Data Article

Spinal cord RNA-seq data after a baclofen treatment in mice with a spinal cord injury

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Nidia de Sousa [a,b,1], Diego Robledo [c,1], Laura González-Llera [d],
Andreia G. Pinho [a,b], Diogo J. Santos [a,b], Susana Monteiro [a,b],
Jonas Campos [a,b], Jorge R. Cibrão [a,b], Nuno A. Silva [a,b],
Laura Sánchez [e,f], António J. Salgado [a,b], Antón Barreiro-Iglesias [d,*]

[a] ICVS/3B's Associate Lab, PT Government Associated Lab, Braga/Guimarães 4806-909, Portugal
[b] School of Medicine, Life and Health Sciences Research Institute (ICVS), University of Minho, Braga 4710-057, Portugal
[c] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian EH25 9RG, UK
[d] Department of Functional Biology, Faculty of Biology, CIBUS, Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain
[e] Department of Zoology, Genetics and Physical Anthropology, Faculty of Veterinary Science, Universidade de Santiago de Compostela, Lugo 27002, Spain
[f] Preclinical Animal Models Group, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela 15706, Spain

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ABSTRACT

Spinal cord injury (SCI) leads to severe functional deficits. Currently, there are no available pharmacological treatments to promote neurological recovery in SCI patients. Recent work from our group has shown that a baclofen treatment can promote functional recovery after a compression SCI in mice [1]. Here, we provide transcriptomic (RNA-seq) data from adult mouse spinal cords collected 7 days after a compression SCI and baclofen (vs vehicle) administration. The Illumina NovaSeq 6000 platform was used to generate the raw transcriptomic data. In addition, we also present bioinformatic analyses

* Corresponding author.
  E-mail address: anton.barreiro@usc.es (A. Barreiro-Iglesias).
  Social media: @Nidia_deSousa (N. de Sousa), @Diego___RS (D. Robledo), @LauraLlerag (L. González-Llera), @BarreiroAnton (A.J. Salgado)
  [1] These authors contributed equally to this work.

including differential gene expression analysis, enrichment analyses for various functional annotations (gene ontology, KEGG and BioCarta pathways or InterPro domains) and transcription factor targets. The raw RNA-seq data has been uploaded to the NCBI Sequence Read Archive (SRA) database (Bioproject ID PRJNA886048). The data generated from the bioinformatic analyses is contained within the article.

Specifications Table

Subject	Neuroscience
Specific subject area	Traumatic spinal cord injury.
Type of data	RNA-seq raw data
	Tables (Excel spreadsheets)
	Figures/graphs
How the data were acquired	RNA sequencing was performed with the Illumina NovaSeq 6000 platform. Software for bioinformatic analyses: FastQC, fastp, kallisto, DESeq2, DAVID and ChEA3.
Data format	Raw and analysed.
Description of data collection	Total RNA was extracted using Trizol from the spinal cord tissue surrounding the lesion site (-0.5 cm to 0.5 cm from the lesion site) of adult (8 to 12 weeks of age) C57BL/6J female mice 7 days after a compression spinal cord injury at T8.
	Experimental groups: control vehicle treated (saline solution) mice and baclofen treated (1 mg/kg) mice. The treatments (saline or baclofen) were administrated after the injury and once a day for the 7 days.
Data source location	• Institution: Life and Health Sciences Research Institute, University of Minho
	• City: Braga
	• Country: Portugal
Data accessibility	Repository names: NCBI SRA and Mendeley Data.
	Raw data identification number: BioProject ID PRJNA886048.
	Analysed data identification number: DOI:10.17632/r7kj2rk2k3.1
	Direct URL to the raw data: https://www.ncbi.nlm.nih.gov/sra/PRJNA886048
	Direct URL to analysed data: https://data.mendeley.com/datasets/r7kj2rk2k3/1
	With the article.

Value of the Data

- These data provide valuable information to understand the genetic/cellular effects of a baclofen treatment after spinal cord injury (SCI).
- The data can be useful for researchers interested in the study of genes and signalling pathways regulating regeneration and/or neuroprotection after SCI.
- The RNA-seq raw data can be used in different workflows and bioinformatic analyses apart from those already provided with the article.
- Data on differentially expressed genes and pathway enrichment can guide future functional analyses in models of SCI.

