

Award Number: ÁÁÁÁW81XWH-32/3/2; 29

TITLE:

Use of GDNF-Releasing Nanofiber Nerve Guide Conduits for the Repair of Conus medullaris/Cauda Equina Injury in the Non-Human Primate

PRINCIPAL INVESTIGATOR:

Kari L. Christe

CONTRACTING ORGANIZATION;
University of California, Davis
Davis, CA 95616

REPORT DATE: ÁÁÁÁÁÁŠ´\~âæãÁG€FF

TYPE OF REPORT;

Annua

PREPARED FOR; U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT;

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Defense position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 1 October 2011		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2010 – 29 Sep 2011	
4. TITLE AND SUBTITLE Use of GDNF-Releasing Nanofiber Nerve Guide Conduits for the Repair of Conus medullaris/Cauda Equina Injury in the Non-Human Primate				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-1-0907	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Kari L.Christe E-Mail: klchriste@ucdavis.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of California, Davis Davis, CA 95616				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Our project studies the effects of lumbosacral ventral root avulsion injury and repair in the non-human primate. During the initial study period, we have made significant progress. We have developed an extensive set of pre-surgical outcome measures, including treadmill locomotor studies, electromyogram (EMG) recordings, MRI studies, urodynamic recordings, and pain behavioral studies. Many of our pre-surgical findings are novel, including assessments of autonomic function. We have, for instance, demonstrated a distinct pattern of EMG activation of the external anal sphincter muscle, an important outcome measure for the studies, which aim to repair cauda equina injuries. The EMG studies show a quiescent baseline at rest but an evoked response may be obtained from gentle stretching of the muscle. The findings have been characterized, an abstract submitted to an international meeting for presentation, and a manuscript is under preparation. We have also demonstrated surgical feasibility of our root injury procedure, with post-operative data collection in progress.					
15. SUBJECT TERMS Cauda equina, non-human primate, ventral root. neural repair, electromyography, magnetic resonance imaging					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1-3
Key Research Accomplishments.....	3
Reportable Outcomes.....	3-4
Conclusion.....	4
References.....	4
Appendices.....	5-9

INTRODUCTION

Cauda equina and conus medullaris forms of spinal cord injury result in paralysis, sensory impairment, and autonomic dysfunction (Hoang and Havton, 2006; Havton and Carlstedt, 2009). The present study investigates the effects of neural repair in the nonhuman primate using a GDNF-releasing nerve guidance channel. The aim of this study is to repair avulsed lumbosacral ventral roots using a bridging strategy. For comparison, the studies will also include the use of a guidance channel without GDNF release and a peripheral nerve graft to bridge the tissue gap. A comprehensive set of electrodiagnostic, imaging, behavioral and anatomical studies will provide detailed information about the outcome of the intervention. We are hopeful that this translational research study may guide future clinical studies on neural repair after cauda equina/conus medullaris injuries. The present report here summarizes the major accomplishments within the first year of this 3-year project.

BODY

Our studies are going well and significant progress has been made. At the beginning of the reporting period, Dr. Havton moved from UCLA to UC Irvine, where he had accepted a position as Professor and Vice Chair for Research in the Department of Anesthesiology & Perioperative Care. Dr. Havton also became a member of the Reeve-Irvine Research Center for Spinal Cord Injury Research. In this collaborative partnership project, Dr. Havton regularly interacts with the participating PIs, Dr. Ahmet Höke at Johns Hopkins University and I (Dr. Kari Christe at the CNPRC). Since we work closely on this project, Dr. Havton and I have developed the same objectives and are in full agreement on the progress of this study and our plans for its completion.

At the onset of the project, adult female rhesus monkeys were selected for pre-surgical testing and enrollment in this study. The selection and screening process has been extensive, as behavioral components are very important for the success of the study. Unfortunately, not all of the subjects that were initially assessed were able to pass the behavioral criteria needed for successful participation. Thus, we have since developed an algorithm to ensure selection of suitable study subjects.

