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Analysis of lumbar dorsal spinal potentials evoked by electrical stimulation of the colon and their changes induced by high-frequency stimulation or ischemia in rats

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Abstract: The clinical-related input and processing of intestinal afferents to the spinal cord is not well known. This study aims to develop an electrophysiological experimental animal model to study spinal cord afferents from the colon during clinical-related conditions such as hyperexcitability or ischemia. Spinal cord evoked potentials (SCEP) were elicited by colonic stimulation in ten male adult Sprague-Dawley rats anesthetized with thiobarbital, 60 mg kg^{-1} i.p. After laminectomy (T11 to L5), a tungsten electrode (500 µm; <50Ω) was placed in the spinal dorsum to record SCEP induced by bipolar electrical stimulation of colon mucosa (basal 30 V; 1 ms) at low (0.2 Hz; 10 min) or high (5 Hz; 5 min) frequency. In 3 experiments, after the basal recording, a respiratory arrest was induced by D-tubocurarine to evaluate the ischemia effects. The SCEPs were stable and reliable (n=310), displaying a N1 wave (delay: 3.9±0.1 ms; amplitude 7.78±0.39 µV) and P1 wave (delay 9.96±0.14 ms; amplitude 2.97±0.21 µV). Colonic high-frequency stimulation induced an amplitude increase in both +11% (N1) and +23.7% (P1) (p<0.001). The ischemia induced a linear decay of both wave amplitudes more intense for the P1 wave. sensitive. These results denote the intense colonic input to the lumbar dorsal spinal cord, the presence of spinal sensory potentiation mechanisms induced by colonic high frequency stimulation, and the high oxygen dependency of the neuronal networks involved in the N1 and P1 wave generation. This experimental model could contribute to the study of visceral pain and inflammation, allowing the electrophysiological evaluation of experimental treatment response in experimental colon disease models.

Keywords: Spinal evoked potentials; Visceral afferences; Colon; Visceral stimulation.

1.0 INTRODUCTION

Acute and/or chronic inflammatory bowel diseases worldwide are 45% to 50% of the patients seen in a medical consultation worldwide. The prevalence of inflammatory bowel disease is distributed between ulcerative colitis (80.7%) and Crohn's disease (15.8%), with differences between countries (Ng et al., 2017; Porter et al., 2020). Visceral pain and somatic pain have distinct characteristics and underlying mechanisms which need to be differentiated to gain a deeper understanding of their unique features (Bielefeldt & Gebhart, 2022; Boezaart et al., 2021; Elsenbruch et al., 2017; Feng & Guo, 2020; Finnerup et al., 2021; Lucarini et al., 2020). However, several experimental models have been used to study pain, but less for visceral pain (Barrett, 2015; Johnson et al., 2020; Regmi & Shah, 2020). Most models focus on recording spinal oligoneuronal activity induced by chemical irritation or mechanical distention (Feng & Guo, 2020; Lucarini et al., 2020). In these experimental models, the neurophysiological approach often involves studying cortical and/or spinal evoked potential (EPs) elicited by electrical or magnetic stimulation of somatosensory or motor pathways, primarily in somatic areas, even in human subjects (Chey et al., 1995; Garvin et al., 2010).

Spinal EPs recorded at the cord dorsum appear as a series of electrical waves with a characteristic shape, characterized by upward negative waves (N) followed by one or more longer-duration positive waves (P) (Kimura, 2013; Shimoji, 1995). The Aα and Aβ myelinated fibers depolarize the spinal dorsal horn interneurons; with increased stimulus intensity, Aδ and C fibers are progressively activated. The depolarization spreads ventrally from the superficial spinal dorsal horn, generating a negative charge (N wave) that continues to the supraspinal projecting interneurons. The experimental evidence supports the notion that the spinal dorsal horn neuronal network generates the P wave in response to the distinct modulation systems triggered by the stimulus (Kimura, 2013; Shimoji, 1995). Identifying spinal evoked potentials (EPs) elicited by visceral stimulation helps pinpoint the specific neural pathways and signal processing involved in transmitting pain signals from the viscera to the central nervous system. This contributes to a deeper understanding of how visceral signals are processed in the spinal cord. Such insights may lead to improved diagnostic techniques and therapeutic interventions. This includes exploring the role of various neurotransmitters, neuromodulators, drugs, and neural circuits in regulating visceral pain. Abnormal spinal cord electrical responses in first-order primary afferent neurons and higher-order sensory processing may occur in visceral pathologies such as ischemia and/or inflammation (Bai et al., 2019; Cohen et al., 2021; Greenwood-Van Meerveld et al., 2015).

