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RESEARCH ARTICLE

Sensorimotor plasticity after spinal cord injury: a longitudinal and translational study

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Abstract

Objective: The objective was to track and compare the progression of neuroplastic changes in a large animal model and humans with spinal cord injury. **Methods**: A total of 37 individuals with acute traumatic spinal cord injury were followed over time (1, 3, 6, and 12 months post-injury) with repeated neurophysiological assessments. Somatosensory and motor evoked potentials were recorded in the upper extremities above the level of injury. In a reverse-translational approach, similar neurophysiological techniques were examined in a porcine model of thoracic spinal cord injury. Twelve Yucatan mini-pigs underwent a contusive spinal cord injury at T10 and tracked with somatosensory and motor evoked potentials assessments in the fore- and hind limbs pre- (baseline, post-laminectomy) and post-injury (10 min, 3 h, 12 weeks). **Results**: In both humans and pigs, the sensory responses in the cranial coordinates of upper extremities/forelimbs progressively increased from immediately post-injury to later time points. Motor responses in the forelimbs increased immediately after experimental injury in pigs, remaining elevated at 12 weeks. In humans, motor evoked potentials were significantly higher at 1-month (and remained so at 1 year) compared to normative values. **Conclusions**: Despite notable differences between experimental models and the human condition, the brain's response to spinal cord injury is remarkably similar between humans and pigs. Our findings further underscore the utility of this large animal model in translational spinal cord injury research.

Introduction

Traumatic spinal cord injury (SCI) is a devastating neurological event, characterized by varying severities of motor, sensory, and autonomic impairment.[1–3] Beyond frank neurological deficits, a hallmark of damage in the spinal cord is robust anatomical and physiological changes in the brain.[4–8] Based on the timeframe by which changes

occur, different mechanisms have been proposed, including unmasking of latent pathways (i.e., immediate) and anatomical sprouting (i.e., progressive).[9]

Current knowledge regarding the progression of cortical reorganization is, however, largely limited to animal studies. As a result, the degree to which similar mechanisms underlie reorganization in humans is mostly unknown. A further complicating factor is that animal studies typically adopt invasive recording techniques, which are rarely applicable in humans.

The primary aim of this study was to investigate the progression of neuroplastic changes in a large animal model (i.e., pigs) and humans with spinal cord injury using common neurophysiological techniques. The fundamental goal was to determine if changes in the brain of pigs were similar to that observed in humans.

Materials and Methods

Ethics, consent, and permissions

Both studies (i.e., human and animal) are in accordance with the Declaration of Helsinki. The human study was approved by the responsible institutional review board (EK-03/2004; PB_2016-00293). The animal study was approved by our institution's animal care committee (University of British Columbia Animal Care Committee, Protocol Number A13-0013).

Human data: data source and selection

Patient data

The European Multicenter Study about Spinal Cord Injury (EMSCI) was reviewed to identify individual with SCI eligible for our study. EMSCI is a longitudinal observational study comprising 19 participating trauma and rehabilitation centers from across Europe. A variety of neurological and functional outcomes are tracked at fixed time points over the first year of injury (i.e., 1, 3, 6, and 12 months). All individuals enrolled in EMSCI obtain standards of rehabilitation care. Further details on the EMSCI database can be found elsewhere (www.emsci.org, ClinicalTrials.gov Identifier: NCT01571531).

To be included in our analysis, individuals with SCI had to meet the following inclusion criteria: (1) SCI as a result of a single traumatic event, (2) the first neurophysiological assessment (i.e., somatosensory [SSEPs] and motor evoked potentials [MEPs]) conducted within 4 weeks following injury, and (3) neurologic level of injury at or below C8. The rational for the selection of low cervical/high thoracic neurological level of injury arises from the study's objective to investigate the changes of brain areas representing intact body parts by performing electrophysiological measurements in the unaffected upper extremities. The ulnar nerve has spinal root entries at C8 and T1 (Fig. 1). Neurological levels of injury at and below C8 allow the assessment of intact upper limb SSEPs

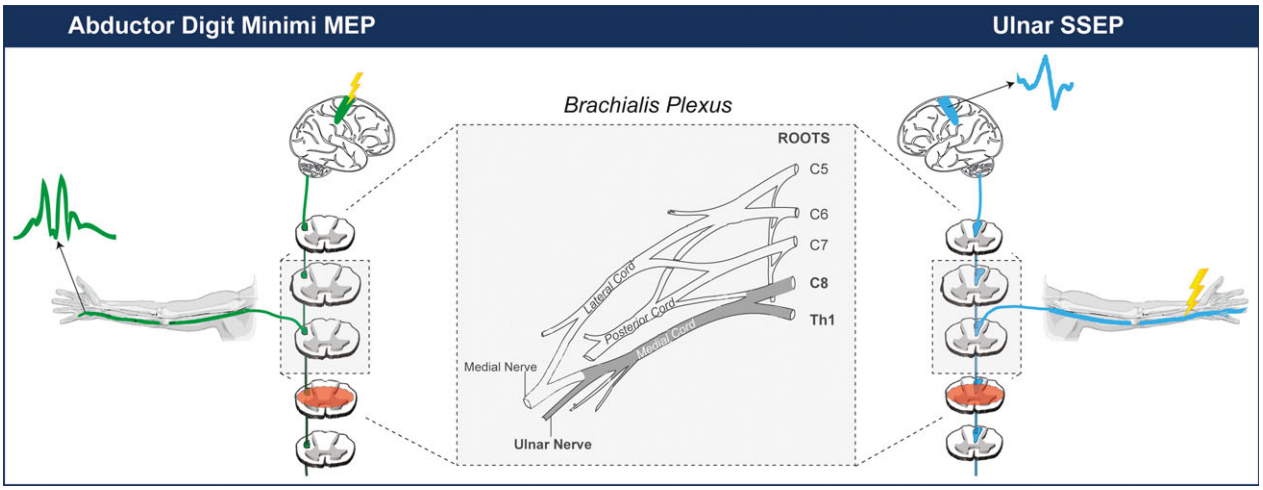

Figure 1. Brachial plexus anatomy and assessments of sensorimotor evoked potentials. The ulnar nerve originates from the C8-T1 nerve roots forming, in part, the medial cord of the brachial plexus. It also innervates the abductor digiti minimi. In response to electrical stimulation, evoked potentials are generated by the transmission of the afferent (somatosensory evoked potentials) or efferent (motor evoked potentials) volleys between the periphery and the cortex. Thus, somatosensory and motor evoked potentials provide unique indices of the integrity of the afferent and efferent volley in spinal, brain-stem, and thalamocortical pathways, as well as primary sensorimotor cortical regions. By virtue of the anatomical arrangement of the ulnar nerve, damage to the spinal cord at or above C8 will result in impaired somatosensory and motor evoked potentials. However, damage below C8 facilitates the recording of normal ulnar somatosensory and motor evoked potentials (i.e., intact pathways).

and MEPs. Exclusion criteria constituted other non-traumatic SCI (e.g., tumor), dementia or severe reduction of intelligence leading to reduced capabilities of cooperation or giving consent, polyneuropathy, and brain injury. Prior to the inclusion in the EMSCI, all individual with SCI gave their written informed consent. The study is in accordance with the Declaration of Helsinki and was approved by all responsible institutional review boards.