Prior to the surgical procedures, each animal undergoes extensive screening and training. Behavioral records and profiles are reviewed to select subjects that are likely to cooperate with training. Next, each animal is introduced to transfer box movement with and/or without using a chute. This is followed by the introduction of the treadmill environment (enclosed by plexi-glass). Animals are trained to walk on the treadmill at various speeds, and the desired behavior is encouraged using various food rewards. Each form of training requires multiple training sessions. In particular, there is extensive variation between subjects with regards to how many sessions are needed for a subject to be a reliable treadmill walker. Occasionally, extra training time is needed to accommodate individual variation. Unfortunately, when a subject requires additional sessions for training, it may then delay the start of one of the other behavioral training sessions, e.g. chair training or pain screening.

In our algorithm, successful treadmill training is a requirement for subsequent chair training, which is in turn needed to perform sensory testing using von Frey hairs and an Electro-von-Frey device to obtain baseline sensory thresholds pre-operatively. In addition, chair training is needed for the application of paint markers over the hip, knee, ankle, distal metatarsal bone, and distal portion of the fifth toe. The paint markers are filmed during treadmill walking to obtain digital recordings for subsequent kinematics analysis.

Electromyography (EMG) recordings of the external anal sphincter are obtained pre-operatively as baseline records. The external anal sphincter muscle was chosen as it is directly affected by the ventral root injury and will undergo partial denervation as a result of the unilateral lumbosacral ventral root avulsion injury in our experimental model. The development of external anal sphincter recordings in the nonhuman primate was developed by us during this project and reflects innovation and a new outcome measure for primate studies. (Initial data from these studies were presented at the Annual Meeting for the Society for Neuroscience in 2011 and the data are presently being prepared for a research manuscript.)

We also developed a method for urodynamic recordings in the nonhuman primate - no previous established methods for cystometrogram recordings and external urethral sphincter EMG recordings in our anesthetized subjects were present in the literature. In addition, we obtain external abdominal wall EMG recordings during these procedures and are therefore able to monitor and screen for the possibility of visceral pain development. These studies are novel and promising and will represent a very useful additional outcome measure. (Similar to our EMG studies of the external anal sphincter, the urodynamic studies are reportable as scientific findings. We are hopeful that during the next reporting period, we will be able to present these findings from control subjects also as a manuscript for scientific publication.)

Magnetic resonance imaging (MRI) of the lower spine is performed to visualize the lumbosacral spinal cord and associated nerve roots as well the bilateral hindlimbs to visualize muscles groups both above and below the level of the knee. The MRI recordings of the spinal cord and nerve roots pre-and post-operatively will allow us to monitor nerve root degeneration and axonal regeneration associated with cauda equina injury and repair. We anticipate that the MRI studies may be able to identify successful muscle reinnervation prior to functional improvement taking place.

An initial eight adult female rhesus monkeys have been selected, screened, and successfully enrolled for full pre-surgical testing, including MRI, treadmill training, chair testing, and pain screening using a von Frey testing approach. An additional 12 animals are currently being identified and evaluated for the project and are at different stages of screening, training, testing, and enrollment for the studies.

Surgical procedures have started. The first two subjects have undergone a unilateral lumbosacral ventral root avulsion procedure according to the original plans. These subjects are doing well post-operatively and post-operative measurements, including behavioral, electrodiagnostic, and imaging studies are ongoing. Next, we are ramping up the surgical procedures with the remaining 18 subjects in

the study scheduled to undergo ventral root injury and repair procedures starting in January and finishing in May, 2012. These subjects will include both experimental and control groups, surgery with ventral root injury plus or minus repair using our GDNF-releasing nerve guidance conduits and peripheral nerve grafts. These animals have been clustered within a relatively narrow time frame to facilitate post-operative data collection for treadmill locomotion, MRI and EMG studies, and pain assessments. The start for the surgical procedures for this larger group of animals (n=18 subjects) was also delayed in order to complete the comprehensive set of treadmill training and chair training with locomotor testing and sensory screening of the participating subjects. We are now ready to initiate the comprehensive surgical series, as the subjects will have undergone the comprehensive pre-surgical screening, selection, and testing prior to undergoing the surgical procedures.

KEY RESEARCH ACCOMPLISHMENTS

Development of an algorithm for selection of animals based on behavioral and treadmill locomotor criteria.

