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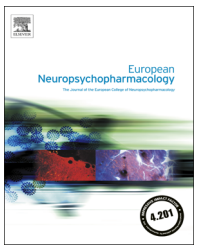

Mesenchymal stem cells protect from sub-chronic phencyclidine insult in vivo and counteract changes in astrocyte gene expression in vitro

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Abstract
Mesenchymal stem cells (MSCs) are an attractive cell source for regenerative medicine strategies in brain diseases. Experimental studies have shown that repeated administration of phencyclidine (PCP) leads to schizophrenia-like behavioral changes in mice. The aim of the present study was to explore the effectiveness of MSC transplantation into the hippocampus in attenuating PCP-induced social behavior deficits. PCP was administered subcutaneously to C57bl mice (10 mg/kg daily) for 2 weeks. On the first day of PCP administration, adult human MSCs were transplanted into the hippocampus. A week after the last PCP dose, the mice underwent social preference testing. MSC transplantation was associated with a significant reduction in the adverse social behavior induced by PCP. Immunohistochemical analysis revealed that the stem cells survived in the mouse brain, and hippocampal Western blot analysis revealed a statistical trend towards a decrease in cleaved caspase 3 protein levels in the stem cell treated group. Upon in vitro co-culture of astrocytes and MSCs, the MSCs, in the presence of PCP, positively regulated astrocyte expression of genes involved in glutamate metabolism and antioxidant defenses. These findings suggest that MSC transplantation into the hippocampus may serve as a novel neuroprotective tool for the treatment of the PCP-induced schizophrenia-like social endophenotype. The mechanism underlying the beneficial behavioral effect may involve

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modulation of host astrocyte functioning, including glutamate processing and antioxidant capacity.

1. Introduction

Stem-cell-based regenerative medicine is a growing field that holds therapeutic promise for diseases for which current methods offer only limited solutions. Stem cell research has yielded important experimental approaches to Parkinson's disease, multiple sclerosis, and cerebrovascular diseases (Lindvall and Kokaia, 2006). The rationale underlying the use of stem cells has evolved from merely replacing cells affected by disease (cell replacement) to enhancing the brain's endogenous protective mechanisms against insult (Boucherie and Hermans, 2009). Animal studies indicate that bone marrow mesenchymal stem cells (MSCs) are a good candidate for this approach because once transplanted into the brain, they support the niche in situ (Horwitz and Dominici, 2008; Prockop, 2009a). Mechanistically, the trophic effect in diseases of the brain was previously reported to be mediated by enhancing neurogenesis, improving availability of neurotrophic factors and inducing an anti-oxidative stress effect (Bao et al., 2011; Kan et al., 2010; Shen et al., 2010; Tfilin et al., 2010; Valle-Prieto and Conget, 2010).

N-methyl-D-aspartate (NMDA) receptors are members of the glutamate receptor channel superfamily. Most neurotransmitter glutamate is recycled through the glutamate–glutamine cycle (Hertz and Zielke, 2004). The steps include conversion of glutamine to glutamate in neurons (via glutaminase), glutamate uptake synaptically by glutamate transporters in astrocytes, conversion of glutamate to glutamine (via glutamine synthetase), and finally, transport of glutamine back to neurons. Studies in transgenic mice have highlighted the relevance of hypoglutamatergic transmission in schizophrenia. Mice expressing low levels of the NDMAR1 subunit of the NMDA receptor exhibit altered patterns of regional brain metabolism, including reduced activity in the medial prefrontal and anterior cingulated cortices and the hippocampus, in addition to deficits in sensorimotor gating and social behavior (Duncan et al., 2004). Transgenic knockout mice lacking genes involved in the glutamate–glutamine cycle display endophenotypes specific to schizophrenia: glutaminase-deficient mice show hippocampal hypoactivity (Gaisler-Salomon et al., 2009), and glutamate-transporter-deficient mice have behavioral deficits resembling negative symptoms (Karlsson et al., 2009).

