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Mechanically evoked cortical potentials: A physiological approach to assessment of anorectal sensory pathways

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ABSTRACT

Background: Normal defaecation involves activation of anorectal mechanoreceptors responsive to pressure and stretch. The aim of this study was to develop selective anal and rectal mucosal light-touch stimulation suitable for measurement of cortical evoked potentials (EPs) in order to explore the sensory arm of these pathways. New method: A novel device was manufactured to deliver selective rectal and/or anal light-touch stimulation using a shielded inter-dental brush mounted on a rotating stepper motor (1 Hz, 1 ms, 15° rotation). Resultant somatosensory EPs recorded with a 32-channel cortical multi-electrode array were compared to those elicited by electrical anorectal stimulation (2 mm anal plug electrode [1 Hz, 1 ms, 10 V]). Results: Eighteen anaesthetized female Wistar rats (body mass 180–250 g) were studied. Electrical and mechanical stimulation provoked similar maximal response amplitudes (electrical anorectal 39.0 μV[SEM 5.5], mechanical anal 42.2 μV[8.1], mechanical rectal 45.8 μV[9.0]). Response latency was longer following mechanical stimulation (electrical anorectal 8.8 ms[0.5], mechanical anal 16.4 ms[1.1], mechanical rectal 18.3 ms[2.5]). The extent of activated sensory cortex was smaller for mechanical stimulation. Sensory inferior rectal nerve activity was greater during anal compared to rectal mechanical in a subgroup of 4 rats. Evoked potentials were reproducible over 40 min in a subgroup of 9 rats. Comparison with

existing methods: Cortical EPs are typically recorded in response to non-physiological electrical stimuli. The use of a mechanical stimulus may provide a more localized physiological method of assessment. Conclusions: To the authors' knowledge these are the first selective brush-elicited anal and rectal EPs recorded in animals and provide a physiological approach to testing of anorectal afferent pathways.

INTRODUCTION

The physiology of defaecation and continence is critically reliant on intact anorectal sensation (Palit et al., 2012). The rectum and anus are richly innervated with mucosal receptors sensitive to temperature, pressure and stretch (Duthie and Gairns, 1960) and disorders of anorectal sensation are associated with a number of functional bowel problems such as faecal incontinence, chronic constipation and irritable bowel syndrome (Mertz et al., 1995; Chanet al., 2005; Vasudevan et al., 2007; Scott et al., 2011; Burgell and Scott, 2012).Cortical evoked potentials (EPs) are widely used to investigate the physiology and pathophysiology of sensory function and cortical representation (Chiappa and Ropper, 1982). In man, they are routinely used for clinical diagnosis of demyelinating conditions (McDonald et al., 2001) and in the rodent they have been applied for study of somatic and visceral pathways (Freeman and Sohmer,1996). Visceral EPs are primarily elicited by electrical stimulation of the target area as this produces potentials of greatest amplitude and clarity, however the use of electrical stimuli is often criticized due to its non-physiological nature (Pratt et al., 1979). An ideal stimulus for assessment of sensory function is one that mimics physiological function as closely as possible, whilst maintaining precision and control. A number of methods using mechanical stimuli for the generation of EPs have been utilized including tapping and the use of air-puffs with some success (Polley et al., 1999; Sosnik et al., 2001); however, application of these methods to the study of luminal organs such as the distal gastrointestinal tract would be technically challenging. Rapid rectal balloon distension has previously been used in both animals and humans for evoking cortical potentials (Nissen et al., 2013). However, this method has limitations, namely the requirement for costly equipment and a high-pressure system with compliant balloons for accurate stimulation. The potentials evoked are usually smaller than electrically evoked potentials and have a longer latency (Hultin et al., 2012). An alternative approach is to deliver light-touch mechanical stimulation. To the author's knowledge there is no previously published method for recording of cortical EPs in response to selective light-touch mechanical stimulation of the rectum and anus. The development of such a technique would allow specific and detailed exploration of physiological pathways involved in neural control of continence.

AIMS

The aim of this study was to develop a method to allow selective physiological stimulation of the rectum and anus in the rat, to allow exploration of resultant cortical responses using EPs and to compare these results with EPs obtained using an electrical stimulus.

