Transcriptional changes in sensory ganglia associated with primary afferent axon collateral sprouting in spared dermatome model

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Abstract

Primary afferent collateral sprouting is a process whereby non-injured primary afferent neurons respond to some stimulus and extend new branches from existing axons. Neurons of both the central and peripheral nervous systems undergo this process, which contributes to both adaptive and maladaptive plasticity (e.g., [1–9]). In the model used here (the “spared dermatome” model), the intact sensory neurons respond to the denervation of adjacent areas of skin by sprouting new axon branches into that adjacent denervated territory. Investigations of gene expression changes associated with collateral sprouting can provide a better understanding of the molecular mechanisms controlling this process. Consequently, it can be used to develop treatments to promote functional recovery for spinal cord injury and other similar conditions. This report includes raw gene expression data from microarray experiments in order to study the gene regulation in spared sensory ganglia in the initiation (7 days) and maintenance (14 days) phases of the spared dermatome model relative to intact (“naïve”) sensory ganglia. Data has been deposited into GEO (GSE72551).

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Additional information

**Value of the data**

- Transcriptomic analysis of this axon growth process is novel and could reveal mechanisms of axon growth.

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**Specifications**

| Specifiers | Value |
|------------|-------|
| Organism/cell line/tissue | Rattus norvegicus |
| Sex | Female |
| Sequence or array type | GeneChip microarray |
| Data format | Rattus norvegicus 230_2 Raw; CEL files |
| Experimental factors | T11 spared DRG, day 7 post injury vs. naïve; T11 spared DRG, day 14 post injury vs. naïve |
| Experimental features | Gene expression profiling of the T11 spared dermatome using naïve rats (n = 5); T11 spared DRG at day 7 post denervation of neighboring dermatomes (n = 7), and T11 spared DRG at day 14 post denervation of neighboring dermatomes (n = 7) |

**Consent**

- Sample source location: Not applicable
- Consent: Not applicable

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Axonal collateral sprouting, modeled here using peripheral nervous system, plays a role in both adaptive and maladaptive neural plasticity in CNS and PNS.

Model provides samples enriched for neurons undergoing collateral sprouting, and impoverished for injured neurons. Provides transcriptomic profile against which other profiles can be compared to determine shared/different mechanisms.

1. Direct link to deposited data

Data is available through the Gene Expression Omnibus (GEO) [10] through the direct link http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72551

2. Experimental design, materials and methods

2.1. Experimental design

All surgical procedures were performed in accordance with published NIH Guidelines and the Institutional Animal Care and Use Committee (IACUC) of Stony Brook University and the University of Louisville (sites where animal work was performed). In this study, we sought to identify gene expression changes associated with collateral sprouting. The experiments were designed to discover global gene expression changes in non-injured DRG neurons undergoing collateral sprouting. Collateral sprouting was induced using the “spared dermatome” model [dermatome is the neuroanatomical term for the area of skin innervated by a single spinal segment]. The focus of the study was the left-side dorsal root ganglion (DRG) of the 11th Thoracic spinal segment (T11). Adult female Sprague-Dawley rats (260–310 g) were anesthetized with pentobarbital (65 mg/kg, i.p.). Left-side T11 DRG neurons remained intact and underwent collateral sprouting after the left-side Dorsal and Lateral cutaneous nerves (DCn and LCn) of the adjacent segments (T9, T10, T12, and T13) were cut and ligated (to prevent regeneration) (Fig. 1A). Thus, the T9, T10, T12, and T13 dermatomes were denervated, but the T11 dermatome remained intact. Axons of the T11 dermatome (and thus derived from the T11 DRG), extended new branches to innervate the T9, T10, T12, and T13 dermatomes (Fig. 1B). [N.B.: This is NOT a spared root experiment. ALL spinal roots were non-injured.] The acute denervation was confirmed using the cutaneous trunci muscle reflex (CTMR) in response to pinch [11–13]. The CTMR could be evoked from innervated areas but not from areas whose innervation had been cut. Thus, the border between innervated (i.e., T11 dermatome) and denervated (i.e., T9, 10, 12, 13 dermatomes) could be defined. Control for a negative CTMR response due to anesthesia levels was the presence of CTMR to pinch of non-denervated skin (T11 dermatome or contralateral side). When cutaneous axons expanded into denervated skin by collateral sprouting, so did the area of skin from which a CTM reflex could be evoked by pinch. Collateral sprouting-mediated expansion of the innervation over time was monitored using

