the same drugs in similar doses were administered (43-47). Furthermore, there were no signs of sedation in the obtained EEG. MPEP, clonidine and pregabalin exhibit, however, anxiolytic-like properties in

rodents which could affect the sensation of pain *per se* (43-47). An anxiolytic effect on the visceral sensitivity can thus not be excluded from these compounds.

In the current study, no sensitization in response to repeated stimulation was apparent, which is in accordance with our previous study (21). This differs from studies using VMR and cardiovascular responses as markers of visceral sensation. In those studies, repeated distensions at 80 mmHg resulted in an increasing response (5,11,12). Direct comparison between studies can be difficult, as different distension paradigms have been utilized. In the current study distensions only lasted 100 ms, whereas distensions in the VMR model typically last up to 30 seconds, which might be explain the demonstrated sensitization. A number of studies have reported visceral hypersensitivity to repeated colorectal balloon distension in patients with IBS (49, 50, 51). Thus, it can be argued, that the present model does not reflect the situation seen in patients with IBS due to the CRD paradigm.

A stimulation pressure of 80 mmHg been used in several previous VMR studies and proven to elicit a painful response in rats (5,11). We have previously shown that rapid distention with 80 mmHg gives robust CEP (21), but no signs of painful behavior such as vocalization, jumping, squeaking or twist was observed. It can be discussed how clinical relevant this very short-lasting stimulus is. Compared to electrical stimulation often used to elicit CEP, the rapid mechanical stimulation is more physiological in nature, as it activated receptors in the colonic wall. However, is could be argued, that the very short stimulation used in the present study does not mimic a clinical realistic stimulus, why direct comparison to a clinical situation should be done with caution.

Previous studies have shown that response to visceral stimulation is influenced by gender (50, 51, 52). However, controversies exist regarding this (53, 54). In the current study only female rats were used. Hence, it should be emphasized, that the results obtained in this study applies to female rats and should be generalized with caution, haven the effect of gender in mind.

5 Conclusion

Comparing the effects of anticipated visceral analgesic compounds on mechanically induced visceral pain from the present CEP experiments with results from previous VMR models indicates that the two models gives rise to similar, but not identical, results. Hence, the findings support the rationale of using a panel of different models and endpoints when evaluating and predicting the preclinical effect of new visceral analgesic drugs. These findings contribute to a more complete assessment and hopefully a more successful translation of preclinical results which may be valuable in future drug development and prediction of efficacy in human experiments.

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Table I: Vehicle (0.9% NaCl) had no significant effect on evoked potentials. The intraclass correlation coefficient (ICC) indicated that each rat produced a similar response before and after administration of the vehicle. All values expressed as mean ±SEM, N=8.

	Latency (ms)		Amplitude (μV)		
	N2	P2	N2	P2	N2-P2
Baseline	47 ±1	113 ±4	-183 ±11	95 ±13	279 ±22
Vehicle	47 ±1	113 ±6	-184 ±10	104 ±15	287 ±22
P-value	0.76	0.93	0.93	0.23	0.39
ICC-value	0.97	>0.99	0.97	0.99	0.99

Table II: Effects of drugs administered i.v. on latency and amplitude. Changes are calculated as the difference between the response at baseline and after the administration of drug. P-values are calculated between vehicle and drug experiments. All values are expressed as mean ±SEM (p-value), N=6.

Drug	Dose (μmol kg^{-1})	Latency change (%)		Amplitude change (%)		
		N2	P2	N2	P2	N2-P2
MPEP	3	3 ±2% (0.50)	3 ±1% (0.10)	-13 ±3% (0.001)	-5 ±2% (0.39)	-11 ±1% (0.01)
MPEP	6	-1 ±2% (0.57)	-2 ±1% (0.56)	-11 ±5% (0.11)	-33 ±5% (0.01)	-19 ±4% (0.02)
MPEP	10	-3 ±11% (0.62)	2 ±3% (0.52)	-29 ±4% (0.004)	-61 ±4% (0.002)	-39 ±5% (0.002)
Baclofen	9	18 ±4% (0.008)	3 ±1% (0.34)	-12 ±3% (0.10)	-1 ±12% (0.85)	-9 ±3% (0.07)
WIN55	0.1	-2 ±8% (0.76)	-8 ±10% (0.52)	-29 ±5% (0.006)	-7 ±20% (0.69)	-23 ±8% (0.046)

Table III: Effects of drugs administered p.o. on latency and amplitude. Latency and amplitude changes are the difference in response between stimulation period 3 from vehicle and drug experiments, relative to the vehicle response. All values are expressed as mean ±SEM (p-value), N=6.

