SUPPLEMENTAL DATA

Engraftment of human nasal olfactory stem cells restores neuroplasticity in mice

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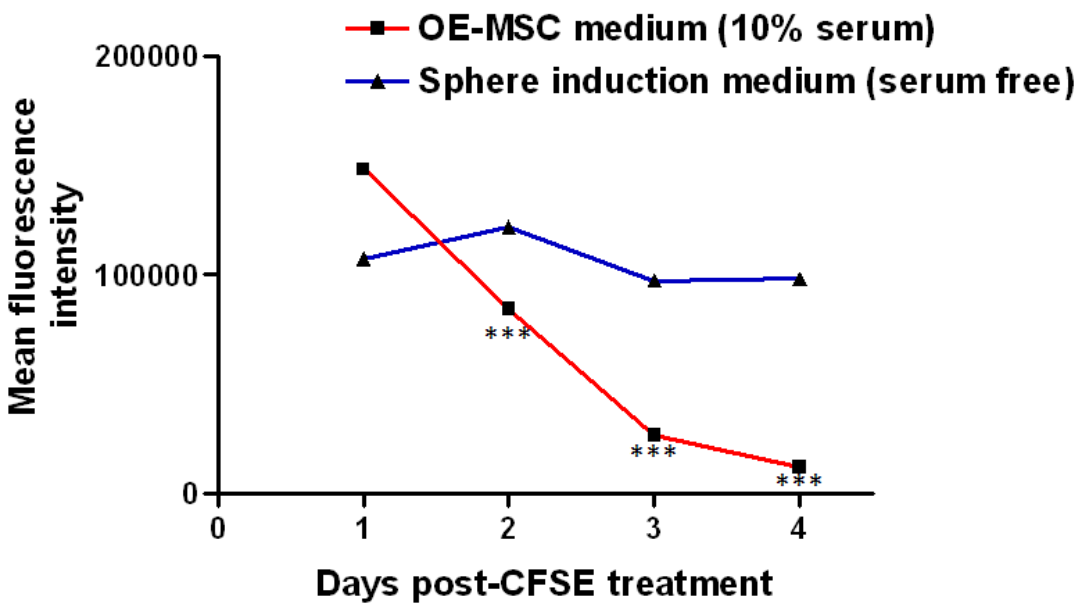
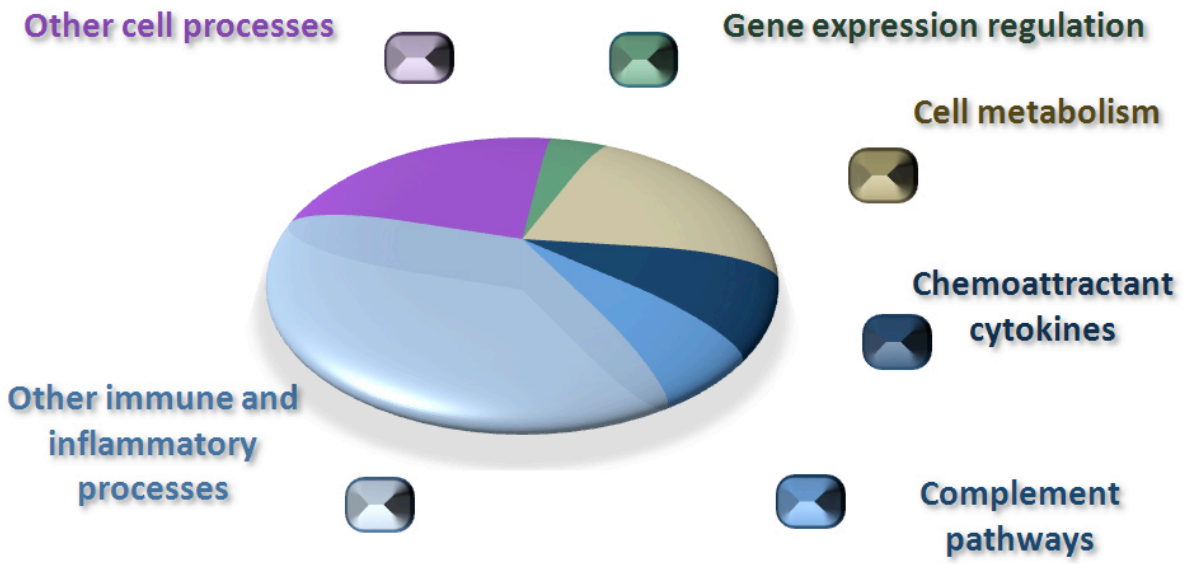


Supplementary Figure 1: OE-MSCs, under the sphere induction medium, prior to transplantation, are not proliferative. CFSE-based cell proliferation assay shows a significant reduction of CFSE fluorescence intensity when OE-MSCs are maintained under serum-based medium (red line) but not when cultivated in sphere induction medium (blue line).



