

Interpreting Cellular Recovery in Acute Spinal Cord Injury: Microglial Marker Return, Progenitor Responses, and Endothelial Transience

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ABSTRACT

Microglial marker restoration, oligodendroglial differentiation-associated expression, and declining endothelial tip-cell representation describe different aspects of the response to spinal cord contusion. This analytical review examines whether they provide equivalent evidence of cellular recovery in GSE162610 and its accompanying cytometric and anatomical observations. The atlas contains 66,178 cells from ten preparations and sixteen female C57BL/6J mice, sampled from uninjured spinal cord and at one, three, and seven days after T8 contusion. Six preparations combined two spinal cords and included astrocyte enrichment. P2RY12-high/MSR1-low microglia constituted more than 90% of microglia before injury and approximately 20% at one day, followed by partial restoration at seven days. CD63-high macrophages separated into CD11b-medium/APOE-low chemotaxis-inducing and CD11b-high/APOE-high inflammatory phenotypes. OPC-A persisted across the sampling interval, whereas OPC-B was most prominent at one day and declined by seven days. Canal-associated *Crym* expression and the *Apln*-positive endothelial response were greatest early after injury. Transition kernels, absorption probabilities, finite-step occupancy, and preparation means are interpreted algebraically; numerical CellRank probabilities are not estimated. The weighted-mean identity establishes that changing state proportions can alter a preparation average without changing probabilities within comparable states. Microglial marker return therefore indicates partial phenotypic restoration, oligodendroglial expression identifies differentiation-associated states, and endothelial transience describes temporary activation. These observations are not equivalent measures of repair. Their overlapping timing neither establishes a common cellular trajectory nor substitutes for measurements of myelin formation, perfusion, or neurological function.

KEYWORDS: spinal cord injury, single-cell RNA sequencing, microglia, macrophages, oligodendrocyte progenitors, endothelial tip cells, CellRank 2, GSE162610

1 Introduction

Recovery after spinal cord injury can refer to restoration of a molecular phenotype, production of differentiated cells, reorganization of damaged tissue, or improvement in neurological function. These outcomes need not occur together. Bellver-Landete and colleagues showed that microglia are an essential component of the neuroprotective scar that forms after spinal cord injury [1]. Brennan and colleagues subsequently demonstrated that microglia coordinate interactions among multiple cell populations during

spinal cord repair [2]. These findings show that departure from homeostatic expression can coexist with a protective tissue response. Conversely, return of a homeostatic marker does not establish recovery of axonal organization, vascular performance, or motor function. Interpretation therefore requires explicit identification of both the cellular property being measured and the tissue outcome to which that property is being related.

GSE162610 permits examination of this distinction

within the first week after thoracic contusion. Its uninjured, one-, three-, and seven-day preparations contain resident microglia, peripheral myeloid cells, oligodendrocyte-lineage populations, astroglia, and vascular cells [3]. The P2RY12-high/MSR1-low microglial phenotype partially returns after its early reduction; progenitors display injury-associated and differentiation-related expression; and the endothelial tip-cell response rises early and subsequently declines. Each comparison uses a different biological reference. The microglial comparison concerns resemblance to an uninjured phenotype, the oligodendroglial comparison concerns progenitor and differentiated identities, and the endothelial comparison concerns persistence of an activated state. Their occurrence in the same tissue interval allows these meanings to be compared without treating the atlas as a test of therapeutic efficacy.

The diversity of the myeloid response argues against arranging all injury-associated cells along one inflammatory-to-reparative axis. Wahane and colleagues identified shared and temporally differentiated transcriptional programs in microglia and macrophages, including distinct proliferative responses associated with HDAC3 activity [4]. Proliferation, inflammatory expression, and other response programs therefore require separate interpretation even within a broadly activated population. Persistent microglial reprogramming after spinal cord injury provides a further distinction: later expression of selected homeostatic features can coexist with an enduring injury-associated molecular state [5]. The return of one marker combination consequently addresses phenotypic resemblance, whereas complete normalization would require agreement across the broader cellular response. Neither the activated nor the homeostatic designation alone specifies the net effect on injured tissue.

Oligodendroglial studies distinguish progenitor activity from both myelin production and functional improvement. Genetic fate mapping after mouse contusion linked resident oligodendrocyte precursor cells to newly myelinating cells over a longer interval than the first post-injury week [6]. This establishes a relationship between cellular origin and a myelin-producing descendant that cannot be obtained simply by detecting progenitor and mature oligodendrocyte transcripts in separate cells. Duncan and colleagues further showed that conditional disruption of *Myrf* inhibited oligodendrocyte

remyelination without preventing spontaneous recovery of stepping in their contusion model [7]. That model-specific dissociation concerns a particular behavioral outcome; it does not establish that remyelination is dispensable under all injury conditions. Together, the studies distinguish differentiation-associated identity, formation of myelin, and recovery of a measured function.

The cellular origin of reactive glia likewise depends on evidence beyond a shared transcript. Ren and colleagues found that ependymal contributions to scar tissue were limited, local, and dependent on direct ependymal injury in the mouse models they examined [8]. O'Shea and colleagues identified local astrocytes as the predominant origin of border-forming astrocytes after crush injury or stroke, with a smaller contribution from oligodendrocyte progenitors [9]. These lineage measurements concern different injury settings and cannot assign ancestry to the *Crym*-associated cells recovered in GSE162610. They instead distinguish three observations that must remain separate: position near the central canal, overlap of astrocytic and ependymal expression, and descent from a labelled precursor. Molecular resemblance and anatomical proximity support a description of a reactive population but do not establish which precursor produced it.

Endothelial tip-cell activity has a different relationship to persistence. A cell can occupy a sprouting position temporarily rather than remain in a permanently differentiated tip-cell state. Dynamic imaging in angiogenic sprouting systems demonstrated exchanges between tip and stalk positions [10]. Although those experiments did not track endothelial ancestry after spinal contusion, they explain why a distinctive sprouting phenotype need not represent an irreversible destination. In GSE162610, *Apln* expression associated with elongated podocalyxin-positive cells is greatest early after injury and decreases thereafter [3]. The temporal pattern identifies an early vascular response. Its decline is consistent with several possibilities, including altered cellular state, survival, or recovery during sampling; distinguishing these possibilities from successful vessel maturation requires vascular measurements rather than transcript prevalence alone.

Anatomical location and injury context further determine which recovery comparison an atlas can support. Matson and colleagues identified a

pro-regenerative transcriptional signature in spinocerebellar neurons beyond the immediate lesion in a mouse spinal cord injury atlas [11]. Fan and colleagues demonstrated region-specific cellular responses after complete spinal cord injury in rhesus monkeys [12]. Skinnider and colleagues integrated single-cell and spatial observations across spinal cord injury conditions in the *Tabulae Paralytica* [13]. Together, these studies show that an injury-associated identity must remain linked to the region and model in which it was measured. Li and colleagues showed that neonatal microglia can support scar-free healing and axonal regrowth after crush injury, providing an age-specific comparison rather than evidence for the outcome of marker return in adult contusion tissue [14]. Differences in age, lesion type, tissue location, and follow-up are therefore biologically consequential; conceptual comparison across studies does not increase the number of GSE162610 preparations.

The preparation history of GSE162610 also affects its cellular representation. The 66,178 retained cells originated from ten preparations, including six two-cord pools with astrocyte enrichment [3]. The expression matrix therefore describes cells recovered under defined isolation and enrichment procedures, rather than an unselected census of the lesion. Dissociation can additionally change transcription: Marsh and colleagues identified *ex vivo* activation signatures in mouse and human brain tissue, particularly in microglia [15]. Those findings establish a relevant technical alternative without identifying any particular GSE162610 state as an artifact. Protein phenotypes and tissue localization provide complementary evidence because they address biological properties that an expression matrix does not establish independently. Their value depends on retaining the measurement type and its sampling denominator.

Changes in population composition must be separated from changes among comparable cells. Büttner and colleagues introduced scCODA to model compositional single-cell observations [16]. Dann and colleagues developed Milo to test differential abundance in graph neighborhoods [17]. Neither approach, by itself, identifies the ancestry of cells in an expanded population. Crowell and colleagues developed a multi-sample framework for detecting subpopulation-specific state changes while retaining sample-level structure [18]. Squair and colleagues showed that inappropriate treatment of cells as

independent replicates can produce false discoveries in single-cell differential-expression analysis [19]. Zimmerman and colleagues likewise demonstrated the importance of addressing pseudoreplication in single-cell studies [20]. Häfner and colleagues further showed that nested designs require explicit attention to the hierarchy between cells and biological units [21]. This distinction is especially important when an injured sample contains a larger fraction of cells resembling a selected endpoint. The overall population can then appear closer to that endpoint even when probabilities or expression profiles within comparable precursor states are unchanged.

Cell-state direction introduces assumptions beyond population representation. Haghverdi and colleagues introduced diffusion pseudotime to order cells relative to a selected starting region [22]. Setty and colleagues developed Palantir to characterize differentiation trajectories and cell-fate probabilities from transcriptional state structure [23]. Saelens and colleagues showed through method comparison that trajectory-inference performance depends on the topology and structure of the underlying biological process [24]. Lange and colleagues formulated CellRank around directional transition matrices and fate probabilities [25]. Weiler and colleagues extended this framework in CellRank 2 to integrate multiple directional views of cellular state change [26]. The event represented by any such probability is determined by the cellular graph, directional premise, and chosen destination. Access to a homeostatic microglial region and access to a mature oligodendrocyte region therefore remain different biological events even when both are expressed on a zero-to-one scale. Numerical comparability does not make their implications for recovery equivalent.

