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®

Methods

1 Automated dissociation of distinct adult brain regions

Brain regions such as olfactory bulb, cortex, cerebellum, and SVZ were dissociated from adult CD1 mouse brains (>6 weeks). The Adult Brain Dissociation Kit was used to disaggregate the tightly connected neural cells successfully. The brain, sophisticated mechanical and enzymatic treatments are required for reliable cellular analysis and cell sorting. In the case of adult rodent brain, and when highest possible viability, recovery, and functionality are served as starting material for the generation of single-cell suspensions to increase the percentage of intact neural cells. Neurons or NSCs were then isolated using a cell sorting device that uses a microchip-based technology for high-purity (<95%) isolation of neural cells using an antibody cocktail that specifically labeled a subset of cells. The whole process including dissection, tissue dissociation, antibody staining, and cell sorting took approximately 3 h.

The dissociation protocol yielded 1-2x 10^6 viable cells in 5 x 10^5 dissociated cells. The experimental procedure including dissection, tissue dissociation, antibody staining, and cell sorting took approximately 3 h.

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 flow chamber of the MACSQuant Tyto™. A novel multiparameter flow cytometry using the MACSQuant Analyzer 10 (fig. 4). Debris, doublets, and dead cells were gated out by flow. After exclusion of cells labeled by the negative markers CD24, Ter-119, and MAP2 20 μm, sorting gates were set on 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 by flow.

2 Isolation of neuronal stem cells from the subventricular zone

Neurons were isolated from SVZ tissue obtained from five mice. Cell sorting was performed using the MACSQuant Tyto™. The negative markers GLAST and glia fibrillary acidic protein (GFAP) were used to exclude glial 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 by flow.

Summary and outlook

- We present a novel approach for the gentle and rapid dissociation of distinct adult mouse brain tissue, which results in high-purity (>95%) isolation of neural cells using an antibody cocktail that specifically labeled a subset of cells.
- The gentle sorting procedure using the MACSQuant Tyto allows enrichment of neurons from distinct brain regions to a purity of up to 95% and NSCs from SVZ to a purity of >90% with a viability of >90%. 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 grew to secondary neurospheres and differentiated into neurons as well as glial cells.
- The experimental procedure including dissection, tissue dissociation, and isolation of neurons or NSCs can be performed in only 3–4 h.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8