Healthy control data

The laboratory at the University Hospital Balgrist established a set of normative data for SSEPs and MEPs amplitudes and latencies in the upper and lower extremities. Derived from this in-house data set (unpublished), a representative sample was used for statistical comparison to observations in the SCI cohort.

Rehabilitation therapy standard of care for patients with SCI within EMSCI

Therapy programs are individually adapted to each patient and depend on their individual functional abilities and deficits. On average and if tolerated by the patient, the total amount of daily therapy time amounts to 180–240 min/day, 5 days a week including training of self-care and independence, physiotherapy and occupational therapy. Patients continuously undergo re-evaluations to assure progress and to adapt the training program to their changing needs. Rehabilitation lasts on average 3–6 month in paraplegic, respectively, 6–9 month in tetraplegic patients.

Outcome measures

Neurophysiological assessments (i.e., tibial and ulnar SSEPs, *abductor digiti minimi* MEPs) were performed at four fixed time points: 1, 3, 6, and 12 months post-injury (Fig. 2A). The N20 latency and N20-P25 amplitude of the ulnar and the N40 latency and N40-P46 amplitude of the tibial SSEPs were the primary outcome variables, assessed separately at each of the four time-points. All assessments were performed by trained examiners and in accordance with international standards.[10,11] Briefly, MEPs were recorded by applying single pulse transcranial magnetic stimulation (TMS) using a routine circular coil magnetic stimulator. For the adductor digiti minimi MEPs (hereafter referred as upper limb MEPs), the stimulation hot spot was determined by stepwise optimizing coil position to obtain a maximum MEP response. Upper limb MEPs were recorded at 1.2 times active motor threshold. Three to five representative upper limb MEPs at the desired stimulus intensity were applied. Tibial and ulnar SSEPs were elicited by single 0.2 msec, repetitive, square wave electrical stimulation (3 Hz) using a Key Point electrophysiological stimulating and recording device (band-pass = 2 Hz to 2 kHz; Medtronic, Mississauga, Ontario, Canada). A total of 600 stimuli (2 × 300) were averaged for the visual detection of the N20-P25 (ulnar) and N40-P46 (tibial) waveforms, respectively. Standard clinical surface gel electrodes (10 mm) were positioned on the tibial nerve at the ankle and ulnar nerve at the wrist. SSEPs were collected at a stimulus intensity that adequately produced a consistent but tolerated muscle twitch.[10,12,13] Lastly, lower extremity motor scores were assessed at each timepoint according to the international Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI).[14]

Animal data: study design

Figure 2B illustrates the study design of the animal experiments. Briefly, the animals underwent behavioral training for 5 days. Subsequently, the baseline behavioral measurement was performed on the day before the surgery. Follow-up behavioral measurements were conducted weekly for 12 weeks starting within 7 days after the surgery to first allow the animals to recover from their initial surgery/injury. On the day of surgery, animals were anesthetized and intubated. Baseline SSEPs and MEPs were recorded prior to initiating the surgical procedures, which included a dorsal exposure to the thoracic spine and laminectomy around T10. Following laminectomy, SSEPs and MEPs were recorded again in order to ensure that the spinal cord was not damaged. The SCI was then induced by contusing and compressing the spinal cord. Specifically, a 50-g weight was dropped from 20 cm height to the exposed spinal cord at the 10th thoracic vertebrae. This was followed by 5 min of compression placing a 150 g weight on the spinal cord. SSEPs and MEPs were recorded immediately after the compression (~10 min after the weight drop contusion injury). All animals underwent follow-up assessments of SSEPs and MEPs at 3 h and 12 weeks post-injury. For all surgical and neurophysiological procedures, animals were anesthetized.

Surgical procedures with animals

Fourteen female Yucatan miniature pigs (Sinclair Bio-Resources, Columbia, MO) were housed, fed, and cared for in accordance with the Canadian Council for Animal Care regulations. The study was approved by our institution's animal care committee (University of British Columbia Animal Care Committee, Protocol Number A13-0013). Female animals were selected as the