| Sample ID | Time point | ng/ul | 260/280 | GEO ID |
|-----------|------------|-------|---------|--------|
| AJP       | Naive      | 27    | 2.13    | GSM1865032 |
| AKG       | Naive      | 58    | 2.02    | GSM1865033 |
| AKJ       | Naive      | 18    | 2.08    | GSM1865034 |
| AKI       | Naive      | 46    | 2.05    | GSM1865035 |
| AKJ       | Naive      | 48    | 2.08    | GSM1865036 |
| AJS       | 7 day      | 53    | 2.06    | GSM1865037 |
| AKB       | 7 day      | 38    | 2.01    | GSM1865038 |
| AJT       | 7 day      | 43    | 2.07    | GSM1865039 |
| AJV       | 7 day      | 36    | 2.08    | GSM1865040 |
| AJR       | 7 day      | 71    | 2.06    | GSM1865041 |
| AKI       | 7 day      | 34    | 2.06    | GSM1865042 |
| AKJ       | 7 day      | 42    | 2.07    | GSM1865043 |
| AIZ       | 14 day     | 33    | 2.04    | GSM1865044 |
| AIV       | 14 day     | 48    | 2.05    | GSM1865045 |
| AIO       | 14 day     | 29    | 2.03    | GSM1865046 |
| AJN       | 14 day     | 51    | 2.07    | GSM1865047 |
| AIV       | 14 day     | 52    | 2.00    | GSM1865048 |
| AIX       | 14 day     | 48    | 1.99    | GSM1865049 |
| AIV       | 14 day     | 32    | 1.92    | GSM1865050 |

Fig. 1. Experimental Design. A) Schematic of the cross-sectional neuroanatomy of the thoracic region of the rat. The DRG is the structure in which the cell bodies of spinal sensory neurons are housed and which was used for microarray. Continuous with the DRG are the structures carrying the axon-branches of the sensory neurons: the dorsal root carrying axons into the spinal cord, and the spinal nerve (or nerve root in human clinical terms) carrying axons out to their peripheral target tissues. B) (Top) Schematic representation of the surgical preparation for the spared dermatome model, showing dorsal cutaneous nerve only (T = thoracic). (Middle, bottom) Mockup representation of the denervated and spared dermatomes, and the expansion of the spared T11 dermatome into the denervated dermatomes by collateral sprouting of the T11 sensory neurons. Black dots represent sensitive (i.e., innervated) areas of skin as defined by activation of the CTM reflex and the yellow/green shaded areas represent areas of denervation induced by axotomy of the T9, T10, T12 and T13 Dorsal and Lateral Cutaneous nerves which become re-innervated by expansion of spared T11 axons by 28 days. C) Graph of the reduction over time in the denervated area of skin (i.e., successful reinnervation by collateral sprouting); n = 6; error bars are SD.
the CTMR to map the border between innervated and denervated skin regions. Collateral sprouting progressed as previously described [14], with expansion barely-detectable in some of the rats by 7 days, but easily detectible in all rats by 14 days. Temporal progression of this process from a separate set of animals is exemplified in Fig. 1C.

The thoracic spared dermatome model was used because it offers an enrichment of spared-sprouting neurons in a single DRG with minimal injury of neurons in the same DRG. This is important for making inquiries into the similarities/differences between axonal collateral sprouting and axonal regeneration induced by nerve injury. The model is an enrichment, but is not perfect — there is some cross-over between dermatomes [15]. However, these cross-overs are limited [16] and there is no true plexus for the thoracic cutaneous nerves used here.

Technical issues dictated that we use whole DRG homogenization and not laser-capture of individual neurons. Principally, it is unclear how many T11 neurons are involved in the collateral sprouting process in this model, but it cannot be a majority. First, not all DRG neurons of the T11 DRG will undergo collateral sprouting. Large neurons do not innervate skin [16,18] and neurons innervating the central region of the T11 dermatome may not be affected (they may be too far from the denervated skin). Second, neuronal tracing from reinnervated skin, which might be used to specifically label those neurons with axons which had undergone sprouting, was not feasible because of the 7–10 days latency required for retrograde transport. Finally, there is no known biomarker for collateral sprouting that could have been employed to reveal those neurons undergoing sprouting. For these reasons, we designed the study using whole DRG homogenate.