Supplementary Figure 2: Pie chart showing functional clustering of genes with a fold change > + 2.5 (related to Table 1). The 114 overexpressed genes in the lesioned hippocampi with a fold change > + 2,5 were grouped in six functional clusters, using DAVID Functional Annotation Clustering Tool. The majority (53%) of over-expressed transcripts are involved in immune and inflammatory processes (grey, light blue and dark blue areas).

Probe trial S1	**Ctrl.**				**Les.**			
Quad-rant	**Q1**	Q2	Q3	Q4	**Q1**	Q2	Q3	Q4
Time (s) in the platform quadrant	22,3 (±1.9)	9,6 (±1.3) ***	17,1 (±1.7)	10,6 (±1.8) **	16,6 (±1.3)	14,5 (±1.8)	14,4 (±1.2)	14,3 (±1.9)

Probe trial S2	**Ctrl.**				**Gr. IH**				**Gr. ICV**				**Sham**				**Dead cells**			
Quad-rant	Q1	Q2	Q3	**Q4**	Q1	Q2	Q3	**Q4**	Q1	Q2	Q3	**Q4**	Q1	Q2	Q3	**Q4**	Q1	Q2	Q3	**Q4**
Time (s) in the platform quadrant	10,1 (±1.5) ***	11,5 (±2.5) **	13,6 (±2) **	25,1 (±1.5)	12,2 (±3.2) **	9,5 (±1.6) ***	10,9 (±2.9) **	27,5 (±3.3)	9,9 (±2) ***	8,5 (±0.8) ***	13,6 (±3.4) **	28,0 (±2.4)	15,7 (±2.7)	14,4 (±3.8)	11,1 (±3.2)	18,1 (±3)	13,8 (±4.5)	10,4 (±2.9) *	18,4 (±4.1)	17,7 (±1.4)

Supplementary Table 1: Time spent in each of the four quadrants during the 1-min probe trial in the Morris water maze at the post-lesioning (S1) and post-grafting (S2) testing period (related to Figure 3). Quadrants Q1 and Q4 were the platform quadrants during S1 and S2, respectively. For each group (Control = Ctrl; Lesioned = Les; Graft IH = Gr.IH; Graft ICV = Gr.ICV; Sham and Dead Cells), selected analyses of variance (ANOVAs) were performed using the time spent in platform quadrant (Q1 for S1; Q4 for S2) and other quadrants (* $p < 0.05$; **$p < 0.01$, ***$p < 0.001$) as parameter.

Supplemental Videos (Legends)

Video 1: Two consecutive trials performed by Balb/C mouse in the olfactory tubing maze (related to Figure 3).

Video 2: Two trials in the Morris water maze performed by C57BL/6 mouse at Day1 and Day5 (related to Figure 3).

Video 3: Three dimensional view, 5 weeks after intra-hippocampal transplantation of exogenous GFP^+ OE-MSCs (green) in the $MAP2^+$ endogenous neuronal network (red) inside cortices above the hippocampus (related to Figure 5).

Video 4: Three dimensional view, 5 weeks after intra-cerebro-ventricular transplantation of exogenous GFP^+ OE-MSCs (green) surrounded by endogenous $GFAP^+$ cells (red) in the granular layers of the dentate gyrus (related to Figure 7).

Supplemental Experimental Procedures

Behavioral procedure

Olfactory tubing maze. This experimental device includes four T-maze testing chambers joined together with plastic elbow tubes (for more details see (Roman et al., 2002)). At the top of each T-maze central cube, inverted fans exhaust the neutral or scented air pulsed at both ends. A well-shaped water port and a buzzer are located above the air and odor port at each extremity of the T-maze. Entry to and exit from the testing chamber are achieved using automated doors and photoelectric cells located 21 cm in front of, and at the entrance to, each testing chamber. An additional photoelectric cell is placed 5 cm in front of each lateral extremity of the testing chamber.

Habituation. All mice were water deprived, weighed, handled for 5 min and put in the olfactory tubing maze for 5 min (day 1), 15 min (day2) and 10 min (day3). The doors were opened, with free access to all areas. All water ports were loaded with 0.1 ml of water. One day later, training sessions started.

Associative memory assessment. When a mouse entered a testing chamber, two different odors were ejected from the right and left tubes, respectively. One odor (citral dimethyl acetal [25.10^{-3} µl/ml], Aldrich) was associated with the positive reward [a drop of water ($\cong$ 0.03 ml)] while the other odor (allyl heptanoate [5.10^{-2} µl/ml], Aldrich) was associated with a 3 sec buzzer sound (non-aversive). Once the choice made, the entrance door closed and the outside door opened, allowing the mouse to get access to the next testing chamber. The animal could not correct an error after a wrong response. During the inter-trial period (15s minimum), neutral air was ejected from all testing-chamber extremities. Every day, each mouse had to perform 20 trials. In each testing chamber, odor delivery was computationally (Hewlett Packard) randomized using Lab VIEW software (National Instruments France). At

the end of the experiment, each mouse had free access to water from 2:00 to 2:05 p.m. Associative memory was assessed by dividing the number of correct responses by the number of odor presentations (20 per day).