Phencyclidine (PCP) is as a non-competitive antagonist of NMDA glutamate receptor. It has also been reported to affect voltage-dependent sodium and potassium channels, the nicotinic acetylcholine receptor, and the sigma receptor (Morris et al., 2005). On the cellular and biochemical level, PCP was reported to selectively induce neurodegeneration in vivo (Wang and Johnson, 2005) and, when administered to cortical organotypic cultures, to cause cell apoptosis in vitro (Wang and Johnson, 2006). In humans, PCP abuse causes behavioral changes characteristic of schizophrenia, including the negative symptoms and the associated cognitive deficits (Javitt, 2007). Rodents treated with subchronic PCP are widely used as hypoglutamatergic models of schizophrenia (Morris et al., 2005; Nabeshima et al., 2006). Subchronic administration of PCP leads to an impairment of social behavior analogous to the specific endophenotype of the common negative symptoms of schizophrenia. Interestingly, NMDA antagonists have previously been reported to impair the Glial-neuronal interactions (Kondziella et al., 2005). This impairment in Glial-neuronal interaction is primarily associated with imbalance in the glutamate homeostasis. One study reported that under specific conditions, the administration of PCP to rats caused a change in the level of glutamate receptors (Fattorini et al., 2008), and another found that repeated PCP administration postnatally was associated with a long-term negative effect on antioxidant defense in the brain (Radonjic et al., 2010).

Prompted by recent findings that MSCs induce a neuroprotective effect by influencing host astrocytes in vivo (Sheikh et al., 2011) and in vitro (Xin et al., 2011), we sought to determine if the transplantation of MSCs into the hippocampus of PCP-treated mice protects against the PCP insult in terms of social behavior. We also examined the effect of MSCs in cultured mouse astrocytes exposed to PCP in vitro.

2. Materials and methods

2.1. In vivo study

2.1.1. Mouse model

A total of 42 male C57BL/6 mice aged 6 weeks (Harlan, Jerusalem, Israel) were used in this experiment. Mice were placed under a 12-h light/12-h dark condition and grown in individual ventilated cages with access to food and water ad libitum. All experimental protocols were approved by the Tel Aviv University Committee of Animal Use for Research and Education.

2.1.2. Experimental design

The mice were divided into 3 groups (n=14 each), as follows. Study group: PCP (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 10 mg/kg dissolved in 100 μl 0.9% normal saline was injected subcutaneously once daily for 14 days. On the first day of PCP administration, MSCs were transplanted into the hippocampus. Sham group: Mice were treated with PCP in the same manner as the study group and underwent the identical surgical procedure, but without implantation of MSCs. Control group: Mice were injected subcutaneously with saline only. To prevent an immune response, all mice received 15 mg/kg subcutaneous cyclosporine A (Novartis, Basel, Switzerland) for 3 days around transplantation. Thereafter, cyclosporine was added to the drinking water (15 mg/kg, according to the expected daily drinking volume per mouse). One day after the last PCP dose, mice underwent the open field behavioral test, and one week after the last PCP dose, they underwent the social preference test, as reported elsewhere (Brigman et al., 2009). Animals were sacrificed one day after completing the social preference test.

2.1.3. Cell culture and transplantation procedure

Human MSCs were purchased from Cambrex Bio Science, Walkersville, Inc. (Walkersville, MD, USA) and cultured and expanded as previously described (Sadan et al., 2009a). On treatment day, the cells were harvested, washed, and prepared for transplantation at a concentration of 40,000 cells/μl. Under chloral hydrate anesthesia,

the mice were placed in a digital stereotactic frame (Stoelting, Wood Dale, IL, USA), and cells (1 μL/injection site) were injected bilaterally to the hippocampus, adjacent to the dentate gyrus (the neurogenic region), at a rate of 1 μL/min (Hamilton 701N syringe) to the following coordinates, (relative to the bregma and dura): anterior–posterior, −2.2 mm; medial–lateral, ±1.8 mm; dorsal–ventral, −2.1 mm. The needle was withdrawn from each location after 5 min. Cell viability was assessed at the end of the transplantation procedure using Trypan blue (Sigma-Aldrich, St. Louis, MO, USA). For cell-tracking purposes, 4 animals in each group were injected with cells labeled with the red fluorescent marker, PKH-26 (Sigma-Aldrich, St. Louis, MO, USA).

