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Development of an algorithm to perform a comprehensive study of autonomic dysreflexia in animals with high spinal cord injury using a telemetry device

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Corresponding Author:	David Popok University of British Columbia Vancouver , British Columbia CANADA
Corresponding Author Secondary Information:	
Corresponding Author E-Mail:	davidpopok@gmail.com;krassioukov@icord.org
Corresponding Author's Institution:	University of British Columbia
Corresponding Author's Secondary Institution:	
First Author:	David Popok
First Author Secondary Information:	
Other Authors:	Chirstopher West, Ph.D. Barbara Friars, Ph.D. Andrei V Krassioukov, M.D., Ph.D.
Order of Authors Secondary Information:	
Abstract:	Spinal cord injury (SCI) is a debilitating neurological condition characterized by somatic and autonomic dysfunctions. In particular, SCI above the mid-thoracic level can lead to a potentially life-threatening hypertensive condition called autonomic dysreflexia (AD) that is often triggered by noxious or non-noxious somatic or visceral stimuli below the level of injury. One of the most common triggers of AD is the distension of pelvic viscera, such as during bladder and bowel distension or evacuation. This protocol presents a novel pattern recognition algorithm developed for a JAVA platform software to study the fluctuations of cardiovascular parameters as well as the number, severity and duration of spontaneously occurring AD events. The software is able to apply a pattern recognition algorithm on hemodynamic data such as systolic blood pressure (SBP) and heart rate (HR) extracted from telemetry recordings of conscious and unrestrained animals before and after thoracic (T3) complete transection. With this software, hemodynamic parameters and episodes of AD are able to be detected and analyzed with minimal experimenter bias.
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October 8th, 2014

Dear Dr. Braiman,

Herewith we submit the article entitled “Development of an algorithm to perform a comprehensive study of autonomic dysreflexia in animals with high spinal cord injury using a telemetry device.” by David Popok, Christopher West, Bárbara Frias and Andrei Krassioukov to be considered for publication in the Journal of Visualized Experiments, JoVE. Dr. Andrei Krassioukov is the corresponding author. All the correspondence should be sent to the International Collaboration on Repair Discoveries, ICORD-BSCC, UBC, 818 West 10th Avenue, Vancouver, V5Z 1M9 British Columbia, Canada. Email: krassioukov@icord.org Tel: 604 675 8819; Fax: 604 675 8820.

This original article outlines the importance of a unique algorithm developed for a detailed analysis of diurnal fluctuations of cardiovascular parameters collected by telemetry. This innovative technology allows for continuous 24 hours monitoring of hemodynamic values, such as blood pressure and heart rate, in order to identify the occurrence of a clinically relevant and life-threatening condition known as autonomic dysreflexia (AD). Hopefully, this method could be adapted in the future for use in human ambulatory bedside detection of AD events.

David Popok is a Masters student and he is the primary author of this paper. He was responsible for the development of the novel algorithm for hemodynamic parameters analysis. Dr. Christopher West was responsible for study conception, surgical procedures, animal care, interpretation and drafting of the manuscript. Dr. Barbara Frias was responsible for interpretation and drafting of the manuscript. Dr. Andrei Krassioukov is the senior author and was responsible for study conception, data interpretation, drafting of the manuscript, and final approval of the manuscript.

All work followed the adequate guidelines for animal use. This work was partially supported by the Canadian Institute of Health Research and the Heart and Stroke Foundation of BC and Yukon. We also state that this is an original paper, which has not been submitted or published in another journal or book. All authors have read and approved the final manuscript.

We would like to invite the following esteemed reviewers: Keith Tansey (Emory University), David S.K. Magnuson (University of Louisville), Veronica Tom (Drexler University), Lawrence Schramm (John Hopkins University), Jill Wecht (Mount Sinai Hospital) and Stephen E. Dicarlo (Wayne State University).

Yours sincerely,

Corresponding author:

Andrei Krassioukov, MD, PhD, FRCPC
Professor, Dep. Medicine, Div. Phys .Med. & Rehab.
Associate Director and Scientist, ICORD,
Director of Autonomic Research Unit,
Staff physician, Spinal Cord Program, GF Strong Rehabilitation Centre,
University of British Columbia

TITLE

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AUTHORS:

Popok, David
International Collaboration on Repair Discoveries (ICORD)
Faculty of Medicine, University of British Columbia
Vancouver, British Columbia, Canada
davidpopok@gmail.com

West, Christopher
International Collaboration on Repair Discoveries (ICORD)
Faculty of Medicine, University of British Columbia
Vancouver, British Columbia, Canada
west@icord.org

Frias, Barbara
International Collaboration on Repair Discoveries (ICORD)
Faculty of Medicine, University of British Columbia
Vancouver, British Columbia, Canada
barbarafrias@gmail.com

Krassioukov, Andrei
International Collaboration on Repair Discoveries (ICORD)
Faculty of Medicine, University of British Columbia
Department of Medicine, Division of Physical Medicine and Rehabilitation
University of British Columbia
GF Strong Rehabilitation Centre
Vancouver, British Columbia, Canada
krassioukov@icord.org

CORRESPONDING AUTHOR:

Dr. Andrei Krassioukov, MD, PhD, FRCPC; International Collaboration on Repair Discoveries, ICORD-BSCC,
University of British Columbia,
Vancouver, British Columbia, Canada.
krassioukov@icord.org
Tel: 604 675 8819
Fax: 604 675 8820

KEYWORDS:

Telemetry; algorithm; spinal cord injury; sympathetic nervous system; autonomic dysreflexia; cardiovascular autonomic dysfunction; heart rate; blood pressure.

SHORT ABSTRACT:
The catheter of a telemetry device is implanted into the abdominal aorta in order to continuously collect beat-by-beat hemodynamic data from animals pre and post-high thoracic spinal cord transection. A novel JAVA software was employed to analyze hemodynamic parameters as well as frequency and intensity of spontaneous episodes of autonomic dysreflexia.

LONG ABSTRACT:
Spinal cord injury (SCI) is a debilitating neurological condition characterized by somatic and autonomic dysfunctions. In particular, SCI above the mid-thoracic level can lead to a potentially life-threatening hypertensive condition called autonomic dysreflexia (AD) that is often triggered by noxious or non-noxious somatic or visceral stimuli below the level of injury. One of the most common triggers of AD is the distension of pelvic viscera, such as during bladder and bowel distension or evacuation. This protocol presents a novel pattern recognition algorithm developed for a JAVA platform software to study the fluctuations of cardiovascular parameters as well as the number, severity and duration of spontaneously occurring AD events. The software is able to apply a pattern recognition algorithm on hemodynamic data such as systolic blood pressure (SBP) and heart rate (HR) extracted from telemetry recordings of conscious and unrestrained animals before and after thoracic (T3) complete transection. With this software, hemodynamic parameters and episodes of AD are able to be detected and analyzed with minimal experimenter bias.