Development of a method for obtaining interpretable quantitative EMG recordings from the external anal sphincter pre- and post-operatively. (Again, the findings from control subjects are novel, were submitted in abstract for to the Annual Meeting of the Society for Neuroscience, and are being prepared for a research manuscript and publication.)

Development of comprehensive urodynamic methods, which allow for screening for visceral pain in addition to obtaining functional micturition data. (These novel data will also be prepared for a research manuscript within the next reporting cycle.)

Collection of comprehensive pre-surgical data, including treadmill locomotor studies with an automated digital recording system, imaging of the lumbosacral spinal cord and lower extremity muscles using MRI, collection of urodynamic recordings, EMG recordings of the external anal sphincter, and sensory threshold testing using manual von Frey hair and Electro-von-Frey approaches.

Start of surgical spine procedures with demonstration of the feasibility of the surgical methods, including identification and avulsion of identified select lumbosacral ventral roots. Animals are tolerating the procedures well and are undergoing scheduled post-operative assessments for data collection, which is ongoing.

REPORTABLE OUTCOMES

Most of the reportable data outcomes will be at the end of the post-operative study period, as longitudinal data are being collected, including locomotor behavior, EMG recordings, pain behavioral monitoring, and MRI studies as well as morphological outcome measures after the collection of nerve root and spinal cord tissues. However, many of our collected pre-surgical data are novel and reflect innovation and new knowledge. For instance, our baseline anal sphincter EMG and comprehensive urodynamic recordings are examples of new and original findings.

In May, 2011, we submitted, an abstract on anal sphincter EMG studies for presentation at the Annual Meeting of the Society for Neuroscience in Washington, DC in November, 2011. The abstract was accepted and presented. (See the appendix for the Society for Neuroscience Abstract and supportive Figures 1-4, respectively.)

Data from our baseline EMG recordings of the external anal sphincter are presently being prepared for a manuscript, which is planned for submission to peer-reviewed scientific journal within the next 1-2 months. We also anticipate preparation of a manuscript based on our development of urodynamic studies in the non-human primate within the next reportable time period.

CONCLUSION

Our studies have made significant progress. We have developed an algorithm for pre-surgical testing of non-human primates including: locomotor treadmill studies, pain behavioral assessments, urodynamic recordings, MRI studies, and anal sphincter EMG studies. We have also demonstrated feasibility of a lumbosacral ventral root avulsion procedure with animals recovering well after the surgical spine and spinal cord procedure. Based on the above experience and studies to date, we will continue our work on the project as planned. Over the next few months, the remaining animals will complete pre-surgical testing and undergo ventral root avulsion injury and repair. Thus far, we have not encountered any significant technical or other problems.

We have as a significant finding documented a distinct pattern of EMG responses from the external anal sphincter in rhesus macaques. These responses are different from the responses in humans, who demonstrate continuous EMG activity at rest and decreased or paused activity when the muscle is stretched. Our findings make possible a quantitative assessment of external anal sphincter EMG recordings in the nonhuman primate and will greatly facilitate the interpretation of our ongoing studies as well as provide future investigations with a new valuable tool and outcome measure.

REFERENCES

- Havton LA, Carlstedt T. Repair and rehabilitation of plexus and root avulsions in animal models and patients. *Curr Opin Neurol* 2009, 22: 570-574.
- Hoang TX, Havton LA. Novel repair strategies to restore bladder function following cauda equina/conus medullaris injuries. *Prog Brain Res* 2006, 152: 195-204.5

APPENDICES



Presentation Abstract

Program#/Poster#: 817.05/UU4

Presentation Title: Evoked electromyographic activity in the external anal sphincter muscle of non-human primates differs from corresponding patterns in humans

Location: Hall A-C

Presentation time: Wednesday, Nov 16, 2011, 8:00 AM - 9:00 AM

Authors: ***U. LEE**¹, H. H. CHANG², K. L. CHRISTE⁴, L. A. HAVTON³;
¹UCLA Dept of Urology, Los Angeles, CA; ²Dept of Anesthesiol. & Perioperative Care, Reeve-Irvine Res. Ctr., ³Dept of Anesthesiol. & Perioperative Care, Dept of Neurology, Reeve-Irvine Res. Ctr., Univ. California Irvine, Irvine, CA; ⁴California Natl. Primate Res. Ctr., Univ. California Davis, Davis, CA