A total of 20 Affymetrix Rat Genome 230 2.0 microarrays were analyzed: six naive controls, seven replicates at day 7 post-surgery (presumed to represent an “initiation phase”), and seven replicates at day 14 post-surgery (presumed to represent a “maintenance phase”). DRGs were NOT pooled onto microarrays. Each animal had its own microarray with T11 DRG mRNA sample which underwent 2-round amplification. After quality control analysis, one of the naive control samples was removed from further analysis yielding a final set of samples from naive (n = 5), 7 day (n = 7), and 14 day (n = 7).

### 2.2. Sample preparation

Animals were euthanized after pentobarbital overdose, exsanguinated by transcardial perfusion with heparinized phosphate-buffered saline, and the tissues (including left-side T11 DRG) extracted and flash-frozen with liquid nitrogen. Total RNA was extracted from the single left T11 DRG using an RNeasy Kit (Qiagen Inc., Valencia, CA); RNA concentration was determined by using spectrophotometry (Nanodrop, Ambion) and quality was assessed by capillary gel electrophoresis (Bioanalyzer, Agilent). RNA yields were in excess of 200 ng and A260/A280 ratio in nuclease-free water ranged between 2.0 and 2.1.

100 ng total RNA per DRG was amplified to produce sufficient biotin labeled antisense RNA for GeneChip analysis. Two round linear amplification was performed using MessageAmp®II (Ambion) according to manufacturer’s instructions. Total RNA was converted into first-strand cDNA by using a T7-Oligo (dT) Primer. After the first round in vitro transcription, 2 μg aRNA was used as input for the second round of amplification using biotin labeled nucleotides during in vitro transcription (ENZO BioArray® HighYield® RNA Transcipt Labeling Kit (T7), Enzo Life Sciences, Farmingdale, NY, USA). The purity and molecular weight range of aRNA products were confirmed by using agarose gel electrophoresis. Amplified RNA was shipped to the Salk Institute (Gage lab) and confirmed to be intact (not degraded) by formamide-gel electrophoresis and spectrophotometry (Table 1). Biotinylated aRNA was fragmented and hybridized to Rat 230 2.0 microarrays (Affymetrix) according to manufacturer’s instructions.

### 2.3. Data acquisition

GeneChips were scanned using an Affymetrix GeneChip® Scanner 3000. All data analysis was performed in R using Bioconductor packages. CEL files were preprocessed and normalized using robust multichip averaging (RMA). Differential expression was performed using Limma.

### 2.4. Results

Analysis of the data using RMA and Limma results in 917 differentially expressed probe sets at day 7 vs. naive and 1920 differentially expressed probe sets at day 14 vs. naive, as defined by fold-change > 1.2 with p-value < 0.05. Some of these were examined by qPCR as validation (Table 2).

#### Conflict of interest

The authors declare they have no conflict of interest.

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**Table 2**

| Gene name | Microarray | qPCR |
|-----------|------------|------|
| Bdnf | 1368767_at | 1.7 | 1.9 |
| Frzb | 1373615_at | −1.1 | −1.1 |
| Grhl3 | 1378402_at | −2.0 | −2.6 |
| Spp1 | 1367581_a_at | 1.5 | 1.6 |
| Enpp3 | 1367905_at | −1.4 | −1.6 |

| | Probe | Fold-change | p-value | Fold-change | p-value |
| | | 7 days | 14 days | 7 days | 14 days |
| Bdnf | 1368767_at | 1.7 | 1.8 | <0.001 | <0.001 |
| Frzb | 1373615_at | −1.1 | −1.5 | 0.5 | 0.003 |
| Grhl3 | 1378402_at | −2.0 | −1.5 | <0.001 | 0.006 |
| Spp1 | 1367581_a_at | 1.5 | 1.7 | 0.004 | <0.001 |
| Enpp3 | 1367905_at | −1.4 | −1.2 | 0.001 | 0.09 |

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Table 2: Comparison of microarray and qPCR results for select transcripts; fold-change is vs. naive. qPCR primers were based on microarray probe sequences.