INTRODUCTION:
Autonomic dysreflexia (AD) is a life-threatening emergency in individuals after acute or chronic spinal cord injury (SCI) at cervical or high-thoracic segments and is usually characterized by episodes of persistent hypertension and bradycardia[1]. AD is principally caused by disruption of descending spinal pathways that usually provide input from supraspinal centers to the spinal sympathetic preganglioninc neurons that control sympathetic activity and vascular tone[1-4]. AD episodes are characterized by a spike in systolic blood pressure (SBP) up to 300 mmHg and if left untreated may lead to seizures, intracranial hemorrhage, myocardial infarction, and even death[5-8]. A variety of noxious and non-noxious stimuli act as a trigger of AD, including bowel and bladder distension, spasms, pressure sores, urinary bladder catheterization or iatrogenic procedures[9-12].

The temporal development of AD in response to SCI has been investigated in both human[9] and animal models[13,14]. Typically these studies have used an 'induced AD' method (i.e., urodynamics, penile vibrostimulations in humans or colorectal distension in animals) to determine the temporal development of AD. Such an approach is limited by the need for repeated assessments at isolated time-points that may preclude an accurate determination of the temporal development of AD. The use of 24-hr blood pressure monitoring in humans allows serial blood pressure measurements to be made at pre-determined intervals. This technique has recently been employed to monitor spontaneously occurring AD in patients with chronic SCI. In animal models, solid-state pressure transducers are being increasingly used to

chronically monitor beat-by-beat arterial blood pressure. Recently, Rabchesvky et al. (2012), developed an algorithm that extracted one second averages of mean arterial pressure (MAP) and compared against a moving average threshold[15]. Spontaneous AD events were characterized based on MAP peaks that are 10mmHg or greater above threshold concurrently with a HR drop of 10bpm or greater.

Here a novel JAVA software that has a built in AD Detection Algorithm is presented. This algorithm works by detecting pre-determined patterns in arterial blood pressure (ABP) and heart rate (HR) that are indicative of a spontaneously occurring AD event. The user is able to manually adjust all input variables to the software such that the 'detection algorithm' can be easily customized to the specific parameters of interest. The software is also able to dichotomize ABP and HR into a given epoch such that diurnal rhythmicity of hemodynamic parameters can be analyzed[16]. In the present manuscript, a detailed explanation is given of the surgical technique that is used to implant the telemetry devices and conduct the SCI surgery. Examples are also provided of the post-processing capabilities of the *AD Detection* software and how cardiovascular function is altered post-SCI. For comparative purposes, the methodology and results obtained from a method of induced AD known as colorectal distension (CRD) is also illustrated.

PROTOCOL:

Male Wistar rats weighing 300-350g were used in this experiment. All rats were maintained on a 12-h light/dark cycle and received standard laboratory rat chow and water ad libitum. All experimental procedures conformed with the guide to the Care and Use of Experimental Animals established by the Canadian Council on Animal Care and granted ethics approval by the University of British Columbia. Surgery and animal care were conducted according to standard procedures in our laboratory (Ramsey *et al.* 2010)[17].

1) Preparation of the animals: surgical procedures

1.1) Implantation of the telemetry device

Note: Protocol was implemented according to the manufacturer's specifications and our previous experience with the implantation of the device: www.datasci.com and Mayorov et al. (2011)[16].

1.1.1) Pre-treat the animals for three days with enrofloxacin (10 mg/kg, s.c.) subcutaneously.

1.1.2) Avoid solid foods for at least 24 hours before the surgery.

1.1.3) Anaesthetize the animals with isoflurane (1.5% with 1.5-2 L/min Oxygen). Shave the abdomen and clean it with 16% iodine.

1.1.4) Make a 5 cm long midline abdominal incision cutting through the peritoneum exposing the intestines.

1.1.5) Retract the intestines (this will expose the descending aorta at the posterior abdominal wall), cover the gut with surgical gauze and hydrate gut with 2-3 mL of Ringers Solution.

1.1.6) Blunt dissect around the aorta and separate the aorta from the inferior vena cava utilizing a blunt instrument.

1.1.7) Place ligatures around the rostral and caudal ends of the aorta, just distal to the renal artery.

1.1.8) Lift the ligatures briefly occluding blood flow and puncture the aorta at the level of 1-2 mm anterior to the iliac bifurcation.

1.1.9) Guide the tip of the catheter of the telemetry device into the aorta using a 20-Gauge curved needle. Advance rostrally to make sure that the tip is just distal to the renal arteries

1.1.9.1) Fix it using a small amount of tissue adhesive. Remove ligatures and ensure there is no blood flow occlusion due to the catheter or tissue adhesive.

1.1.10) Secure the body of the telemetry device into the abdominal wall using 4-0 silk sutures. Use 4-0 Vicryl and 4-0 Prolene sutures to close the muscle and skin, respectively.

1.2) Post-Surgical Care

1.2.1) Feed the animals with fruit, assortment of cereals, meal replacement, water ad libitum and ringers upon symptoms of dehydration, for 5 days after telemetry device implantation and 14 days post T3 transection. House the animals in specialized cages with low-reaching water bottles and rubber matting to facilitate movement of the forelimbs of the animal.

1.2.2) Administer enrofloxacin (10 mg/kg, s.c.), buprenorphine (0.02 mg/kg, s.c.), and ketoprofen (5 mg/kg, s.c.) once a day during the following three days after surgery.

1.2.3) Empty the bladder manually three to four times daily until spontaneous voiding returns (about 10 days post telemetry implantation and post-injury).

1.3) Complete transection of the spinal cord

Note: Allow animals to recover from telemetry implantation surgery for at least 2 weeks before T3 complete transection surgery.

1.3.1) Treat animals with prophylactic enrofloxacin (10 mg/kg, s.c.) for three days before SCI surgery.

1.3.2) On the day of the surgery administer subcutaneously enrofloxacin (10 mg/kg), buprenorphine (0.02 mg/kg), and ketoprofen (5 mg/kg) pre-operatively.

1.3.3) Anaesthetize the animals with ketamine hydrochloride (70 mg/kg, i.p.) and medetomidine hydrochloride (0.5 mg/kg, i.p.).

1.3.4) Make a dorsal midline incision, 2 cm in length, in the superficial muscle overlying the C8-T3 vertebrae.

1.3.5) Blunt dissect in order to reveal the dura layer.