The cortical EPs reflect the cumulative involvement of peripheral and central afferent pathways, while spinal EPs measure conduction characteristics of the first-order primary afferent neurons, particularly the initial sensory processing and the influence of different pain modulation systems (Kimura, 2013; Shimoji, 1995). EPs at both cortical and spinal levels have been recorded in response to mechanical gut distention using a balloon inserted into the gastrointestinal tract, including the oesophagus, colon, and rectum (Burma et al., 2017; Johnson et al., 2020; Regmi & Shah, 2020). However, this model has limitations, as the timing of the distention overlaps with the recording. As a result, two research groups have used simultaneous recordings of cortical and spinal EPs in response to visceral electrical stimulation in humans (Chey et al., 1995; Garvin et al., 2010), suggesting potential clinical applications for this technique. However, further insights into the neurophysiological, pharmacological, visceral pathological processes and visceral pain are needed, with more controlled experimental approaches that are difficult to achieve in human research.

This study describes a reliable experimental model in rats to record spinal EPs in response to electrical intraluminal stimulation in the colon. This model allows for investigating visceral pain and inflammation in a controlled laboratory environment, which is not always possible in clinical trials. We aim to evaluate these spinal EPs and their changes induced by high-frequency electrical stimulation, mimicking increased visceral afferents in colitis or, during visceral ischemia, or both common clinical scenarios.

2.0 MATERIALS AND METHODS

2.1 Experimental design and animal handling

An experimental, cross-sectional, and descriptive study was carried out in adult (weight 300-350 g) male Sprague Dawley rats fed *ad libitum* and with a lighting scheme light: dark 12: 12 hours. Animals were handled according to the ethical guidelines for handling laboratory animals in accordance with guidelines or information provided by the American Association for Laboratory Animal Science, the International Association for the Study of Pain (IASP) and the National Law for the Protection of Wild and Captive Fauna, with the endorsement of the Institutional Bioethics

Committee (Approval CDBBUC 012-2018, code BASÑJKG).

2.2 Animal surgery and monitoring

Rats (*n*=10) were anesthetized with sodium thiopental (60 mg kg^{-1} i.p.) until reaching a deep level of anaesthesia evidenced by the absence of corneal and tail withdrawal reflexes; 0.25 mg of atropine diluted in 0.5 ml of physiological solution was administered subcutaneously to reduce respiratory secretions. Throughout the experiment, the body temperature was kept constant (37.5±0.5°C; mean ± SD) by a thermal DC blanket placed on the ventral surface of the animal and feedback regulated with continuous monitoring of the animal's temperature using a paravertebral thermistor (Chagín-Nazar & Eblen-Zajjur, 2015; Eblen-Zajjur & Sandkühler, 1996).

2.3 Lumbar laminectomy

A dorsal laminectomy was performed from T11 to L5, exposing the lumbar enlargement of the spinal cord, which was covered with mineral oil to prevent it. The animal was fixed to a spinal stereotaxic frame, so the exposed spine remained firm, straight and without movement. Using the dorsal skin folds, a container was formed that was filled with mineral oil (**Figure 1A**) (Chagín-Nazar & Eblen-Zajjur, 2015; Eblen-Zajjur & Sandkühler, 1996).

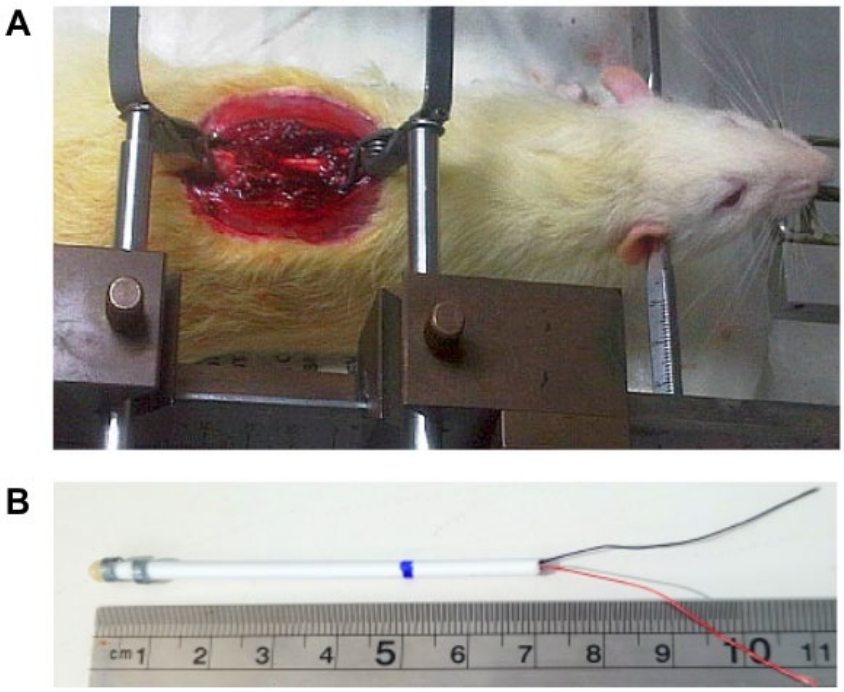

Figure 1. (A) The adult rat is fixed to a spinal stereotaxic frame. A dorsal laminectomy from T11 to L5 was performed, exposing the lumbar enlargement of the spinal cord. A container was formed around the spinal cord that was filled with mineral oil to avoid drying by using the dorsal skin folds and the spinal fixing clamps. **(B)** Diagram of the colon stimulation electrode with its measurements. Bipolar contacts are near the non-traumatic rounded tip (pearl pin head) of the array, and its wires are running through the tube. The cathode (red) is the near-tip contact. The blue mark denotes fixed penetration distance; a remanent external tube is used to fix it to the rat tail with an adhesive strip.