Abstract:

Introduction

Concentric needle electromyography (EMG) of the external anal sphincter (EAS) muscle is useful in the diagnosis of sacral neuropathic lesions. Rodent studies of the anal sphincter complex with EMG have been described, and demonstrate quiescence at baseline. Concentric needle EMG of the external anal sphincter in humans demonstrates an active interference pattern at baseline, associated with fecal continence, decreased amplitude after deinnervation injury, and EMG silence during defecation. Detailed anal sphincter EMG firing activity in the non-human primate model is unknown. In the present study, we aimed to develop a method to assess electromyographic activity for the external anal sphincter muscle in non-human primates.

Methods

Electromyography (EMG) of the EAS muscle was performed in neurologically intact adult female rhesus macaque (*Macaca mulatta*) monkeys (n=4 subjects). A pair of EMG electrodes was inserted into the EAS bilaterally. EAS recordings were obtained using ketamine/dexmedetomidine or propofol anesthesia. Baseline EMG recordings were obtained, and EMG activity was induced by inserting a glass probe (10mm, 13 mm, 16 mm, or 20 mm in diameter) into the rectum for a period of 5 seconds.

Results

All baseline EMG recordings showed a pattern of quiescence with absence of any tonic or continuous EMG activity. Introduction of glass probes of increasing size produced a brief period of stretching of the EAS muscle which induced burst-like EMG activity during the insertion and removal of the probe. After the probe removal, the EAS muscle showed continued EMG activity, which gradually declined in amplitude and frequency over the subsequent 10-30 seconds. The evoked response depended in part on the probe size with individual subjects showing a stimulus-response relationship to probes of increasing diameter. Repeated evoked responses were consistent and similar in pattern and duration. Evoked EMG activations were followed by baseline electromyographic silence.

Conclusion

The above pattern of EAS EMG activity in non-human primates is different from the corresponding pattern of continued EMG activity during rest and EMG silence during defecation in humans.

Disclosures: **U. Lee:** None. **H.H. Chang:** None. **K.L. Christe:** None. **L.A. Havton:** None.