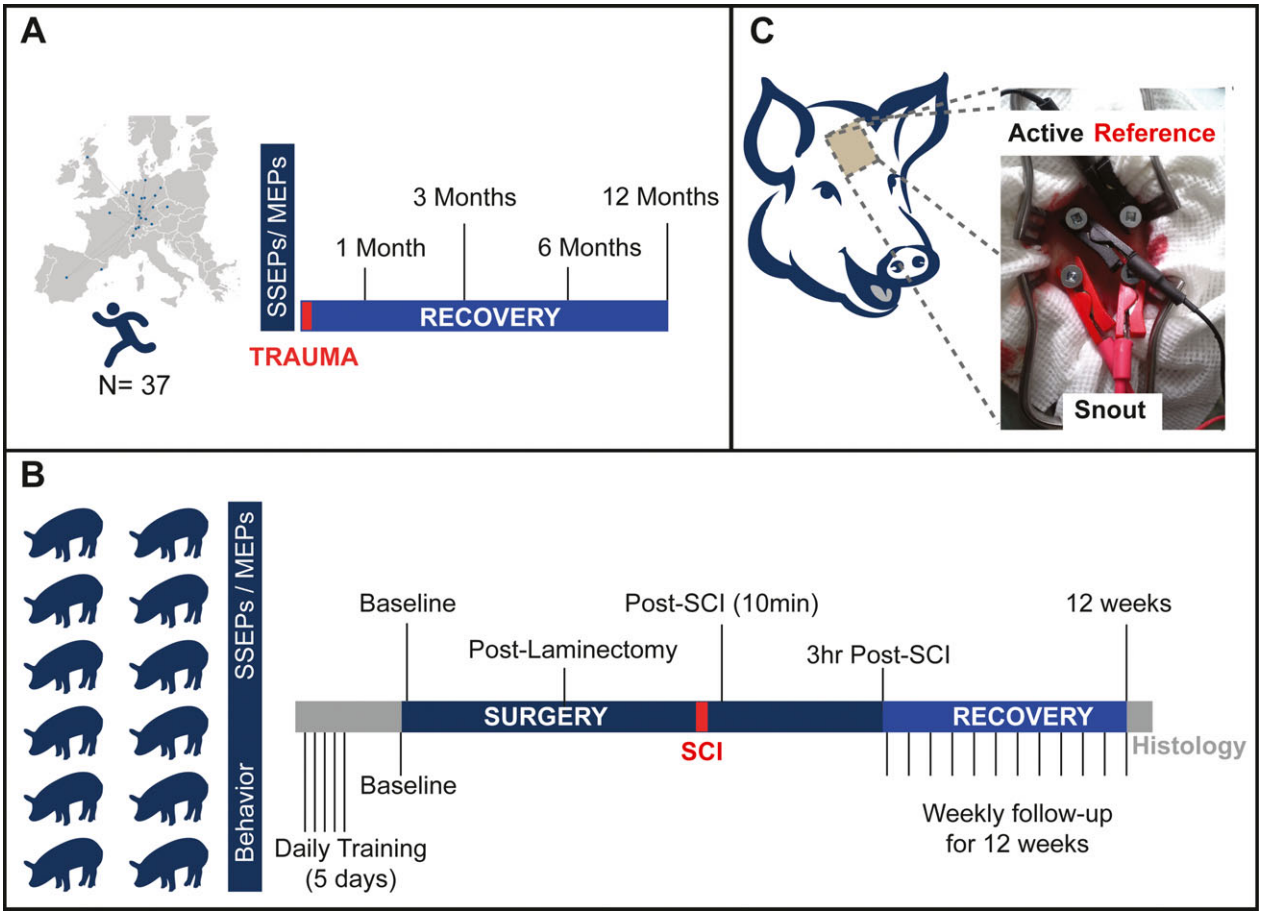

Figure 2. Design of human and animal study. (A) A total of 37 patients with spinal cord injury were enrolled in the study and meticulously followed up for a year. Neurophysiological (somatosensory and motor evoked potentials) and behavioral assessments (sensory and motor score) were performed 1, 3, 6, and 12 months post-injury. (B) Twelve female Yucatan miniature pigs underwent behavioral training for 5 days. Subsequently, the baseline measurement was conducted on the day before the surgery. Follow-up measurements were conducted weekly for 12 weeks starting 7 days after the surgery allowing the animals to recover. On the day of surgery, animals were anaesthetized and intubated. Prior to the surgical procedures baseline somatosensory and motor evoked potentials were recorded. Following laminectomy, somatosensory and motor evoked potentials were recorded again in order to ensure that the spinal cord was not damaged. The spinal cord injury was then induced by contusing and compressing the spinal cord. Somatosensory and motor evoked potentials were recorded immediately after the compression. All animals underwent follow-up assessments of somatosensory and motor evoked potentials at 3 h and 12 weeks post-injury. (C) Experimental set-up of the neurophysiological assessment in pigs. Four screws served as recording and stimulation electrodes (black = active, red = reference). To reduce the high impedance (i.e., due to the thickness of the pig's skull) the screws were drilled in to skull.

management of the bladder and urethra necessitated due to SCI is far less complicated in females compared to the male animals. Anesthesia was induced with propofol (1.6–7 mL). Pigs were intubated, ventilated, and maintained with a combination of fentanyl (20 ± 2.8 μg/kg/h), propofol (21.4 ± 6.1 mg/kg/h), and ketamine (9.8 ± 1.7 mg/kg/h). After the animal was anesthetized, the external jugular vein was catheterized (i.e., central line catheter) for the delivery of drugs and a rectal temperature probe was inserted to monitor the core temperature. The animals were covered with a surgical drape with a window above the surgical site.

Screw placement for neurophysiological recordings

For placement of the scalp electrodes, a 10-cm midline longitudinal incision was made at the level of the ears extending anteriorly toward the snout. Dissection was carried to the level of the bone until the sagittal and coronal sutures were visualized. Four electrodes were attached 1 cm lateral to the sagittal suture and 1 cm anterior and posterior to the coronal suture (Fig. 2C). To reduce the high impedance (i.e., due to the thickness of the pig's skull) and improve both motor cortex stimulation and signal recordings from

the somatosensory cortex, screws were placed into the skull. The skull was carefully drilled bi-cortically to a standard depth of 4 mm. Sterilized stainless steel screws (15 mm length, 1.1 mm diameter) were inserted and connected to alligator clips. To avoid complications such as infection, the electrode screws were removed at the end of neurophysiological recordings (i.e., 3 h post-injury) and the skin was approximated with reverse cutting prolene suture. The electrode screws were reinserted at the same location for the 12-week follow-up measurement.

Laminectomy and induction of SCI

The laminectomy has been previously described in detail.[15] After the T10 laminectomy, the weight-drop device was rigidly secured to the pedicle screws and positioned so that the impactor (mass: 50 g) would fall directly on the exposed dura and spinal cord at vertebrae T10. The tip of the impactor (diameter: 9.53 mm) was instrumented with a load cell (LLB215, Futek Advanced Sensor Technology, Irvine, CA) to record the force at impact. Immediately following the contusion injury (drop height: 20 cm), compression was applied by placing a 100 g mass on top of the impactor for 5 min. Subsequently, the weight-drop apparatus was removed.

Somatosensory evoked potentials

SSEPs were recorded in response to the stimulation of the left and right tibial nerve (i.e., below the level of contusion) as well as the right median nerve (i.e., above the level of contusion). Electrical stimulation comprised repetitive square wave (0.5 msec) pulses delivered at 3.1 Hz using sub-dermal needle electrodes (1.2 cm, 27 Gauge, Neuroline twisted pair, Ambu, Copenhagen, Denmark). A total of 600 stimuli (2 × 300) were delivered using a Keypoint electro-diagnostic device (Medtronic, Mississauga, Ontario, Canada; bandpass = 2 Hz–2 kHz). Stimuli were given at intensities four times the threshold required to visualize twitching in the muscles distal to the stimulation point. For each individual animal, the stimulation intensity was determined at baseline (i.e., control condition). The same intensity was applied for all testing sessions. SSEPs were recorded unilaterally from the skull screws of the cortex contralateral to the stimulated nerve.

Motor evoked potentials

Bilateral depolarization of the motor cortex was achieved by delivering stimulation trains through stainless steel alligator clips clamped to the electrode screws. Stimuli intensities ranged from 80 to 100 mA in 10–15 trains of 5 pulses (pulse duration = 0.5 msec, Interstimulus Interval = 0.5 msec) and were delivered by a Keypoint electro-diagnostic device (Medtronic, Mississauga, Ontario, Canada). Small bipolar needles (1.2 cm, 27 Gauge, Neuroline twisted pair, Ambu, Copenhagen, Denmark) were placed in the muscles of the right forelimb (*Extensor Carpi Radialis*) as well as left and right hindlimbs (*Tibialis Anterior*). Signal recorded from the muscles was amplified and then band-pass filtered at 30 Hz to 1 kHz, which is in accordance with set-up of human MEP recordings.[10]

Porcine thoracic injury behavior scale

Upon 4 days of acclimatization and habituation to the large animal facility, animals were handled daily for 5 days (each day 15 min) to become familiar with experimental handling. For the subsequent 5 days, animals were trained daily (each day 15 min) to walk non-stop up and down a rubber mat (width 1.22 m, length 5 m). For the behavioral assessment, animals walked back and forth on the mat five times. The hindlimb function was analyzed while walking and given a score of 1–10 according the Porcine Thoracic Injury Behavioral Scale.[16] Animals did not undergo any additional physical training (e.g., treadmill) pre- or post-injury.