1. Objective

Spinal cord injury (SCI) is a devastating condition leading to permanent functional deficits in the affected individuals. Loss of neuronal/glial cells and lack of axon regeneration are the main

contributors to the absence of recovery and currently there are no pharmacological treatments to promote neurological recovery after SCI. Interestingly, Romaus-Sanjurjo et al. [2,3] showed that baclofen (a GABAB receptor agonist) administration inhibits caspase activation and promotes axon regeneration in descending neurons of lampreys after a complete SCI. More recently, our group also showed that an acute baclofen treatment (starting on the day of injury) improves locomotor recovery and control of bladder function after SCI in mice [1]. Importantly, this correlates well with observational data in human patients revealing a positive association between baclofen administration and neurological recovery after SCI [4,5]. However, the molecular and cellular mechanisms by which baclofen promotes functional recovery in injured mice are not fully understood yet. Here, we used one of the baclofen treatment conditions (1 mg/kg administrated intraperitoneally once a day) previously used by de Sousa et al. [1] and performed an RNA-seq analysis of the spinal cord of control and baclofen treated mice 7 days after a T10-T11 compression SCI.

2. Data Description

Total RNA was extracted from the spinal cord surrounding the lesion site (0.5 cm above and 0.5 cm below the lesion) of control ($n = 6$) and baclofen treated ($n = 6$) mice. Thereafter, libraries were prepared and checked for quality using the Bionalyzer 2100 (all samples had RIN values higher than 7). The raw RNA-seq reads were obtained with the Illumina NovaSeq 6000 platform and deposited in the NCBI SRA repository (BioProject ID PRJNA886048; baclofen treated samples are named AC-1 mg in the repository). Then, we carried out different bioinformatic analyses of the raw RNA-seq data including: quality control, pseudo-alignment against the mice transcriptome, principal component analysis, differential expression and functional enrichment. Fig. 1A shows the principal component analysis showing the degree of clustering of control and baclofen treated samples. Analysis of differential gene expression between vehicle treated control animals and baclofen treated animals revealed a total of 992 differentially expressed genes (FDR < 0.05) (Fig. 1B; Supplementary File 1). Of these, 347 genes showed decreased expression and 645 genes showed increased expression in the injured spinal cord after the baclofen treatment (Fig. 1B; Supplementary File 1). Fig. 1C shows the expression differences of the 10 genes showing the most significant differential expression (see Supplementary File 1). Enrichment analyses revealed 95 significantly enriched terms, including GO terms, KEGG pathways and Inter-Pro domains (Supplementary File 2). Similar pathways from enrichment analyses were grouped together and are shown in Supplementary File 3. A transcription factor target enrichment analysis was performed on the list of differentially expressed genes (see Supplementary File 4). Supplementary files 1 to 4 were deposited in Mendeley Data (DOI:10.17632/r7kj2rk2k3.1).
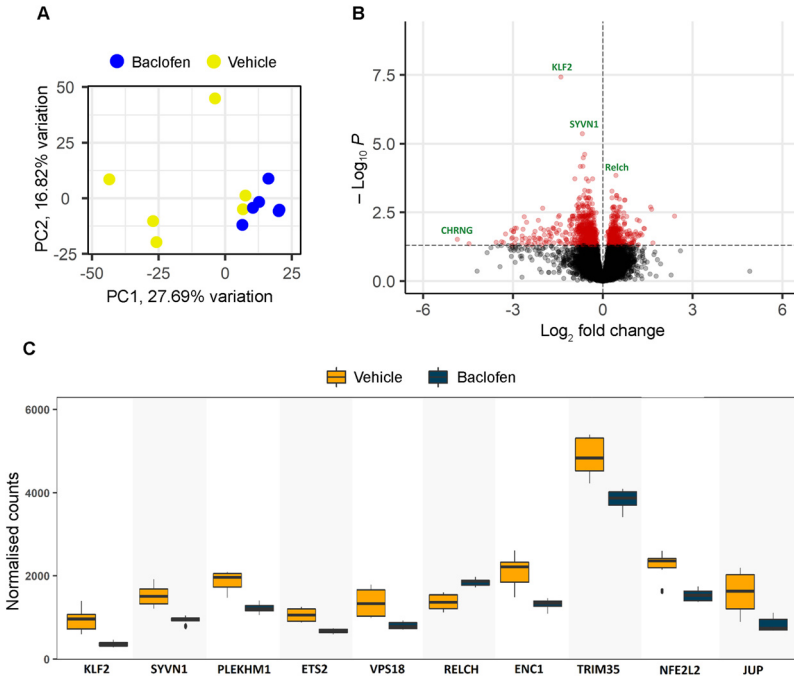
Fig. 1. *A.* Principal component analysis showing the vehicle control (yellow dots) and baclofen (blue dots) treated samples clustered according to their gene expression. Each dot represents one mouse. *B.* Volcano plot displaying the results of the RNA-seq analysis (statistical significance vs. magnitude of change) and highlighting some of the most significant genes. Each dot represents one gene. A positive fold change indicates increased expression after the baclofen treatment. *C.* Boxplot displaying the expression of the 10 genes showing the most significant differential expression after the baclofen treatment. Full gene names and functions (Uniprot annotations) of the genes highlighted in graphs B and C: *Chrng* (Acetylcholine receptor subunit gamma): After binding acetylcholine, the AChR responds by an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane; *Klf2* (Krueppel-like factor 2): Transcription factor that binds to the CACCC box in the promoter of target genes such as HBB/beta globin or NOV and activates their transcription; *Syvn1* (E3 ubiquitin-protein ligase synoviolin): Component of the endoplasmic reticulum quality control (ERQC) system also called ER-associated degradation (ERAD) involved in ubiquitin-dependent degradation of misfolded endoplasmic reticulum proteins. During the early stage of B cell development, supports further differentiation into mature B cells; *Plekhm1* (Pleckstrin homology domain-containing family M member 1): Acts as a multivalent adapter protein that regulates Rab7-dependent and HOPS complex-dependent fusion events in the endolysosomal system and couples autophagic and the endocytic trafficking pathways; *Ets2* (Protein C-ets-2): Transcription factor activating transcription. Binds specifically the GGA DNA motif in gene promoters and stimulates transcription of those genes; *Vps18* (Vacuolar protein sorting-associated protein 18 homolog): Plays a role in vesicle-mediated protein trafficking to lysosomal compartments including the endocytic membrane transport and autophagic pathways. Believed to act as a core component of the putative HOPS and CORVET endosomal tethering complexes which are proposed to be involved in the Rab5-to-Rab7 endosome conversion. Involved in dendrite development of Pukinje cells; *Relch* (RAB11-binding protein RELCH): Regulates intracellular cholesterol distribution from recycling endosomes to the trans-Golgi network through interactions with RAB11 and OSBP; *Enc1* (Ectoderm-neural cortex protein 1): Actin-binding protein involved in the regulation of neuronal process formation and in differentiation of neural crest cells. Down-regulates transcription factor NF2L2/NRF2 by decreasing the rate of protein synthesis; *Trim35* (E3 ubiquitin-protein ligase TRIM35): E3 ubiquitin-protein ligase that participates in multiple biological processes including cell death, glucose metabolism, and in particular, the innate immune response; *Nfe2l2* (Nuclear factor erythroid 2-related factor 2): Transcription factor that plays a key role in the response to oxidative stress: binds to antioxidant response (ARE) elements present in the promoter region of many cytoprotective genes. Also plays an important role in the regulation of the innate immune response. It is a critical regulator of the innate immune response and survival during sepsis by maintaining redox homeostasis and restraint of the dysregulation of pro-inflammatory signaling pathways like MyD88-dependent and -independent and TNF-alpha signaling; *Jup* (Junction plakoglobin): Common junctional plaque protein. The presence of plakoglobin in both the desmosomes and in the intermediate junctions suggests that it plays a central role in the structure and function of submembranous plaques. Acts as a substrate for VE-PTP and is required by it to stimulate VE-cadherin function in endothelial cells. Can replace beta-catenin in E-cadherin/catenin adhesion complexes which are proposed to couple cadherins to the actin cytoskeleton.