Keyword(s): ELECTROPHYSIOLOGY , PRIMATE, SPINAL CORD

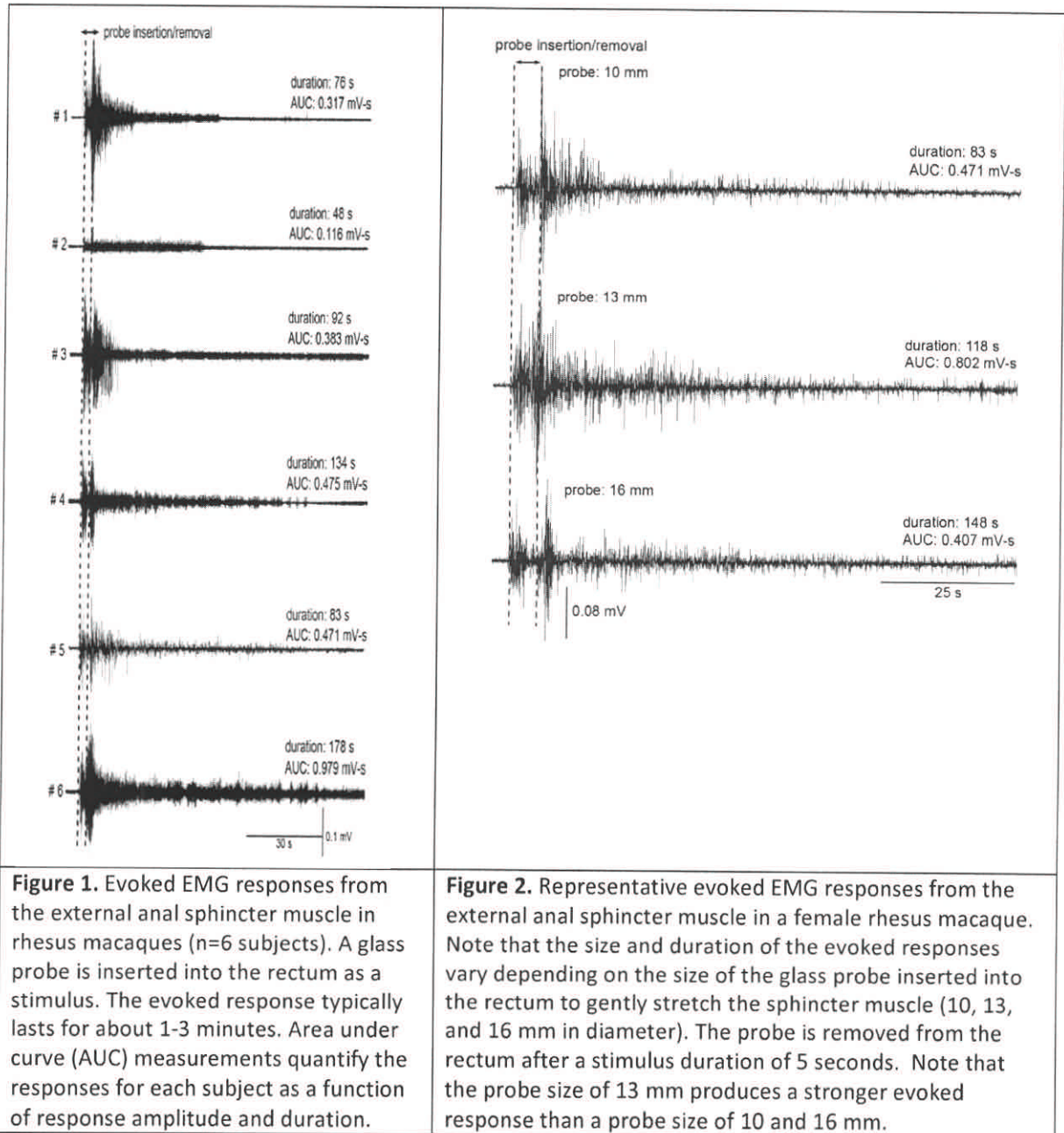
Support: Department of Defense Spinal Cord Injury Research Program

Adelson Medical Research Foundation

[Authors]. [Abstract Title]. Program No. XXX.XX. 2011 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2011. Online.

2011 Copyright by the Society for Neuroscience all rights reserved.
Permission to republish any abstract or part of any abstract in any form must be obtained in writing by SfN office prior to publication.

