Supplementary Materials and Methods

Treatment and glia-like differentiation of MSCs

Human undifferentiated adipose tissue-derived MSCs (ScienCell Research Laboratories, CA) were propagated and maintained in DMEM with FBS (10%), gentamicin (50 µg/ml), nonessential amino acid (5mM) and glutamine (5mM) (37°C, 5% CO_2 humidified atmosphere). For glia-like differentiation, MSCs were treated with DMEM/Opti-MEM with F12 – B 27.5%, FGFb (50ng/ml), SHH (250ng/ml) and EGF (50ng/ml), for 3 weeks (37°C, 5% CO_2).

GNP synthesis and characterization

Spherical GNPs (20nm) were synthesized using sodium citrate as a reducing agent, based on a well-established procedure (Betzer *et al*, 2015; Popovtzer *et al*, 2008). GNPs were conjugated to MDDA linker (12-mercaptododecanoic acid, Sigma) then coated with D-(þ)-glucosamine hydrochloride (Sigma-Aldrich, Israel), due to its stability and high cell-uptake rate.

GNP labeling of cells

D-(+)-Glucosamine hydrochloride (3mg) was added to activated linker-coated GNPs (30 µg/ml), added in excess to MSCs (one million particles/cell), and incubated (37°C, 3 hours). GNPs undergo endocytosis (Kalivas and Volkow, 2011; Uys *et al*, 2011) through a receptor-mediated endocytosis internalization mechanism (Roberts-Wolfe and Kalivas, 2015). After incubation, the medium was washed twice with PBS, followed by trypsin treatment; cells were centrifuged twice (7 minutes in 1000 rpm) to wash out unbound

nanoparticles. GNP uptake was measured using Flame Atomic Absorption Spectroscopy (FAAS; below) and was found to be $1.1\times10^6 \pm 0.12$ per cell.

Real-time quantitative PCR analysis

Total RNA was isolated from cultured MSCs growing on astrocytic or control mediums using QIAzol reagent (Qiagen, CA) according to the manufacturer's protocol. 0.5µg of RNA was employed to synthesize cDNA by Thermoscript (Invitrogen) with oligo dT primers. To detect EAAT-1 and EAAT-2 mRNA, SYBR green qPCR method was employed using the following primers: EAAT-1, upstream ATGACTAAAAGCAATGGAGAAGAGC; downstream TCTACATCTTGGTTTCACTGTCG; EAAT-2, upstream CTAATGAGATCCAGGAAGGCA; downstream TTCACGTTTCCAAGGTTCTTC. Control S12 mRNA levels were used: upstream TGCTGGAGGTGTAATGGACG; downstream CAAGCACACAAAGATGGGCT. Quantitative mRNA expression data was acquired and analyzed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Grand Island, NY). Data was further analyzed by Comparative CT (ΔΔCT) method, and results expressed in arbitrary units.

Western blot analysis

Cell lysates from control and differentiated MSCs (30µg protein) were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Rabbit anti-EAAT1 and EAAT2 antibodies (Santa Cruz Biotechnology, USA)) and goat anti-actin antibody (Sigma, USA) were used. Following incubation with the primary and secondary antibodies, immunoreactive bands were visualized by ECL Western blotting detection kit (Amersham, USA).

MSCs [^{3}H]glutamate uptake

Cells were plated at 2.0×10^5 cells per well. Following 2 washes in DMEM, cells were transferred to Krebs buffer (1h, 37°C). Cells were then incubated with 1 ml L-[3,4-3H]glutamate for 10, 60 and 90 min and uptake was terminated by addition of ice-cold PBS (3ml). Cells were washed three times with PBS, collected with a rubber policeman, and [^{3}H]-Glutamate levels were determined with liquid scintillation counter.

MTT cell proliferation assay

Cells were plated at 100,000/well in a 24-well plate and allowed to adhere overnight. GNPs were suspended in media at 1 mg Bi/mL, sonicated, and 10-fold dilutions in media were made down to 0.1µg/mL. GNP solution (500µL) was added to each well. Controls received fresh media only. After 2 days of co-incubation, GNP solution was replaced with mixture of 400µL media and 40µL of MTT powder (1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan, Sigma) at 5mg/mL in PBS. Two hours later, media was aspirated and 1mL of DMSO (Fisher) was added to each well. 50µL was immediately transferred to a 96-well plate and absorbance was measured at 560nm (SpectraMax 190, Molecular Devices), using DMSO-only wells to establish baseline. For each experiment, 3 wells were tested per bismuth concentration, and experiments were repeated twice. Optical density measurements were normalized to wells containing cells unexposed to GNPs.