METHODS

Experiments were carried out in accordance with a protocol approved by the University College Dublin Animal Ethics Research Committee. The licence was granted by the Irish department of Health and Children (reference: B100/4435). Animals were kept at a 12/12-h light/dark cycle and had access to water and a rodent standard diet ad libitum. A total of 18 female Wistar rats were used. Animals were anaesthetically induced with isoflurane (4%) in oxygen (1 L/min) and then surgically anaesthetized with a 20% solution of 1.5 g/kg i.p. urethane (Sigma, Arklow, Ireland). Femoral vein cannulation was performed to allow administration of fluids and additional i.v. urethane as required. Anaesthetic depth was monitored regularly using both the pedal withdrawal to toe pinch and corneal reflex. A tracheostomy and intubation were performed to prevent airway obstruction.

Anal and rectal mechanical stimulation

A novel device was developed for mechanical light-touch stimulation. A commercially available interdental brush (2 mm Ø, 1 cm length for anal stimulation and 5 mm Ø, 1 cm length for rectal stimulation, TePe®, Malmö, Sweden) was mounted on a bipolar stepper motor (15M020D1B, Radionics, Dublin, Ireland) with a driver that set a rotation of 15 degrees when triggered (Somotronic101, Radionics, Dublin, Ireland). The shaft of the brush was placed within a 3D-printed (Ultimaker, Geldermalsen, Netherlands) customized shielding device (constructed from polylactic acid) that allowed selective stimulation of the rectum (Fig. 1). Anal canal stimuli were delivered with the 2 mm brush stimulating the distal 3 mm of the rat anal canal. Rectal stimuli were delivered using the 5 mm brush, and the anal shield in situ.

Electrical stimulation

For electrical anorectal stimulation, a gold-plated plug cathode (diameter: 2 mm) was placed in the anal canal and a silver wire anode (diameter: 500 µm) introduced subcutaneously lateral to the external anal sphincter on the left side. Stimulation was delivered at10 V amplitude with a pulse frequency of 1 Hz and pulse duration of 1 ms as previously reported (Griffin et al., 2011; Evers et al., 2014).

Recording of somatosensory evoked potentials

A 4 mm × 4 mm craniotomy centred over the area of maximal anal representation (anteroposterior coordinate −0.6 mm, medio-lateral +2 mm from Bregma (Griffin et al., 2011)) was made over the right somatosensory cortex. Evoked potentials were recorded using an extradural multi-electrode array (flexMEA, Multi Channel Systems, Reutlingen, Germany) consisting of 32 recording electrodes, two reference and two ground electrodes covering an area of 1830 μm × 1830 μm. Sampling frequency was 10,000 Hz and electrode impedance approximately 50 kΩ. Recordings were amplified and filtered (USB-ME-FAI System, Multi Channel Systems) prior to display (MC Rack 4.3.0). Evoked potentials to electrical stimulation (1/s) were recorded for and averaged over 200 s. For mechanical stimulation the rotor was set to rotate 15° every second and each 15° rotation of the brush elicited a potential. Because of the small voltage of potentials and level of background noise recordings consisted of a 200 sweeps average and averaged data was used for further analysis.

Nerve recordings

The anal canal and rectum have separate innervations (the anus is a somatic structure innervated by the inferior rectal nerve (IRN)whereas the rectum is visceral, innervated by the pelvic autonomic nerves) thus selective stimulation of the rectum in the absence of anal canal stimulation can be confirmed by recording activity in the IRN. The IRN was exposed and placed on a bipolar recording electrode. Recordings were taken while the anal canal and rectum were mechanically stimulated. For recording, Spike 2.06 (C.E.D., Cambridge, U.K.) was used and recording was averaged over 200 s. Because recording from the IRN, which is a very small nerve with only 40–50 axons (Peirce et al., 2009), is technically difficult to perform and the expected size of effect was large, this was only performed in 4 rats. Two types of control experiments were performed: evoked potentials were recorded post mortem and the effect of submucosally injected lidocaine (20%) on EPs wastested.4.

Study design

The study was composed of 3 parts1. Comparison of mechanical and electrical anorectal EPs – in all 18rats, EPs in response to electrical stimulation of the anorectum, mechanical stimulation of the rectum and mechanical stimulation of the anal canal were performed in a random order.2. Confirmation of selective rectal stimulation – in a subgroup of 4rats, sensory stimulation of the rectum without significant activation of anal afferents was confirmed using nerve recordings from the IRN.3. Evoked potential stability – in a further subgroup of 9 rats, stability studies of cortical responses to anal electrical and anal mechanical stimulation were performed comparing 4 recordings acquired over a 40 min period.