2.4 Recording system

The EPs were recorded with a tungsten monopolar electrode (100 µm Ø; impedance < 5 kΩ) placed in the left spinal cord dorsum using a triaxial micromanipulator and penetrating 100 µm deep from the cord dorsum. The final position of the recording electrode was chosen by the maximum amplitude of the N wave of the EPs, in response to the application of a single pulse stimulus. The needle-ground electrode was inserted into the paravertebral musculature 1 cm distal to the recording electrode (Meléndez-Gallardo & Eblen-Zajjur, 2016, 2018).

2.5 Electrical stimulation of the colon

The colon was intraluminal electrically stimulated using a bipolar electrode of Ag/AgCl wires of 500 µm in diameter each, placed in an exposed wire ring, 4 mm apart each (distal cathode) at the end of a semi-rigid

polyethylene tube 3 mm Ø and 7 cm long, inside this tube runs the connection cables toward the stimulator (**Figure 1B**). This configuration is simpler than that used in humans with a spiral hook shape (Chey et al., 1995; Garvin et al., 2010). The electrode was implanted in the colon lumen 5 cm deep in the same way that the temperature probe of the electric blanket is routinely implanted, which, for these experiments, was relocated in the paravertebral musculature. The external part of the electrode was fixed to the rat tail with clinical adhesive tape.

2.6 Stimulation protocol

An electrical square pulse of 1 ms duration and 30 V amplitude was applied (A-M Systems Inc. Stimulator, Model 1200) every 20 seconds (0.05 Hz) for 10 minutes (total of 30 pulses). The frequency was increased 100 times, that is, to 5 Hz for 5 minutes (total 1500 pulses). The aim of these two stimulation patterns was not the evaluation of the stimulation/response relationship but the application of the low- (0.05 Hz, Basal) or high (x100) frequency stimulation to generate neuronal potentiation (Chagín-Nazar & Eblen-Zajjur, 2015; Cohen et al., 2021; Valenzuela et al., 2021).

2.7 Ischemia protocol

In 3 animals, the spinal EPs were recorded with stimulation at 0.05 Hz for 10 minutes as a baseline immediately after a respiratory arrest was induced by D-tubocurarine (0.2 mg kg^{-1} i.p.); (Wix-Ramos & Eblen-Zajjur, 2011a, 2011b), and the spinal EPs were recorded at the same stimulation frequency (0.05 Hz) up to 30 minutes of ischemia.

2.8 Spinal EPs recording and processing

The spinal EPs were differentially amplified (10,000x; A-M System. Inc. Model 3000 Amplifier), bandpass filtered (10-500 kHz) and digitized at 1.8 kHz sampling rate (DATAQ, 148U), visualized using a conventional personal computer and the Dataq Browser XLTM recording program. The signals were stored on the hard drive and offline analyzed using the open access software DataView v.11.15.4 (Heitler, 2007) for measuring the amplitudes (in µV) and latencies and durations (in ms) of the N and P waves of the spinal EPs. Animals were sacrificed at the end of the experiment by administration of intracardiac thiopental overdose.

2.9 Statistical analysis

Mann-Whitney bivariate nonparametric tests were applied to compare values before and during high-frequency stimulation or during global ischemia. Curve fitting between the ischemic time versus amplitude of the N and P waves was calculated, and their slopes were compared using the Kolmogorov-Smirnov test and their regression coefficients. The results are described by their values of central tendency (median) and dispersion (25-75 percentiles and coefficient of variation). For all cases, $p<0.05$ was assigned as the level of statistical significance. Data processing was performed using the PAST v3.17 statistical package (Hammer et al., 2018).

3.0 RESULTS

3.1 Spinal EPs triggered by electrical stimulation of the colon

A total of 10 animals and 450 basal EPs (recorded at 0.05 Hz) were evaluated. **Table 1** presents the descriptive statistical values of the latency and duration of the N1 and P1 waves for basal condition recording. The reliability of N1 and P1 waves during basal recordings evaluated by the statistical dispersion over the recording time shows a maximum of 2.6% and 7.1% of the dispersion coefficient for delay and amplitude, respectively (**Table 1**). It should be noted that the delay of the P1 wave is 2.55 times that of the N1 wave and slightly less than half of its amplitude ($p<0.05$). The similarity of the values of the arithmetic mean and the median, as well as those corresponding to the asymmetry and kurtosis, suggests a Gaussian distribution of both delay and amplitude values. The peak-to-peak amplitude (Δ N1/P1) was 4.81 µV (**Table 1**).