Drug	Dose (µmol kg^{-1})	Latency change (%)		Amplitude change (%)		
		N2	P2	N2	P2	N2-P2
Clonidine	0.05	-4 ±2% (0.07)	-13 ±5% (0.07)	-21 ±11% (0.09)	-29 ±11% (0.048)	-25 ±8% (0.048)
Pregabalin	200	-18 ±3% (0.011)	-30 ±5% (0.007)	-48 ±7% (0.010)	-43 ±14% (0.037)	-47 ±6% (0.002)

Legend:

Figure 1: A: Position of skull electrodes. Three electrodes were placed 1.5 mm lateral to the sagittal line with the most anterior electrode 1.5 mm anterior to bregma. The following electrodes were separated by 3 mm. The reference electrode was placed 2 mm posterior to lambda. **B:** Stimulation protocol. Colorectal distension was done by giving 100 ms long pressure pulses at 80 mmHg with an inter-stimulus-interval of 5 ±2 s. Five periods of stimulation were recorded for each experiments. Stimulation period 3 and 4 were separated by 10 minutes due to administration of either drug or vehicle. All other stimulation periods were separated by 6 minutes.

Figure 2: Effect of vehicle (0.9% NaCl) on evoked potentials. The depicted evoked potentials are a representative example form one rat. The different components are labelled, but only the most stable and pronounced peaks (N2 and P2) are used to evaluate the evoked response. When averaging the response from all rats, the CEP appears smoother and small peaks are no longer apparent.

Figure 3: Effect of drugs on mechanical evoked responses. Solid black lines represent the pre-drug response (S3) and gray dashed lines represent the response after administration of drugs (S5). MPEP shows a dose dependant effect, whereas baclofen had on significant effect on amplitude, but increased latency to the first negative peak. WIN55 caused a reduction of the amplitude. Orally administrated clonidine and pregabalin caused a reduction of the amplitude. Evoked potentials represent grand mean average based on recordings from all rats, n=6.

Figure 1:

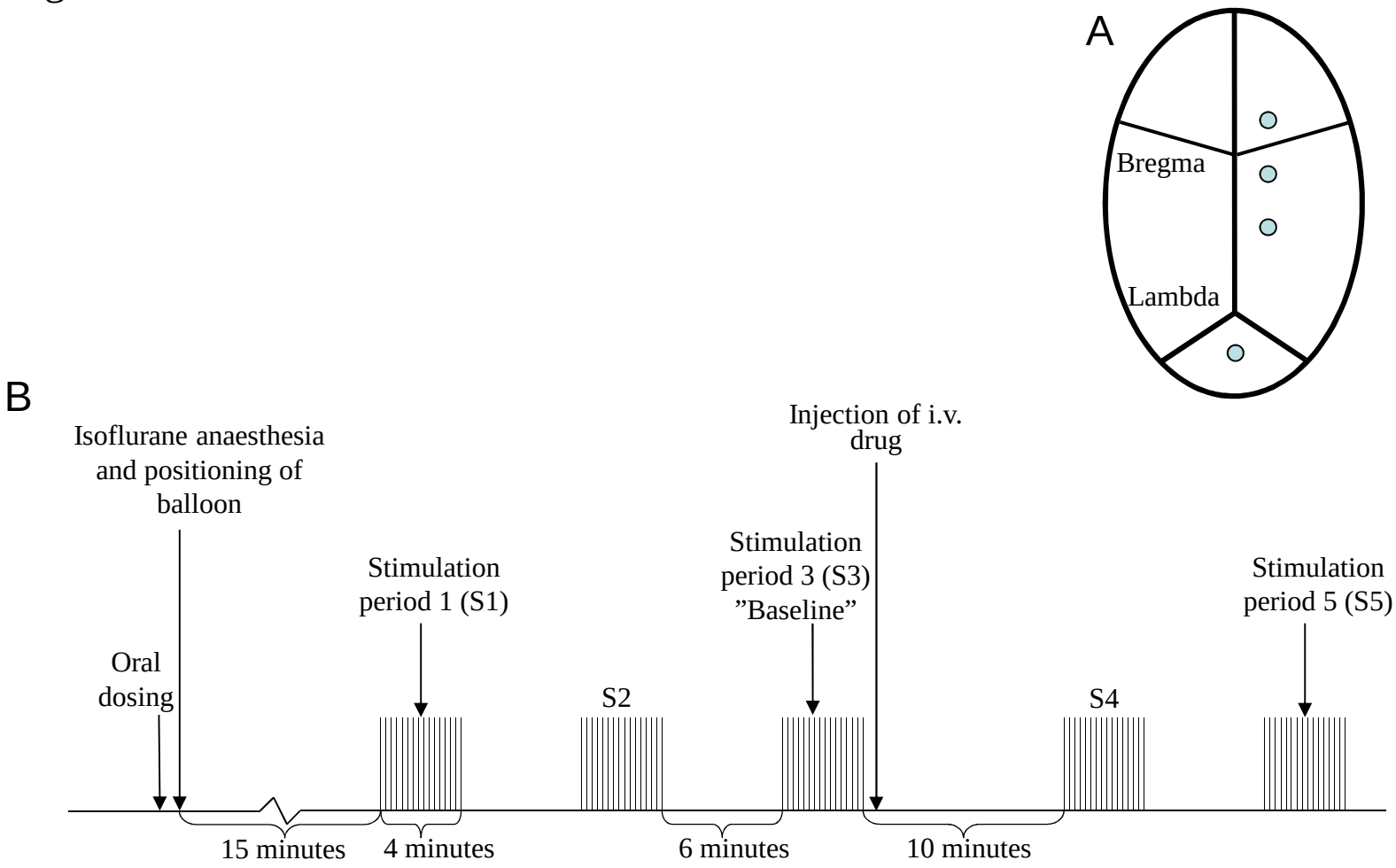


Figure 2:

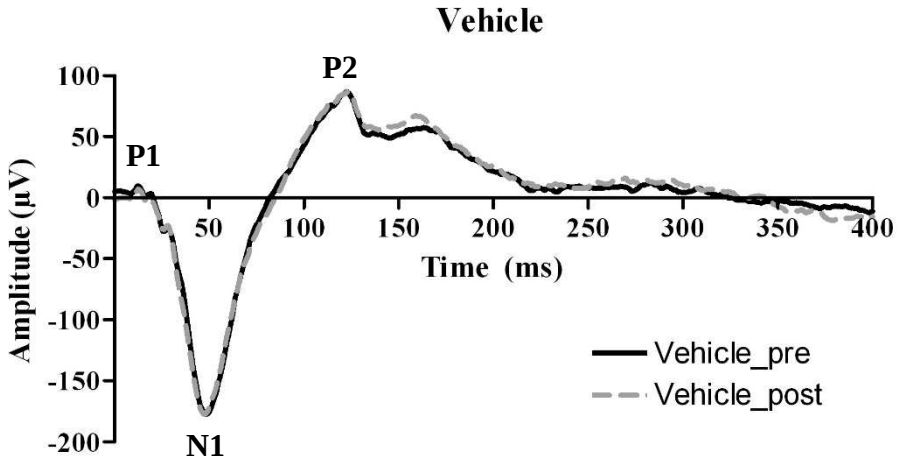
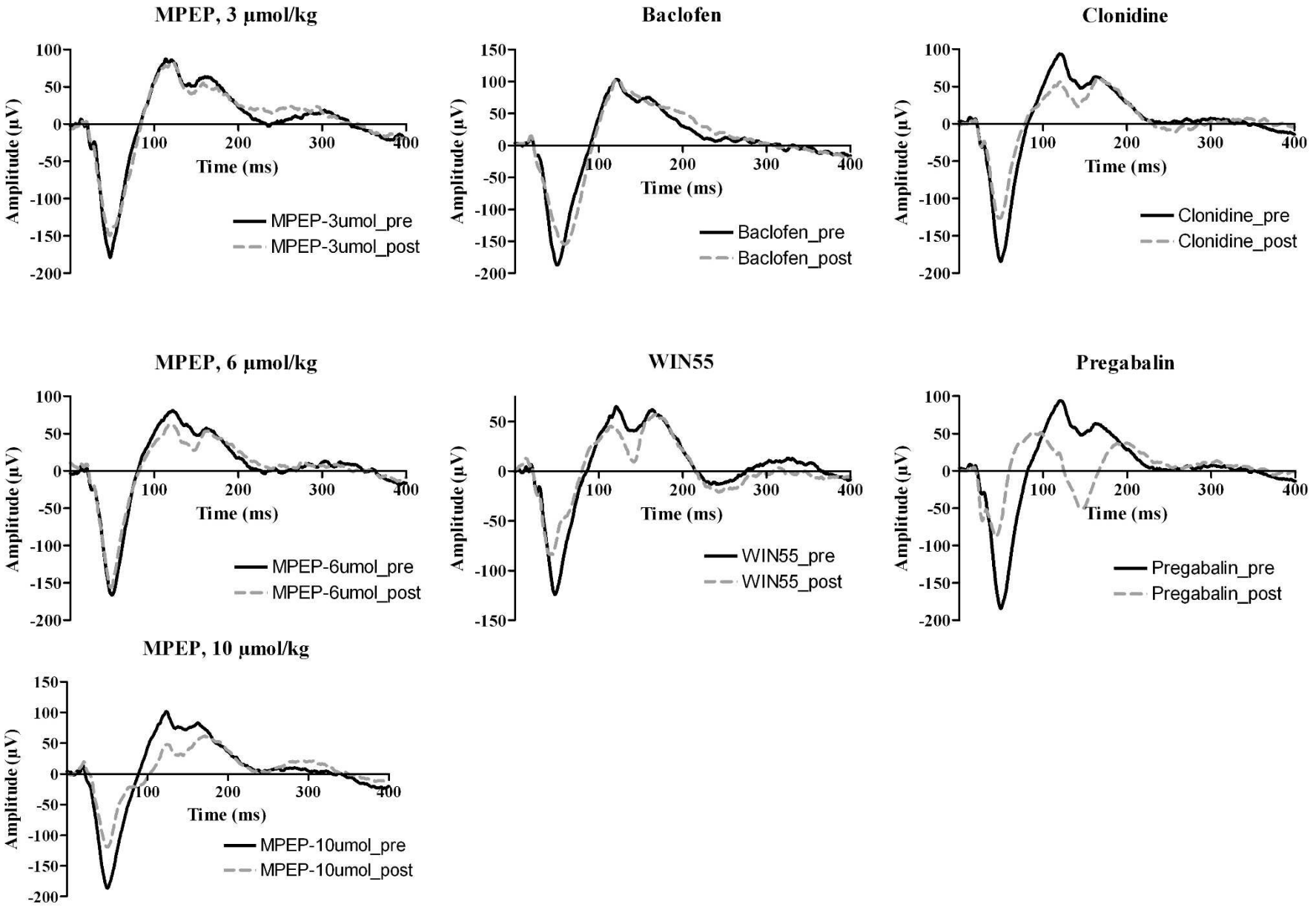

Vehicle
P2
P1
N1
100
50
0
-50
-100
-150
-200
Amplitude (µV)
50
100
150
200
250
300
350
400
Time (ms)
Vehicle_pre
Vehicle_post

Figure 3:


MPEP, 3 µmol/kg
Amplitude (µV)
Time (ms)
MPEP-3umol_pre
MPEP-3umol_post
Baclofen
Baclofen_pre
Baclofen_post
Clonidine
Clonidine_pre
Clonidine_post
MPEP, 6 µmol/kg
MPEP-6umol_pre
MPEP-6umol_post
WIN55
WIN55_pre
WIN55_post
Pregabalin
Pregabalin_pre
Pregabalin_post
MPEP, 10 µmol/kg
MPEP-10umol_pre
MPEP-10umol_post

Pharmacological modulation of colorectal distension evoked potentials in conscious rats

Highlights: (3-5 bullet points, <85 words)

- MPEP, WIN55, clonidine, and pregabalin reduce visceral nociceptive input to the brain.
- The obtained results suggest that the effect of baclofen on CRD-induced VMR is through a different mechanism than used by MPEP, WIN55, clonidine, and pregabalin.
- Brain responses to colorectal distension provide a useful tool to evaluate the pharmacological effect in rats and may serve as a valuable model for evaluating and predicting the preclinical effect of visceral analgesic drugs.