A novel approach for efficient dissociation of distinct adult mouse brain regions and subsequent sorting of neurons and neural stem cells using the MACSQuant® Tyto®

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Introduction

Careful tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris are prerequisites for reliable cellular analysis and cell sorting. In the case of adult rodent brain, sophisticated mechanical and enzymatic treatments are required to disaggregate the tightly connected neural cells successfully. The process becomes even more challenging when only small brain regions serve as starting material for the generation of single-cell suspensions and when highest possible viability, recovery, and functionality are crucial for successful downstream processing.
In the past, we set up elaborated technologies for the automated dissociation of whole adult rodent brain. The process is based on mechanical dissociation using the gentleMACS™ Octo Dissociator with Heaters and an optimized enzymatic treatment, followed by an efficient clearing procedure to eliminate cell debris and erythrocytes. The protocol was now further refined for highly effective generation of single-cell suspensions from small brain regions at high viability rates and neural cell yield and a minimum of contaminating debris.

Subsequently, neurons or neural stem cells (NSCs) were isolated from the cell suspensions by the MACSQuant® Tyto®, a novel multiparameter cell sorting device that uses a microchip-based technology for sterile and gentle cell isolation. This protocol resulted in highly pure (80-99%) and viable (>90%) neurons from cerebellum, cortex, and olfactory bulb. Furthermore, NSCs from the subventricular zone (SVZ) were identified with a new marker combination and successfully sorted at high purity (>95%) and viability (>90%). Isolated NSCs formed a large number of neurospheres, which gave rise to secondary neurospheres and differentiated into different neural cell types.
In summary, we present a novel approach for the isolation of neurons from various brain regions and NSCs from the SVZ. The process combines an elaborated method for gentle and efficient dissociation of small brain regions with a sophisticated cell sorting technology.

Methods

1 Automated dissociation of distinct adult brain regions

Brain regions such as olfactory bulb, cortex, cerebellum, and SVZ were dissected from adult CD1 mouse brains (>6 weeks). The Adult Brain Dissociation Kit, mouse and rat was used to dissociate cerebellum, cortex, and olfactory bulb, and the Neural Tissue Dissociation Kit (T) to process SVZ tissue. Both kits were applied in combination with the gentleMACS Octo Dissociator with Heaters. After dissociation, debris and optionally erythrocytes were removed from the single-cell suspensions to increase the percentage of intact neural cells. Neurons or NSCs were then isolated using the microchip-based microfluidic cell sorter MACSQuant Tyto (fig. 1).

The dissociation protocol yielded $1.03\times10^6\pm6\times10^5$ cells (n = 5) per cerebellum, whereas $6.3\times10^5\pm 2.8\times10^5$ cells were obtained from one cortical hemisphere (n = 4) and $1.9\times10^5\pm9\times10^4$ cells from olfactory bulbs of one mouse (n = 4). Viability rates were between 70 and 80%, depending on the brain region. Dissociation of SVZ tissue derived from one mouse yielded $3.69\times10^5\pm9.3\times10^4$ cells with a viability rate of >97% (n = 12).