Table 1. Descriptive statistics of delays and amplitude values of the N and P waves of the lumbar spinal cord potentials evoked by basal colonic visceral electrical stimulation (1 ms; 30 V; 0.05 Hz), $n=10$ animals, values obtained from $n=310$ EPs.

	N1 Wave		P1 Wave	
	Delay (ms)	Amp (µV)	Delay (ms)	Amp (µV)
Mean	3.9	7.78	9.96	2.97
Std. error	0.02	0.07	0.03	0.04
Stand. Dev.	0.1	0.39	0.14	0.21
Median	3.91	7.78	9.94	2.98
P25	3.83	7.55	9.87	2.85
P75	3.98	8.06	10.08	3.07
Skewness	-0.28	-0.29	-0.38	-0.21
Kurtosis	-0.51	1.13	0.34	1.42
Coeff. Var.	2.56	5.03	1.39	7.1

The high-frequency (5 Hz) colon electrical stimulation increased the N1 and P1 amplitude by 11% (U=23.4; $p<0.01$) and 23.7% (U=28.5; $p<0.01$), respectively (**Figure 2A**). However, no statistically significant changes were observed in the latencies of both waves. We observed a damping effect during low stimulation

frequency for the amplitude of N1 to N2 (-87.7%) and P1 to P2 (-71.6%) waves but also during high stimulation frequency N1 to N2 (-73.8%) and P1 to P2 (-68.1%) (**Figure 2A**).

3.2 Effect of global ischemia

Global ischemia due to respiratory arrest induced by D-tubocurarine linearly decreased the amplitude of N1 and P1 waves evoked by the electrical stimulation of the colon (**Figure 2B**). The amplitude decay of both waves N1 and P1 was highly linearly correlated (r^2 = 0.945 and 0.845 respectively; p<0.00001). The decay regression equation for the N1 wave was -0.4110 (minutes of ischemia) + 12.67, and for the P1 was -0.2568 (minutes of ischemia) + 6.005. However, the wave P1 reached zero amplitude 7 minutes before the N1 wave (**Figure 2B**).