Spinal cord histology

Twelve weeks post-injury all animals were euthanized and the spinal cord was harvested, post-fixed, and cryoprotected as described previously.[15] Subsequently, spinal cords were cut into 1 cm segments centered on the injury site, embedded in OCT blocks and frozen at −80°C before being cut into 20 μm thick cross cryosections. Sections were serially mounted onto adjacent silane-coated SuperFrost-Plus slides (Fisher Scientific, Pittsburgh, PA) such that sections on the same slide were obtained from tissue 400 μm apart and stored at −80°C. For differentiating gray and white matter, Eriochrome Cyanine R histochemistry was performed. Neutral Red was used as a counterstain. ECR-stained sections were examined, and pictures (5× objective) were taken of sections at 800 lm intervals throughout the lesion site (Zeiss AxioImager M2 microscope, Carl Zeiss Canada Ltd., Toronto, ON, Canada). Images were analyzed using Zen Imaging Software (Carl Zeiss Canada Ltd., Toronto, ON, Canada), by manually tracing the spinal cord perimeter and spared tissue for each image captured. The spared white matter was defined as the areas that were stained for Eriochrome Cyanine R, whereas gray matter was considered spared when it was a stereotypic light gray color with a consistent neuropil texture. The percentages of white matter and gray matter were calculated by dividing the spared

white or gray matter by the total area of the spinal cord on a given section.

Statistical analyses

All statistical procedures were performed using IBM's Statistical Package for the SocialSciences (SPSS) version 23.0 (Armonk, New York, USA). For all analyses, $P < 0.05$ was considered as statistical significance. To take into account the longitudinal nature of the data (animal and human) and adjust for potential confounders, as well as to handle missing data, the primary analysis comprised a linear mixed effects model. Post-hoc analyses were performed and Bonferroni corrections were applied to adjust for multiple comparisons.

For the human data, the analysis focused on various primary-dependent variables: Latencies and amplitudes of MEPs, tibial and ulnar SSEPs (6 models). The time-points of assessment (i.e., 1, 3, 6, and 12 months), age at baseline, and lesion completeness (i.e., complete or incomplete) were included as independent variables. In a planned sub-analysis, the MEP parameters of the individuals with SCI at all time points were compared to the healthy control cohort using a linear mixed model.

The primary outcomes of the animal study were SSEP and MEP latencies and amplitudes (both hind- and forelimb) at pre-defined time-points: baseline, post-laminectomy, as well as 10 min, 3 h, and 12 weeks post-injury. In separate linear mixed models, latencies and amplitudes of SSEPs and MEPs were set as dependent variable, while time-points were included as independent variables. We further examined the amount of motor recovery following SCI employing a linear mixed model. In all models, subjects were included as random factor. Pearson correlation analyses were used to identify relationships between neurophysiological (SSEPs and MEPs), behavioral (Porcine Thoracic Injury Behavioral Scale Score), and immunohistochemistry outcomes (spared white and gray matter).

Results

Human data

Basic demographics and other characteristics of the study sample at all time-points after injury are summarized in Table 1. A total of 37 individuals with SCI from the EMSCI database were included in the analysis.

Table 1. Demographics and neurophysiological data of all individuals with spinal cord injury.

Characteristics		
Total	37	
Sex, *n* (%)		
Male	28 (75.7)	
Female	9 (24.3)	
Age at Injury		
mean (SD)	51.8 (18.9)	
AIS* at baseline, *n* (%)		
A	6 (16.2)	
B	5 (13.5)	
C	4 (10.8)	
D	22 (59.5)	
Neurological level of injury at baseline, *n* (%)		
C8	26 (70.3)	
T1	8 (21.6)	
T2	2 (5.4)	
T3	1 (2.7)	
Functional data	Mean (SD)	
Total lower extremity motor score at		
1 month	23.1 (19.0)	
3 months	28.5 (20.3)	
6 months	29.5 (20.6)	
12 months	30.5 (20.1)	
Neurophysiological data	Mean (SD)	Mean (SD)
Motor evoked potentials (ADM)	Amplitude [mV]	Latency [msec]
1 month	2.3 (0.8)	23.6 (6.6)
3 months	2.4 (1.3)	22.9 (4.1)
6 months	2.8 (0.4)	24.0 (5.0)
12 months	2.7 (0.6)	22.6 (2.3)
Sensory evoked potentials (Tibial)	Amplitude [μV]	Latency [msec]
1 month	1.0 (1.3)	54.9 (1.0)
3 months	1.0 (0.9)	55.1 (0.9)
6 months	1.4 (1.2)	55.5 (1.4)
12 months	1.2 (1.1)	54.2 (1.3)
Sensory evoked potentials (Ulnar)		
1 month	1.7 (0.8)	26.2 (3.6)
3 months	2.2 (0.9)	26.6 (3.3)
6 months	2.6 (0.8)	26.1 (3.0)
12 months	2.7 (0.9)	26.3 (4.7)

ADM, abductor digiti minimi, SD, standard deviation.

*American Spinal Injury Association Impairment Scale: A, no sensory or motor function is preserved; B, sensory function is preserved below the level of the injury, but there is no motor function; C, motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade of <3; D, motor function is preserved below the neurological level, and at least half of the key muscles below the neurological level have a muscle grade of ≥3.

Somatosensory and motor evoked potentials above the spinal cord injury level

Ulnar SSEP amplitudes increased significantly over time ($F = 4.6$, df: 3, $P = 0.004$), while the SSEP latencies remained unchanged ($F = 1.0$, df: 3, $P = 0.392$). Pair-wise comparisons yielded an increase in amplitudes at 3 and 6 months compared to the recordings at 1 month (Fig. 3A). The average increase in amplitudes was +33.0%, +47.8%, and +65.2% at 3, 6, and 12 months, respectively.