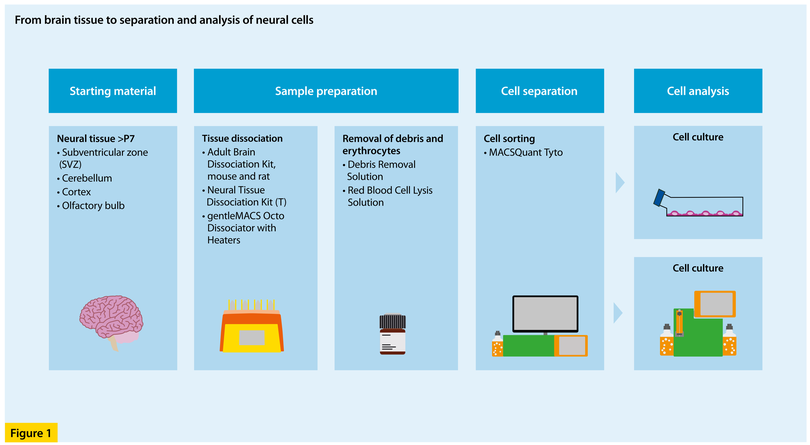


Figure 1

2 Sorting of neurons and neural stem cells using the MACSQuant Tyto

The entire cell sorting process of the MACSQuant Tyto occurs within the closed environment of the MACSQuant Tyto Cartridge (fig. 2). This single-use cartridge contains a sorting microchip, which allows for completely aseptic conditions without cross-contamination between samples. Unlike in conventional droplet sorters, cells do not experience high pressures, and no charge is applied, thus ensuring gentle sorting conditions for high cell viability and maintained functionality. In this study, the MACSQuant Tyto enabled high-speed fluorescence-based isolation of neurons and NSCs.
Neurons were separated from dissociated cerebellum, cortical hemisphere, and olfactory bulb from adult mice by the depletion of non-neuronal cells using an antibody cocktail that specifically labeled the non-neuronal cells.
NSCs were identified and sorted based on the positive markers GLAST and plexin-B2 and the negative markers CD24, Ter119, and CD45 to exclude erythrocytes, leukocytes, microglia, neurons, ependymal cells, and neuroblasts[1] (table 1). The whole process including dissection, tissue dissociation, antibody staining, and cell sorting took approximately 3 h.

Antibodies for identification and sorting of neural stem cells	Marker expression on neural stem cells
Anti-GLAST (ACSA-1)-APC	High
Anti-Plexin-B2-PE	Mid to high
CD24-VioBlue®	Negative
CD45-VioBlue	Negative
Anti-Ter-119-VioBlue	Negative

Table 1

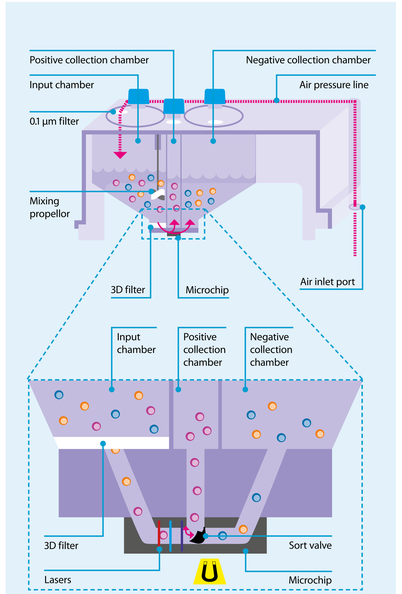


Figure 2

Results

1 Isolation of neurons from distinct brain regions

Neurons were isolated from dissociated cerebellum, cortical hemisphere, and olfactory bulb from adult mice as described above. Fractions of cells before and after sorting were analyzed by flow cytometry using the MACSQuant Analyzer 10. One cerebellum delivered 1.2×10^4 neurons with a purity of 80% and a viability of 97%, while one cortical hemisphere yielded 4.4×10^3 neurons (purity: 99%; viability: 95%), and two olfactory bulbs resulted in 4.8×10^3 neurons (purity: 95%; viability: 96%). Figure 3 shows a representative result. Debris, doublets, and dead cells were excluded from the analysis.

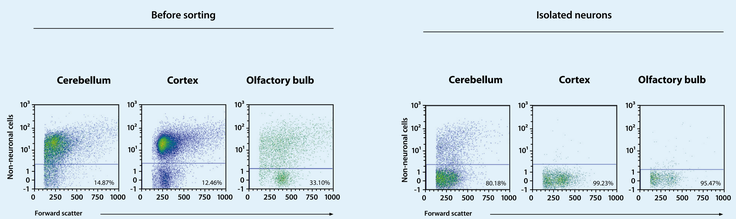


Figure 3

2 Isolation of neural stem cells from the subventricular zone

NSCs were isolated from SVZ tissue obtained from five mice. Cell sorting was performed using the MACSQuant Tyto based on the positive markers GLAST and plexin-B2 and the negative markers CD24, Ter119, and CD45.
Upon completion of the sorting process, samples of the non-sorted cells as well as the positive and negative cell fractions were analyzed by flow cytometry using the MACSQuant Analyzer 10 (fig. 4). Debris, doublets, and dead cells were gated out (not shown). After exclusion of cells labeled by the negative markers CD24, CD45, and Ter119 (left column), a gate was set on plexin B2⁺ cells (middle column), which then allowed the identification of plexin-B2⁺GLAST⁺ NSCs (right column).
Sorting of $1.85\times10^6\pm4.65\times10^5$ total cells resulted in 36,000±8,000 plexin-B2⁺GLAST⁺ NSCs with a purity of >95% and a viability rate of >93% as analyzed by PI staining (n = 6).

