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TITLE: Use of GDNF-Releasing Nanofiber Nerve Guide Conduits for the Repair of Conus Medullaris/Cauda Equina Injury in the Nonhuman Primate

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Progress Report Summary of USAMRMC Protocol Number SC090273P2, Award Number W81XWH-10-1-0907 entitled, "Neural Repair in a Primate Model of Cauda Equina Injury"

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 non-human primate using a GDNF-releasing nerve guidance channel. The studies aim 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 planning of future clinical studies on neural repair after cauda equina/conus medullaris injuries. The present report will summarize the major accomplishments within the third year of this 3-year project.

BODY

Our studies have gone well and significant progress was 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 joined, as a member, the Reeve-Irvine Research Center for Spinal Cord Injury Research. In this collaborative partnership project, all three of the participating PIs (Leif Havton at University of California, Irvine, Ahmet Höke at Johns Hopkins University, and I) interact on a regular basis. In particular, Dr. Havton routinely comes to the California National Primate Research Center and has been present for all surgeries, EMGs, and urodynamic studies. Dr. Havton interacts regularly with the participating PIs (myself, Dr. Kari Christe at the California National Primate Research Center at UC Davis and Dr. Ahmet Höke at Johns Hopkins University).

At the onset of the project, research subjects in the form of adult female rhesus monkeys were selected for pre-surgical testing and enrollment in these studies. The selection and screening process was extensive, as behavioral components were very important for the success of these studies. Not all subjects that are initially assessed were able to pass the behavioral criteria needed for successful participation in our study. During the first year of the project, we developed an algorithm to ensure selection of suitable subjects for these studies. While the pre-surgical selection, screening, and testing of our subjects was extensive and time consuming, we were successful in developing a method for optimal subject selection, trained staff to perform the behavioral screening and evaluation, as well as successfully implementing our procedures for animal enrollment and testing. This algorithm for animal selection was also followed during years 2 and 3.

Prior to the start of surgical procedures, each animal underwent extensive screening and training. Behavioral records and profiles are reviewed to select subjects that are likely to cooperate with training.

Next, each animal was introduced to transfers from cage to a carrying cage using a chute. This was followed by the introduction of the treadmill environment, which is enclosed by a plexi-glass cage. Animals were trained to walk on the treadmill belt at various speeds, and the desired behavior was encouraged using various food rewards. The training requires multiple training sessions. Extensive variation between subjects existed with regards to how many sessions that were needed for a subject to be a reliable treadmill walker. Extra time for training was sometimes allocated to accommodate for this individual variation. However, when a subject required additional sessions for treadmill training, delayed the start of the other behavioral training sessions, e.g. chair training for sensory threshold and pain screening.

Successful treadmill training was a requirement for subsequent chair training, which was needed in order to perform sensory testing using von Frey hairs and an Electro-von-Frey device to obtain baseline sensory thresholds pre-operatively. In addition, the chair training was 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 were filmed during treadmill walking to obtain digital recordings for subsequent kinematics analysis.

Electromyography (EMG) recordings of the external anal sphincter were 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 non-human primate was developed by us during this project and reflects innovation and a new outcome measure for future studies in monkeys. The data from control subjects are presently in the late stages of preparation for a research manuscript aimed at being submitted this fall. The analysis of the control data were expanded over the past year by development of customized computer programs to allow for also quantitative analyses of firing frequency as a component of the EMG studies. As a result, our data interpretation has strengthened and markedly improved conclusions made in our research manuscript.

We have also developed a method for urodynamic recordings in the non-human primate. The continued development and refinement of the urodynamic procedures primarily took place during year 2 of the project. This was an achievement as well, as no previously established methods are present in the literature. We developed a method for performing cystometrogram recordings, urethra pressure recordings, and external urethral sphincter EMG recordings in anesthetized subjects. In addition, we developed a method for obtaining external abdominal wall EMG recordings during the 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 presently analyzing the data from the pre-surgical series with the goal of preparing a research manuscript on the normal physiology of the lower urinary tract in non-human primates aimed at being submitted within the next few months. Data collected during the post-operative phase are being collected as well and will be prepared for subsequent manuscripts on injury and repair studies in this translational research model. For manuscripts that include the urodynamic and other behavioral or

physiological data collected from post-operative subjects, the final data interpretation and preparations of research manuscripts will follow the completion of data collection from the last post-operative animals, and this last post-operative data collection will take place in March, 2014.