Morris Water Maze. It consisted of a circular black pool (diameter 170 cm; height 60 cm) filled with water (20 °C) to a height of 40 cm. The pool was located in an experimental room with ample surrounding visual cues (e.g. balloons, computer, animal cages, sink, etc.) which could be used by the mouse to guide its navigation. The pool was virtually divided into four quadrants of equal surface and 4 different starting points were identified. A circular platform (diameter 10 cm) was placed in the pool, 0.5 cm underneath the water surface. It was camouflaged by creating a nearly invisible platform-to-background color match.

Spatial acquisition. For each trial, the mouse was released from the side of the pool, facing the wall at a randomly assigned starting point, and given 60 s to reach the submerged platform. Each day, each mouse was given four trials, the starting point differing for each trial. Different starting points were assigned randomly each day. When the mouse found the platform, it was left there for 15 s before the next trial was started. When the mouse did not find the platform within 60 s, the experimenter placed it there for 15 s before the next trial was run. During 5 consecutive days, the platform was placed in the SW quadrant (Q1) or the NE quadrant (Q4) for the training before and after transplantation respectively. Using a video-tracking system, multiple parameters (e.g. the latency to reach the platform, distance swum, etc.) were recorded for each trial. At the end of the four trials, the mouse was removed from the pool, towel dried, and returned to its home cage.

Probe trial. When the last trial of the last day was completed, the platform was removed and the mouse was given a probe trial for 60 s. The testing procedure used before the probe trial is

considered to provide a measure of spatial reference memory, while the probe trial is considered to measure the strength of spatial learning.

Microarray experiments

Four weeks after ibotenic acid-induced injury, lesioned mice (n=3) and control mice (n=3) were decapitated. Brains were quickly removed and hippocampi dissected under RNAse free conditions before being immediately placed in RNA later (Qiagen) and stored at -80°C until analysis. Total RNA was isolated and treated with DNAse using the Rneasy Lipid Mini Kit (Qiagen). RNA concentration and purity were determined using a NanoDrop-1000 Spectrophotometer (NanoDrop Technologies). RNA quality was assessed by the Agilent 2100 Bioanalyser (Agilent Technologies). For each sample, cDNA was generated from 600 ng of total RNA with Agilent Quick Amp Kit (Agilent Technologies) then synthesis, amplification and labeling of complementary RNA with Cyanine 3 dye were performed according to manufacturer's protocols. Cyanine 3 labeled cRNA (1.65 µg) was fragmented and hybridized to the Agilent Whole Mouse Genome Oligo Microarray 4x44k at 65°C for 17 hours. After washing, fluorescence intensity at each spot was assayed using G2565BA Microarray Scanner (Agilent). After data extraction with Feature Extraction Software 9.5.3 (Agilent), they were normalized (background subtraction and quantile normalization) using AgiND library developped under R software for agilent microarrays data normalization and vizualisation. Then fold change ratio (each lesioned hippocampus against pooled control hippocampus) was calculated for every spot. Genes with a fold change superior to 2.5 were selected. Means and SEM for each fold change were calculated. Selected genes were clustered into functional groups with the help of DAVID Functional Annotation Clustering Tool (1, 2); (http://david.abcc.ncifcrf.gov/home.jsp).

CFSE cell proliferation assays

CFSE stainings (CellTrace CFSE Cell Proliferation Kit, C34554, Molecular Probes) were conducted according to the manufacturer's instructions with a final concentration of 2.5 μM. Briefly, proliferative capacity of OE-MSCs have been assessed across a 4 day cell culture period in two different media: 1) OE-MSC medium containing Dulbecco's Modified Eagle Medium/ Ham F12 supplemented with 10% Foetal Bovine Serum and 1% Penicillin/Streptomycin (Invitrogen); 2) Sphere induction medium containing Dulbecco's Modified Eagle Medium/ Ham F12 supplemented with insulin, transferrin, selenium (ITS-X, 1%; Invitrogen), EGF (50 ng/mL), and FGF2 (50 ng/mL). At the end of each time point (i.e. from Day 1 to Day4 post CFSE treatment), CFSE fluorescence intensity was determined by Flow cytometry. A minimum of 10,000 cells in the living population were analyzed.

Supplemental References

1. Dennis, G., Jr., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., and Lempicki, R.A. 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4:P3.
2. Huang da, W., Sherman, B.T., and Lempicki, R.A. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4:44-57.