1.3.6) Pierce the dura layer with microscissors or with the tip of a 25 gauge needle.

1.3.7) Open the dura at the T2-T3 intervertebral gap and perform a complete transection using microscissors. Open the microscissors but not laterally enough to burst vertebral arteries and veins. In swift motion transect the spinal cord and search for an exaggerated twitch in the animal hind limbs. Confirm a complete transection *via* visual separation of the rostral and caudal spinal cord stumps.

1.3.8) After confirmation, place gelfoam between the stumps to achieve hemostasis. Apply sufficient gelfoam to stop bleeding and to separate the stumps of the spinal cord.

1.3.9) Use 4-0 Vicryl and 4-0 Prolene sutures to close the muscle and skin, respectively. After SCI surgery, give animals pre-warmed Lactated Ringer's solution (5 mL, s.c. at 30 °C) and allow them to recover in a temperature-controlled environment.

1.3.10) Apply post surgery treatment as done in step 1.2.

2) Telemetry monitoring of hemodynamic parameters

2.1) Collect beat-by-beat arterial blood pressure (ABP) and core body temperature every day for 24 hours, pre and post SCI. Sample the arterial blood pressure and core body temperature at 1000 Hz in 24 hour blocks using the telemetry device.

2.2) Place a SmartPad under the animal cage for wireless recharging of the transducer and for receiving of digital telemeter signals from the transducers.

Note: The output voltage is subsequently passed on to a Configurator connected to a computer, which organizes the data flow from each channel. The voltage is converted to continuous arterial blood pressure and temperature recordings. Visualize the sampled data utilizing data acquisition software, such as LabChart.

2.3) Discard the telemetry data collected during animal monitoring, as well as the 10 minutes immediately prior to monitoring and 10 min after monitoring.

3. Assessment of Spontaneous Incidences of Autonomic Dysreflexia (AD)

Note: The frequency, severity, and duration of spontaneous AD events were assessed using an algorithm developed for our own novel *AD Detection* JAVA platform software (Figure.4). A novel algorithm has been developed to automatically detect spontaneous AD events based on 24 hour SBP and HR telemetry recordings before and after SCI utilizing parameters specified in **Figure. 2**.

3.1) Extract SBP and HR values from raw telemetry data over period of interest using acquisition software.

Note: Acquisition software detects and determines the value of the SBP peaks from continuous ABP recordings. Heart rate is extrapolated by determining the duration distance between adjacent SBP peaks.

3.2) Upload a CSV file of the telemetry recordings with interbeat interval in column A, SBP values in column B, MAP values in column C and time of day in column D **(Figure. 4A).**

3.3) Using the software **(Figure. 4B-D)**, specify the relevant physiological HR range (i.e. R to R interval) for the SBP recordings between 180 bpm and 625 bpm. Specify the relevant HR range on the software under the *Heart Rate Minimum* and *Heart Rate Maximum* panels.

3.4) Create a threshold for SBP and HR through a moving average window of 240 seconds. Indicate the window length of the moving average threshold for the analysis in the *AD Duration Threshold* panel.

3.5) Set a SBP transposed threshold at 20 mmHg above the moving average baseline. Indicate the transposition value in the *Moving Average Threshold Transposition BP* panel.

3.6) Isolate SBP peak clusters that exceed the transposed threshold with a peak to peak interval less than 2 seconds and for a duration of greater than 10 seconds. Specify the peak to peak interval using the *Interpeak Interval BP* and the duration interval using the *Peak Cluster Interval BP* panels.

3.7) Group SBP peak clusters that are within 120 seconds of each other.

Note: Detected potential AD events that are within 120s of each other would be grouped as a single event. Specify the maximum duration allowed between consecutive SBP clusters to differentiate separate AD events in the *AD Duration Threshold* panel.

3.8) Confirm if group of SBP peak clusters are associated with a potential spontaneous AD event by detecting a drop in HR of 40 bpm or greater.

3.8.1) Average 10% of the HR values upon onset of the potential event *(specify this percentage in the upper heart rate drop average range panel).* Average 75% of the heart rate values from the end of the potential event *(specify this percentage in the lower heart rate drop average range panel).*

3.8.2) Subtract the lower heart rate threshold from the higher heart rate threshold, in order to ensure a corresponding drop of 40 bpm or greater. Specify the heart rate drop restriction using the *heart rate drop restriction* panel.

3.9) Once the panels have been filled, press *OK*. A graphical presentation of the detected AD events are presented, which include the spikes in SBP and associated HR data (Figure. 4C-D). An output excel file is also generated with the pressor response (mmHg), duration (seconds), max systolic blood pressure (mmHg), minimum HR (bpm) and HR drop (bpm) of each detected AD event.

4. Colorectal distension to intentionally elicit AD

Note: The severity of induced AD can be determined through colorectal distension (CRD), a clinically relevant stimulus that mimics the bowel routine[3,18,19].

4.1) Verify if the balloon of a French catheter, 10 mm in length, is functionally feasible by inflating it with air.

4.2) Place lubricant on the tip of the catheter. Insert the deflated balloon tip into the rectum and position it 2 cm from the anal opening. Secure the catheter to the tail with surgical tape. Place a custom tube around the outside of catheter to prevent rats from biting/pulling out the catheter.

4.3) After insertion of the catheter, infuse the balloon with 2 mL of air over 10 s, allow BP and HR to stabilize. Maintain distension for 1 min. Animals are allowed to move freely in their respective cages. Make sure the tip of the catheter remains inside the rectum.

4.4) Repeat distension 3 times per trial, repeating trials daily with a minimum interval of 10 min between trials.

4.5) Average beat-by-beat data over 1 s intervals and report the maximum increase in SBP and maximum decrease in HR over multiple trials.

REPRESENTATIVE RESULTS:

Using telemetry, arterial blood pressure is sampled at a frequency of 1000 Hz continuously for 24 hours. An illustrative recording of arterial blood pressure (ABP) using LabChart is shown in

Figure 1B. The sample ABP was monitored by a solid state pressure sensor inserted into the descending aorta. The novel JAVA platform *AD Detection* software is able to extract relevant SBP (mmHg) peaks (**Figure. 1C**). We may also extract the HR (bpm) from the time interval between adjacent SBP peaks (**Figure. 1D**).

SBP and HR extracted from beat by beat ABP sampled at 1000Hz are chosen as the input variables for the *AD Detection* software. The software characterizes spontaneous AD events by an increase in SBP greater than 20 mmHg accompanied by a decrease in HR of 40 bpm or greater, as presented in **Figure. 2**. These events are initially identified utilizing a 240 second SBP moving average baseline transposed vertically 20mmHg. The *AD Detection* software ensures the detected AD event is 'real' by checking for a HR drop of 40 bpm or greater with respect to the onset of the AD event. The software detects and characterizes spontaneous AD events from continuous beat by beat, 24 hour SBP and HR telemetry recordings. Along with the time of day and frequency of these AD events, the *AD Detection* software provides the following tabular information: the max SBP, pressor response, duration of the AD event, min HR and HR drop upon onset of the spontaneous AD event.