Animals

Adult male FSL and Sprague-Dawley (SD) rats (>230g) were maintained under conditions of unvarying temperature (23ºC) and humidity (50%), in a 12:12h light/dark cycle and with free access to food and water, as approved by the Animal Care

Committee of Bar-Ilan University and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

MSC treatment

FSL and SD rats were habituated to the animal housing room for 1 week. Baseline behavioral measurements (detailed below) were conducted. Twenty-four hours later, animals were anesthetized with ketamine hydrochloride (100mg/kg, i.p.) and xylazine (10mg/kg, i.p.), then placed in a stereotaxic apparatus (David Kopf). A hole was drilled through the skull and a 10µl Hamilton syringe was inserted unilaterally into the left lateral ventricle (anterior −0.8, lateral 1.5, ventral -4.0mm from the bregma). FSL rats received either 210^5 MSC-EAAT labeled with GNPs (total volume 10µl; n=10), or vehicle with free GNPs as control (10µl; 30mg/mL; n=10); SD rats (n=7) received vehicle. Injection rate was 2µl/min, over 5 min. Antibiotics (Baytril, 0.4ml, s.c.) and analgesics (Rimadyl, 0.05ml, s.c.) were administrated for 3 consecutive days.

Locomotor Activity Test

Tests were conducted 24 hours before injections, and on day 19 after treatment. Rats (n=7 from each group, in this and in subsequent behavioral tests) were placed in a plastic polymer box (60×60×30 cm), for 5 minutes. Illumination intensity in the room was 5 lx. A camera placed above the box recorded behavioral parameters. Total mobility time and average velocity of each animal were analyzed using the Noldus Ethovision 7.0 XT camera and video tracking software package. Video and computer equipment and recording and observation analysis were situated in a separate room.

Novelty Exploration Test

Tests were conducted 24 hours before injections, and on day 20 after treatment. Rats were allowed to explore a new object for 5 min in a plastic polymer box (60×60×30 cm). The object, with a complex texture surface (glass ball, 15×6×5 cm) was placed in the center of the exploration area. A camera was placed above the box and recorded the animals' naïve behavioral parameters, measuring the total duration of time spent exploring the object. The video and computer equipment were situated in a separate room, in which all recording and observation analysis was conducted. Behavior in the novelty exploration test was measured using Noldus Ethovision 7.0 XT camera and video tracking software package.

Sucrose self-administration

Tests were conducted 24 hours before injections, and on days 14, 20 after treatment. The experiment was conducted as in (Friedman *et al*, 2009). Briefly, this test assesses whether the treatment increases basic reward behavior ('anhedonia', used as a measure of depressive-like behavior (De La *et al*, 2005; De La *et al*, 2004)). Rats were transferred daily into operant conditioning chambers (Med-Associates Inc.; St Albans, Vermont) for 30 min sessions, during their dark cycle, and allowed to self-administer sucrose (10% sucrose solution; 0.13ml per infusion) delivered into a liquid drop receptacle for oral consumption. During the infusion, a light located above the active lever was lit for 20s. During the 20s intervals of sucrose infusion, active lever presses were recorded, but no additional sucrose reinforcement was provided. Presses on the inactive lever were recorded, but they did not activate the infusion pump and light. Rats were returned to the home cages at the end of each session. Animals had free access to water and food, ensuring that the test measured only anhedonic behavior.

Forced Swim Test

Depressive-like behavior (despair) was measured by calculation of immobility time in the modified version of the Porsolt Forced Swim Test (Friedman *et al*, 2009). Tests were conducted 24 hours before MSC treatment, and on day 21 after treatment. For the ketamine-treated control rats (n=3), tests were conducted at baseline (before surgery, during which ketamine (100mg/kg, i.p.) was injected), and then 24hrs, 3 days, 4 days, 7 days and 12 days after surgery. Rats were placed in a cylindrical tank (height: 40cm, diameter: 18cm) containing just enough water (at 2°C above room temperature) so that the rat could not touch the bottom with its hind paws. The amount of time that each rat either swam or stayed immobile during a 5min period was recorded. Immobility was defined as suspension of swimming, in such a manner that both hind paws were immobile. Recording and analysis were conducted using Noldus Ethovision 7.0 XT.

It is important to note that because our model is a genetic one, herein the swim test is not used to induce depressive behavior as in other models of depression. Rather, it serves as a test to examine the efficacy of MSC-EAAT treatment on depressive-like behavior.

***In-vivo* micro-CT scans**

Animals (n=3 for treated FSLs and free-GNP injected FSLs) were scanned in-vivo with a micro-CT scanner to screen for the location of the cells, at two timepoints – one day and one month after injection were taken for CT scanning. Images were obtained using a micro-CT device (Skyscan High Resolution Model 1176, Bruker micro-CT, Kontich, Belgium) with a nominal resolution of 35μm, a 0.5 mm aluminum filter, and applied x-ray tube voltage of 45 kV. The projection images were reconstructed into

cross sectional slices (NRecon v.1.6.9, Bruker micro-CT). Surface-rendered three-dimensional (3D) models were constructed for 3D viewing of the analyzed brain regions. Volume rendered 3D images were generated using an RGBA transfer function in SkyScan CT-Volume ("CTVol") software. At the end of the experiment, brains where taken for further ex-vivo scans and FAAS analysis. The detection limit was approximately 1000 GNP-labeled cells.