Developmental potential provides another premise for assigning direction. Gulati and colleagues introduced CytoTRACE, which relates transcriptional diversity to developmental potential [27]. Kang and colleagues later developed CytoTRACE 2 as a distinct predictive framework for estimating cellular potency [28]. In injured tissue, however, increased RNA complexity can accompany activation or proliferation rather than developmental immaturity. A potential-based ordering may therefore emphasize a different biological property from an ordering rooted in homeostatic microglia. Moreover, two orderings calculated from related expression measurements can share the same sensitivities to transcript content and

cell recovery. Their agreement is not independent observation of a cell's future. The probability definitions considered below make these dependencies explicit while preserving the distinction between a molecularly plausible direction and a measured cellular history.

The research question is whether partial restoration of microglial homeostatic markers, oligodendroglial differentiation-associated expression, and declining endothelial tip-cell representation provide equivalent evidence of cellular recovery during the first week after thoracic contusion. The comparison uses the sequencing, cytometric, and anatomical observations associated with GSE162610 together with algebraic interpretation of transition, absorption, occupancy, and preparation averages. It separates the effect of changing state proportions from the meaning of the state-specific quantities being averaged. This distinction allows each observation to be related to its appropriate biological outcome: microglial phenotypic restoration, oligodendroglial differentiation-associated identity, or transient vascular activation. The conclusion concerns the equivalence of these readouts, not a numerical estimate of tissue repair.

2 Materials and Methods

2.1 T8 contusion, tissue sampling, and study design

This analytical review examines the single-cell, cytometric, and anatomical observations of Milich and colleagues associated with GSE162610 [3]. Female C57BL/6J mice aged eight to ten weeks received a 65-kilodyne contusion at the T8 vertebral level. Tissue collection used an eight-millimeter segment centered on the lesion or the corresponding uninjured region. The analysis relates these observations to the transition and averaging equations below; it does not include additional animal experiments, expression-matrix processing, statistical testing, or fitted CellRank estimates. Publications dated before 2026 provide biological and mathematical comparisons. Their interventions, cell counts, and outcomes remain attributed to their respective experiments and are not pooled with GSE162610.

Sampling comprised uninjured tissue and one, three, and seven days post-injury (dpi). Table 1 distinguishes the preparations, contributing animals, and retained cells at each stage. Four preparations contained a single spinal cord, and six contained two

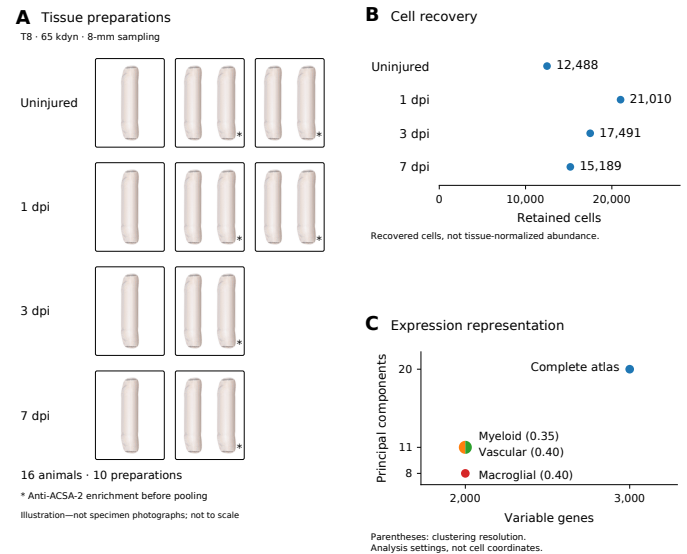


Figure 1. Sampling and expression representation. (A) Cord contributions within preparations. (B) Retained cells. (C) Variable-gene and principal-component settings; parentheses indicate clustering resolution. Cell yield is not tissue-normalized abundance.

cords. In every two-cord preparation, one suspension underwent enrichment with anti-ACSA-2 microbeads before combination with the other suspension. Submitted suspensions met a viability threshold of at least 80% [3]. The nested cord groups in Figure 1(A) represent this sampling hierarchy; their appearance carries no measurement of specimen morphology or lesion severity.

The three uninjured and three one-day preparations each represent five contributing animals, whereas the two preparations at each later stage represent three animals (Table 1). Single-cord and pooled preparations therefore occur at every stage, but the separately observed sequencing units remain unequal in number. Pooling increases the tissue contribution while combining the expression of two animals within one preparation label. Enrichment changes which cells enter that preparation. Consequently, a preparation average describes its recovered population rather than either animal separately or an unselected tissue sample. This distinction limits interpretation of stage differences without invalidating the identification of marker-defined populations within the recovered cells.

2.2 Expression processing and cellular classification

GSE162610 expression processing comprised preparation-specific filtering, normalization, integration, and cell annotation. Quality assessment

Table 1. Tissue preparations, contributing animals, and retained cells in GSE162610 [3].

Injury stage	Preparations	Animals	Retained cells	Tissue preparation
Uninjured	3	5	12,488	One single-cord and two pooled preparations
1 dpi	3	5	21,010	One single-cord and two pooled preparations
3 dpi	2	3	17,491	One single-cord and one pooled preparation
7 dpi	2	3	15,189	One single-cord and one pooled preparation
Total	10	16	66,178	Four single-cord and six pooled preparations

Table 2. Expression-representation and clustering settings in GSE162610 [3].

Cell population	Variable genes	Principal components	Clustering specification
Complete atlas	3,000	20	Seurat default graph-clustering parameters
Myeloid compartment	2,000	11	Resolution 0.35
Vascular compartment	2,000	11	Resolution 0.40
Macroglial compartment	2,000	8	Resolution 0.40

considered unique molecular identifier counts, detected genes, mitochondrial and ribosomal transcript fractions, and doublet predictions. Preparation-specific cutoffs used three median absolute deviations around the median. Scrublet identified potential doublets separately within each preparation, with removal affecting approximately one to five percent of cells. Low-transcript filtering preferentially affected endothelial cells, while high mitochondrial fractions disproportionately affected astrocytes [3]. These cell-type sensitivities mean that a common numerical filtering criterion need not select equivalent biological populations across compartments. The processing description concerns construction of the annotated atlas, not fitting of the probability equations.

Normalization and integration used Seurat version three. Genes detected in at least ten cells were retained within each sample, and SCTransform supplied normalization and variance stabilization. Table 2 gives the feature counts, principal-component dimensions, and clustering specifications for the full atlas and its separately processed compartments. Annotations combined established markers with spinal cord reference datasets. Neurons were excluded from detailed interpretation because dissociation was expected to select against their survival [3]. Resident microglia, peripheral myeloid cells, oligodendrocyte-lineage cells, and endothelium therefore form distinct compartments for interpretation. Astroglial, ependymal-associated, stromal, and pericyte observations provide anatomical and molecular context without defining transitions between these compartments.

The atlas used 3,000 variable genes and 20 principal components, whereas each selected compartment used 2,000 variable genes with either 11 or 8 components (Table 2). The myeloid and vascular points coincide in Figure 1(C) because both used 11 components, although their clustering resolutions differ, at 0.35 and 0.40. The macroglial compartment used 8 components and resolution 0.40. These coordinates describe expression representation rather than cell positions or a ranking of biological complexity. In particular, fewer components do not establish less developmental diversity. Interpretation of a subdivision depends on its molecular content—activation, cell-cycle activity, or differentiation-associated expression—rather than on its numerical clustering specification alone.

The 16 cord contributions, four retained-cell totals, and compartment-specific parameter coordinates in Figure 1 describe three different levels of the experiment. Panel A identifies the ten separately prepared tissue collections. Panel B shows how many expression profiles remain at each stage, with the greatest yield at one dpi. Panel C specifies the dimensions used to classify the atlas and its compartments. More retained cells increase the material available to describe cellular heterogeneity; they do not increase the number of independent preparations. Likewise, coincident analysis parameters do not imply coincident lineages. These distinctions determine what subsequent comparisons of cell states can represent.

Technical explanations for an unexpected marker include doublets and ambient RNA. Wolf and

colleagues introduced Scanpy as a framework for large-scale single-cell expression analysis and graph-based processing [29]. Heumos and colleagues summarized current best practices for single-cell analysis across modalities, including quality control and integration decisions [30]. Germain and colleagues developed scDbtFinder specifically for identifying doublets in single-cell sequencing data [31]. Young and Behjati introduced SoupX to estimate and remove ambient RNA contamination from droplet-based data [32]. Fleming and colleagues developed CellBender to model and remove systematic background signal using a different probabilistic strategy [33]. These publications describe possible technical contributions but do not represent additional procedures performed on GSE162610 in the present analysis. Luecken and colleagues showed that atlas integration must balance improved mixing of preparations against preservation of genuine biological differences [34]. A cross-compartment marker can therefore have more than one explanation. Neither its presence nor a theoretical technical explanation justifies reassigning an annotated population without examining the corresponding measurements.