The representative graphs of SBP and HR of **Figure 3** present the severity and duration of an induced AD episode during CRD. As the balloon of the catheter is inflated, there is a rapid and persistent increase in SBP, accompanied by pronounced bradycardia. There is a gradual stabilization of the cardiovascular parameters as distension progresses, and normalization of hemodynamic parameters as the pediatric Foley catheter is deflated. After the insertion of the Foley catheter, SBP and HR indices are averaged for 60s before inflation to generate a baseline. From the baseline, the severity or pressor response associated with the spike in SBP (mmHg) and drop in HR (bpm) can be determined.

Figure 1. Schematic of telemetry device implantation and representative trace of arterial blood pressure (ABP). Schematic illustration of the telemetry transducer implantation with the tip of the catheter directed to the occluded descending aorta and the body of the telemetry device secured to the abdominal wall[16]. Example data of ABP sampled at 1000 Hz from the telemetry device for a 24 hour period. From this recording, it is possible to extract cardiovascular indices, such as systolic blood pressure (SBP; mmHg; **Figure. 1B**) and heart rate (HR; bpm; **Figure. 1C**).

Figure 2. A spontaneous episode of autonomic dysreflexia (AD) in spinal cord injured animals. The novel JAVA platform software detected spontaneous AD events from 24 hour telemetry recordings of systolic blood pressure (SBP; **Figure. 2A**) and heart rate (HR; **Figure. 2B**). An increase of 20 mmHg or greater in SBP together with a drop in HR of 40 bpm or greater upon onset of the spike in SBP is considered an AD event. The *upper HR threshold* consists of the mean 10% of the HR values upon onset of the potential event. The *lower HR threshold* consists of the mean 75% of the heart rate values from the end of the potential event. Subtract the *lower HR threshold* from the *higher HR threshold* in order to ensure a corresponding drop of 40bpm or greater.

Figure 3. Representative trace of induced autonomic dysreflexia (AD) by colorectal distension (CRD) in spinal cord injured animals. Upon induction of CRD, there is a sudden and persistent increase in SBP (**Figure. 3A**) accompanied by a marked drop in HR (**Figure. 3B**).

Figure 4. JAVA platform spontaneous autonomic dysreflexia (AD) detection software. A CSV format continuous arterial blood pressure excel file with interbeat interval (IBI), systolic blood pressure (SBP), mean arterial pressure (MAP) and time (**Figure. 4A**) is uploaded to the program and the AD detection parameters are specified (**Figure. 4B**) as outlined in the protocol. The program presents the temporal phenotype of these spontaneously occurring AD events (**Figure. 4C-D**).

DISCUSSION:

The protocol describes a detailed implementation of a novel JAVA platform *AD Detection* software which would be combined with a telemetry device, for a long-term thorough analysis of ABP in SCI-animals (**Figure. 1B**). This is the first software that allows for the characterization of ABP patterns to detect spontaneous AD events as they occur sporadically throughout the duration of the day. A well-characterized T3 SCI animal model can illustrate the functional capacity of the software to detect the frequency, pressor response and duration of spontaneous AD. Along with the detection of spontaneous AD events, the software can analyze oscillations in ABP and discern diurnal variations of hemodynamic parameters.

The novel "AD Detection" software efficiently, reliably and accurately detected and characterized spontaneous AD events in acute and chronic SCI animals. An AD event is considered to occur upon an increase in SBP of 20 mmHg or greater (**Figure. 2A**) and is often associated with pronounced bradycardia[9]. In this particular study, SBP fluctuations were considered a primary indicator for the appearance of a dysreflexic episode, although the software has the ability to also use MAP should the investigator wish. The software has the ability to discern the pronounced bradycardia by detecting the HR drop of a user determined amount upon onset of the AD event **(Figure. 2B)**. Rabchevsky et al. utilized a characteristic drop of 10bpm as the threshold upon which a hypertensive event is classified as a spontaneous AD event. This must be questioned, as rodents have a very high resting heart rate; hence, 10bpm is likely insufficient to define bradycardia. HR drop restrictions may be altered by the user to not only determine drops in HR but also increases in HR depending on the level and completeness of the SCI.

Prior to telemetry, CRD-induced AD was found to be a robust method of induced AD that is both experimentally feasible and well characterized (**Figure. 3A, 3B**)[15,20]. Repetitive induction of AD in rat animal models with high SCI was initially found to mimic the spontaneous and frequent episodes of AD associated with high thoracic SCI[4]. CRD is a potent, noninvasive stimulus for AD in rats with high thoracic SCI[14] which emulates some of the most common causes of AD clinically such as constipation and fecal impaction[3]. CRD-induced AD does not account for the wide variety of afferent stimuli that may also evoke these reflex mediated hypertensive events. Thus CRD-induced AD in conjunction with detection and characterization of spontaneous AD provide the ideal scope in which we can study the temporal phenotype of

AD.

Telemetry is a state-of-the-art method for monitoring physiological functions in awake and freely moving animals while minimizing stress-associated artifacts, such as distress, handling and anesthesia[21]. In this case, a solid-state pressure transducer made of a lightweight biosilicone material is used. As opposed to the low pressure response of fluid based catheters, a *solid state pressure sensor* can monitor subtle changes in cardiovascular parameters. These transducers can sample continuous, beat by beat blood pressure and core body temperature at frequencies up to 2kHz. Solid state sensors also have the benefit of preventing motion artifacts commonly encountered in fluid based catheters. Though telemetry devices are invasive and costly, it accurately monitors the diurnal rhythm of hemodynamic parameters[15,16,21,22]. The recovery time after implantation is critically important for the survival of the animal, since mechanical obstruction to the blood flow in the abdominal aorta by the implant itself may result in insufficient blood supply to the hind body[15].

Telemetric detection of spontaneous AD, in addition to CRD induced AD, account for the wide spectrum of stimuli associated with the onset of these life threatening episodes. Therefore, detecting and characterizing AD events is critical for considering treatments for SCI patients. There are currently no feasible noninvasive techniques available for humans that allow for chronic, beat by beat, monitoring of hemodynamic parameters. Ambulatory blood pressure monitoring is insufficient due to the low temporal resolution. Animals models are necessary in order to accurately detect and characterize the onset of these spontaneous AD events utilizing the novel *AD Detection* software. Upon advent of new continuous hemodynamic monitoring technologies, the software may be applied as a vital tool clinically to monitor the onset of these events. The use of telemetry devices in conjunction with the *AD Detection* software might be a useful future strategy for clinical ambulatory monitoring of acute AD.