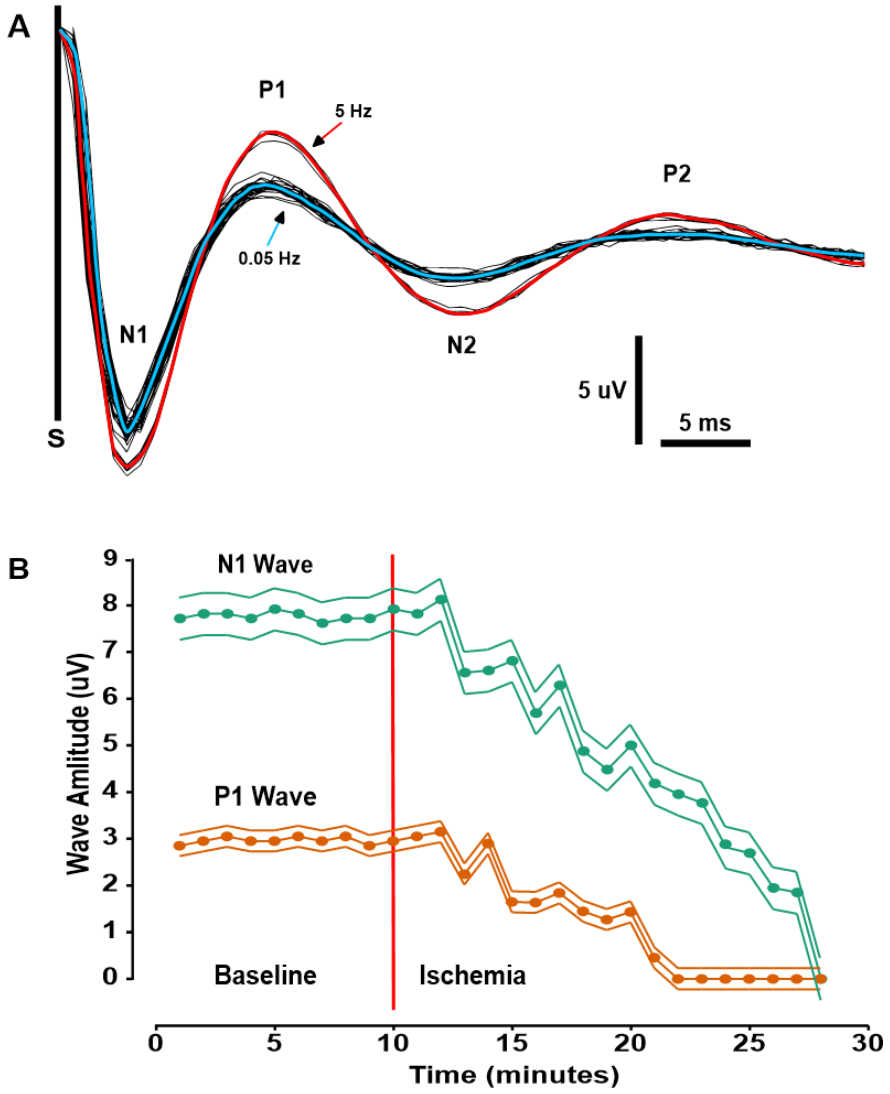

Figure 2. (A) Overplot of the N1, P1, N2 and P2 waves of the lumbar evoked potentials by stimulation of the colon mucosa (1 ms and 30 V) at low (0.05 Hz; lower arrow) or high frequency (5 Hz; upper arrow), S: stimulus artifact. Mean trace is overplotted for 0.05 Hz stimulation (light blue) and for 5 Hz (red). **(B)** Effect of global ischemia induced by D-tubocurarine-respiratory arrest (vertical red line at 10 minutes of the experiment) on the N1 (green, upper trace) and P1 (red, lower trace) waves. Amplitude points are 1-minute arithmetic mean ± standard deviation.

4.0 DISCUSSION

In the present study, we developed a detailed animal experimental model for the neurophysiological investigation of spinal evoked potentials elicited by electrical stimulation of the rat colon. This model includes using an intraluminal colon electrode, specific stimulation parameters, and a recording setup for cord dorsum potential. We report reference values for the

delay, amplitude, and patterns for N1, P1, N2, and P2 components of the cord dorsum potentials, as well as the changes in spinal EPs induced by two frequent clinical situations such as colitis where an increased activity of visceral afferents emulated experimentally by high-frequency colon stimulation and, visceral ischemia.

Previous studies have reported the simultaneous recording of stable spinal and cortical evoked potentials (EPs) in response to electrical stimulation in the human rectum (Chey et al., 1995; Garvin et al., 2010). These studies utilized a stimulation pattern with pulses of 0.2 ms duration and intensities up to 100 mA, allowing the recording of spinal EP in >80% of the human subjects. The spinal EP were dependent on stimulus intensity and could be blocked by the application of lidocaine jelly at the stimulation site. In contrast, our animal model significantly improves the signal-to-noise ratio, which involves direct implantation of the recording electrode into the lumbar spinal dorsal horn through a laminectomy. As a result, each electrical stimulating pulse applied to the colon reliably induced a spinal EP.

Our baseline stimulation frequency (0.05 Hz) falls within that reported (Chey et al., 1995; Garvin et al., 2010) and low enough to avoid spinal potentiation and/or sensibilization processes (Chagín-Nazar & Eblen-Zajjur, 2015; Cohen et al., 2021). This was confirmed by increasing the colon stimulation frequency to 5 Hz, which increased the amplitude of the N1, N2 and P1, P2 waves. This effect was previously associated only with increased stimulation intensity but was not tested for higher stimulation frequency (Chey et al., 1995; Garvin et al., 2010). The delay from the stimulus artifact to the peak/valley values of the spinal N and P waves during baseline recordings was significantly shorter (by -18.75% to -19.3%) compared to human data (Chey et al., 1995; Garvin et al., 2010). This shorter latency is consistent with the shorter conduction distance from the stimulation site to the spinal cord and the slower conduction velocity of visceral afferents (Brierley et al., 2018; Brookes et al., 2013).

Recording cortical evoked potentials (EPs) during acid infusion into the distal esophagus in humans reduces the stimulus-response delay (Sarkar et al., 2001). In contrast, our results, using a different nociceptive stimulus (high-frequency electrical stimulation) and spinal EPs, did not show a change in this parameter. However, we observed increased amplitude of the N1 and P1 waves by this high-frequency stimulus, also reported in the same human study (Sarkar et al., 2001) but by noxious acid esophageal infusion. Taken together, these results strongly support the notion of central hypersensitivity processes.

The amplitude differences between the N1 and P1 waves of the recorded EPs align with those described for somatosensory EPs elicited by peripheral nerve stimulation, such as the sural or sciatic nerve. In these cases, the synchronous depolarization of the sensory interneurons of the dorsal horn generates the N wave, which is of higher voltage due to the greater number of neurons involved and synchronization of their discharges. The P wave, on the other hand, is generated by lesser neuronal or axonal synchronicity, leading to a lower amplitude (Meléndez-Gallardo & Eblen-Zajjur, 2016, 2018).

The stability of N and P waves observed during baseline recordings allowed us to detect changes induced by increased stimulation frequency that mimics a nociceptive visceral input to the spinal horn. This led to increased synchronization of the neuronal population, resulting in a higher N1 wave, followed by activation of descending modulation systems, which enhanced the P1 wave (Bannister et al., 2015; Kimura, 2013; Shimoji, 1995).