2.3 Directional probabilities and the biological meaning of an endpoint

Diffusion pseudotime assigns an ordering relative to a selected starting region, as formulated by Haghverdi and colleagues [22]. Developmental potential supplies direction through a different property of expression; CytoTRACE operationalizes this idea through transcriptional diversity [27]. Homeostatic microglia and oligodendrocyte progenitors therefore represent different starting identities and require compartment-specific biological interpretation. Bergen and colleagues generalized RNA velocity to transient cellular states through dynamical modeling of spliced and unspliced RNA [35]. Gorin and colleagues subsequently analyzed the assumptions and identifiability limitations that underlie RNA-velocity inference [36]. These requirements distinguish RNA velocity from an ordering based on ordinary expression counts. The equations below concern the definitions and consequences of directional probabilities; no velocity estimates or fitted transition matrices are available within this analysis.

Let $T^{(P)}$ and $T^{(D)}$ be row-stochastic matrices on the same cells, derived respectively from pseudotime and

developmental potential. Their convex combination is

$$T_\alpha = \alpha T^{(P)} + (1 - \alpha) T^{(D)}, \quad 0 \leq \alpha \leq 1. \quad (1)$$

Eq. (1) preserves the nonnegative entries and unit row sums of its component matrices. Equal weighting gives their arithmetic average, and the limiting weights recover the two components. These properties establish a valid transition matrix but do not determine which biological process its direction represents. For microglia, an ordering away from homeostasis may differ from one emphasizing RNA complexity; combining them does not make activation equivalent to maturation. The parameter α remains symbolic rather than selected or optimized for GSE162610. CellRank 2 separates this construction of directional kernels from the subsequent calculation of cell-state probabilities [26], allowing the biological meaning of direction and destination to be distinguished.

Generalized Perron cluster analysis identifies macrostates from strongly connected regions of a transition structure. The corresponding Markov-state formulation accommodates nonreversible processes [37]. Such a macrostate describes transition behavior and need not coincide with an expression cluster or an irreversible fate. For specified absorbing sets A_f , let Q contain transient-to-transient transitions and R contain transitions from transient cells into those sets. The absorption probabilities satisfy

$$(I - Q)B = R, \quad B = (p_{if}), \\ p_{if} = \Pr(X_{\tau_A} \in A_f \mid X_0 = i), \quad (2)$$

The matrix I is the identity, each row of B corresponds to a transient cell, and each column corresponds to a designated endpoint. The first entry time into the union of absorbing sets is τ_A . Complete absorption requires reachable absorbing sets and the absence of a separate recurrent region that retains probability indefinitely. Under these conditions, Eq. (2) quantifies eventual access to the selected endpoints within the specified Markov process. It does not record direct descent in spinal tissue. A mature oligodendrocyte endpoint concerns differentiation-associated identity, whereas a homeostatic microglial endpoint concerns resemblance to an uninjured phenotype. Endpoint selection is therefore part of the biological definition of the probability, even though either calculation produces a dimensionless value.

A reactive state with uncertain terminal behavior can instead be described by finite-step occupancy,

$$o_{if}(h) = e_i^T T_\alpha^h \chi_f. \quad (3)$$

The vector e_i selects the starting cell, and χ_f represents membership in macrostate f . The integer h counts graph transitions rather than days after injury. Eq. (3) concerns state membership at a finite horizon and does not require the state to be a permanent destination. This distinction is relevant to reactive microglia and the early endothelial tip-cell response. A temporary state can be biologically important even when eventual absorption into it is low, while occupancy of that state does not establish completion of vascular repair. Absorption and finite-step occupancy consequently answer different questions about the same transition model, without identifying the histories of individual cells in tissue.

2.4 Preparation averages and state representation

For a preparation containing n_s eligible cells within one lineage, the mean endpoint probability is

$$\bar{p}_{sf} = \frac{1}{n_s} \sum_{i \in s} p_{if}. \quad (4)$$

The denominator n_s in Eq. (4) counts eligible recovered cells within a preparation, not contributing animals. The resulting average therefore describes preparation s . Equal weighting of preparation averages would avoid assigning more biological weight solely to a preparation with a larger cell yield, but neither averaging operation makes barcodes independent replicates or separates the contributions of pooled animals. Squair and colleagues demonstrated that cell-level replication cannot substitute for the biological replication required for valid differential-expression inference [19]. Häfner and colleagues extended this concern to nested single-cell designs in which cells are explicitly embedded within higher-level biological units [21]. At three and seven dpi, only two preparation-level observations are available for each stage, so agreement between preparations is more informative for a stage comparison than the number of cell-level values within either preparation. Numerical precision at the cellular level does not remove this replication constraint.

The same average can be written exactly as a mixture over states,

$$\bar{p}_{sf} = \sum_k w_{sk} \bar{p}_{skf}, \quad w_{sk} = \frac{n_{sk}}{n_s}. \quad (5)$$

In Eq. (5), n_{sk} counts the cells in state k , w_{sk} is their proportion within the recovered lineage, and $\bar{p}_{skf}$ is their mean endpoint probability. This weighted-mean identity separates state representation from values within states. A preparation mean can differ because the state proportions differ, the state-specific means differ, or both differ. Increasing the proportion of cells with a higher endpoint probability raises the overall mean even when the probabilities of comparable precursor cells remain unchanged. Thus, a shift in an aggregate probability cannot by itself identify a change in precursor behavior. The distinction follows from the definition of the average rather than from a fitted biological effect.

A comparison restricted to common states addresses the cells represented in both preparations under a comparable biological definition. A state not recovered at one stage has no observed state-specific mean at that stage; assigning a value of zero would instead assert that its cells cannot reach the selected endpoint. Those are different statements. Likewise, an identically named state cannot be assumed comparable if its defining expression differs between preparations. The algebraic decomposition identifies these requirements without supplying numerical state weights, state-restricted contrasts, or reweighted outcomes for GSE162610. It clarifies whether an aggregate comparison concerns the recovered lineage mixture or a specified population within that mixture.

2.5 Sampling time, protein phenotypes, and illustration provenance

The four collection stages order separate tissue observations rather than repeatedly observing the same cells. An ordering rooted in uninjured cells can agree with injury time partly because of that starting choice. Klein and colleagues developed moscot to infer time- and space-aware correspondences between cell populations under an optimal-transport framework [38]. Van den Berge and colleagues developed tradeSeq to test trajectory-associated expression patterns along specified lineages [39]. These methods address different questions and rest on different assumptions; neither is performed on GSE162610 in this analytical review. The sampling schedule establishes when tissue was collected, whereas individual transition rates, branch assignments, and regulatory effects remain distinct quantities that collection time alone does not measure. Cytometry distinguishes microglia by P2RY12 and

MSR1 and macrophages by CD63, CD11b, and APOE. Milich and colleagues reported that, within CD63-high macrophages, APOE-low/CD11b-medium cells correspond to the chemotaxis-inducing population whereas APOE-high/CD11b-high cells correspond to the inflammatory population [3]. A later published correction accompanies the original article and is therefore retained in the bibliographic record [40]. Expression profiles, protein measurements, and tissue localization are compared while retaining the cell population and sampling denominator of each observation. Anatomical localization establishes where a marker-defined population occurs; it neither converts a protein category into an ancestry record nor measures the functional effect of that population on the lesion.

The cellular and fluorescence-like tissue artwork is AI-generated illustration, not specimen photography or experimental microscopy. Its apparent morphology, fluorescence, spatial distribution, and colocalization are not biological measurements. Numerical panels display the cell counts or phenotype proportions specified by the cited observations. Temporal entries without an explicit stage-specific observation remain unassigned rather than being set to zero. Mathematical panels depict Equations (1)–(5) symbolically, without fitted probabilities or optimized weights. The figures therefore distinguish quantitative observations, qualitative biological descriptions, and algebraic relationships; their interpretation rests on the measurements and definitions stated in the text.

3 Results

3.1 Retained cells and preparation-level replication

GSE162610 contains 12,488 uninjured cells, 21,010 cells at one dpi, 17,491 at three dpi, and 15,189 at seven dpi: 66,178 cells in total, including 53,690 from injured tissue [3]. The largest point coordinate in Figure 1(B) therefore belongs to one dpi. It identifies the largest retained expression collection, not the greatest tissue cellularity or capacity for repair. The one-day stage contains three preparations, compared with two at each later stage, and all stages include both single-cord and enriched pooled preparations. Total cell recovery must consequently remain separate from a tissue-normalized comparison of inflammatory recruitment, glial abundance, or vascular density.

The sixteen contributing mice produce ten separately observed preparation-level collections after pooling

(Table 1). This hierarchy allows detailed molecular classification to coexist with limited biological replication. Many cells from a rare state in one preparation could define that state accurately while providing little information about its recurrence across preparations. A smaller population distributed consistently across preparations would answer a different question about biological recurrence. Stage totals cannot distinguish those possibilities for each lineage. Their contribution is to define the amount of retained material and its preparation structure, not to estimate lineage-specific agreement or variation between animals concealed within a pool.

Fifteen broad clusters identify the recovered populations before subdivision of the myeloid, vascular, and macroglial compartments [3]. The different feature and component settings in Table 2 and Figure 1(C) indicate that these subdivisions describe compartment-specific expression structure. They separate resident immune, recruited myeloid, progenitor, differentiated glial, and vascular identities relevant to different injury responses. The resulting classifications are not positions on one developmental scale. Neither their principal-component counts nor proximity between lineages in an embedding establishes a transition from inflammatory tissue to repaired tissue. Cellular identity and preparation membership must therefore remain attached to every subsequent comparison.