DISCLOSURES:

The authors have nothing to disclose.

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Figure 1
Click here to download Figure: Figure 1 - Blood Pressure and Heart Rate Extraction.pdf

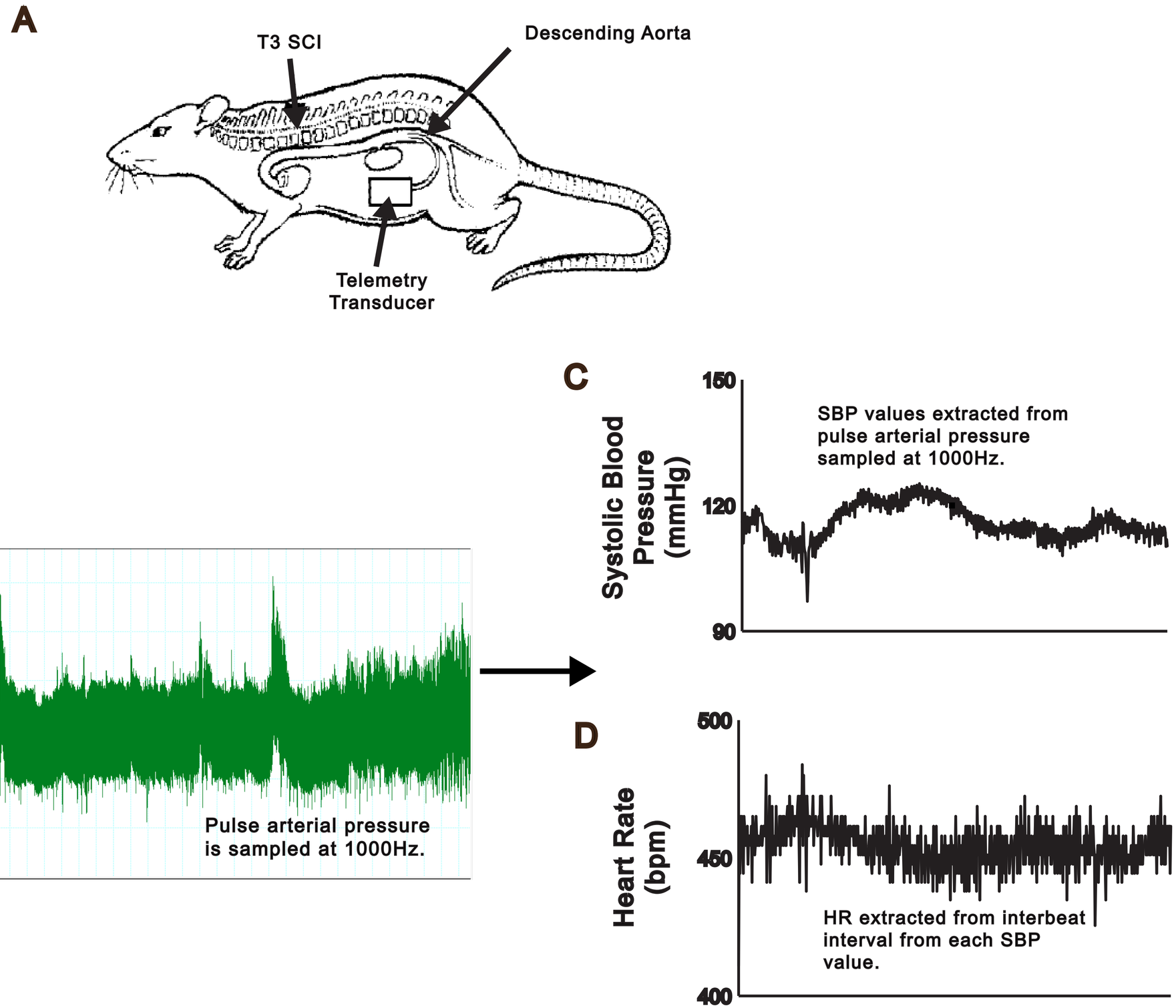

Figure 1

Figure 2
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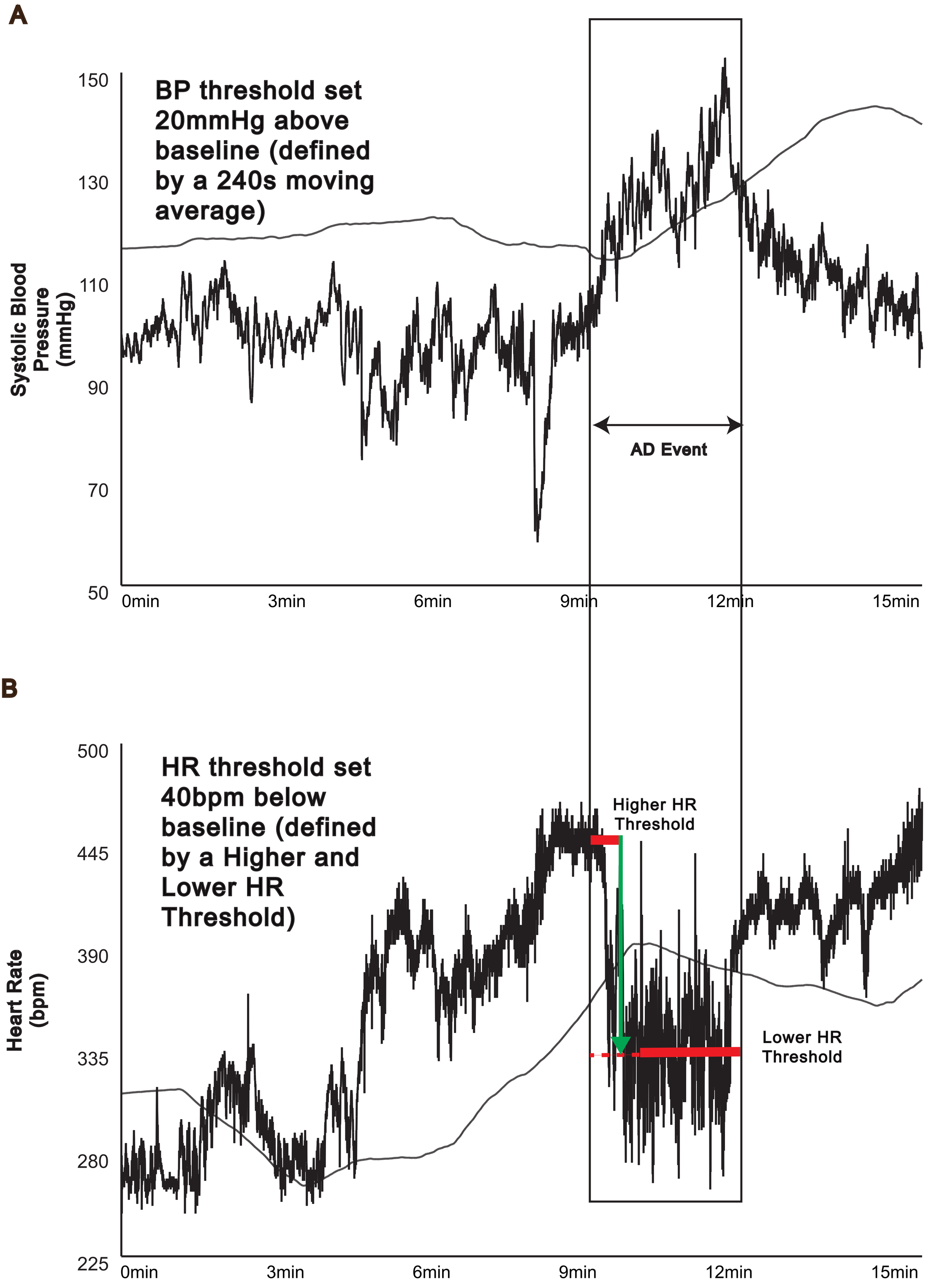