At the start of our experimental protocol, we administered a single dose of atropine to reduce respiratory tract secretions during anaesthesia. Although atropine is a known inhibitor of the intestinal smooth muscle, its half-life in rats is approximately 43 minutes, and the single dose we used (0.25 mg) was 5 times lower than the minimal effective dose reported in rats (Harrison et al., 1974). Additionally, spinal EP recording began 50 - 60 minutes after the spinal surgery, during which plasma atropine levels would have been undetectable (Harrison et al., 1974).

We also tested the effect of global ischemia (respiratory arrest) on spinal EPs elicited by electrical colon stimulation. This resulted in a linear decay in the amplitude of the N1 and P2 waves, which can be attributed to progressive depletion of the intracellular ATP, ionic pump failure, and reduced neuronal synchronicity in both the spinal dorsal horn and supraspinal structures (Wix-Ramos & Eblen-Zajjur, 2011a, 2011b). Interestingly, the P1 wave was abolished 7 minutes before the N2 wave, suggesting that the spinal dorsal horn neuronal population (N1 wave) remains active longer than the supraspinal structures responsible for the P1 wave. This is likely due to higher oxygen demand and metabolism of supraspinal structures (Wix-Ramos & Eblen-Zajjur, 2011a, 2011b).

These findings may have clinical relevance, as colonic ischemia in humans is associated with high mortality rates (24% to 94%) (Yadav et al., 2015), and spinal EPs could have diagnostic potential.

In previous studies, D-tubocurarine has been used to induce global ischemia in rats by blocking nicotinic receptors at the motor end-plate of the respiratory muscles, testing the effects of drugs aimed at reducing brain cytotoxic oedema (Wix-Ramos & Eblen-Zajjur, 2011a, 2011b). In the present study, we employed the same ischemia protocol to observe its impact on the spinal EPs elicited by electrical colon stimulation. In rodents, the nicotinic receptor of the spinal dorsal horn provides tonic stimulation to inhibitory GABAergic and glycinergic interneurons, resulting in analgesia (Matsumoto et al., 2007). D-tubocurarine might reduce these inhibitory signals, increasing spinal neuronal excitability, which should be reflected in enhanced EPs amplitude. However, the rapid onset of the ischemia likely overrides any excitatory effect of nicotinic receptor blockade. This hypothesis requires further research.

5.0 CONCLUSIONS

In summary, our experimental model of colon nociception provides a valuable tool for evaluating pathological processes, interventions, drugs, and their effects on the spinal cord as the primary centre for sensory processing and descending modulation systems, which is difficult to assess in humans.

Limitations and further research

Recording spinal EP by rectal electrical stimulation can assess potential abnormalities in primary afferent neural pathways innervating the rectum in several neurodegenerative and functional pain disorders (Chey et al., 1995; Garvin et al., 2010). The model described here could demonstrate central sensitization in the spinal cord, which, similar to somatic sensibility and chronic pain, may explain the visceral pain experienced by patients with chronic colitis through spinal neuronal hyperexcitability. Human experimentation shows limitations compared to animal experimental models, i.e., complex behaviour masking effect, result interpretation, not controlled or hidden variables, difficulties conducting longitudinal studies, discomfort or pain-inducing protocols. However, it is important to note that while animal experiments have certain advantages, such as cross-species differences in pain processing, behaviour, and immune response (Devinsky et al., 2018), they are also subject to ethical concerns, as well as the need to promote the principles of replacement, reduction, and refinement principles to ensure that animal testing is carried out as humanely and sparingly as possible, but reducing the statistical power of their results. Another limitation is the use of D-tubocurarine to induce global ischemia, and its blocking effect on the nicotinic receptors could reduce spinal inhibitory signals. Other experimental methods for ischemia should be tested to verify our results in this respect. Additional experiments should be conducted to investigate clinical-like colitis, including conditions such as infections, altered colonic microbiota, intestinal prokinetic or anti-kinetic drugs, and post-surgical akinesia, among other common clinical scenarios directly related to colitis.

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Author Contributions: AEZ designed the experiments and protocols; MD, MG and AA performed animal care and handling; MD, MG, AA and AEZ performed the experiment, and collected, processed and analyzed the data; AEZ prepared the manuscript. All authors reviewed the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

Bai, Y., Chen, Y. B., Qiu, X. T., Chen, Y. B., Ma, L. T., Li, Y. Q., Sun, H. K., Zhang, M. M., Zhang, T., Chen, T., Fan, B. Y., Li, H., & Li, Y. Q. (2019). Nucleus tractus solitarius mediates hyperalgesia induced by chronic pancreatitis in rats. *World Journal of Gastroenterology*, *25*(40), 6077–6093. https://doi.org/10.3748/wjg.v25.i40.6077

Bannister, K., Patel, R., Goncalves, L., Townson, L., & Dickenson, A. H. (2015). Diffuse noxious inhibitory controls and nerve injury: Restoring an imbalance between descending monoamine inhibitions and facilitations. *Pain*, *156*(9), 1803–1811. https://doi.org/10.1097/j.pain.0000000000000240