3.2 Microglial marker redistribution and partial restoration

Homeostatic, inflammatory, dividing, and migrating microglia differ in overlapping marker combinations. Homeostatic cells express *P2ry12*, *Tmem119*, and *Siglech*. Inflammatory cells show reduced *P2ry12* and increased *Igf1*; dividing cells express cell-cycle genes with increased *Msr1*; and migrating cells combine reduced *P2ry12* with *Msr1* and *Igf1* [3]. The four identities in Figure 2(A) are therefore distinguished without imposing a compulsory order. Their shared markers show that cell-cycle activity, inflammatory expression, and growth-factor-associated transcription do not define mutually exclusive functions. In particular, *Igf1* expression in an inflammatory identity prevents that transcript alone from serving as an exclusive indicator of a reparative population.

The P2RY12-high/MSR1-low protein phenotype accounts for more than 90% of microglia before injury

and approximately 20% at one dpi. During the early response, many MSR1-high cells retain high P2RY12. By seven dpi, the double-high population decreases and the homeostatic compartment partially returns [3]. Figure 2(B) preserves the lower-bound notation before injury and the approximate one-day proportion. The seven-day finding is a qualitative partial restoration, not a measured percentage in this comparison. These observations establish an early redistribution followed by partial recovery of a selected phenotype, while leaving its exact later magnitude and the histories of individual cells unresolved.

Reduced *P2ry12* transcripts and retained P2RY12 protein at one dpi identify different aspects of the microglial response. Substituting one measurement for the other would change which cells are assigned to a homeostatic category. The P2RY12-high/MSR1-high population also demonstrates that acquisition of an activation-associated protein can coexist with retention of a homeostatic protein feature. A larger homeostatic fraction at seven dpi may consequently reflect returning marker expression, persistence of cells that retained the phenotype, changes in the representation of other states, or a combination of these processes. The protein observations establish population-level phenotypic restoration but do not distinguish those cellular histories or demonstrate complete reversal of the injury response within each microglial cell.

The selected marker combinations and their temporal or anatomical associations are collected in Table 3. This comparison places microglial phenotypes beside peripheral myeloid, progenitor, and endothelial observations while retaining their different cell identities and measurement types.

The entries in Table 3 connect each biological claim to a particular observation. The microglial entry concerns partial return of a protein phenotype; the monocyte entry concerns prominence among recovered peripheral cells; the macrophage entries include lesion-core localization; and the tip-cell entry describes an early response followed by decline. These findings share collection stages but not a common numerical denominator or measure of recovery. Marker combinations also retain essential context: *Msr1* occurs in more than one microglial state, whereas *Apln* requires vascular localization to support tip-cell identification. The table therefore

distinguishes what changes, where or when it changes, and which population is being described.

3.3 Monocyte representation and macrophage protein identities

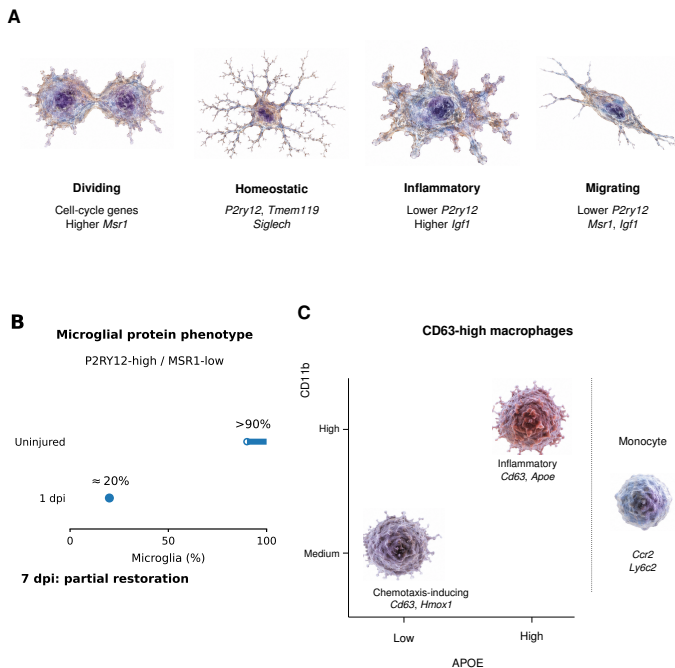
Peripheral monocytes express *Ccr2* and *Ly6c2*. The two principal macrophage populations share *Cd63* but differ in preferential *Hmox1* and *Apoe* expression, associated respectively with chemotaxis-inducing and inflammatory identities. Neutrophils, monocytes, and chemotaxis-inducing macrophages are prominent at one dpi; chemotaxis-inducing and inflammatory macrophages are prominent at seven dpi [3]. The later myeloid composition therefore differs from the early recruited population. This temporal redistribution identifies which phenotypes are represented at the sampled stages, while the proportion of early monocytes producing each later macrophage population remains unmeasured.

Within CD63-high macrophages, the chemotaxis-inducing phenotype is CD11b-medium/APOE-low and the inflammatory phenotype is CD11b-high/APOE-high. Milich and colleagues reported five biological cytometry replicates per time point and identified both APOE-low and APOE-high CD11b-positive/CD63-positive populations within the lesion core at seven dpi [3]. The later correction to the original report is cited separately to preserve the publication record [40]. These observations provide protein and anatomical support for the expression-defined identities. Their categorical positions in Figure 2(C) distinguish the phenotypes without representing calibrated fluorescence distances. Monocytes are shown separately because their identification by *Ccr2* and *Ly6c2* does not specify a measured conversion into either macrophage category.

The coexisting transcripts in Figure 2(A), the microglial protein proportions in panel B, and the macrophage categories in panel C describe different properties of the myeloid response. The first distinguishes expression programs; the second measures representation of a selected phenotype within microglia; and the third identifies peripheral macrophage protein combinations. Partial return of P2RY12-high/MSR1-low microglia therefore does not quantify peripheral macrophage recruitment. Conversely, prominence of APOE-high macrophages does not establish a change in microglial ancestry.

Table 3. Selected molecular identities and temporal or anatomical observations in GSE162610. The primary cellular assignments are reported by Milich et al. [3]. The later published correction is cited separately [40].

Cell state	Molecular distinction	Temporal or anatomical observation
Homeostatic microglia	<i>P2ry12</i> , <i>Tmem119</i> , <i>Siglech</i>	Predominate before injury; partial return by 7 dpi
Dividing/migrating microglia	<i>Msr1</i> ; cell-cycle genes in dividing cells; <i>Igf1</i> in migrating cells	Nonhomeostatic populations during the acute response
Monocytes	<i>Ccr2</i> , <i>Ly6c2</i>	Prominent among peripheral myeloid populations at 1 dpi
Chemotaxis-inducing macrophages	<i>Cd63</i> , preferential <i>Hmox1</i> ; APOE-low phenotype	Present with inflammatory macrophages in the lesion core
Inflammatory macrophages	<i>Cd63</i> , preferential <i>ApoE</i> ; APOE-high phenotype	Most represented macrophage phenotype in flow cytometry at 7 dpi
OPC-A	<i>Tnr</i> , <i>Pdgfra</i> , <i>Cspg4</i>	Present at all sampled stages
OPC-B	<i>Tnc</i> ; lower <i>Pdgfra</i> and <i>Cspg4</i>	Appears after injury; greatest representation at 1 dpi
Endothelial tip cells	<i>Apln</i> ; vascular localization with podocalyxin	Early increase at 1 dpi followed by decline

**Figure 2.** Myeloid identities and protein phenotypes. (A) Microglial states. (B) P2RY12-high/MSR1-low proportions. (C) Macrophage APOE/CD11b categories reported in the original study [3]. The later correction to the source article is cited separately [40]. Uninjured representation exceeds 90%; seven-day restoration is qualitative.

Reading these measurements separately preserves the early phenotypic redistribution and the later macrophage coexistence without treating either as a direct observation of a shared cell-fate trajectory.

The agreement between transcript identity, protein category, and lesion localization strengthens identification of the two macrophage populations. Each measurement nevertheless retains its own biological denominator. Five cytometric replicates per time point do not increase the number of expression preparations in Table 1, and tissue presence does not determine whether later representation results from recruitment, selective survival, proliferation, or phenotype acquisition. The supported comparison is

between observed myeloid compositions and molecular identities. Interpreting it as differentiation efficiency would require a relationship between specific precursor cells and their descendants that neither the phenotype categories nor the anatomical observations supply.

Both macrophage populations express overlapping sets of conventional polarization-associated genes, with *Arg1* and *Chil3* more strongly associated with the chemotaxis-inducing population [3]. A binary M1/M2 assignment would conceal this overlap and incorrectly suggest that the APOE categories directly quantify opposing tissue effects. The phenotype labels instead identify the accompanying chemotaxis-associated and inflammatory expression programs. Their biological contribution must remain related to timing and lesion context. Early monocyte prominence and later coexistence of macrophage phenotypes do not establish a compulsory progression between two mutually exclusive functional states.

