ELSEVIER

Journal of Neuroscience Methods 162 (2007) 215–221

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Is it possible to generate cerebral evoked potentials with a mechanical stimulus from the duodenum in rats?

Joanna C. Murrell [a,*], Sheryl L. Mitchinson [a], Alastair C. Johnstone [a], Craig B. Johnson [a], Geoffrey R.G. Barnes [b]

[a] *Institute of Veterinary Animal and Biomedical Sciences, College of Sciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand*
[b] *Institute of Fundamental Sciences, College of Sciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand*

Received 2 November 2006; received in revised form 17 January 2007; accepted 20 January 2007

Abstract

The study aim was to develop a model to generate cerebral evoked potentials (CEPs) by mechanical distention of the duodenum in rats. Twenty Sprague–Dawley rats were anaesthetized and the EEG recorded from the left and right somatosensory cortices (S1L, S1R). A balloon catheter was implanted into the duodenum. A pneumatic device, triggered by data acquisition software, inflated the balloon for 200 ms every 3 s to deliver a repeatable noxious stimulus. EEG was recorded for 100 ms before and 500 ms after onset of inflation and the response to 512 stimuli averaged to generate a CEP. Two CEPs were generated in each animal and data summed to calculate a single CEP for each channel.

Data were excluded when the signal to noise ratio was <2, therefore data are presented from 11 animals. A repeatable CEP was identified in waveforms recorded from S1L. The mean (S.D.) CEP comprised a triphasic waveform (P1, N1, P2) with latencies of 246.0 (24.7), 289.3 (12.8) and 321.5 (13.2) ms, respectively. We are the first group to have generated and characterized a CEP following mechanical stimulation of the duodenum. This model can be applied to further elucidate the mechanisms leading to visceral pain perception.

Keywords: Cerebral evoked potential; Visceral; Pain; Rat; Electroencephalogram

1. Introduction

Visceral pain is the most common form of pain produced by disease and one of the most frequent reasons why patients seek medical attention (Cervero and Laird, 1999). It originates from internal organs such as the stomach, kidney, gall bladder and intestines and results from various insults to these organs, such as distention from impaction or tumors, ischaemia, inflammation or mesenteric traction (Al-Chaer and Traub, 2002). Despite the huge clinical significance of visceral pain in man, the underlying mechanisms leading to visceral pain perception are poorly understood. Historically, the majority of investigations into the pathophysiology of pain have focused on somatic pain, and differences between the somatic and visceral pain pathways have only recently been recognized.

Evoked potentials are fragments of brain electrical activity time-locked to a specific sensory stimulus. Evoked potential studies investigating pain usually utilize somatosensory evoked potentials (SEPs), evoked by short stimulation of peripheral somatosensory fibers. SEPs evoked by high intensity stimulation represent neural processing of noxious stimuli (Bromm and Lorenz, 1998; Stienen et al., 2003, 2004). Carefully controlled laboratory studies in human beings have shown that amplitudes of late SEPs elicited by short noxious stimuli are correlated to the intensity of pain sensation (Bromm and Scharein, 1982; Chudler and Dong, 1983; Kochs et al., 1996; Miltner et al., 1989).

Techniques to generate evoked potentials from the gastrointestinal tract in man have recently been developed (Drewes et al., 2002, 2005; Hobson et al., 2000a,b). These investigations, commonly carried out in awake human volunteers, aim to model cerebral generators following stimulation of the gastrointestinal tract, in order to further elucidate the mechanisms of visceral pain perception. The generated potentials are referred to as cerebral evoked potentials (CEPs) and are described in terms of the latencies and amplitudes of positive and negative deflections in the waveforms. Electrical stimuli are commonly used to stimulate the colon (Arendt-Nielsen et al., 1997; Hobday et al., 2001) and a mechanical stimulus (distention) has been used

* Corresponding author. Tel.: +64 6 350 5799x4565; fax: +64 6 350 5699.
E-mail address: J.C.Murrell@massey.ac.nz (J.C. Murrell).

0165-0270/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2007.01.014

in some studies investigating oesophageal and rectal pain sensation (Harris et al., 2006; Hobson et al., 2000a). Recently a multi-modal pain assessment tool able to deliver mechanical, electrical, cold and warm noxious stimuli has been developed to mimic clinical pain in the human oesophagus (Drewes et al., 2002). CEPs resulting from noxious stimulation of the gastrointestinal tract have never been recorded in animals. The aim of this study was to develop a model to generate CEPs by mechanical distention of the duodenum in rats. The objective of the research program was to develop a clinically relevant model of visceral pain in rats that could be used to record CEPs using intracerebral electrodes placed at target loci. Use of intracerebral recording electrodes, where the site of placement can be verified post-mortem using histological techniques, provides a robust technique to verify the generators of neurophysiological signals. Non-invasive neurophysiological methods used to identify generators of CEPs in man include dipolar source modelling and magnetoencephalogram (MEG). However MEG suffers from lacking identification of deep sources and radial orientated dipoles and modelling techniques are inherently reliant on assumptions to build generator models. Invasive animal models can be used to overcome the limitations of these techniques.

