Automated adult and neonatal mouse brain dissociation and magnetic isolation of neurons increases efficiency and sensitivity for single-cell RNA sequencing and gene expression profiling

Sandy Reiß[1], Stefan Tomiuk[1], Ruth Kläver[1], Jana Soyka[1], Maria Delso Vallejo[1], Cole Jones[2], Andreas Bosio[1], and Melanie Jungblut[1]
[1]Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany [2]10x Genomics, Pleasanton, CA, USA

Introduction

Single-cell RNA sequencing has emerged as a powerful technology for the analysis of different cell types at high resolution. Especially in case of the huge diversity of neural cells, the technology can help to better understand their phenotype and function. A prerequisite for single-cell analysis of tissue-derived cells is the preparation of single-cell suspensions with high cell viability and a minimum of cell debris, which is particularly challenging in case of highly sensitive neurons.
In this study, we dissociated adult and neonatal mouse brain tissue by a combination of an ideal mechanical dissociation process using the gentleMACS™ Octo Dissociator with Heaters (Miltenyi Biotec) and an optimized enzymatic treatment. After tissue dissociation, adult neurons were isolated using the Neuron Isolation Kit, mouse (Miltenyi Biotec) to increase the percentage of target cells for single-cell transcriptomics.
Gene expression profiling of adult unsorted cells and isolated neurons was performed using the Chromium™ Single Cell 3′ Reagent Kits v3 (10x Genomics) for preparation of single-cell RNA-seq libraries. Furthermore, we compared single-cell sequencing data from manually and automatically dissociated neonatal mouse brain.
Isolation of neurons resulted in substantial enrichment of target cells and allowed for the identification of different neuronal subpopulations that could not be distinguished in the unsorted brain cell fraction. Analysis of automatically and manually dissociated neonatal mouse brain tissue led to the identification of the same neural cell clusters with comparable cell numbers, proving that the automated gentleMACS Technology–based dissociation did not have any negative influence on the cells and ultimately on the analysis.
In summary, we show that an optimized automated tissue dissociation protocol based on the gentleMACS Octo Dissociator and subsequent magnetic isolation of neurons significantly improves sensitivity and resolution of single-cell sequencing for the characterization of neuronal subtype complexity.

Results

1 Isolation of neuronal cells improves sensitivity for single-cell analysis

After dissociation of whole adult mouse brain tissue using the Adult Brain Dissociation Kit, mouse and rat in combination with gentleMACS Technology, neurons were magnetically isolated from the resulting cell suspensions using the Neuron Isolation Kit, mouse (fig. 1A). Unseparated cells and the isolated neuronal cell fraction were then analyzed by flow cytometry to assess the purity of the isolated neurons.
Two samples were individually processed, and separation of 7.2–8.1×10[6] neural cells yielded 0.8–1.0×10[5] neurons with a viability of approximately 86% and a purity of 97% (fig. 1B). Single-cell RNA-seq libraries of adult unsorted cells and isolated adult neurons were generated using the Chromium Single Cell 3′ Reagents Kits v3 (10x Genomics). In detail, 16,000 cells per sample (n = 2) were loaded onto the Chromium Chip B Single Cell and processed according to the manufacturer's instructions. All single-cell RNA-seq libraries were pooled and sequenced using two NextSeq™ 500 High Output Cartridges on the NextSeq 500 Instrument (Illumina®). For processing and visualization of the single-cell RNA sequencing data, the Seurat package (v3.0.0) was used[1]. The analysis resulted in the identification of 5,500–7,900 cells per sample with an average of 12,000 reads per cell.

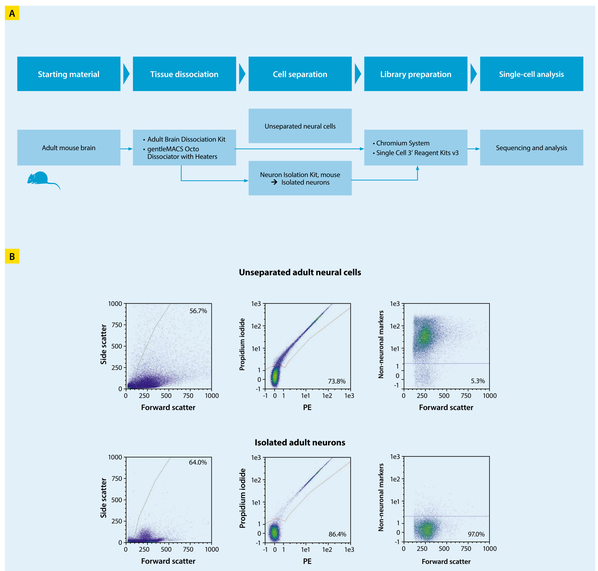

Figure 1

Single-cell analysis of non-sorted adult neural cells led to identification of 24 different subclusters, representing neuronal and different glial cell types, i.e., astrocytes, microglia, oligodendrocytes, vascular cells, and immune cells. The majority of neural cells were identified as glial cells and only a subset, represented by cluster 14 and 18, showed expression of immature and mature neuron markers (fig. 2A and B). Two individually processed samples showed comparable cell counts and percentages in the different clusters, pointing to the reproducibility of the experimental procedure (fig. 2B and C).