3.4 OPC heterogeneity and differentiation-associated expression

The oligodendrocyte lineage contains OPC-A, OPC-B, dividing progenitors, pre-oligodendrocytes, and mature oligodendrocytes. OPC-A is present at every sampled stage and expresses *Tnr*, *Pdgfra*, and *Cspg4*. OPC-B appears after injury, reaches its greatest representation at one dpi, and declines by seven dpi. It expresses *Tnc* with lower *Pdgfra* and *Cspg4* than OPC-A [3]. These differences distinguish a persistent progenitor identity from a predominantly early injury-associated identity. Their parallel placement in Figure 3(A) does not imply that OPC-B must intervene between OPC-A and a mature oligodendrocyte.

OPC-A is associated with neural-development and myelination-related expression, while OPC-B shows growth- and proliferation-associated expression.

Differences involving *Ascl1* in OPC-A and *Sox9* in OPC-B accompany lower-level *Crym* and *Vim* expression in OPC-B that overlaps with other macroglial identities [3]. The combination is more specific than a simple loss of canonical progenitor markers: reduced *Pdgfra* and *Cspg4* occurs alongside an early growth-associated response. That profile cannot be interpreted as completed maturation from the reduced markers alone. Independent oligodendrocyte-lineage analysis also identifies heterogeneity after spinal cord injury [41], supporting separate consideration of activation, proliferation, and differentiation-associated expression within the lineage.

The temporal expression associations differ between astrocytes and progenitors (Figure 4(D)). At one dpi, both populations show translation and biosynthetic responses. At three dpi, astrocytic changes emphasize mitochondrial processes, whereas progenitor changes emphasize mitosis. Later comparisons identify lipid processing in astrocytes and glial development, differentiation, and migration in progenitors [3]. Thus, similar early biosynthetic activity is followed by distinct population-associated processes. These qualitative associations describe the molecular content of the response; they neither give counts of cells completing differentiation nor measure the number of functional myelin sheaths formed. Shared timing is therefore compatible with different cellular tasks rather than a synchronized recovery sequence.

A mature oligodendrocyte identity provides a biologically interpretable differentiation-associated destination within the lineage. Its presence among recovered cells does not identify which sampled progenitors gave rise to it. Dividing cells can occupy a transient cell-cycle state, while other cells retain progenitor characteristics. The pre-oligodendrocyte and mature identities in Figure 3(A) consequently describe classified populations rather than descendants of the illustrated OPCs. Oligodendrocyte differentiation and successful remyelination remain separate outcomes [42]. Molecular maturation describes the cell, whereas effective myelin repair additionally concerns axonal association, sheath formation, anatomical distribution, and function. Those outcomes cannot be quantified from the coexistence of progenitor and mature expression profiles.

3.5 Canal-associated *Crym* expression and glial matrix transcripts

The astroependymal population combines *Crym* with astrocyte-associated *Gfap* and *Timp1* and ependymal-associated *Vim* and *Tm4sf1*. It is not detected in the uninjured classification, increases at one dpi, and decreases over the subsequent sampling interval. It also lacks the cilia-associated expression characteristic of conventionally annotated ependymal cells [3]. This combination of shared and distinguishing transcripts defines an injury-associated molecular identity. The expression distinction does not establish physical loss of cilia, and none of the shared markers independently identifies a cellular precursor. Molecular classification and ancestry therefore remain separate even when astrocytic and ependymal features occur within the same population.

The anatomical observations locate the strongest *Crym* signal at one dpi in cells surrounding the central canal near the lesion, with a decrease by three dpi. GFAP-positive cells expressing *Crym* also occur in gliotic tissue around the injury [3]. Figure 3(B) distinguishes these canal-adjacent and parenchymal contexts through illustrations. The cited tissue measurements establish a localized injury-associated response rather than uniform acquisition of this phenotype by all astrocytes. Shared *Crym* labeling identifies molecular resemblance between the two locations but does not establish migration between them or descent from the same precursor population.

The OPC populations preferentially express the matrix-associated transcripts *Acan*, *Bcan*, *Ncan*, and *Vcan* relative to astrocytes and astroependymal cells in the atlas [3]. Matrix-associated expression is therefore not confined to the population labelled reactive astrocytes. The comparison identifies which recovered cells express these molecules, rather than assigning deposited matrix protein to tissue compartments in the same proportions. Transcript prevalence, protein accumulation, and spatial arrangement describe different quantities. Consequently, this expression distribution cannot determine the amount of matrix produced or its net effect on axonal growth. The biological finding is a broader distribution of matrix-related transcription across glial identities.

OPC-B and *Crym*-associated astroependymal cells share selected transcripts and are both most apparent early after injury. Their resemblance identifies related

components of the acute glial response, but it is compatible with common activation without direct conversion between the populations. Conversely, absence of a measured lineage connection does not establish that conversion is biologically impossible. The sequencing and localization observations distinguish molecular identities and tissue contexts while leaving the precursor relationship unresolved. Astrocyte enrichment further affects interpretation of their recovered proportions: expression and localization can support the presence of a phenotype even when the available cell fractions do not provide an unbiased estimate of its frequency in intact tissue.

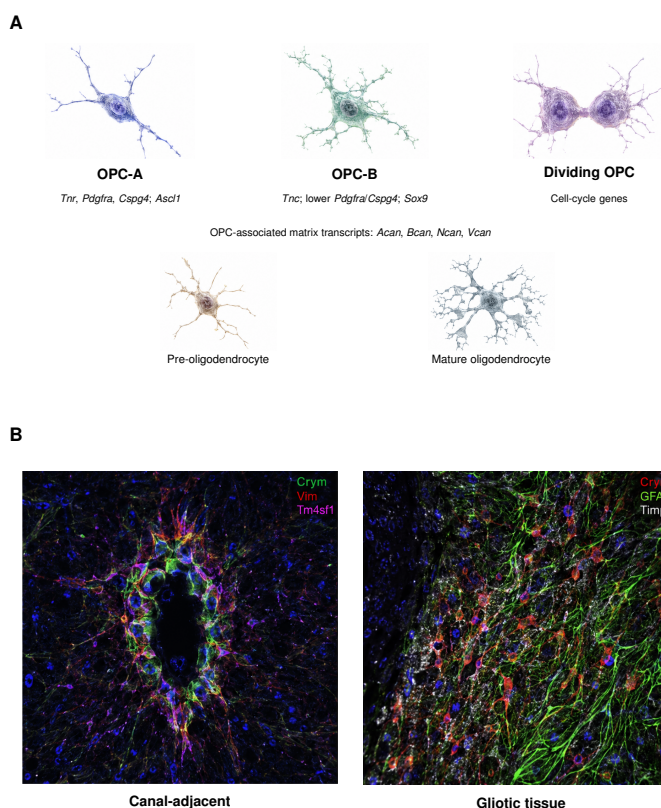


Figure 3. Glial identities and anatomical context. (A) Oligodendrocyte-lineage states. (B) Canal-adjacent and gliotic *Crym*-associated responses [3].

The progenitor, dividing, and differentiated identities in Figure 3(A) contrast with the two anatomical contexts in panel B. The first comparison concerns expression-defined populations; the second concerns locations in which the *Crym*-associated response occurs. Neither the shared *Crym*/*Vim* features nor an early increase makes these populations successive stages of a universal glial transition. Their combined interpretation is that injury-associated programs can occur in different glial identities and anatomical locations while remaining distinct from completed

myelination or scar resolution. The illustrations organize those molecular and anatomical distinctions but add no measurements of cell shape, fluorescence, or spatial relationships.

3.6 Early *Apln*-positive endothelium and angiopoietin expression

The endothelial compartment distinguishes arterial, venous, capillary, and tip-cell identities. Arterial cells express *Gkn3* and *Stmn2*, venous cells express *Vwf* and *Vcam1*, and capillary cells express endothelial markers including *Ly6a* and *Cldn5* without the selective arterial or venous pattern. Tip cells express *Apln*, are not detected in the uninjured endothelial classification, and increase at one dpi before declining [3]. These observations distinguish an early injury-associated endothelial state from the other vascular identities. Their placement in Figure 4(C) does not establish an arterial-to-venous sequence or track tip cells into capillary descendants.

Apelin transcript detection together with podocalyxin staining locates the early *Apln* signal in elongated vascular-associated cells near the lesion. The response is greatest at one dpi and decreases by three dpi. Because apelin transcripts also occur outside the vascular compartment, transcript positivity alone is insufficient to identify every positive cell as an endothelial tip cell [3]. Vascular labeling, morphology, and sampling time jointly support the assignment. The biological conclusion derives from those tissue observations; the fluorescence-like illustrations in Figure 4(C) are not acquired channels or measurements of colocalization.

The decline after the early endothelial response establishes temporal restriction of the observed phenotype, not the fate of individual tip cells. A lower representation can accompany altered expression or a change in the cells surviving and entering the recovered population. The same observation does not establish either failure of the vascular response or successful formation of perfused vessels. A temporary sprouting state could contribute to tissue reorganization while becoming less frequent. Interpreting persistence as improvement, or decline as resolution, would therefore require an independently defined vascular outcome. Perfusion, barrier integrity, and descendant identity remain different properties from the prevalence of *Apln*-associated cells.

Early tip-cell *Angpt2* expression and later astrocytic

Angpt1 expression associate different cell populations with vascular-related transcription over the injury interval [3]. Their timing is compatible with changing endothelial and glial participation in vessel organization, while secretion, receptor activation, and physiological stabilization remain unmeasured by these transcript associations. The one-, three-, and seven-day spacing in Figure 4(D) retains the collection schedule. Equal-sized symbols indicate qualitative observations rather than abundance. An unassigned stage-specific entry records the limit of the temporal description, not an observed absence of the population.