Analysis

Spike 2.06 (C.E.D.) was used to display acquired data from all 32 channels. Traces were automatically averaged by the software and used for all further analysis. The channel with the greatest amplitude was used for detailed analysis of waveform morphology. Latency and amplitude of the EPs, based on the first positive deflection following stimulation, were measured utilizing Spike 2.06software tools. Colour maps expressing activated spatial extent were generated in Excel (Microsoft Corporation, Redmond, U.S.A.). Traces were not blinded for analysis, because the stimulus artefact unmistakably marks traces recorded from electrical stimulation.

Normally distributed numeric data were expressed as mean and standard error of the mean (SEM). The one-way repeated-measures ANOVA was used to quantify differences in EP waveform amplitude and latency for both comparison of techniques and stability studies. A Bonferroni post-test was performed where adequate. GraphPad Prism 4 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analyses. The criterion for statistical significance was $P < 0.05$.

For quantification of the spatial extent of activated area on the somatosensory cortex, all channels expressing amplitudes greater than 90% of the maximal amplitude were counted in each animal. The activated cortical areas of anal electrical and anal mechanical EPs were compared.

RESULTS

Comparison of mechanical and electrical anorectal evoked potentials

Evoked potentials in response to anal electrical, anal mechanical and rectal mechanical stimulation were successfully recorded in all18 rats. Maximum EP response amplitude was similar between techniques (anal electrical 39.0 ± 5.4 µV; anal mechanical 42.2 ± 8.1 µV; rectal mechanical 45.8 ± 9.0 µV, $F(2,34) = 0.98, p = 0.38$); however inter-animal variation was high, especially for mechanical stimulation. The coefficient of variation was 59.1% for electrical, 81.2% for anal mechanical and 82.8% for rectal mechanical stimulation. EP response onset following both anal and rectal mechanical stimulation was significantly delayed in comparison with EP responses to electrical stimulation (anal electrical 8.8 ± 0.5 ms; anal mechanical 16.4 ± 1.1 ms; rectal mechanical 18.3 ± 2.5 ms, $F(2,34) = 14.80$, $p < 0.0001$). A Bonferroni post-test showed that compared to anal electrical EPs, both anal mechanical and anal rectal EPs had longer onset latencies. Amplitudes and latencies are shown in Supplemental Figure1. Channels showing the maximal amplitude for all locations were close together on the MEA (Supplemental Figure 3). The total number of channels with greater than 90% of the maximal amplitude was smaller in the mechanical anal EP group(N = 40) than in the electrical anal EP group (N = 73) indicating a smaller extent of activated sensory cortex (Fig. 2a and

b). Post mortem recorded mechanical EPs showed a flat line (N = 3). Application of lidocaine reduced EP amplitude in 4/5animals, but a large reduction was only seen in 2 animals (−82%, −94%).

Confirmation of selective rectal stimulation

Direct recordings from the IRN performed during intra-anal brush stimulation demonstrated a significant electrical response (1.6 ± 0.5 μV). By contrast, stimulation of the rectum, with the anal shield in situ resulted in minimal axonal activity (0.3 ± 0.1 μV) con-firming a relative absence of IRN activation during selective rectal stimulation. Introduction of the anal shield reduced the peak-to-peak amplitude by 64%, 83%, 86% and 89% in the 4 animals (Fig. 2c and d).

Evoked potential stability

Evoked potentials repeated at 10-min intervals over a 40-minperiod demonstrated the reproducibility of EP recordings. The coefficient of variation over time for each animal was 11.9% ± 3.9%for anal electrical and 9.6% ± 1.9% for anal mechanical stimulation. There was a no effect of time for either anal electrical or anal mechanical EPs ($F(2,16) = 1.15$, $p = 0.34$) and ($F(2,16) = 1.76$,$p = 0.20$) respectively, confirming stability of recorded responses (Supplemental Figure 2).