2.1.4. Behavioral test procedure

For the open field test, mice were placed in a 50 cm^2 arena and videotaped for 60 min. The social preference test was performed as previously described (Nadler et al., 2004). The apparatus consisted of a plastic arena divided into one central and two lateral compartments. The lateral compartments each contained a single plastic cup, and the lateral walls of the central compartment had openings for the mice to pass through. Mice were habituated for 20 min/day for 2 consecutive days prior to the test day, when they were free to explore the middle chamber. On the test day, the test mouse was initially habituated to the arena for 10 min, during which time two black partitions completely covered the sides of the arena containing the cups. Thereafter, the Plexiglas partitions were removed and an unfamiliar male C57BL/6 mouse was placed in one cup (social stimulus). The other cup remained empty (inanimate stimulus).The mice were free to explore all chambers for 10 min, during which time they were videotaped. Video files were analyzed with Ethovision 7 software (Noldus, Wageningen, The Netherlands). Behavioral parameters analyzed in the social preference test were total time spent in each chamber, total nose pokes to the cups, and total time spent in proximity to the cups (representing social/non-social object exploration). To calculate the results, we used the preference index (S−NS)/(S+NS), where S is the time spent in the social chamber and NS is the time spent in the non-social chamber.

2.1.5. Immunohistochemistry

At the end of the experiment (23 days after transplantation), 4 animals from each group were anesthetized with chloral hydrate and transcardially perfused with cold phosphate-buffered saline (PBS), followed by paraformaldehyde 4% in phosphate buffer. The brains were immersed in 4% paraformaldehyde for 24 h at 4 °C followed by cryoprotection in 30% sucrose for an additional 48 h. The brains were frozen in chilled 2-methylbutane (Sigma-Aldrich), stored at −70 °C, and subsequently sectioned into slices measuring 10 μm. For microglial labeling, fluorescein isothiocyanate (FITC)-conjugated Bandeiraea simplicifolia isolectin B4 (IB-4; 1:50 in PBS; Sigma-Aldrich) was added for 1 h. Nuclei were counterstained with 4′,6-Diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO, USA). Sections were mounted with fluorescent mounting solution (Dako, Glostrup, Denmark), covered with a cover slide, and sealed. Digital images were obtained with a fluorescence Olympus BX52TF microscope.

2.1.6. Western blot

At the end of the experiment, based on proximity to the average behavioral data, 4 animals from each group were sacrificed using CO_2. Immediately thereafter, the brains were crudely dissected, and whole hippocampus tissues were separated and cryopreserved at −70 °C. The tissue was then thawed, and total protein was produced as previously described (Grunbaum-Novak et al., 2008). Protein concentration was determined using the bicinchoninic acid (BCA) kit (Thermo Scientific, Rockford, IL, USA). Protein samples were analyzed by Western blot, as described previously (Taler et al., 2008); 50 μg were loaded in each lane. The following antibodies were employed: anti-glutamine synthetase, anti-cleaved caspase-3, anti-emerin, anti-actin (Millipore). Band intensities were visualized and analyzed with the Odyssey system (LI-Cor, Lincoln, NE, USA).

2.1.7. Enzyme-linked immunosorbent assay (ELISA)

Quantification of BDNF levels was conducted using a BDNF specific ELISA kit (Millipore) according to the manufacturer's instructions. Protein extracts were loaded on the ELISA plate (in quadruple samples, 25 μg protein in each well). The absorbance at 450 and 570 nm was recorded on a Microplate Reader (Labsystems Multiscan MS, USA). Results were normalized to total amount of protein.