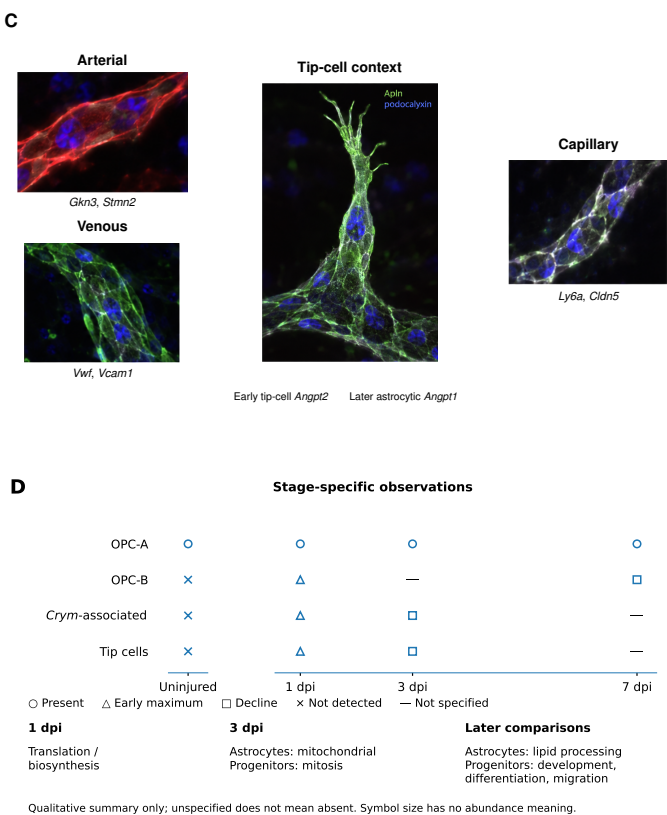


Figure 4. Glial and vascular observations (continued). (C) Endothelial identities and angiopoietin expression. (D) Qualitative temporal observations reported by Milich et al. [3].

The early OPC-B, *Crym*-associated, and endothelial tip-cell responses overlap in Figure 4(D), whereas the later expression associations concern different populations and processes. This common timing identifies concurrent lesion responses rather than a measured chain of signaling or ancestry. The vascular identities in panel C supply cellular context for *Apln* and angiopoietin expression without establishing vessel performance. Declining tip-cell representation cannot replace a perfusion measurement, just as

progenitor differentiation-associated expression cannot replace measurement of myelin formation. The shared time axis allows the responses to be compared but does not make their biological endpoints equivalent.

3.7 Conditional probabilities and state-dependent preparation means

The three recovery comparisons concern different properties. Partial return of P2RY12-high/MSR1-low microglia describes population-level phenotypic resemblance to uninjured tissue. Progenitor and mature oligodendrocyte identities describe differentiation-associated molecular organization. The early endothelial tip-cell increase and subsequent decline describe a transient injury response. These findings distinguish restoration, differentiation-associated identity, and temporary activation; none is a numerical estimate of CellRank absorption. Even a probability assigned to each compartment would retain the different biological definition of its event. A common numerical range would not make the events interchangeable measures of repaired tissue.

The kernel combination in Figure 5(A) represents the directional premise in T_α , while panel B distinguishes eventual access to absorbing sets from occupancy at a finite graph horizon. The matrix $\tilde{T}_\alpha$ denotes a modification that imposes absorbing endpoints; it does not identify permanent destinations observed in spinal tissue. Direction and the event being evaluated are therefore separate modeling choices. Access to a homeostatic microglial region concerns one phenotype, access to mature oligodendrocytes concerns a differentiated identity, and occupancy of a tip-cell region concerns a potentially temporary state. Equal numerical values would describe those distinct events, not equal amounts of biological recovery.

The weighted-mean identity in Eq. (5) introduces another distinction. With state-specific means held fixed, increasing the weight of a state whose endpoint probability is higher increases the preparation mean. With state weights held fixed, changes in the state-specific means can also change the preparation mean. The two constructions in Figure 5(C) represent these alternatives symbolically. An aggregate difference alone cannot distinguish their contributions, because both enter the same average. This conclusion is algebraic: it requires neither selected numerical weights nor an estimated effect in

GSE162610. The biological interpretation depends on whether the changing quantity is state representation or probability within a comparable state.

The distinction has different implications across compartments. Conditional on a selected macrophage endpoint, a preparation with more endpoint-like macrophages could have a higher mean while probabilities among comparable monocytes remain unchanged. A change in the relative representation of progenitor and differentiated oligodendroglial states could similarly alter a lineage average. Temporary enrichment of tip-like endothelial cells could alter an occupancy summary without establishing stable vascular maturation. These are consequences of the stated averaging identity, not estimates of the magnitude or direction of such effects in GSE162610. They identify which biological explanation an overall mean leaves unresolved.

recovery comparison. Kernel construction determines favored transitions, the endpoint set or finite horizon determines the event, and state composition determines the preparation average. Each component changes the biological interpretation independently of the numerical range. The microglial, progenitor, and endothelial observations therefore require their own cellular reference before an average can be related to recovery. Their current interpretation is a comparison of partly overlapping responses with different molecular and temporal meanings, not an estimated common trajectory or a quantified restoration of tissue function.

4 Discussion

4.1 Phenotype restoration, differentiation, and transience

The partial return of P2RY12-high/MSR1-low microglia, the coexistence of progenitor and differentiated oligodendroglial identities, and the decline of an early *Apln*-associated endothelial response are not equivalent observations of recovery. The microglial comparison refers to an uninjured phenotype within the same broad lineage. The oligodendroglial comparison refers to molecular identities associated with differentiation. The endothelial comparison refers to the persistence of an activated state. Figures 2 and 3 therefore describe different biological changes rather than interchangeable indicators of one repair process. Their temporal overlap establishes concurrent cellular responses but does not establish equal functional benefit or a common direction toward restored tissue.

Each comparison nevertheless supports a positive biological interpretation. An uninjured reference makes partial microglial phenotype restoration identifiable. Recognizable progenitor and mature identities make oligodendroglial expression interpretable in relation to differentiation. The early maximum and later reduction of the *Apln*-associated response establish restricted timing of vascular activation. The limitation arises when these objects are replaced by a broader claim about tissue recovery. Marker expression concerns phenotype, differentiated identity concerns the cell's molecular condition, and tip-cell prevalence concerns a transient vascular state. None of those measurements independently establishes the corresponding structural or physiological outcome.

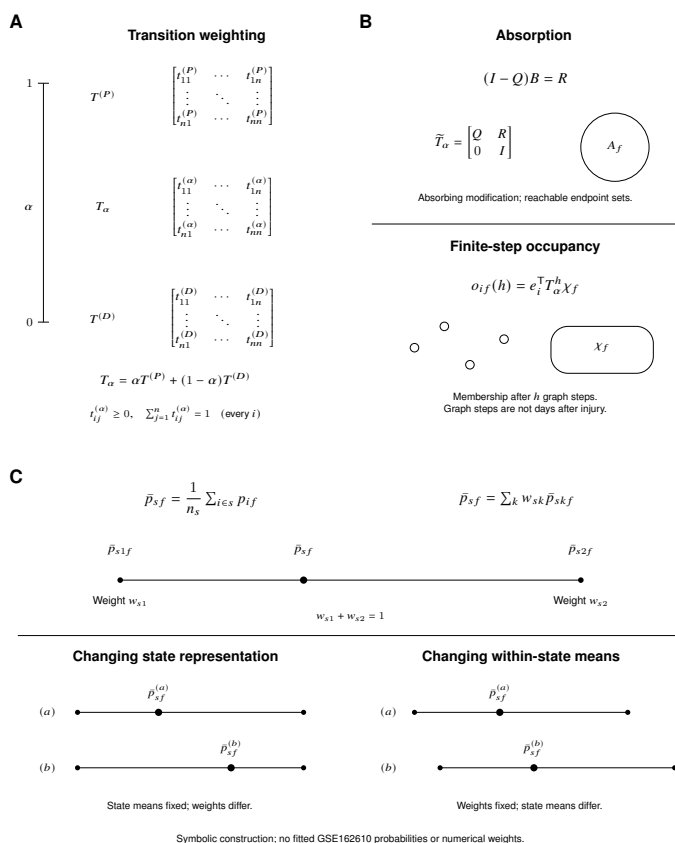


Figure 5. Conditional probabilities and preparation means.

(A) Kernel weighting. (B) Absorption and finite-step occupancy. (C) State proportions and within-state means, Equations (1)–(5). The CellRank fate-mapping framework is described by Lange et al. [25]. Its multiview extension in CellRank 2 is described by Weiler et al. [26].

The three components of Figure 5 explain why a shared probability scale cannot standardize the

The molecular and temporal columns of Table 3 retain the information needed to distinguish these interpretations. A phenotype defined by *P2ry12* transcripts is not identical to one defined by P2RY12 protein. Neither shares the biological definition of a mature oligodendrocyte or a functional vascular endpoint. Likewise, lesion-core localization identifies tissue presence rather than differentiation efficiency. The relevance of a particular marker therefore depends on its cell identity, measurement modality, and accompanying anatomical evidence. This organization preserves the specific findings while preventing an observation from serving as an untested substitute for another merely because both are associated with injury responses.