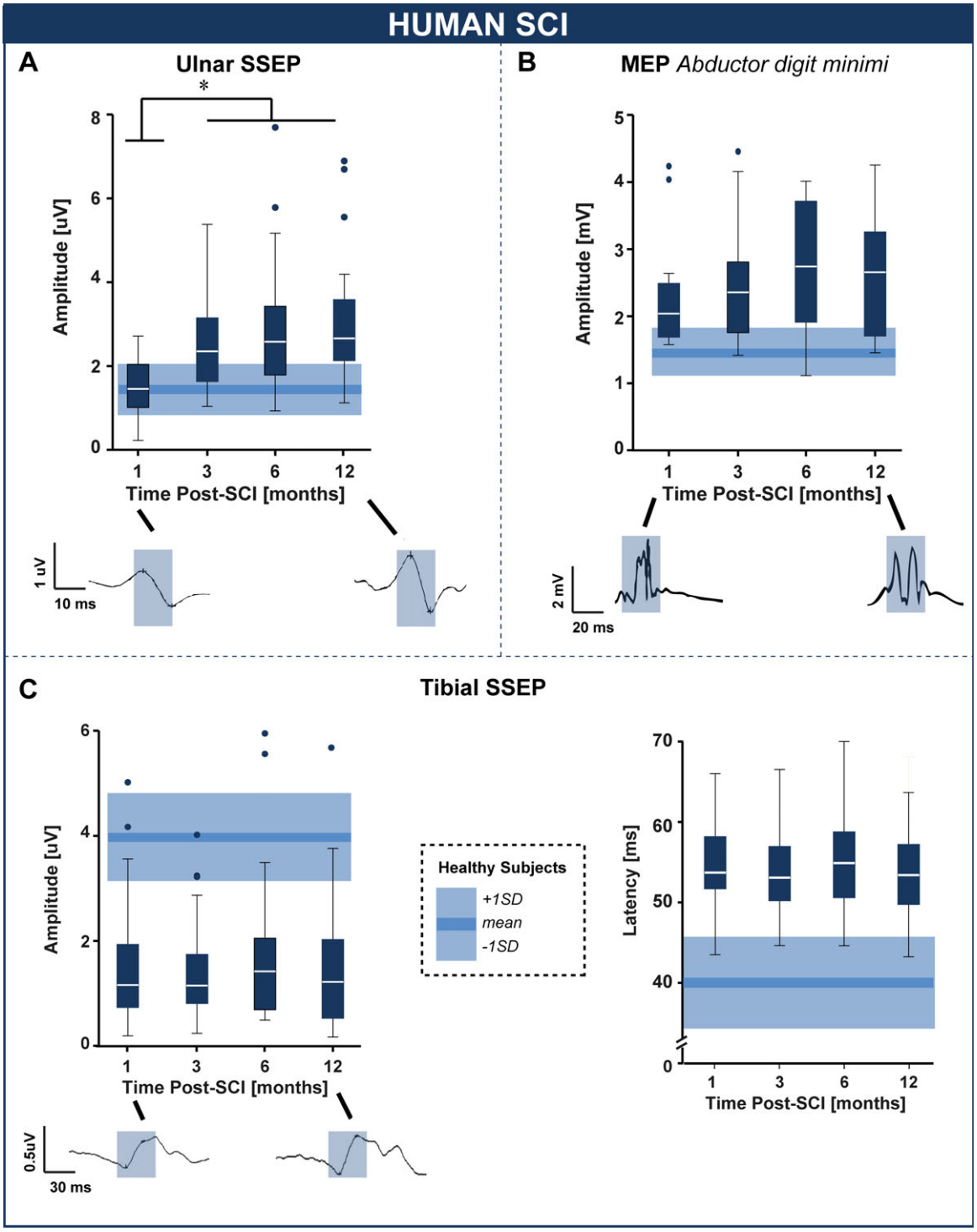

Figure 3. Neurophysiological assessments in human patients. (A) Human ulnar somatosensory evoked potential amplitudes increased over time independent of the injury severity. (B) Motor evoked potentials remained stable independent of the injury severity. In comparison to healthy controls, the motor evoked potential amplitudes in patients was elevated. (C) Temporal progression tibial somatosensory evoked potentials in patients with spinal cord injuries. In comparison to healthy controls, the tibial somatosensory evoked potentials remained impaired over time. Specifically, smaller amplitudes and prolonged latencies hallmarked the patient population. AIS Scale: A – no motor or sensory function preserved below the level of lesion, B – sensory but not motor function is preserved, C and D – Motor and sensory function is preserved, but impaired to variable degree.[14]

There was no main effect of injury completeness on ulnar SSEP amplitudes ($F = 1.3$, df: 1, $P = 0.256$) and latencies ($F = 0.119$, df: 1, $P = 0.730$), suggesting independence from injury severity. There was also no interaction effect between injury completeness and time on amplitudes ($F = 0.536$, df: 2, $P = 0.586$) and latencies ($F = 0.129$, df: 2, $P = 0.879$). This indicates that severity did not impact amplitudes and latencies as a function of time.

There was no main effect of time or injury completeness on the upper limb MEP amplitudes (Time: $F = 1.571$, df: 3, $P = 0.200$; Completeness: $F = 2.400$, df: 3, $P = 0.124$) or latencies (Time: $F = 0.536$, df: 3, $P = 0.155$; Completeness: $F = 0.915$, df: 3, $P = 0.549$) (Fig. 3B). At the first assessment (1-month post-injury), the upper limb MEP amplitudes were already significantly higher in individuals with SCI (mean = 2.3, SD = 0.8) compared to healthy controls (mean = 1.4, SD = 0.6; Conditions $t = 2.88$ and $P = 0.02$). Over time during subsequent assessments there was no significant change. MEP latencies were not significantly different from healthy controls at 1 month post-injury ($t = 0.891$, $P > 0.05$) and also did not change significantly over time.

Somatosensory evoked potentials below the spinal cord injury level

We found a main effect of lesion completeness on tibial SSEP amplitudes ($F = 7.3$, df: 1, $P = 0.031$), but not on latencies (Time: $F = 0.8$, df: 3, $P = 0.506$; Completeness: $F = 0.22$, df: 1, $P = 0.882$). Post-hoc analyses revealed a significant increase in amplitudes at 3, 6, and 12 months compared to 1 month (Fig. 3C). Individuals with SCI with sensory incomplete lesions (AIS B-D) exhibited an increase in tibial SSEP amplitudes, while the amplitudes did not change over time in individuals with SCI with complete lesions (AIS A). This observation is in line with previous reports.[12]

Motor recovery

In the present cohort of patients, there was a significant main effect of time on the LEMS scores ($F = 9.54$, df:3, $P < 0.001$). That is, motor function recovered on averaged by 6.4 points from 2 weeks to 6 months post-injury (Table 1). The effect of recovery persisted after adjusting for level of lesion and completeness (i.e., AIS Score) ($F = 7.34$, df: 3, $P < 0.001$).

Animal data

A total of 14 animals underwent experimental SCI. All animals received a contusion injury by dropping a 50-g mass from 20 cm height at the T10 level of the spinal cord. The mean (standard deviation) impact force applied to the exposed spinal cord measured at the tip of the impactor was 2731 (514) kdynes. All animal characteristics are summarized in Table 2. Two animals had to be euthanized within the first 24 h after surgery due to unexpected upper airway compromise and thus, were excluded from the data analysis. Complications related to the cortical stimulation procedures, such as damage due to screw placement or neurological deficits, were not observed upon post-mortem examination. In order to prevent a drug-effect on the neurophysiological outcomes, animals received the same anesthesia on both days (i.e., induction of SCI and follow-up at 12 weeks). Individual animal data are presented in Table S1.

Somatosensory and motor evoked potentials above the spinal cord injury level

Determined at baseline, the stimulation intensities for the acquisition of SSEPs ranged between 4.0 and 6.5 mA (kept the same for the consecutive measurements). There was a significant main effect of time on ulnar SSEP amplitudes ($F = 5.7$, df: 4, $P = 0.001$, Fig. 4A). Pair-wise comparisons yielded a significant increase (+64.3%) in amplitudes at 12 weeks post-injury compared to baseline ($P = 0.006$). The latencies of ulnar SSEPs in acutely injured animals (10 min and 3 h post-injury) were comparable to the pre-injury recordings ($F = 0.78$, df: 4, $P = 0.895$). The latencies in the chronic state of injury (12 weeks) remained comparable to the pre-injury recordings ($F = 0.82$, df: 4, $P = 0.771$).

Immediately following the weight drop contusive SCI, the forelimb MEP amplitudes (i.e., *Extensor Carpi Radialis*) increased 365.7% ($P = 0.033$) compared to both pre-injury time-points (Fig. 4B). The amplitudes remained higher at all post-injury time-points (3 h post-SCI: +337%, $P = 0.049$, 12 weeks post SCI: +334%, $P = 0.046$). The linear mixed model confirmed that there was indeed a statistically significant main effect of time on forelimb MEP amplitude ($F = 5.0$, df: 4, $P = 0.006$). No change in latencies were observed at any time-point (all $P > 0.05$).