DISCUSSION

To the best of the authors' knowledge this is the first report of a simple, cheap and reliable technique for mechanical light-touch stimulation of the anal canal and rectum in the rat. This new method has two significant benefits over existing approaches: first, the significant advantage of allowing selective stimulation of either the anal canal or rectum as demonstrated by minimal IRN activation when using the customized shielding device; and second, the physiological nature of the stimulus. It is appreciated that electrical stimulation of an area is more widespread than the location of the cathode due to current spread with neuronal activation caused by direct axonal depolarization. By contrast, a mechanical stimulus provides a more focal assessment of the afferent pathways, and therefore provides a more accurate assessment of sensory stimuli. Evoked potentials recorded in response to mechanical stimulation are likely to reflect more selective activation of light touch and vibration pathways. Previously described methods for mechanical anorectal stimulation in the rat have not been demonstrated to be selective in nature (Hultin et al.,2012).

One other group has reported on a rodent model of mechanical rectal EPs using balloon distention. The morphology of those EPs showed a small positive peak at 23 ms followed by a large negative peak at 55 ms. In our experiments, only one peak corresponding to the second (large negative) peak

was observed. This may reflect the different stimuli (distention vs. light-touch) or different recording technique (awake animals vs. anaesthetized animals in the current study) (Nissen et al., 2013).

The cortical EPs did not represent artefactual pick up of stepper motor noise. Three lines of evidence support this conclusion. First, post mortem traces showed no cortical deflections. Second, anal mechanical EPs were reduced after application of lidocaine (how-ever, this experiment was difficult to perform because only small volumes could be injected and the high mucosal blood flow presumably cleared the injected drug quickly; previous studies have shown that the application of lidocaine to the bowel has inconsistent effects on cortical EPs (Gener et al., 2009; Hultin et al., 2012)). Third, anal canal stimulation produced EPs in the nerve of supply (IRN) confirming that afferent volleys were being produced by the stimulus. When results were compared with electrically elicited EPs, the resultant cortical response location and amplitude were similar suggesting a similar number of neurons activated. Latency of response following mechanical stimulation was prolonged and more variable when compared with electrically elicited potentials, a finding which has been demonstrated in studies of mechanically EPs in humans (Hobday et al., 2000), and is likely to be representative of a combination of: (1) time taken for the brush to turn; (2) activation of the entire sensory pathway including the receptor; (3) more selective neuronal activation. The authors acknowledge a number of drawbacks of this technique. First, there are technical difficulties in correct placement of brush and shield within the anal canal. Some care is needed to ensure that the brush and shielding device are placed in such a way to prevent movement of the anal canal during turning. To minimize inadvertent stimulation of the anal canal the brush was fixed centrally within the anal shield. The second difficulty is that prolonged mechanical brush stimulation can cause irritation and damage to the lining of the rectal wall. This limits protracted use of this technique. A further problem is inability to directly visualize the rectal brush during stimulation. During this study, the brush size was selected according to the size/weight group of the rodent being studied, with ability to record EPs during stimulation taken as confirmation of stimulation of rectal afferents. Changes in anal or rectal sensation are prominent in defaecatory disorders. While rectal hyposensitivity is often present in constipation (Scott et al., 2011) and regularly in spinal cord injury (Burgelland Scott, 2012), both rectal hypo- and hypersensitivity can be present in irritable bowel syndrome (Mertz et al., 1995). Faecal incontinence patients can present with rectal and anal hyper- or hyposensitivity (Chan et al., 2005; Vasudevan et al., 2007; Burgelland Scott, 2012). As in some conditions both hypo- and hypersensitivity may be present, abnormal sensation is often tied to more severe symptoms and affects treatment options, sensitivity testing is an important part of patient evaluation (Burgell and Scott,2012).This model could be used in two ways: (1) In combination with other rodent models of diseases and their treatment (Healy et al., 2008; Griffin et al., 2011; Qin et

al., 2011), the study of the afferent somatosensory pathways of the anus and rectum may provide new insights into the pathophysiology and treatment of those disorders. (2) The method may also work in the human anal canal and rectum. Previous human studies have used rapid balloon inflation methods to produce EPs. Mucosal light-touch stimulation could be achieved with a rigid fenestrated collar of sufficient diameter to permit fixed dilation that allows light mucosal stimulation via small apertures in the collar although other difficulties in relation to human translation would have to be overcome.

CONCLUSION

This technique allows selective mechanical light-touch stimulation of the anal canal and rectum in the rat that can be applied to study the physiology and pathophysiology of anorectal sensation in defaecatory disorders.

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FIGURES

Figure 1:

Photograph of brush stimulator (A) and diagrammatic representation of brush placement during stimulation of the rectum (B), and anus (C).