Figure 2

Figure 3
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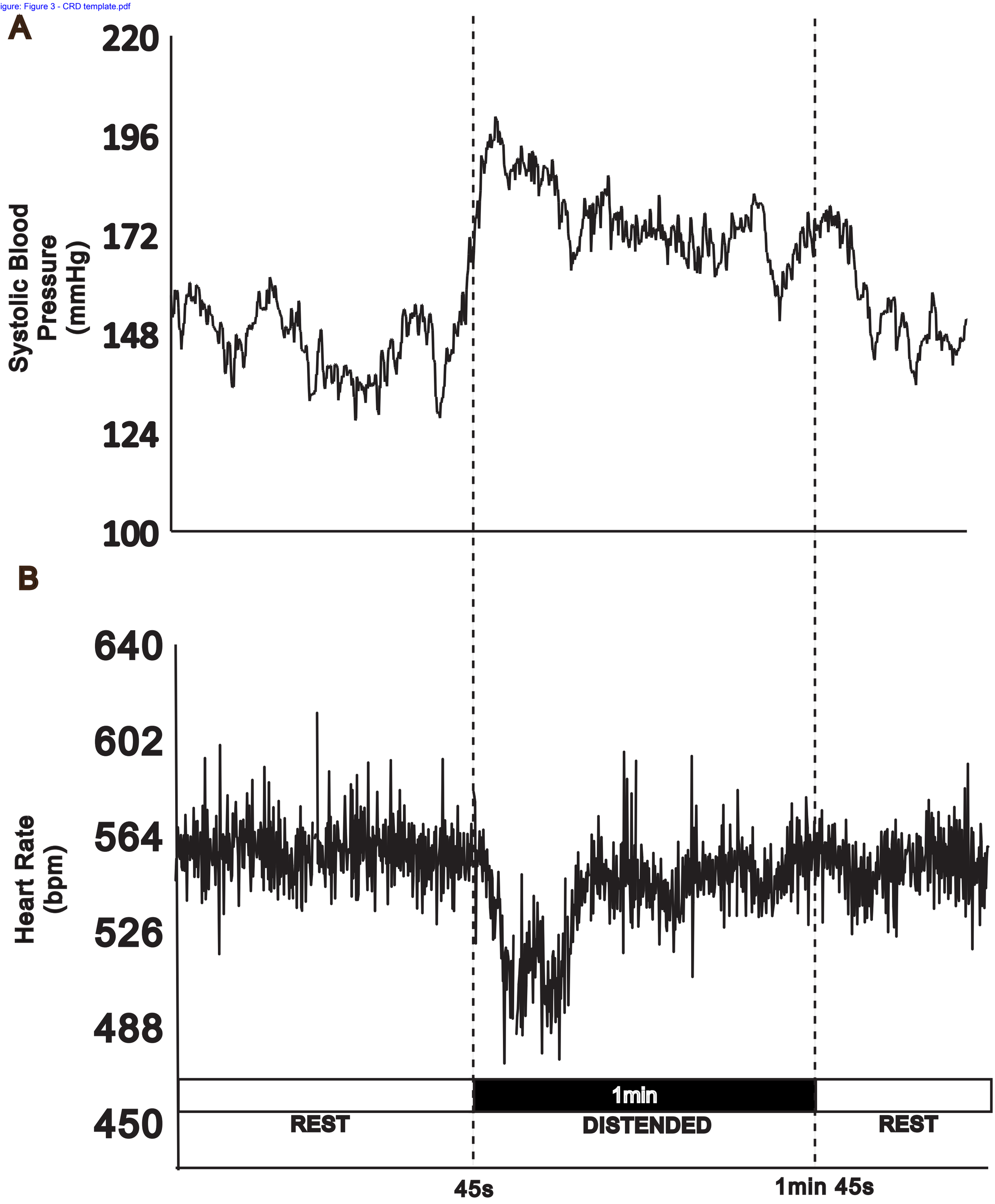


Figure 3

Figure 4

Click here to download Figure: Figure 4 - JAVA platform spontaneous autonomic dysreflexia (AD) detection software.pdf