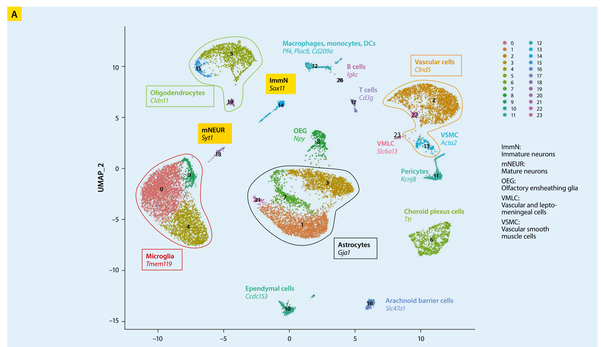

Figure 2

2 Isolated neurons show neuronal subtype diversity

Isolation of neurons substantially enriched the number of neuronal cells and increased the resolution of analysis. This allowed for the identification of several neuronal subpopulations containing both immature and mature neurons, which could not be distinguished in the unsorted brain fraction. At the same time, all non-neuronal cell–specific transcripts were significantly decreased, and 88% of the identified cells were defined as neurons. Only minor subsets of contaminating non-neuronal cells were identified (fig. 3A and B).
Cell counts and percentages in two individually processed samples were comparable (fig. 3A and B).
Figure 3C and D highlight Sox11- and Gad2-positive neuronal cells in the neuronal population, indicating that the majority of neurons were Sox11-positive immature GABAergic neurons.
In summary, the results demonstrate that neuronal cell isolation significantly improves sensitivity and resolution of single-cell sequencing for analysis of different neuronal subtypes in adult mouse brain samples.