3. Experimental Design, Materials and Methods

3.1. Animals

Female C57BL/6 J mice (Charles River, USA), 8 to 12 weeks of age, were maintained at the animal facilities of the Institute of Life and Health Sciences (ICVS, Braga, Portugal) under standard laboratory conditions (12 h light: 12 h dark cycles, 22 °C, relative humidity of 55%, ad libitum access to standard food and water). The 12 mice used in this study were randomly assigned to the baclofen ($n = 6$) or saline (vehicle control; $n = 6$) treatment groups.

3.2. SCI Surgery

Animals were handled every other day for 1 week prior to the surgeries, for stress reduction and habituation to the experimenter presence. Animals were anesthetized with an intraperitoneal injection of a mixture containing ketamine (Imalgene; 75 mg/kg; Merial, France) and medetomidine (Dormitor; 1 mg/kg; Pfizer, USA). Once anesthetized, buprenorphine (Bupaq; 0.05 mg/kg) was also administrated subcutaneously for analgesia. To confirm the surgical plane of anesthesia, mice were pinched on the lower limb toes. Mice were placed under a warm lamp before and after surgery and placed on a warm surgical blanket to avoid dropping the body temperature. Vaseline was applied onto the eyes to prevent drying and corneal damage. Surgeries were performed following aseptic procedures. A dorsal midline incision was made at the level of the thoracic spine (T5-T12). The paravertebral muscles were retracted and the spinous and laminar arc of T8-T9 were removed, exposing the spinal cord. The spinal cord was completely compressed using forceps (Dumont #5/45° angled; FST, USA) for 10 s to produce a severe lesion at the T10-T11 spinal cord segment level. Lesions were performed consistently with the same forceps and always by the same experimenter to reduce variability in the applied force. After SCI, the muscle and skin were closed with 4.0 polyglycolic absorbable sutures (Safil, G1048213). Atipamezole (Antisedan; Pfizer) was administered subcutaneously to revert the anesthesia, and animals were allowed to completely recover from the anesthesia under a warm lamp. Animals were then placed on individual cages with hydrogel and moisturized food pellets on the floor to allow easy access to water and food. Buprenorphine (Bupaq, 0.05 mg/kg), vitamins (Duphalyte, Pfizer), saline, and enrofloxacin (Bayer, Germany) was administrated twice a day for post-operative care. Manual voiding of bladders was performed twice a day. During post-operative care, animals were also monitored for body temperature and recovery of general activities. Basso Mouse Scale (BMS) was used 48 h post-injury (hpi) as previously described [1] to confirm the SCI led to no motor movement in both hind paws (BMS score = 0). BMS scoring was performed as a quality control procedure for the surgery to exclude eventual partial lesions.