A

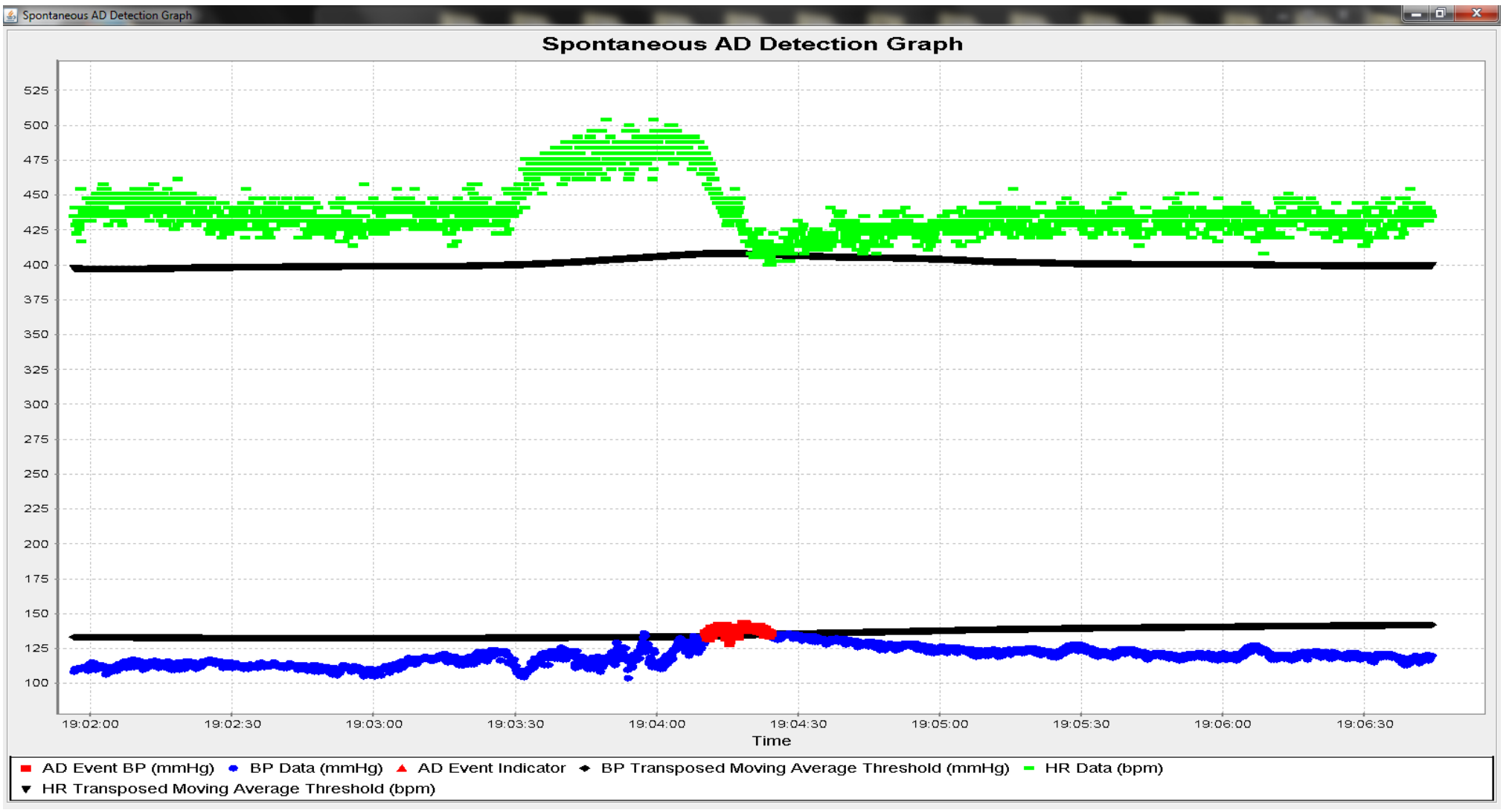
IBI	SBP	MAP	TIME
0.047	130.1875	101.8958	10:36:54.602
0.118	130.0313	101.1667	10:36:54.649
0.125	128.2813	100.5729	10:36:54.767
0.119	127.2656	95.57813	10:36:54.892
0.116	125.75	99.6875	10:36:55.011
0.125	130.2188	100.4479	10:36:55.127
0.12	129.3906	98.06771	10:36:55.252
0.117	127.625	100.7396	10:36:55.372
0.12	131.6563	103.3438	10:36:55.489
0.119	134.6406	105.7865	10:36:55.609
0.123	135.4063	106.0521	10:36:55.728
0.123	134.9688	101.7604	10:36:55.851
0.117	134.25	105.25	10:36:55.974
0.12	133.8594	104.2135	10:36:56.091
0.12	132.1875	103.0833	10:36:56.211
0.12	132.1406	103.1719	10:36:56.331
0.122	132.6875	103.5938	10:36:56.451
0.119	131.4375	100.2188	10:36:56.573
0.12	131.6875	102.5625	10:36:56.692
0.117	130.5625	101.5833	10:36:56.812
0.121	130.8125	100.5417	10:36:56.929
0.118	133.5313	102.9583	10:36:57.050
0.115	133.7344	102.5573	10:36:57.168
0.126	134.6563	104.2813	10:36:57.283
0.12	134.0469	102.0573	10:36:57.409
0.121	134.8438	104.1667	10:36:57.529
0.117	134.1875	104.75	10:36:57.650
0.114	133.5	101.25	10:36:57.767
0.121	133.5938	103.4479	10:36:57.881
0.121	134.9063	105.1771	10:36:58.002
0.12	134.5625	104.4792	10:36:58.123

B

Blood Pressure type	SBP
Animal Number	
Date of Assessment - use 'dd-mm-yyyy' format	dd-mm-yyyy
Moving Average Window BP (seconds)	240
Moving Average Window HR (seconds)	240
Moving Average Threshold Transposition BP (mmHg)	20.0
Moving Average Threshold Transposition HR (bpm)	-40.0
Interpeak Interval BP (seconds)	2
Peak Cluster Interval BP (seconds)	10
AD Duration Threshold (seconds)	120
Heart Rate Drop Restriction (bpm)	40.0
Upper Heart Drop Average Range (initial +/-% of AD Event)	5.0
Lower Heart Drop Average Range (final % of AD Event)	75.0
Heart Rate Minimum (bpm)	180.0
Heart Rate Maximum (bpm)	625.0
Report Filename	AD Event Detection Report