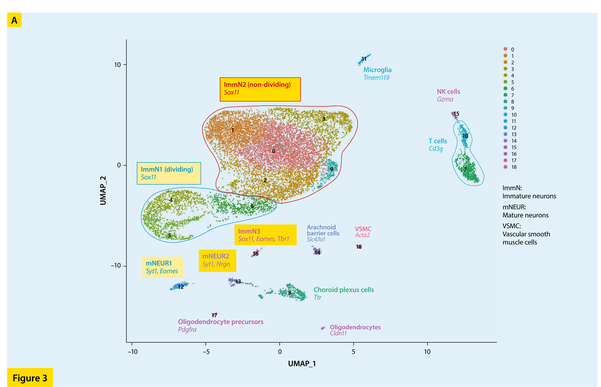

Figure 3

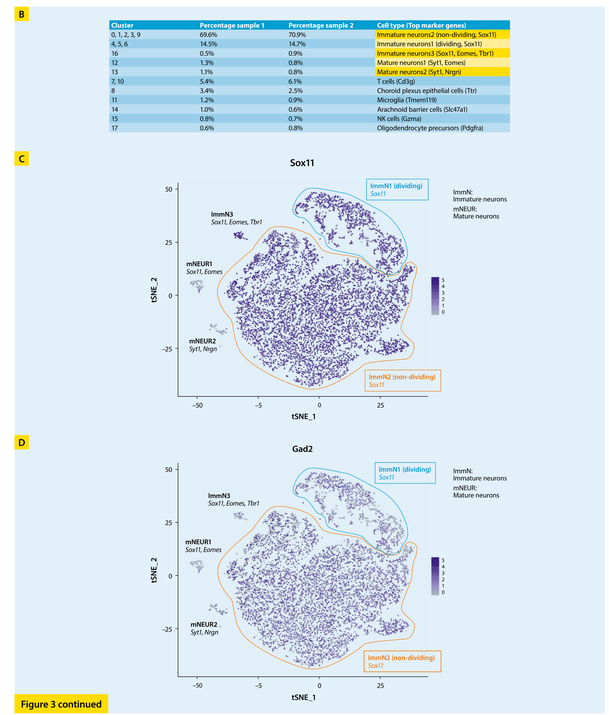

Figure 3 continued

3 Single-cell analysis of automatically and manually dissociated mouse brain tissue

Total neonatal mouse brains (P3) were dissected and subjected either to an automated or a manual dissociation procedure (fig. 4A).
In case of the automated process, brain tissue was dissociated using the Neural Tissue Dissociation Kit – Postnatal Neurons in combination with the gentleMACS Octo Dissociator with Heaters.
In case of the manual protocol, fire-polished glass Pasteur pipettes with decreasing tip diameters were used for mechanical dissociation and the components of the Neural Tissue Dissociation Kit – Postnatal Neurons for enzymatic treatment at 37 °C.
Automated dissociation of one mouse brain yielded 1.15×10[7] cells with a viability of 95%. Likewise, 1.47×10[7] total cells with a viability of 95% were obtained from one manually dissociated mouse brain (fig. 4B). 8,000 cells of the manually and automatically dissociated tissue samples were loaded and single-cell analysis was performed as described above. Approximately 6,000 cells were identified per sample with an average of 20,000 reads per cell.

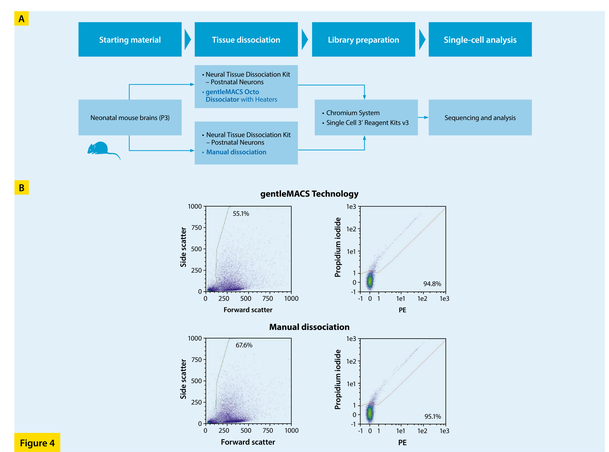

Figure 4

Single-cell analysis of both automatically and manually dissociated whole neonatal mouse brain tissue led to the identification of distinct cell populations. The UMAP plot shown in figure 5A depicts 23 cellular clusters that were assigned to different neural cell types by identification of the top marker genes, including different immature neuronal subtypes, astrocytes, oligodendrocytes, microglia, ependymal cells, vascular cells, macrophages, and epithelial cells (fig. 5A and B).
Analysis of automatically and manually dissociated tissue led to the identification of the same neural cell clusters with comparable percentages of cells (fig. 5C), demonstrating that the time-saving, standardized automated process does not compromise the quality of the results.

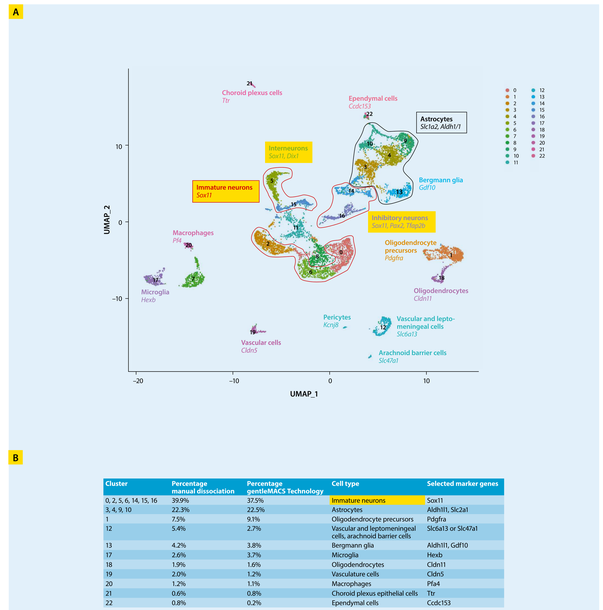

Figure 5

Conclusion

- The Adult Brain Dissociation Kit, mouse and rat in combination with the gentleMACS Octo Dissociator with Heaters allowed for efficient dissociation of adult mouse brain tissue and identification of a variety of different adult neural cell types by single-cell sequencing.
- Isolation of neurons using the Neuron Isolation Kit, mouse led to substantial enrichment of neuronal cells and significantly improved sensitivity and resolution of single-cell sequencing for analysis of different neuronal subtypes.
- Replicates showed comparable cell counts and percentages in the different clusters, pointing to the reproducibility of the experimental procedure.
- Single-cell analysis of automatically and manually dissociated neonatal mouse brain led to identification of the same cellular clusters, including all different major neural subpopulations.

Reference
1. Butler, A. *et al.* (2018) Nat. Biotechnol. 36: 411–420.