SUPPORTIVE DATA



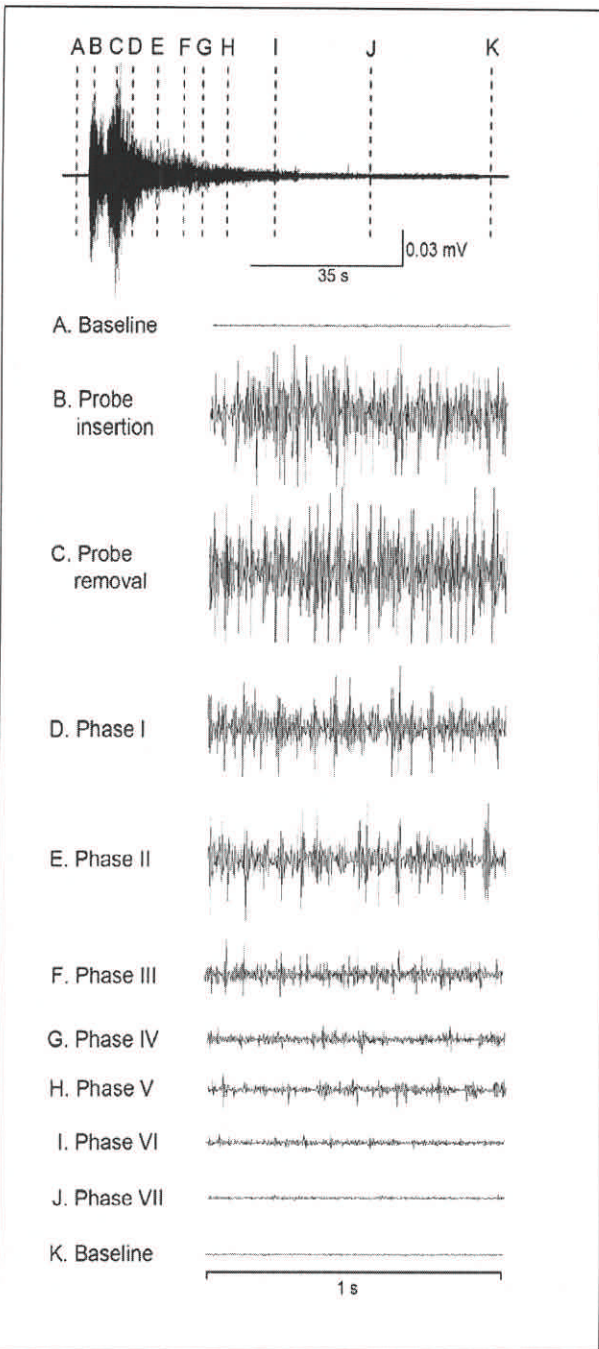


Figure 3. Evoked EMG responses from the external anal sphincter muscle following rectal insertion and removal of a glass probe (10 mm diameter). Stimulus duration was 5 seconds. A-K indicate different time points of stimulus and evoked responses. Note step wise decrease in EMG amplitude until return of quiescent baseline.

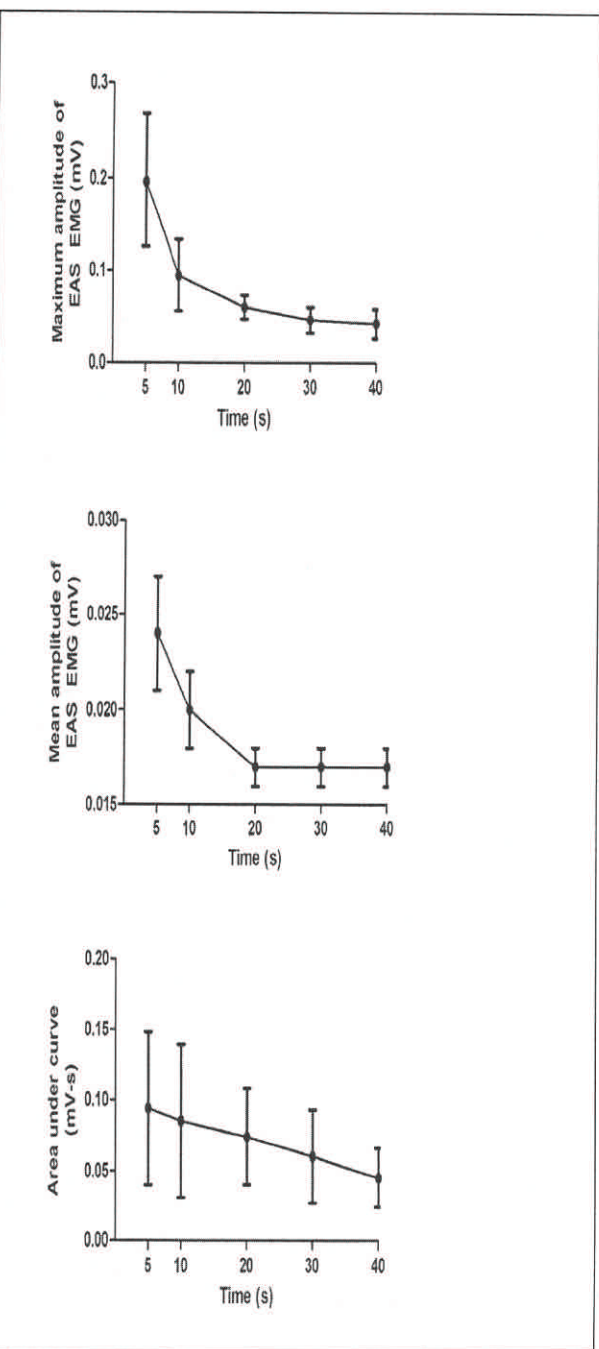


Figure 4. Quantitative studies of evoked EMG responses from the external anal sphincter muscle. The responses are presented as maximum and mean amplitude as well as area under curve measurements over the first 40 seconds after rectal probe presentation. Note gradual decrease of all three outcome measures over time.