OK Cancel

C



D

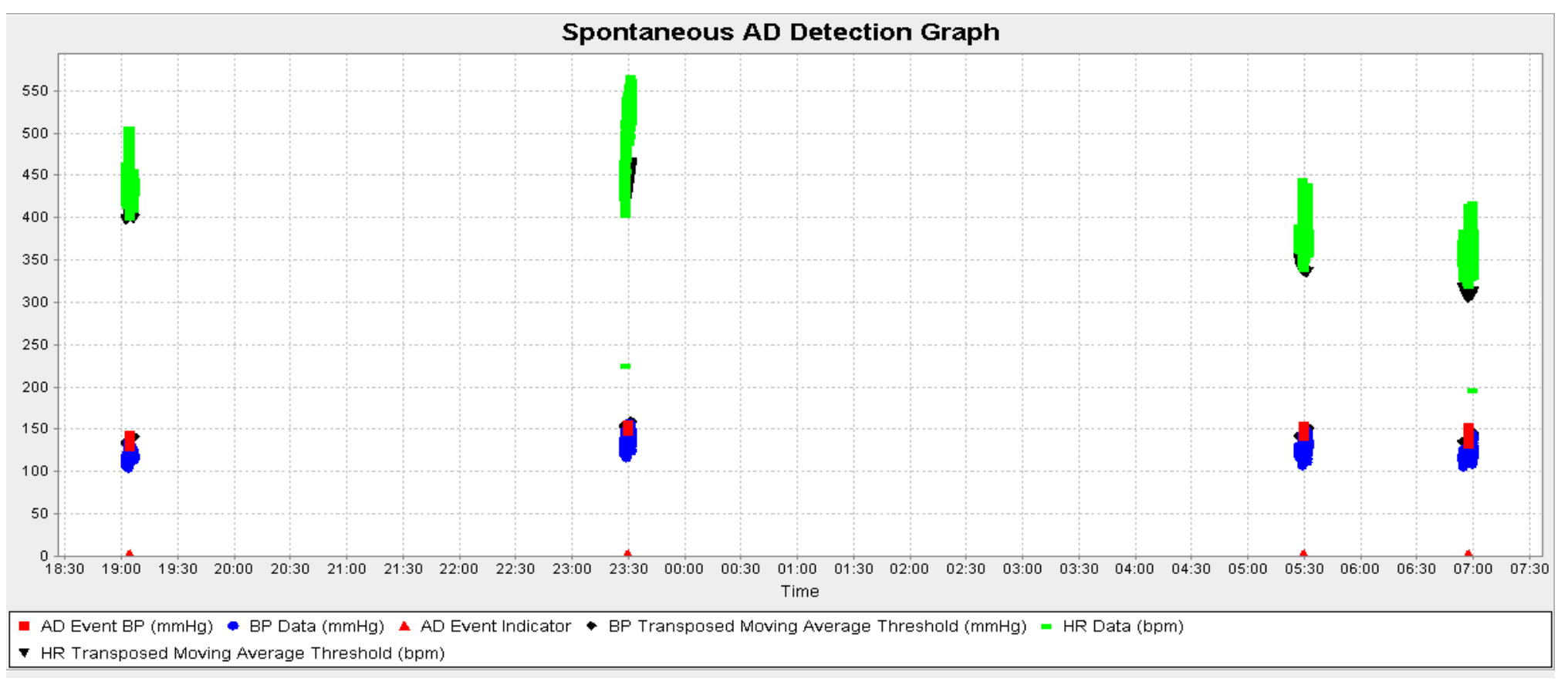


Figure 4

Excel Spreadsheet- Table of Materials/Equipment

Click here to download Excel Spreadsheet- Table of Materials/Equipment: JoVE Materials.xls

Name of Material/ Equipment	Company	Catalog Number
11 Male Wistar Rats (250-300g)	Harlan Laboratories	141
Lab Chart (PowerLab® Data Acquisition System)	AD Instruments	
Pressure Telemeter	Milar	RP-TRM54P
Configurator	Milar	TR190
SmartPad	Milar	TR180
Isoflurane	Baxter Corp.	DIN: 02225875
Baytril	Bayer Healthcare	DIN: 02169428
20-Gauge Curved Needle		
4-0 Silk Sutures	eSutures.com	A182
4-0 Vicryl Subcuticular	eSutures.com	J385
Buprenorphine	Reckitt Benckiser	DIN: 0281250
Ketoprofen	Merial	DIN: 02150999
Ketamine Hydrochloride	Bioniche	DIN: 01989529
Medetomidine Hydrochloride	Pfizer	DIN: 02333929
Lactated Ringer's Solution	Braun	DIN: 01931636
Gelfoam	Pharmacia & Upjohn Company	03603-14-1
Microscissors	Fine Science Tools	15003-008
Matlab	MathWorks	
10 mm Catheter	Coloplast	AA6110

Comments/Description

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Author(s): David Rabchak, Christopher West, Barbara Frias, Andrei V Krassioukov

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Name: Dr. Andrei V. Krassioukov

Department: Medicine, Division of Physical Medicine and Rehabilitation

Institution: University of British Columbia.

Article Title: Development of an algorithm to perform a comprehensive study of autonomic dysreflexia in animals with high spinal cord injury using a telemetry device.

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Rebuttal Comments - Development of an algorithm to perform a comprehensive study of autonomic dysreflexia in animals with high spinal cord injury using a telemetry device.

Changes made by the Science Editor:

1. There have been edits made to the manuscript.

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

2. 1.1.1 - How is the drug administered?

The enrofloxacin drug is administered subcutaneously (i.e. s.c).

3. 1.1.4 - Is there anything that must be moved or dissected to expose the descending aorta?

W hen making the abdominal incision, cut through the peritoneum to expose the intestines. Hydrate the gut tissue with 2-3mL ringers and retract the intestines. The descending aorta will be exposed at the posterior abdominal wall. Blunt dissect around the aorta and separate the aorta form the inferior vena cava using a blunt instrument.

4. Please describe software usage in section 3 in more step-wise detail.

We would like to thank reviewer for their comments. We have rephrased section 3 as to enhance reader's understanding of the different panels within the BP analysis software and the recommended settings that we had used for our algorithm. As a results of the science editor's previous comments, we have previously added Figure 4 to illustrate the user interface of the software and the output (Figure 4C and 4D)

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
This manuscript describes a novel algorithm to assess spontaneously occurring episodes of autonomic dysreflexia after a spinal cord injury. This will significantly impact the field.

Major Concerns:
None

Minor Concerns:
None

Additional Comments to Authors:
N/A

We would like to thank reviewer for their comments.

Reviewer #2:
Manuscript Summary:
This protocol presents a novel pattern recognition algorithm developed for a JAVA platform software to study the fluctuations of cardiovascular parameters as well as the number, severity and duration of spontaneously occurring autonomic dysreflexia events. The software is able to apply a pattern recognition algorithm on hemodynamic data such as systolic blood pressure and heart rate extracted from telemetry recordings of conscious and unrestrained animals before and after thoracic (T3) complete transection.

Major Concerns:
None

Minor Concerns:
None

Additional Comments to Authors:
None

We would like to thank reviewer for their comments.

Reviewer #3:

Manuscript Summary:

This is a very interesting study and also very useful if can be translated into human being. The experimental design is reasonable. The description of technique is very details. It can be accepted in current format. It may be better and more understandable if the author can provide a diagram to illustrate how the device is set in the animal.

Major Concerns:

N/A

Minor Concerns:

It may be better and more understandable if the author can provide a diagram to illustrate how the device is set in the animal.

We would like to thank the reviewer for their comments. We have added a simplified diagram (derived from Mayorov et al. 2001) outlining the anatomical landmarks associated with the telemetry device implantation (Figure 1A), with respect to the T3 SCI surgery.

Additional Comments to Authors:

N/A