Barrett, J. E. (2015). The pain of pain: Challenges of animal behavior models. *European Journal of Pharmacology*, *753*, 183–190. https://doi.org/10.1016/j.ejphar.2014.11.046

Bielefeldt, K., & Gebhart, G. F. (2022). Visceral pain. In *Clinical Pain Management* (pp. 355–365). Wiley. https://doi.org/10.1002/9781119701170.ch34

Boezaart, A. P., Smith, C. R., Chembrovich, S., Zasimovich, Y., Server, A., Morgan, G., Theron, A., Booysen, K., & Reina, M. A. (2021). Visceral versus somatic pain: An educational review of anatomy and clinical implications. *Regional Anesthesia & Pain Medicine*, *46*(7), 629–636. https://doi.org/10.1136/rapm-2020-102084

Brierley, S. M., Hibberd, T. J., & Spencer, N. J. (2018). Spinal afferent innervation of the colon and rectum. *Frontiers in Cellular Neuroscience*, *12*(December). https://doi.org/10.3389/fncel.2018.00467

Brookes, S. J. H., Spencer, N. J., Costa, M., & Zagorodnyuk, V. P. (2013). Extrinsic primary afferent signalling in the gut. *Nature Reviews Gastroenterology and Hepatology*, *10*(5), 286–296. https://doi.org/10.1038/nrgastro.2013.29

Burma, N. E., Leduc-Pessah, H., Fan, C. Y., & Trang, T. (2017). Animal models of chronic pain: Advances and challenges for clinical translation. *Journal of Neuroscience Research*, *95*(6), 1242–1256. https://doi.org/10.1002/jnr.23768

Chagín-Nazar, M., & Eblen-Zajjur, A. (2015). Effect of noxious bucal trigeminal high frequency electrical stimulation on lumbar spinal cord evaluated by evoked potentials in the rat. *Archivos de Neurociencias*, *20*(4), 258–264. https://doi.org/10.31157/an.v20i4.101

Chey, W. D., Beydoun, A., Roberts, D. J., Hasler, W. L., & Owyang, C. (1995). Octreotide reduces perception of rectal electrical stimulation by spinal afferent pathway inhibition. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *269*(6 32-6), 821–826. https://doi.org/10.1152/ajpgi.1995.269.6.g821

Devinsky, O., Boesch, J., Cerda- Gonzalez, S., Coffey, B., Davis, K., Friedman, D., Hainline, B., Houpt, K., Lieberman, D., Perry, P., Prüss, H., Samuels, M., Small, G., Volk, H., Summerfield, A., Vite, C., Wisniewski, T., & Natterson- Horowitz, B. (2018). A cross-species approach to disorders affecting brain and behaviour. *Nature Reviews Neurology*, *14*, 677–686. https://doi.org/10.1038/s41582-018-0074-z

Eblen-Zajjur, A. A., & Sandkühler, J. (1996). Synchronicity of nociceptive and non-nociceptive adjacent neurons in the spinal dorsal horn of the rat: Stimulus-induced plasticity. *Neuroscience*, *76*(1), 39–54. https://doi.org/10.1016/S0306-4522(96)00286-2

Elsenbruch, S., Icenhour, A., & Enck, P. (2017). Visceral pain - A biopsychological perspective. *Neuroforum*, *23*(3), 105–110. https://doi.org/10.1515/nf-2017-A029

Feng, B., & Guo, T. (2020). Visceral pain from colon and rectum: the mechanotransduction and biomechanics. *Journal of Neural Transmission*, *127*, 415–429. https://doi.org/10.1007/s00702-019-02088-8

Finnerup, N. B., Kuner, R., & Jensen, T. S. (2021). Neuropathic pain: Frommechanisms to treatment. *Physiological Reviews*, *101*(1), 259–301. https://doi.org/10.1152/physrev.00045.2019

Garvin, B., Lovely, L., Tsodikov, A., Minecan, D., Hong, S., & Wiley, J. W. (2010). Cortical and spinal evoked potential response to electrical stimulation in human rectum. *World Journal of Gastroenterology*, *16*(43), 5440–5446. https://doi.org/10.3748/wjg.v16.i43.5440

Greenwood-Van Meerveld, B., Prusator, D. K., & Johnson, A. C. (2015). Animal models of gastrointestinal and liver diseases. Animal models of visceral pain: Pathophysiology, translational relevance, and challenges. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *308*(11), G885–G903. https://doi.org/10.1152/ajpgi.00463.2014

Hammer, O., Harper, D. A. T., & Ryan, P. D. (2018). PAST: Palaeontological statistics package for education and data analysis. *Paleaeontol. Electron*, *4*(9).

Harrison, S., Bosin, T., & Maickel, R. (1974). Physiological disposition of atropine in the rat. *Pharmacology Biochemistry and Behavior*, *2*, 843–845. https://doi.org/10.1016/0091-3057(74)90120-8

Heitler, W. J. (2007). DataView : A Tutorial Tool for Data Analysis . Template-based Spike Sorting and Frequency Analysis. *The Journal of Undergraduate Neuroscience Education*, *6*(1), 1–7.