Magnetic resonance imaging (MRI) was performed of the lower spine to visualize the lumbosacral spinal cord and associated nerve roots as well as 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 allow us to monitor nerve root degeneration and axonal regeneration associated with the cauda equina injury and repair. The MRI studies may be able to identify successful muscle reinnervation prior to functional improvement taking place.

All 20 subjects, adult female rhesus monkeys, were 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.

Surgical procedures were with the majority of subjects operated on in year 2. These subjects included both experimental and control groups, including subjects undergoing surgery with ventral root injury and repair using our GDNF-releasing nerve guidance conduits and peripheral nerve grafts. Our comprehensive outcome measures, included pain testing, locomotor treadmill studies, urodynamic studies, EMG recordings, and MRI studies.

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. Development of comprehensive urodynamic methods, which allow for screening for visceral pain in addition to obtaining functional micturition data.

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.

Demonstration that surgical use of peripheral nerve grafts and of GDNF-releasing nerve guidance conduits are feasible as bridges between the spinal cord and avulsed ventral roots in the non-human primate. The surgical procedures were tolerated well.

REPORTABLE OUTCOMES

Most of the reportable data outcomes are currently being analyzed; they include 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, do not forget many of our collected pre-surgical data were 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.

We are currently preparing a manuscript describing evoked activation of external anal sphincter EMG activity in rhesus macaques and provide support and rationale for these studies to serve as quantifiable outcome measures in primate spinal cord injury and repair models. Detailed analyses of signal processes here have allowed for both amplitude and frequency based quantitative studies.

We are also preparing a manuscript that demonstrates feasibility of performing comprehensive urodynamic studies in non-human primates, and that such functional outcome measures of micturition reflexes are suitable for studies of spinal cord injury and repair in non-human primates. Here, evoked bladder contractions and voiding are determined using both cystometrogram recordings and urethra pressure recordings, and subsequent calculations for voiding efficiency is performed.

During year 3, we presented at two national scientific meetings:

Lee U, Chang HH, **Christe KL**, Havton LA. Evoked voiding contractions and corresponding urethral sphincter electromyography in non-human primates differ from rodents. American Urological Association Annual Meeting, San Diego, CA, May 4-8, 2013.

Ohlsson M, Nieto JH, Villablanca P, **Christe KL**, Havton LA. Perioperative imaging studies and intra-operative EMG monitoring as guide for surgical repair of avulsed ventral roots using intercostal nerve grafts in rhesus macaques. Annual Meeting of the Society for Neuroscience, 2013, 43.

During year 3, we published one paper with a number of other papers in preparation.

Christe, Kari L, Lee Una L, Lemoy, Marie-Josee, and Havton, Leif A, "Generalized Seizure Activity in Association with Ketamine Anesthesia and Urodynamic Studies in an Adult Rhesus Monkey (*Macaca mulatta*), Comparative Medicine, October 2013, Vol 63, No 5; 1-3.

CONCLUSION

Our studies have made significant progress and the data collected is still being analyzed for more information. We developed an algorithm for pre-surgical testing of nonhuman primates, including locomotor treadmill studies, pain behavioral assessments, urodynamic recordings, MRI studies, and anal sphincter EMG studies. We demonstrated feasibility of a lumbosacral ventral root avulsion procedure with animals recovering well after the surgical spine and spinal cord procedure. We also demonstrated

the feasibility of using peripheral nerve grafts and GDNF-releasing nerve guide conduits to bridge tissue gaps between the spinal cord and avulsed ventral roots. All surgeries for the studies (20 subjects) were completed in year 2. During year 3, we continued our ongoing collection of comprehensive functional and imaging data for the planned study time of 18 months for each subject. The data collection will be completed by March, 2014, and ongoing data analysis of collected and incoming behavioral, physiological, imaging, and anatomical data will be prepared for manuscripts involving post-operative subjects in the Spring, 2014. Manuscripts involving new data collected from control subjects are presently being prepared for manuscripts, which are aimed for submission to peer-reviewed journals in the Fall and Winter, 2013.

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.

SUPPORTIVE DATA (next page)