The temporal pattern also resists a compulsory inflammatory-to-regenerative sequence. Early glial biosynthesis coincides with vascular activation, but subsequent progenitor, astrocytic, and microglial responses remain distinct: mitosis and later differentiation-associated expression in progenitors, mitochondrial and lipid-processing associations in astrocytes, and partial restoration of a microglial protein phenotype. These differences allow coordination among compartments without implying synchronized movement toward one measured endpoint. Molecular and anatomical compatibility can identify populations participating in the same lesion interval; establishing a causal chain from that compatibility to tissue repair requires a different type of observation. The coexistence of the responses should therefore remain distinct from a demonstrated mechanism linking them.

4.2 Myeloid phenotype, cellular origin, and tissue function

The decline from more than 90% to approximately 20% P2RY12-high/MSR1-low microglia is a change in representation within the microglial protein comparison, not a direct count of resident-cell loss. The early P2RY12-high/MSR1-high population is particularly important because it combines a retained homeostatic feature with increased MSR1. Figure 2(B) thus supports redistribution of marker combinations rather than complete replacement of one cellular identity by another. The partial return at seven dpi could arise through several histories, including marker re-expression and altered state proportions. None of these histories can be selected from the observed percentages alone.

Persistent microglial reprogramming after spinal cord injury explains why selected marker return need not imply complete molecular normalization [5]. The temporally differentiated programs identified by Wahane and colleagues further separate proliferative activity from other myeloid responses [4]. These findings support interpreting the homeostatic, inflammatory, dividing, and migrating identities in Figure 2(A) as different combinations of cellular programs. They do not establish one-to-one equivalence between subpopulations carrying similar names in different experiments. Injury models, sampling intervals, and classification choices remain relevant to any such comparison. The agreement concerns heterogeneity of the response, not a universally fixed sequence of microglial states.

Microglial function also depends on the tissue effect under examination. Bellver-Landete and colleagues showed that adult microglia contribute to organization of a neuroprotective scar after spinal cord injury [1]. Brennan and colleagues demonstrated that adult microglia coordinate interactions among surrounding cell populations during repair [2]. Li and colleagues, in a distinct neonatal crush model, showed that microglia can participate in scar-free healing and axonal regrowth [14]. The distinction among these models prevents the adult seven-day protein phenotype from being interpreted as a surrogate for neonatal repair. It also prevents either a homeostatic or activated label from specifying a universally favorable cellular condition. Molecular state must be related to the functional response measured in the corresponding tissue and injury context.

The APOE/CD11b comparison concerns peripheral macrophage identity rather than restoration of resident microglia. Milich and colleagues identified two lesion-associated macrophage populations using shared CD63 expression, APOE-low/CD11b-medium and APOE-high/CD11b-high protein categories, and accompanying *Hmox1/Apoe* transcriptional differences [3]. The correction published for that article is retained as a separate bibliographic item rather than combined with the primary evidence citation [40]. The coexistence of these populations at seven dpi is compatible with different tasks or different cellular histories, but the phenotype measurements do not distinguish those alternatives. Early monocyte prominence followed by later macrophage prominence therefore establishes a

population comparison, not a conversion efficiency. Partial return of a microglial protein phenotype cannot resolve the abundance, origin, or activity of the recruited compartment, even when inflammatory transcripts overlap.

The sample counts across modalities must remain separate for the same reason. Five cytometric replicates per time point support the protein comparison within that experiment; tissue labeling supports lesion localization; and the ten sequencing preparations support the expression atlas. These complementary observations cannot be summed into a larger number of independent sequencing samples or treated as repeated measurements of the same fate probability. Agreement strengthens specific assignments of transcript identity, protein phenotype, and tissue presence. It does not identify preparation-specific cell histories or resolve which process produced the observed later macrophage composition.

4.3 Progenitor differentiation, glial ancestry, and myelin repair

The OPC-A/OPC-B comparison distinguishes progenitor responses that are not reducible to one maturation coordinate. OPC-B combines lower *Pdgfra/Cspg4* with *Tnc* and growth-associated expression during the early injury response. The reduction in canonical progenitor markers therefore cannot alone establish advancement toward a mature oligodendrocyte. In Figure 3(A), the dividing population concerns cell-cycle activity, while pre-oligodendrocytes and mature cells concern differentiation-associated identity. The presence of these populations defines the range of molecular states recovered within the lineage. It does not measure the rate at which dividing or nondividing progenitors produce functional oligodendrocytes.

Lineage and myelin measurements over longer intervals address outcomes different from the acute expression comparison. Assinck and colleagues traced resident progenitors to oligodendrocytes and myelinating Schwann cells after contusion, with myelin outcomes assessed over weeks to months [6]. Their lineage labels establish cellular origin in a way that separate first-week progenitor and mature profiles cannot. Duncan and colleagues, by contrast, disrupted oligodendrocyte remyelination while preserving spontaneous stepping recovery in their mouse contusion model [7]. The two studies

distinguish progenitor contribution, formation of myelin, and recovery of a particular behavior. They neither make these outcomes universally independent nor allow one outcome to be substituted automatically for another.

Perturbation experiments also distinguish a myeloid influence on progenitor differentiation from simultaneous expression changes. In a separate mouse study, macrophage GIT1 deficiency increased TNF α , reduced mature oligodendrocyte representation, and impaired remyelination and functional outcomes; soluble TNF inhibition reversed the adverse effects [43]. Those intervention and rescue observations provide causal evidence beyond the coexistence of macrophages and OPCs. They support the biological plausibility of myeloid regulation of progenitor differentiation without identifying the APOE-defined macrophage populations in GSE162610 as the mediators of that mechanism. The GSE162610 marker comparison contains neither a GIT1 perturbation nor a corresponding treatment contrast.

The canal-associated population requires equally precise interpretation of origin. Ren and colleagues found localized ependymal contributions dependent on direct injury, cautioning against assigning every canal-adjacent reactive cell to an ependymal lineage [8]. O'Shea and colleagues identified a smaller oligodendrocyte-progenitor contribution alongside the predominant local-astrocyte origin of border-forming astrocytes after crush injury or stroke [9]. These findings also rule out a blanket assumption that cross-lineage contribution is biologically impossible. For the *Crym*-associated contexts in Figure 3(B), the question remains specific: the localization and shared transcripts do not identify the precursor of the cells recovered in GSE162610. Lineage measurements in another injury model cannot assign that ancestry retrospectively.

The preferential OPC expression of *Acan*, *Bcan*, *Ncan*, and *Vcan* broadens the molecular description of matrix-associated glia beyond astrocytes alone [3]. It identifies recovered populations expressing matrix-related transcripts, not measured contributions to extracellular protein deposition. Tissue matrix organization and its effect on axonal growth depend on properties that are not contained in the relative transcript distribution. The progenitor panel in Figure 3(A) should consequently be read as a

comparison of molecular identities rather than a reconstruction of scar structure. Progenitor activity is relevant to differentiation and tissue response, but it does not quantify restored myelin or the structural resolution of the lesion.

4.4 Endothelial transience and glial signaling compatibility

Endothelial transience can be consistent with participation in repair rather than its failure. A temporary *Apln*-associated state could contribute to vascular reorganization even if fewer cells later occupy it. Dynamic imaging of angiogenic sprouting shows that endothelial cells can exchange tip and stalk positions [10], providing a reason to distinguish tip identity from a permanent cellular fate. Those experiments do not identify the descendants of tip cells after spinal contusion. They support interpretation of Figure 4(C) as a comparison of vascular identities and an injury-associated response, rather than a sequence in which tip cells form the final irreversible destination.

The association of *Apln* transcripts with elongated podocalyxin-positive cells restricts the vascular assignment because *Apln* also occurs outside endothelium [3]. Localization thus strengthens cell identification without measuring vessel performance. The temporal observations in Figure 4(D) establish an early maximum and subsequent decrease at the stages for which those findings are available. An unassigned observation cannot be interpreted as a measured zero, and decline cannot determine whether vessels became perfused or responding cells disappeared. The distinction prevents cellular prevalence from being treated as either anatomical maturation or physiological restoration.

The early endothelial *Angpt2* and later astrocytic *Angpt1* associations place vascular-related transcription in different populations over time [3]. Such compatibility concerns potential intercellular relationships rather than descent. Browaeys and colleagues developed NicheNet to link candidate ligands with downstream target-gene programs [44]. Jin and colleagues developed CellChat to infer and analyze cell–cell communication networks from expression data [45]. Dimitrov and colleagues showed that communication inferences depend materially on the method and interaction resource used [46]. A later study by Dimitrov and colleagues introduced LIANA+ as a framework for integrating

multiple communication-inference approaches and data modalities [47]. These literature comparisons concern interpretation; communication analyses are not performed on GSE162610 in this article. A compatible ligand–receptor transcript pair remains distinct from ligand secretion, receptor occupancy, downstream activity, and vessel stabilization. The angiopoietin observations therefore identify molecular and temporal context without establishing those signaling events.

State markers must also be distinguished from regulators of state acquisition. *Apln*, *Tnc*, and *Msr1* identify particular responses or populations, but their association with an endpoint does not establish control over entry into that endpoint. Van den Berge and colleagues developed tradeSeq to describe gene-expression changes along specified trajectories [39]. Bravo González-Blas and colleagues developed SCENIC+ to infer enhancer-driven gene-regulatory networks from multiomic measurements [48]. These approaches therefore address different quantities. An RNA-only observation does not contain measured enhancer activity, and a plausible molecular association is not a perturbation result. The marker combinations in the glial and vascular figures support cellular classification while leaving regulatory causation separate from identification.