3.3. Drug Treatment

After SCI, mice were randomly assigned to each of the 2 experimental groups. Baclofen ((±)-β- (Aminomethyl)-4-chlorobenzenepropanoic acid; Lioresal; (±)-Baclofen; Sigma-Aldrich, USA; CAS number 1134470; 1 mg/kg) or saline for the Vehicle treated group (Saline Solution 0.9%; B. Braun, Germany; SKU 06063042) were administrated intraperitoneally (IP). Baclofen or saline were administrated after the lesion and once a day for 7 days. The first IP injection was performed 10 min after the SCI surgery.

3.4. Tissue Processing and Total RNA Extraction

At 7 dpi mice were anesthetized as above and euthanized. Spinal cords (-0.5 cm to 0.5 cm surrounding the lesion epicenter) were dissected out and immediately put in RNAlater (Ambion,

USA). RNA extraction was performed using the RNeasy mini kit (Qiagen, Germany) with DNase treatment following the manufacturer's instructions. Isolated RNAs were eluted in nuclease free water. RNA quality and quantity were evaluated in a Bioanalyzer (Bonsai Technologies, Spain) and in a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA). RNAs were stored at −80 °C until use for RNA-seq.

3.5. RNA-seq and Bioinformatic Analyses

The Illumina Truseq mRNA stranded RNA-Seq Library Prep Kit protocol was followed. Libraries were checked for quality and quantified using the Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA), before being sequenced on one S1 lane of the Illumina NovaSeq 600 instrument using 150 base paired-end sequencing at Edinburgh Genomics (UK). The quality of the sequencing output was assessed using FastQC v.0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Quality filtering and removal of residual adaptor sequences was conducted on read pairs using Fastp v.0.20.0 [6]. Illumina specific adaptors were clipped from the reads and leading and trailing bases with a Phred score <20 were removed; only reads where both pairs were longer than 36 bp post-filtering were retained. Transcript abundance was quantified using Kallisto v0.46.1 [7]; filtered reads were pseudo-aligned to the transcriptome of the mouse genome assembly GRCm39 (GCA_000001635.9). Differential expression (DE) analyses were performed using R v.4.1.1 (https://www.r-project.org/). Gene count data were used to estimate differential gene expression using the Bioconductor package DESeq2 v.3.4 [8]. Briefly, size factors were calculated for each sample using the "median of ratios" method and count data was normalized to account for differences in library depth. Next, gene-wise dispersion estimates were fitted to the mean intensity using a parametric model and reduced toward the expected dispersion values. Finally, a negative binomial model was fitted for each gene and the significance of the coefficients was assessed using the Wald test. The Benjamini–Hochberg false discovery rate (FDR) multiple test correction was applied, and genes with FDR < 0.05 were considered differentially expressed. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), BioCarta pathways and InterPro domain enrichment analyses were performed using the DAVID bioinformatics resource Functional Annotation tool [9], using the mouse transcriptome as background. Transcription factor enrichment analysis was performed using ChEA3 [10]. Gene functions shown in Fig. 1C come from Uniprot annotations [11].

Ethics Statements

The animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the EU Directive 2010/63/EU for animal experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Raw RNA-seq data (Original data) (NCBI SRA).

CRediT Author Statement

Nidia de Sousa: Investigation, Writing – review & editing; **Diego Robledo:** Writing – original draft, Formal analysis; **Laura González-Llera:** Formal analysis; **Andreia G. Pinho:** Investigation; **Diogo J. Santos:** Investigation; **Susana Monteiro:** Investigation; **Jonas Campos:** Conceptualization, Writing – original draft; **Jorge R. Cibrão:** Investigation; **Nuno A. Silva:** Investigation; **Laura Sánchez:** Investigation; **António J. Salgado:** Writing – review & editing, Supervision; **Antón Barreiro-Iglesias:** Conceptualization, Writing – original draft, Supervision.

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Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2022.108809.

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