Somatosensory evoked potentials below the spinal cord injury level

Tibial SSEPs were abolished following SCI induction and did not recover, confirming the completeness of injury (Fig. 4C). All animal latencies and amplitudes of medial and tibial SSEPs are summarized in Table 2.

Table 2. Animal characteristics, behavioral, and neurophysiological outcomes.

Characteristics		
Total, *n*	12	
Weight, kg		
Range	18.5–25	
Age, days		
Range	125–142	
Anesthesia (SCI induction), mean (SD)		
Hydromorphone (mg/kg/hr)	0.1 (0)	
Propofol (mg/kg/h)	19.5 (1.4)	
Ketamine (mg/kg/h)	9.8 (1.7)	
Fentanyl (μg/kg/h)	20.0 (2.8)	
Anaesthesia (Follow-up), mean (SD)		
Hydromorphone (mg/kg/hr)	0.1 (0)	
Propofol (mg/kg/h)	21.7 (5.5)	
Ketamine (mg/kg/h)	9.8 (1.6)	
Fentanyl (μg/kg/h)	19.8 (2.7)	
Force applied for SCI [Kdynes]		
mean (SD)	2731 (514)	
PTIBS, mean (SD)		
Baseline	10 (0)	
Follow – up: 1 week	1.9 (1.0)	
Follow – up: 2 week	2.8 (1.2)	
Follow – up: 3 week	2.8 (1.1)	
Follow – up: 4 week	3.0 (0.9)	
Follow – up: 5 week	3.3 (0.7)	
Follow – up: 6 week	3.5 (1.4)	
Follow – up: 7 week	3.6 (1.2)	
Follow – up: 8 week	3.5 (1.2)	
Follow – up: 9 week	3.6 (1.2)	
Follow – up: 10 week	3.8 (1.2)	
Follow – up: 11 week	3.8 (1.0)	
Follow – up: 12 week	3.8 (1.0)	
Motor evoked potentials (Extensor Carpi Radialis)	Amplitude [μV], mean (SD)	Latency [msec], mean (SD)
Baseline	385 (249)	21.4 (2.0)
Laminectomy	474 (588)	21.2 (2.4)
Post-SCI	1108 (980)	20.0 (1.3)
3 h Follow-up	1400 (916)	20.8 (2.2)
12 Weeks follow-up	1296 (894)	20.9 (2.1)
Somatosensory evoked potentials (Median right)		
Baseline	7.6 (4.2)	17.6 (2.5)
Laminectomy	7.2 (4.7)	18.2 (2.5)
Post-SCI	7.0 (4.2)	17.6 (1.9)
3 h Follow-up	6.9 (4.7)	17.8 (2.3)
12 Weeks follow-up	14.7 (5.3)	17.8 (1.2)
Somatosensory potentials (Tibial right)		
Baseline	3.8 (1.6)	27.9 (2.0)
Laminectomy	3.5 (0.7)	26.0 (2.4)
Post-SCI	0 (0)	0 (0)
3 h Follow-up	0 (0)	0 (0)
12 Weeks follow-up	0 (0)	0 (0)
Somatosensory potentials (Tibial left)		
Baseline	3.4 (1.7)	26.7 (1.6)
Laminectomy	2.7 (0.4)	25.7 (1.8)
Post-SCI	0 (0)	0 (0)
3 h Follow-up	0 (0)	0 (0)
12 Weeks follow-up	0 (0)	0 (0)

PTIBS, porcine thoracic injury behavioral scale; SCI, spinal cord injury; SD, standard deviation.