Johnson, A. C., Farmer, A. D., Ness, T. J., & Greenwood-Van Meerveld, B. (2020). Critical evaluation of animal models of visceral pain for therapeutics development: A focus on irritable bowel syndrome. *Neurogastroenterology and Motility*, *32*(4), 1–8. https://doi.org/10.1111/nmo.13776

Kimura, J. (2013). *Electrodiagnosis in Diseases of Nerve and Muscle*. Oxford University Press. https://doi.org/10.1093/med/9780199738687.001.0001

Lucarini, E., Parisio, C., Branca, J., Segnani, C., Ippolito, C., Pellegrini, C., Antonioli, L., Fornai, M., Micheli, L., Pacini, A., & Bernardini, N. (2020). Deepening the mechanisms of visceral pain persistence: An evaluation of the gut-spinal cord relationship. *Cells*, *9*, 1772. https://doi.org/10.3390/cells9081772

Matsumoto, M., Xie, W., Inoue, M., & Ueda, H. (2007). Evidence for the tonic inhibition of spinal pain by nicotinic cholinergic transmission through primary afferents. *Molecular Pain*, *3*, 41. https://doi.org/10.1186/1744-8069-3-41

Meléndez-Gallardo, J., & Eblen-Zajjur, A. (2016). Noxious mechanical heterotopic stimulation induces inhibition of the spinal dorsal horn neuronal network: analysis of spinal somatosensory-evoked potentials. *Neurological Sciences*, *37*(9), 1491–1497. https://doi.org/10.1007/s10072-016-2613-y

Meléndez-Gallardo, J., & Eblen-Zajjur, A. (2018). Thermo-dependence of noxious mechanical heterotopic stimulation-dependent modulation of the spinal dorsal horn response to somatosensory stimulation. *Journal of Integrative Neuroscience*, *17*(3–4), 413–424. https://doi.org/10.3233/JIN-180076

Ng, S. C., Shi, H. Y., Hamidi, N., Underwood, F. E., Tang, W., Benchimol, E. I., Panaccione, R., Ghosh, S., Wu, J. C. Y., Chan, F. K. L., & others. (2017). Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a

systematic review of population-based studies. *The Lancet*, *390*(10114), 2769–2778. https://doi.org/10.1016/s0140-6736(17)32448-0

Porter, R. J., Kalla, R., & Ho, G.-T. (2020). Ulcerative colitis: Recent advances in the understanding of disease pathogenesis. *F1000Research*, *9*. https://doi.org/10.12688/f1000research.20805.1

Regmi, B., & Shah, M. K. (2020). Possible implications of animal models for the assessment of visceral pain. *Animal Models and Experimental Medicine*, *3*(3), 215–228. https://doi.org/10.1002/ame2.12130

Sarkar, S., Hobson, A. R., Furlong, P. L., Woolf, C. J., Thompson, D. G., & Aziz, Q. (2001). Central neural mechanisms mediating human visceral hypersensitivity. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *281*(5), G1196–G1202. https://doi.org/10.1152/ajpgi.2001.281.5.G1196

Shimoji, K. (1995). Origins and properties of spinal cord evoked potentials. Atlas of human spinal cord evoked potentials. Washington: Butterworth-Heinemann, 1-25. In M.R. Dimitrijevic & J.A. Halter (Eds.), *Atlas of human spinal cord evoked potentials.* (pp. 1–25). Butterworth-Heinemann.

Valenzuela, F., Rana, M., Sitaram, R., Uribe, S., & Eblen-Zajjur, A. (2021). Non-Invasive Functional Evaluation of the Human Spinal Cord by Assessing the Peri-Spinal Neurovascular Network with near Infrared Spectroscopy. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, *29*, 2312–2321. https://doi.org/10.1109/TNSRE.2021.3123587

Wix-Ramos, R., & Eblen-Zajjur, A. (2011a). Time course of acute neuroprotective effects of lidocaine evaluated by brain impedanciometry in the global ischemia model. *Pharmacology*, *88*(5–6), 316–321. https://doi.org/10.1159/000334274

Wix-Ramos, R., & Eblen-Zajjur, A. (2011b). Time course of acute neuroprotective effects of lithium carbonate evaluated by brain impedanciometry in the global ischemia model. *Canadian Journal of Physiology and Pharmacology*, *89*(10), 753–758. https://doi.org/10.1139/y11-073

Yadav, S., Dave, M., Edakkanambeth, J Harmsen, W., Tremaine, W., Zinsmeister, A., Sweetser, S., Melton, L., Sandborn, W., & Loftus, E. (2015). A population-based study of incidence, risk factors, clinical spectrum, and outcomes of ischemic colitis. *Clinical Gastroenterology and Hepatology*, *13*(4), 731–738. https://doi.org/10.1016/j.cgh.2014.07.061