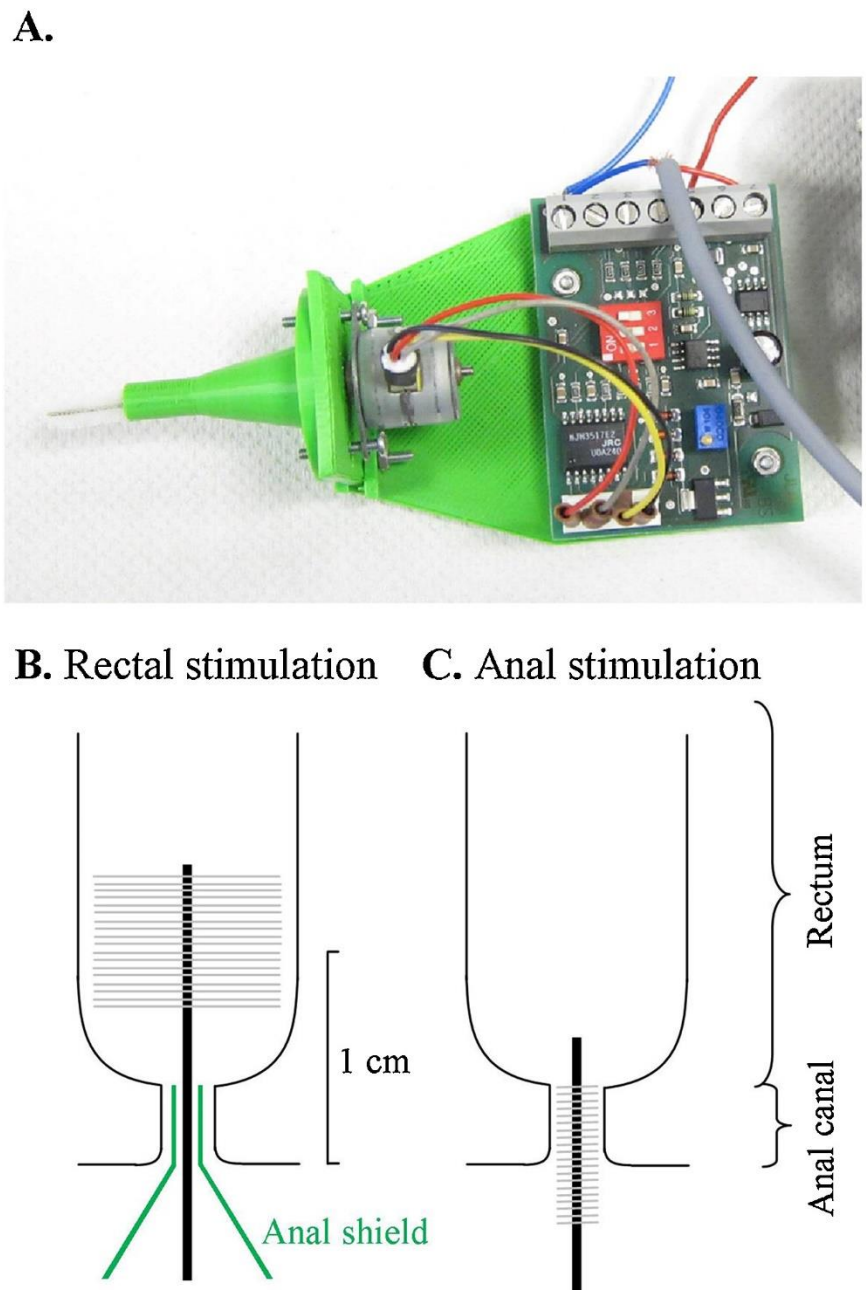

Figure 2:

(A) Average EP traces of electrical anorectal stimulation (N = 18, blue), mechanical anal stimulation (N = 18, red) and mechanical rectal stimulation (N = 18, green). Traces consist of one upward and

one downward deflection. (B) Representative colour maps of anal electrical and anal mechanical stimulation in one animal. Note the smaller extent of mechanical stimulation. (C) IRN recordings during mechanical anal stimulation and mechanical rectal stimulation. A clear response at 8 ms is seen during stimulation of the anal canal, a finding that is absent during rectal stimulation. (D) Peak-to-peak amplitudes from IRN recordings during mechanical anal stimulation and mechanical rectal stimulation.