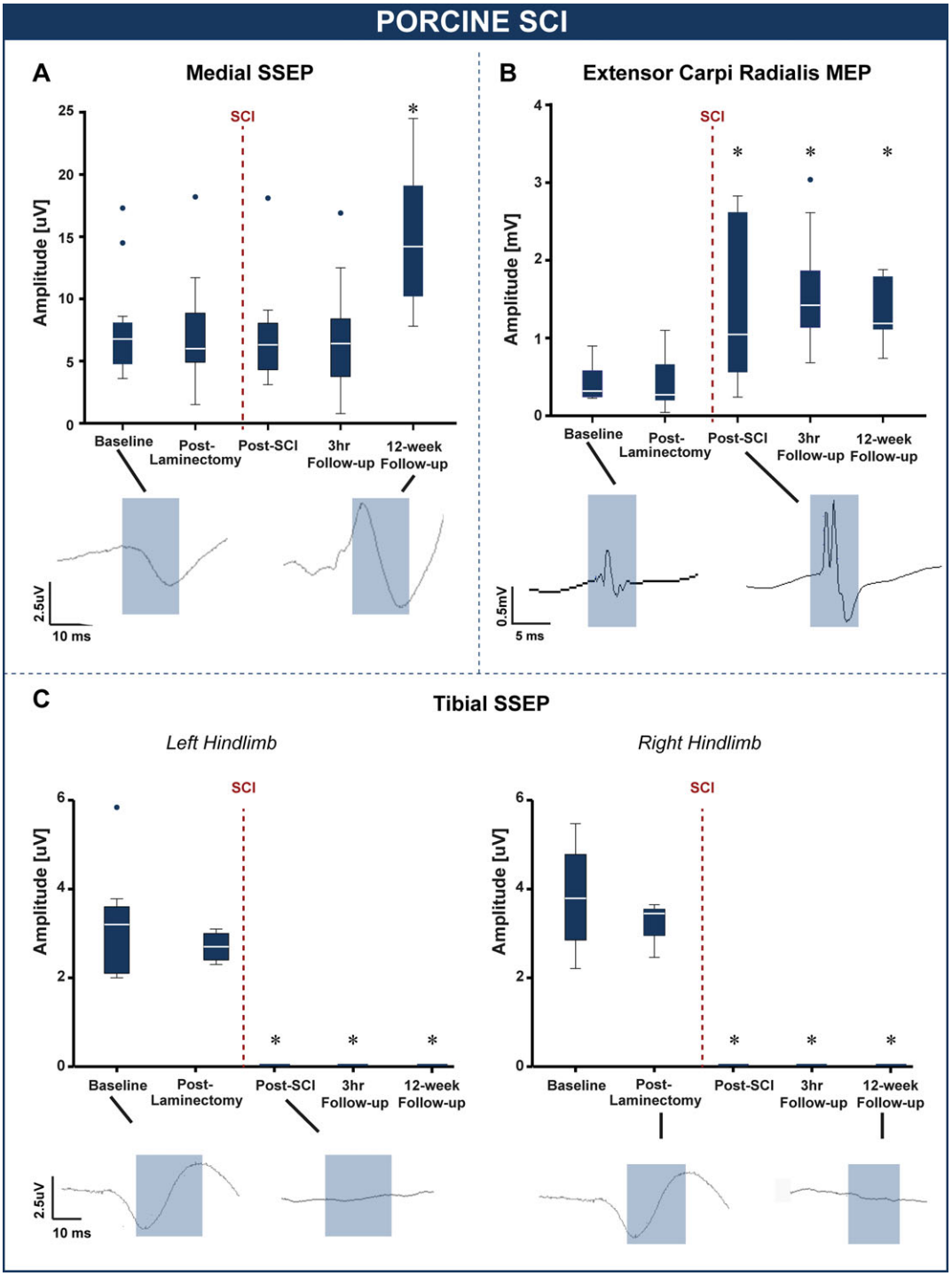

Figure 4. Neurophysiological assessments in yucatan miniature pigs. (A) The medial somatosensory evoked potentials were not affected by the laminectomy and spinal cord injury and remained stable up to 3 h post-injury. An increase in amplitudes was evident 12 weeks post-injury alluding to potential reorganization of the somatosensory cortex. (B) Motor evoked potentials were unaffected by the laminectomy. However, the spinal cord injury induced a massive increase in motor evoked potential amplitudes likely due to an increase in cortical excitability. The motor evoked potentials remained elevated over the follow-up period (12 weeks). (C) Temporal progression of left and right tibial somatosensory evoked potentials. The somatosensory evoked potentials remained stable after laminectomy confirming that the surgical procedure did not harm the spinal cord. Following the contusion, the somatosensory evoked potentials were abolished and did not recover over a period of 12 weeks reflecting the severity of the injury.

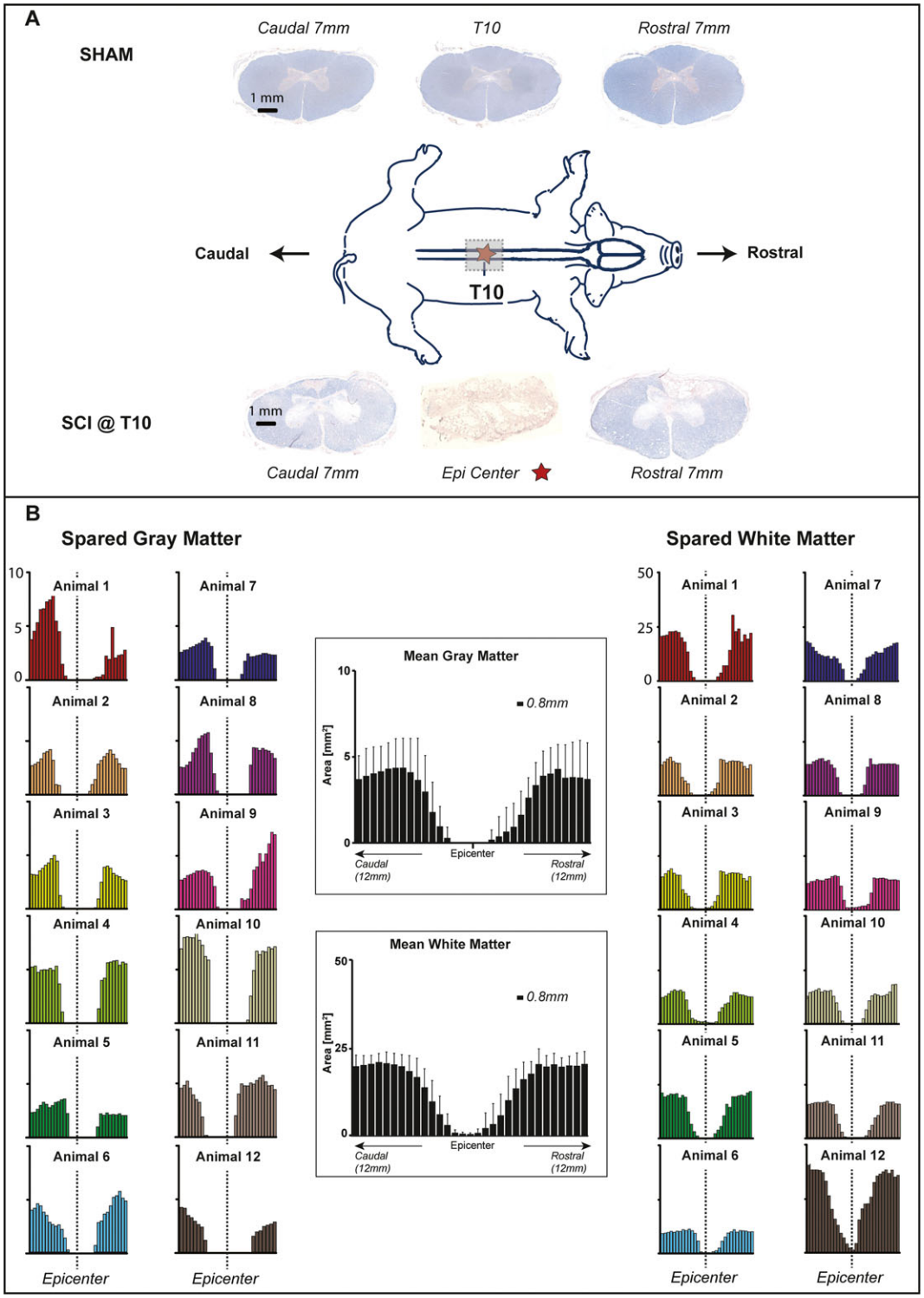

Figure 5. Immunohistochemistry findings. (A) Representative Eriochrome cyanine R–stained images of axially sectioned spinal cords. Cross-sectional sections of spinal cord tissue, at 12 weeks post-injury, stained with Eriochrome cyanine R to detect tightly packed myelin in SHAM (top row) and spinal-cord–injured pigs (bottom row). Scale bar = 1 mm. Spinal cord injury results in the loss of myelin, large cavitation, and tissue disorganization extending away from the lesion epicenter. Total spared gray matter (left panel) and spared white matter (right panel) determined by area measurements taken from axial sections of spinal cord tissue 800 μm apart in all spinal cord injured animals.

Motor recovery - porcine thoracic injury behavioral score

At baseline, all animals reached the highest PTIBS Score (10 ± 0). One week after SCI, hindlimb motor function was severely impaired, resulting in a mean PTIBS Score of 2.0 ± 0.9. Motor recovery was observed in all animals over time ($F = 11.4$, df: 11, $P < 0.001$). The average recovery was 2 ± 1.1 points from 1 to 12 weeks post-injury. The amount of motor recovery was not correlated with the observed changes in forelimb MEPs ($F = 1.2$, df: 11, $P = 0.289$) and SSEPs ($F = 0.8$, df: 11, $P = 0.354$).

Immunohistochemical quantification of injury severity

In order to quantify the extent of spared tissue at the lesion epicenter as well as the rostro-caudal spread of the injury, quantification of spared gray and white matter was performed on serial sections stained with Eriochrome cyanine R (Fig. 5A). At week 12 post-SCI, both white and gray matter at the lesion epicenter was completely abolished (Fig. 5B). There was no relationship between neurophysiological parameters related to the forelimbs and spared tissue in the thoracic lesion site (all comparisons $P > 0.05$).