2. Materials and methods

2.1. Animals and anaesthesia

This study was approved by the Massey University Animal Ethics Committee. Twenty adult female Sprague–Dawley rats, weight [mean (S.D.)] 273 (34) g were studied. Anaesthesia was induced and maintained with halothane. Orotracheal intubation was carried out with an 18 gauge cannula (Advantive, Viuret, Morelos, Mexico) and ventilation was controlled using IPPV (V valve ventilator, Vetronics, Bioanalytical Systems Inc., W La Fayette, IN, USA), adjusted to maintain end-tidal carbon dioxide concentration (ET [CO_2]) between 5 and 5.5 kPa. A 22 gauge cannula (Advantive, Viuret, Morelos, Mexico) was placed in the right lateral tail vein and Hartmann's solution (Baxter, Toongabbie, Australia) was administered at a rate of 10 ml kg^{-1} h^{-1} throughout anaesthesia. Monitoring during anaesthesia comprised end-tidal halothane concentration (ET[hal]), ET [CO_2] (Hewlett Packard M1025B gas monitor, Palo Alto, California, USA), and rectal temperature using a digital thermometer (Q 1437, Dick Smith Electronics, New Zealand). Body temperature was supported throughout anaesthesia with a circulating warm water blanket heating device (T pump, Gaymar Industries Inc., NY, USA). ET[hal] was maintained constant at $0.95 \pm 0.05\%$ during the experimental recording period.

2.2. Placement of EEG recording electrodes

The head of the rat was secured in a stereotaxic apparatus (Dual ultra precise small animal stereotaxic instrument, David Kopf Instruments, Tujunga, California, USA) and the periosteum was exposed by a midline skin incision. Four silver–silver chloride electrodes (0.4 mm diameter × 8 mm Ag–AgCl segment, In Vivo Metric) were placed in contact with the dura through holes drilled in the skull. Two active electrodes were placed over the left and right primary somatosensory cortices at loci corresponding to afferent sensory input from the cranial abdomen (S1R, S1L) (3 mm caudal to bregma, 2.5 mm left and right of midline) (Chapin and Lin, 1984). The reference electrode was placed over the fontal sinus (10 mm cranial to bregma, 1 mm left of midline) and a ground electrode was placed in the right caudal quadrant (7 mm caudal to bregma, 2.5 mm right of midline).

2.3. Apparatus to deliver a repeated mechanical stimulus to the duodenum

A pneumatic pump device, capable of repeatedly inflating a balloon catheter was designed and built in-house. This device comprised a 20 ml syringe that was mounted in a metal case and fixed in a stable position. The head of the syringe plunger was attached to a moving metal bar, driven by compressed air, which moved the syringe plunger in and out in a repeatable controlled manner. This allowed the syringe to act as a pump, providing a controlled system to inflate a balloon catheter attached to the nozzle of the syringe. The volume of inflation was controlled by locknuts on a threaded rod, which could be adjusted to limit the movement of the syringe plunger in either direction. The air supply was delivered at a constant pressure of 80 psi to drive the pump mechanism. The pneumatic device was triggered by data acquisition software (Scope 4 v 4.01, PowerLab AD Instruments), which also controlled the dwell time (duration) of the inflation.

The balloon catheters were also designed and built in-house, a new balloon catheter was used in each rat. A 14 gauge triple lumen polyurethrane catheter (Hydrocath^{TM}, Ohmeda, Swindon, UK) was converted into a balloon catheter by securing the distal (blind) end of a long latex balloon over the distal opening at the tip of the catheter. The balloon was secured to the catheter by a cotton suture wrapped around the catheter and open end of the balloon over a length of 5 mm to ensure an airtight seal. This site was covered in a thin film of urethrane repair adhesive and sealant (Aquaseal, McNett Corporation, Bellingham, USA) and allowed to dry for 24 h. The length of balloon that could become inflated varied between 11 and 13 mm.

The balloon catheter was implanted in the anterior duodenum, just distal to the pylorus. A midline incision was made in the abdominal wall and the stomach was exteriorized. A purse string suture was placed in the lateral wall of the forestomach, just proximal to the margo plicatus. The distal tip of the balloon catheter was lubricated and inserted into the stomach through a stab incision in the center of the purse string suture. The catheter was gently manipulated through the pylorus so that the balloon and distal tip of the catheter were located in the anterior duodenum. The purse string suture was secured to prevent both leakage of stomach contents into the abdomen, and catheter movement. The stomach was repositioned and the abdominal incision closed, leaving the proximal catheter protruding from the incision site. The catheter port corresponding to the distal catheter opening was connected to the nozzle of the syringe.