Ex-vivo micro-CT scans

An additional contrast agent was used for *ex vivo* scans, due to minimal differences in density and X-ray absorption between different brain tissue types (i.e., no native CT contrast). A procedure based on previous studies by de Crespigny *et al* (2008) and Saito (2012) was used and modified in order to be applied to rat brains. This procedure is based on the usage of a nonionic iodinated contrast agent (Iopamidol, Bayer Schering Pahrma, Japan). Brains were removed and placed in 10% buffered formalin for 5 days of fixation, then soaked in Iopamidol (150mg/ml), diluted with 7.5% paraformaldehyde at 4°C for 14 days. Prior to CT imaging, brains were removed from the solution, blotted dry and placed in a sample holder for imaging. The sample holder was sealed with plastic film to prevent dehydration. Brains were then scanned in the micro-CT at a nominal resolution (pixel size) of 9μm, employing an aluminum filter 0.2mm thick and an applied x-ray tube voltage of 45 kV. Camera pixel binning of 2×2 was applied. The scan orbit was 180 with a rotation step of 0.4 degrees. Reconstruction was carried out with a modified Feldkamp (1984) algorithm using the SkyScan™ NRecon software accelerated by GPU (Yan *et al*, 2008). Gaussian smoothing (2%), ring

artifact reduction and beam hardening correction (20%) were applied. The cross-section slices were stored in 8-bit BMP format (256 shades of gray).

FAAS analysis

FAAS (SpectrAA 140, Agilent Technologies) was used to determine amounts of gold in each region, and thus quantify the exact amount of cells that reached each brain region. Brains were placed in a rat brain matrice, cryosectioned at 1mm in the coronal plane. Punches from 30 different brain regions were taken with a brain punch set with an inner diameter of 1mm/0.5mm, depending on brain region size. Tissues obtained from experimental animals were melted with aqua regia acid (mixture of nitric acid and hydrochloric acid in a volume ratio of 1:3). Samples were evaporated, filtered and diluted to 5 ml. Au lamp was used to determine the gold concentration. A calibration curve with known gold concentrations was prepared (commonly: 0.1, 1, 2 and, 5mg/ml). Gold concentration was determined according to the absorbance value with correlation to the calibration curve. Samples were analyzed in triplicate; averages and standard deviations of the resulting data were taken. The amount of cells per region was calculated based on the amount of gold per region divided by the number of GNPs uptaken into the cells (detailed above in *GNP labeling of cells*, and in (Betzer *et al*, 2014)).

Real-time quantitative PCR of EAAT and BDNF brain

Animals were sacrificed by rapid decapitation (day 21). Brains placed in a rat brain matrice were cryosectioned at 1mm in the coronal plane, bi-hemispheric hippocampal brain punches were dissected and snap frozen in liquid nitrogen. Total RNA was isolated using QIAzol reagent (Qiagen, Germantown, MD) according to manufacturer's

protocol. 0.5µg RNA was used to synthesize cDNA by Thermoscript (Invitrogen) with oligo dT primers. Expression of human EAAT1 and rat BDNF were determined by TaqMan stem-loop RT-qPCR method. Primers and appropriate probes were obtained from Life Technologies. EAAT1 primers were used as above, and BDNF primers were based on a coding sequence of the rat BDNF gene: upstream: 5′-GCGGCAGATAAAAAGACTGC-3′ and downstream: 5′-GCCAGCCAATTCTCTTTTTG-3′. S12 mRNA was used as internal control. Quantitative mRNA expression data were acquired and analyzed with ABI Prism 7000 Sequence Detection System (Applied Biosystems, NY). Data were further analyzed by Comparative CT method.

Immunohistochemistry

After conclusion of the behavioral tests (day 21), brains were removed for immunohistochemical staining or RT-qPCR (below). Animals under deep anesthesia were transcardially perfused with PBS×1 followed by 4% paraformaldehyde (n=2). Excised brains were post-fixed in paraformaldehyde (48h, 4°C), then equilibrated in PBS containing 30% sucrose (48h, 4°C), and gradually frozen in dry ice. Tissues were cryosectioned (30µm) in the coronal plane. Ten slices per brain were washed in PBS, blocked with a blocking solution (1h), then stained overnight with primary antibodies: mouse anti-human mitochondria monoclonal (millipore MAB1273), rabbit anti-human/mouse/rat BDNF monoclonal (abcam ab108383) and rabbit anti-human/mouse/rat EAAT1 polyclonal (abcam ab416) primary antibodies. Slices were washed and stained (1h) with secondary antibodies for visualization: Alexa Fluor 568 goat anti-mouse (mitochondria), Alexa Fluor 488 goat anti-rabbit (EAAT1) and Alexa Fluor 555 goat anti-rabbit (BDNF). Slices were also stained (2 min) with a DAPI solution

(0.0005mg/ml) to visualize cell nuclei. For control, slices were not incubated with primary antibody, with all other parameters identical to experimental slices. Fluorescent images were acquired with Olympus FV-1000 confocal microscope.

Statistical Analysis

For behavioral tests, one-way ANOVA or two-way ANOVA with repeated measures followed by Bonferroni's multiple comparison or Tukey's post hoc was performed. Differences between two groups were analyzed using Student's t-test.