Discussion

The present study characterizes the brain's response to an acute SCI in humans and pigs. In both species, the amplitudes of upper limb SSEPs, rostral to the level of lesion, progressively increased over time. In contrast to SSEPs, motor responses increased immediately after experimental injury in pigs and remained elevated out to 12 weeks. Along similar lines, MEPs were significantly greater compared to the healthy control cohort at 1-month post-injury and remained higher throughout recovery. Using a common neurophysiological technique, our results demonstrate robust changes in the brain that occur independent of species, suggesting shared mechanisms of cortical reorganization.

Evidence of cortical reorganization in humans has been derived primarily from cross-sectional studies, chiefly by way of evaluating motor pathways. In a small cohort of acutely injured individuals ($n = 4$, 6–17 days post-injury), MEPs were greater in amplitude compared to healthy controls in upper limb muscles rostral to the level of injury.[17] Similar changes in MEPs rostral to the level of lesion were observed in chronic stages of injury ($n = 2$ and $n = 6$, respectively)[4,18] and correspond with functional neuroimaging studies.[5] Our findings bridge acute[17] and chronic cross-sectional observations,[4,5,18] for the first time revealing early (1-month) and persistent increases in MEP amplitudes in a larger sample ($n = 37$) of individuals with SCI.

Compared to the motor cortex, less is known in humans with SCI regarding reorganization in primary sensory areas. A recent functional magnetic resonance imaging study revealed no major changes in the primary somatosensory cortex in a cohort of individuals with chronic SCI stimulated by touch or noxious heat.[8] Others, adopting similar neuroimaging approaches, have shown subtle shifts in topography related to the presence of chronic SCI neuropathic pain.[19] None, to the best of our knowledge, have reported reorganization based on SSEPs. In stark contrast to MEPs, observations in both humans and pigs indicate that reorganization in the sensory cortex develops over time. The progressive nature of these changes is in agreement with previous rodent studies applying SSEPs,[20] and functional magnetic resonance imaging.[21]

Based on the timing of motor and sensory reorganization, there are a number of potential mechanisms to consider. Immediate changes in the brain could be related to a reduction in spontaneous cortical activity occurring directly in response to injury. Such a shift has been reported in experimental rodent models,[22] and in chronic phases of human spinal cord injury.[23–25] Reductions in spontaneous cortical activity, which drive slow-wave activity, give rise to increased local field potentials following stimulation of the forepaw[22,26] and larger motor evoked potentials.[27] Our findings, however, suggest that the modulation of SSEP amplitudes does not happen immediately after injury, but rather delayed. The discrepant results can be partially explained by methodological differences, such as stimulation frequency. While rodent studies typically employ low-frequency stimulation (0.5 Hz) to avoid adaption, our experimental approach involved continuous peripheral stimulation at 3.1 Hz (in both pigs and humans). Castro-Alamancos and colleagues previously demonstrated that stimulation frequencies above 2 Hz lead to a "steady state" in response magnitudes (i.e., comparable SSEP amplitudes pre and post-injury).[28] Alternatively, trauma-induced changes in the brain may be attributable to state-independent mechanisms. One such mechanism is unmasking of latent pathways, resulting from decreased cortical inhibition.[26] Evidence of reduced cortical inhibition has been reported for motor function in the chronic phase of spinal cord injury.[29] Moreover, increases in somatosensory evoked potentials have been ascribed to unmasking of latent pathways immediately following reversible nerve block.[30] In contrast to the immediate changes, delayed reorganization in the brain following spinal cord injury is in line with the state-independent concept of anatomical

reorganization (e.g., sprouting).[31] Anatomical reorganization in ascending and descending CNS pathways is evident in various animal models of spinal cord injury.[32–34] Further investigation is needed to elucidate these state-independent mechanisms, including studies applying detailed neurophysiological and neuroimaging techniques in the very early stages of injury.

An important observation of our study is that cortical reorganization measured above level is functionally insignificant to patients with SCI. In neither humans or pigs was a relationship with injury severity and the extent of functional recovery observed. This suggests that sensory and motor reorganization, as measured by SSEPs and MEPs, is occurring independently of long-term neurological recovery. Future studies should, however, address the relationship with other functionally and highly meaningful outcomes. For example, sensory reorganization may be related to the onset of neuropathic pain; symptoms of which tend to develop over a similar time-frame as changes in SSEPs.[35]

Limitations of this study relates to the measurement of cortical plasticity in humans after SCI. First, a relatively small number of individuals with neurological level of injury at or below C8 ($n = 37$), in which upper limb SSEPs and MEPs were recorded for clinical evaluation, were included in our analysis. These individuals were selected a priori because: (1) lower thoracic injuries (e.g., T4 and below) are not routinely examined with upper limb SSEPs and MEPs, and (2) according to International Standards[14] the C8 spinal segment is intact, thereby allowing for conduction of ascending and descending signals (i.e., SSEP and MEP, respectively). However, subclinical sensorimotor deficits in C8 (i.e., missed by muscle strength and sensory testing), and partial recovery in the adjacent T1 spinal segment may also have facilitated increased SSEP and MEP amplitudes. Additionally, SSEPs and MEPs were not examined in humans with SCI until 1-month. This is related to difficulties performing very early neurophysiological assessments in individual with SCI with acute, traumatic SCI. Consequently, increased MEP amplitudes at 1-month post-injury are compared to normal control values. There were also notable differences in how neurophysiological outcomes were recorded in pigs and humans. Both SSEPs and MEPs were acquired in anesthetized pigs, while humans were conscious; electrical stimulation was applied in pigs, while transcranial magnetic stimulation was delivered in humans to acquire MEPs. Despite these differences, outcomes of both SSEPs and MEPs were very similar. Finally, there are important similarities between pigs and humans in comparison to rodents, including anatomic and physiologic characteristics as well as life expectancy.[36] Taking into account these shared characteristics, our findings highlight the importance of large animal species such as the Yucatan mini-pig in translational research and in the examination of trauma-induced neuroplasticity.

Conclusion

In summary, the progression of sensory and motor reorganization, in cortical areas non-directly affected by spinal lesion (i.e., above the lesion level) was characterized in humans and pigs following traumatic SCI. Findings derived from our translational approach indicate that the reorganization of the motor system begins immediately after injury, while sensory reorganization occurs over time. Collectively, our findings highlight the importance of large animal species, such as the Yucatan mini-pigs, in translational research and development of spinal cord repair strategies to examine neuroplasticity.

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Author Contributions

CRJ contributed substantially to the conception and design of the study, the data acquisition, analysis, and interpretation. Furthermore, she drafted the research article. FS performed the surgeries and was involved in the data collection, data analysis, and revising the research article. JA was substantially involved in data analysis and revised the research article critically for important intellectual content. KS was involved in the data collection, data analysis, and revising the research article. NM performed the surgeries, was involved in the data collection, and revised the manuscript. EO was involved in the data collection (immunohistochemistry) and revised the manuscript. MH was involved in the human data collection and revised the manuscript. AC made substantial contributions to study conception, design, and data collection as well as participated in revising the research article critically for important intellectual content. BK made substantial contributions to study conception and design as well as participated in revising the research article critically for important intellectual content. JLKK contributed substantially to study design, the data acquisition, analysis, and interpretation, and was involved in drafting the research article.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Type and dosages used to induce anesthesia on both experimental days.