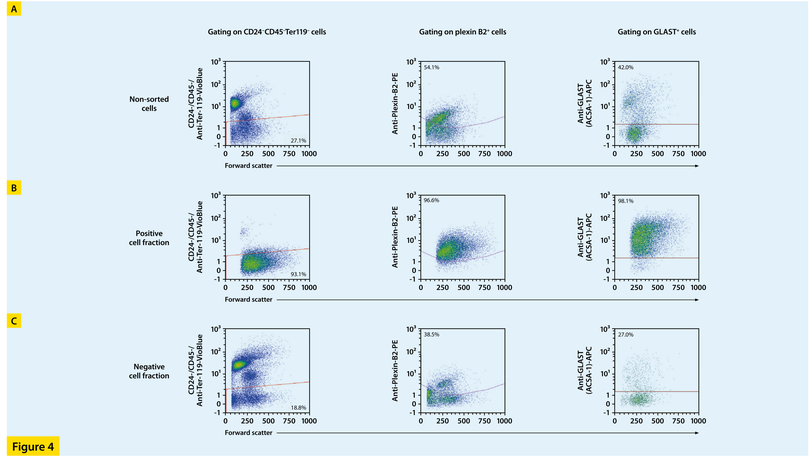


Figure 4

3 Isolated neural stem cells give rise to primary and secondary neurospheres and differentiate into glial cells and neurons

Neurosphere assays are widely used to identify stem cells based on their capacity for self-renewal and their ability to differentiate. Isolated cells were cultivated on ultra-low attachment plates in MACS® Neuro Medium supplemented with MACS NeuroBrew®-21 (w/o Vitamin A), EGF (20 ng/mL), and FGF-2 (20 ng/mL) for 7–10 days. To assess the self-renewal capacity, generation of secondary neurospheres was tested. To that end, primary neurospheres were dissociated using the Neurosphere Dissociation Kit (P) and plated again for 7–10 days in the same medium.
For differentiation, neurospheres were plated on poly-L-lysine–coated 24-well glass bottom plates in MACS Neuro Medium supplemented with MACS NeuroBrew-21. After 7 days of culture, cells were fixed and subjected to immunocytochemical analysis using antibodies specific for the astrocyte markers GFAP, nestin, and GLAST, the neuronal cell marker MAP2, and the oligodendrocyte marker O4.
Cultivation of isolated NSCs under the specified assay conditions led to formation of a large number of neurospheres (fig. 5A, B), which gave rise to secondary neurospheres. Furthermore, neurospheres differentiated into glial cells as well as neurons as shown by expression of GFAP, nestin, GLAST, MAP2, and O4 (fig. 5C–F).

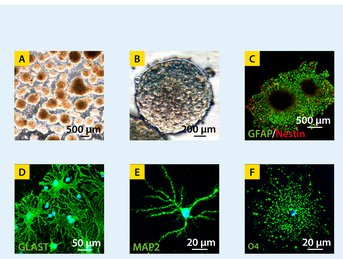


Figure 5

Summary and outlook

- We present a novel approach for the gentle automated and rapid dissociation of distinct regions of adult rodent brain tissue, which results in single-cell suspensions with high cell viability.
- The gentle sorting procedure using the MACSQuant Tyto allows enrichment of neurons from distinct brain regions to a purity of up to 99% and NSCs from SVZ to a purity of >95% with a viability of >93%. Cells are therefore suitable for further functional and molecular studies.
- Capacity of the isolated NSCs for self-renewal and differentiation was tested, and it was shown that the cells formed a large number of primary neurospheres, which gave rise to secondary neurospheres and differentiated into neurons as well as different glial cells.
- The experimental procedure including dissection, tissue dissociation, and isolation of neurons or NSCs can be performed in only 3–4 h.

Reference: 1. Mich, J.K. *et al.* (2014) eLife 3: e02669. Doi: 10.7554/eLife.02669

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