4.5 Preparation composition and endpoint-dependent averages

Preparation identity remains relevant after restricting analysis to one lineage. Selection of microglia, macrophages, progenitors, or endothelial cells removes comparisons between fundamentally different identities but does not establish equal recovery of every state within the selected population. Anti-ACSA-2 enrichment directly alters astrocytic representation, and tissue pooling prevents animal-specific expression from being separated within six preparations. Figure 1 and Table 1 consequently define the population to which a summary refers: cells recovered from ten separately processed tissue preparations under the stated procedures. A modeled probability assigned to any of those cells retains that sampling context. Restricting the lineage cannot restore information removed by pooling or selective recovery.

An overall lineage mean and a mean restricted to comparable states describe different populations. The former averages the mixture recovered from a

preparation; the latter concerns a defined molecular identity within that mixture. Figure 5(C) shows why both quantities can behave differently. Greater representation of mature-like cells can increase an oligodendroglial mean without changing the mean among progenitors, while a progenitor-specific difference can be diluted by changes in other state proportions. Eq. (5) establishes this dependence algebraically but does not determine which component dominates in GSE162610. An empirical attribution to state composition or within-state change would require the corresponding measured or fitted values.

Endpoint selection also determines the meaning of a concentrated probability. In a finite model with one reachable absorbing destination and certain absorption, an eventual-entry probability of one follows from the model construction; it does not independently establish irreversible biological commitment. Lange and colleagues emphasized that CellRank fate probabilities are conditional on the transition model and selected terminal regions [25]. Weiler and colleagues retained this conditional interpretation while extending CellRank 2 to multiview directional information [26]. Adding another endpoint therefore changes the event under consideration, whereas diffuse probabilities may reflect incomplete directional information, unrepresented alternatives, or access to multiple destinations. The distinction between the transition matrix and its absorbing modification in Figure 5(B) identifies the endpoint assumption rather than treating permanence as a property directly observed in the cells.

A graph constructed from multiple preparations introduces shared dependence before the preparation means are calculated. Each preparation can influence neighborhoods and directional relationships, so excluding its values from a final average does not remove its earlier contribution to the estimated transition structure. Refitting after exclusion addresses a different question about model dependence, but no such computation forms part of this analysis. Crowell and colleagues showed why multi-sample single-cell analyses should preserve sample-level structure [18]. Squair and colleagues demonstrated the false-discovery risk created when cell-level observations are treated as independent biological replicates [19]. Zimmerman and colleagues provided a practical framework for addressing

pseudoreplication bias in single-cell studies [20]. Häfner and colleagues extended these principles to nested condition comparisons [21]. Biological replication, numerical estimation, and endpoint definition are therefore separate uncertainties that cannot be inferred from the precision of a cell-level value alone.

The four collection stages likewise do not determine the biological duration of one transition in Eq. (3). Klein and colleagues developed moscot to map cell populations across time and space using optimal transport [38]. Lange and colleagues developed moslin to integrate lineage-tracing information when mapping cells across time points [49]. These methods use additional assumptions or lineage information that are absent from the present algebraic interpretation. Chronological tissue collection must therefore remain distinct from the events of a graph-based process. Absorption concerns eventual access to selected destinations, whereas occupancy concerns state membership after a finite number of transitions. Neither quantity is a measurement of days to recovery. Relating either to vascular restoration, myelin formation, or treatment benefit requires observations connecting the modeled event to the corresponding biological outcome.

4.6 Biological scope and measurement limitations

The biological scope is female C57BL/6J mice aged eight to ten weeks, a 65-kilodyne T8 contusion, an eight-millimeter lesion-centered tissue segment, and uninjured tissue plus the first seven days after injury. These observations do not establish equivalent responses in males, neonatal animals, complete transection, primates, or chronic injury. Matson and colleagues showed that cellular responses can differ according to neuronal population and anatomical position within a mouse spinal cord injury atlas [11]. Fan and colleagues demonstrated regional heterogeneity after complete spinal cord injury in rhesus monkeys [12]. Skinnider and colleagues further integrated single-cell and spatial datasets across multiple spinal cord injury conditions in the *Tabulae Paralytica* [13]. These broader comparisons reinforce the need to keep tissue location and experimental context explicit, but they do not enlarge the sample size of GSE162610. The dissociation-associated limitation on neuronal recovery also prevents the detailed immune and glial classification from being treated as a complete

description of the circuitry responsible for neurological function.

Dissociation-associated transcription provides another limit on interpreting activation. Marsh and colleagues demonstrated preparation-induced signatures in brain tissue, especially in microglia [15], but this does not identify which GSE162610 cells, if any, share those artifacts. Protein phenotypes and anatomical localization corroborate specific assignments without validating every expression difference or supplying an unselected tissue frequency. The complementary measurements therefore strengthen the identification of a population while leaving the size of between-preparation variation and the histories of its individual cells unresolved. Two preparations at each later sequencing stage remain two preparation-level observations regardless of how many other biological features are described.

The need to keep measurement modalities distinct is also evident outside spinal cord injury. Ahmed and Ibrahim combined biochemical markers with histopathological examination in a rat toxic-injury model and reported that treatment-related responses varied across the biochemical and structural outcomes [50]. Their study does not provide evidence about spinal cord cell states; rather, it offers a methodological example of why molecular, biochemical, and tissue-level readouts should not be treated as interchangeable measures of recovery.

The present analysis does not evaluate an implanted scaffold, but material context remains relevant when cellular observations are extrapolated to tissue-engineering strategies. Sáenz and colleagues reviewed how biomaterial classes differ in composition, processing, biocompatibility, and biomedical application [51]. That materials perspective reinforces a boundary of the present study: transcriptional state alone cannot determine how a future cell-based intervention would interact with an implanted material or host tissue environment.

The quantitative panels, temporal annotations, and mathematical constructions have distinct evidential roles. Retained-cell totals and microglial protein proportions retain their numerical denominators; qualitative timing describes the stages at which a response is identified; symbolic equations specify conditional model events and averages. Cellular and fluorescence-like artwork is illustrative rather than a

measurement of morphology, intensity, colocalization, or distance. The amount of visual detail therefore does not increase the biological evidence.

Conclusions depend on the attributed cellular observations and the stated algebraic identities, each interpreted at the level it addresses.

The combined interpretation distinguishes partial microglial protein-phenotype restoration, heterogeneous oligodendroglial identities relevant to differentiation, and a restricted period of endothelial activation. The preparation-average identity further explains why a future aggregate probability could mix changing state representation with changing values among comparable cells. These conclusions identify the cellular property that changes in each compartment. They do not classify repair of the lesion as successful or unsuccessful; instead, they establish that this outcome cannot be determined by treating the three cellular observations as equivalent measurements. Structural and physiological recovery remains a separate question from the molecular and population comparisons.

5 Conclusion

Partial microglial marker return, oligodendroglial differentiation-associated expression, and endothelial transience do not provide equivalent evidence of recovery after spinal cord contusion. The P2RY12-high/MSR1-low population decreases from more than 90% of microglia before injury to approximately 20% at one dpi and partially returns by seven dpi, establishing restoration of a selected population-level phenotype. OPC-A, OPC-B, dividing progenitors, and differentiated oligodendroglial identities distinguish injury-associated activity from maturation-related expression. The early *Apln*-positive endothelial maximum followed by decline establishes temporal restriction of vascular activation. These are different cellular changes rather than successive observations of one demonstrated repair sequence.

The ten preparations, including six two-cord pools, remain the separately observed sequencing units despite recovery of 66,178 cells. The weighted-mean identity shows why an overall endpoint probability can change through state representation alone, without a change among comparable cells. Endpoint selection and finite-step occupancy also define different biological events, and graph transitions have no established duration in days. The probability

equations are interpreted algebraically rather than fitted to GSE162610, so they clarify the recovery comparison without supplying numerical fate estimates or individual-cell histories.

The answer to the research question is therefore compartment-specific: microglial marker return indicates partial phenotypic restoration, oligodendroglial profiles identify differentiation-associated states, and endothelial transience describes temporary activation. Their overlapping timing supports concurrent myeloid, glial, and vascular responses, not a common quantitative measure of repaired tissue. Complete microglial normalization, formation of functional myelin, restored perfusion, and neurological recovery remain distinct outcomes requiring their corresponding measurements. Neither visual resemblance between cell states nor averaging conditional probabilities removes those biological distinctions.

Data Availability

All data and analytical information used in this paper are included within the manuscript and its cited source material. The underlying single-cell expression data and metadata are publicly available in NCBI GEO under accession [GSE162610](#), with sequencing reads available under SRA accession SRP295673. Milich and colleagues provide the source sequencing, cytometric, and anatomical observations analyzed in this review [3].

Ethics Statement

This analytical review involved no new animal or human experimentation.

Funding

No external funding was received for this work.

Conflicts of Interest

The author declares no conflicts of interest.

Use of Artificial Intelligence

Artificial intelligence tools were used solely for English-language improvement, grammatical editing, and presentation refinement. No AI tool was used to generate or alter the underlying research data, fabricate references, perform the reported scientific analyses, or determine the scientific conclusions. The

author reviewed and accepts full responsibility for the final manuscript.

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