2.2. In vitro study

2.2.1. Co-culture of astrocytes and MSCs

Primary mouse astrocytes were isolated and cultured as previously described (Aprico et al., 2004). In brief, the cortex tissue was dissected from newborn mice (postnatal day 1–3), and the meninges were removed. Purified tissues were mechanically dissociated in cold PBS (Biological Industries, Beit Haemek, Israel) and digested by incubation with 1/5 V/V trypsin (Biological Industries) for 10 min at 37 °C. The reaction was stopped by the addition of complete medium [Dulbecco's Modified Eagle's Medium (DMEM); Biological Industries], supplemented with 10% fetal bovine serum (Biological Industries), 100 μg/ml streptomycin, 100 U/ml penicillin, 12.5 units/ml nystatin (SPN; Biological Industries), 2 mM L-glutamine (Biological Industries), and 50 μg/ml DNAse (Sigma). At this point, the tissue was mechanically dissociated once more to ensure full dissociation into single cells. The cells were washed once with complete medium and centrifuged at 1100 rpm for 7 min. The supernatant was removed, and the pellet was resuspended in complete medium. The cells were plated at a density of 10^4 cells/cm^2. Cell cultures were grown in complete medium and maintained at 37 °C and 5% CO_2. The cells were allowed to proliferate until confluence was achieved. Microglial cells were then eliminated by shaking at 250 rpm for 18 h on a horizontal orbital shaker, followed by removal of the microglia-containing medium. The cells were trypsinized and replated several hours later and, after 2 passages, were seeded onto 24 mm wells (3×10^5/well). After 48 h, 5×10^5 MSCs were introduced into the well using a 0.4 μm Polyester membrane transwell (Corning, NY, USA). PCP was added (10 μm) for 4 days starting on the first day of co-culture. Medium containing PCP was changed every other day.

2.2.2. RNA extraction and real-time polymerase chain reaction (PCR)

After 4 days of co-culture, transwells harboring MSCs (on the upper chambers) were removed. The astrocytes in the lower chambers were washed twice with PBS, and total RNA was extracted using a commercial TRI reagent (Sigma), as previously described (Barzilay et al., 2008). The expression levels of the following genes were analyzed: glial fibrillary acidic protein (*GFAP*), excitatory amino acid transporters 1 and 2 (*EAAT1*, *EAAT2*), nuclear factor erythroid 2-related factor (*Nrf2*), Parkinson disease (autosomal recessive, early onset) 7 (*DJ-1*/PARK7), and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Real-time semiquantitative PCR was performed in an ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA) using Platinum® SYBR® Green qPCR SuperMix UDG with ROX (Invitrogen, Grand Island, NY). PCR amplification was stopped at 40 cycles (program: 2 min at 50 °C; 2 min at 95 °C; 40 repeats of 15 s at 95 °C and 30 s at 60 °C). For each gene, the specificity of the PCR product was assessed by verifying a single peak on melting curve analysis. PCR analyses were conducted in triplicate for each sample. The reaction was performed in a total volume of 20 μL containing 1 μL of the previously described cDNA, the 3′ and 5′ primers at a final concentration of either 250 nM or 500 nM each, and 10 μL of Sybr Green Mix.

Quantitative calculations of the gene of interest were performed against *GAPDH* using the ΔΔCT method.

2.3. Data analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) 17.0 software (SPSS Inc., Chicago,IL). Comparisons between groups were performed using two-tailed analysis of variance (ANOVA) with Tukey post hoc test. The results were considered significant at $p<0.05$. All results are expressed as mean ± SEM.

3. Results

3.1. Behavioral effect of MSCs transplantation on PCP treated mice

On the behavioral test performed one day after the last PCP treatment, findings for total moving distance (locomotor activity) over 60 min for were similar in all 3 groups of mice (Figure 1A). Compared to the control group, the sham group spent significantly (33%) more time not moving ($p=0.01$). The study group also spent more time not moving than the controls (20.5%), but the difference did not reach statistical significance ($p=0.063$) (Figure 1B).

On the social preference test, performed one week after the last PCP treatment, there was no difference in total locomotor activity among the groups in the first 10 min, when the mice were limited to the central zone with no social/nonsocial stimulus (Figure 1C). Thereafter, when they were allowed to explore both the chamber with the social stimulus and the chamber with the nonsocial stimulus, the control mice and the study mice showed a similarly significant preference for the social stimulus whereas the sham mice did not. For all 3 parameters of total time spent in each chamber (Figure 1D), total number of nose pokes towards the stimuli (Figure 1E), and total time in close proximity to the stimulus (Figure 1F), the preference index of the sham mice was significantly lower than for both the control mice and the study mice ($p<0.01$, Figure 1D-F).

3.2. Survival of transplanted cells

Using fluorescent microscopy, we detected that the MSCs pre-labeled with PKH-26 in the hippocampus, near the site of transplantation (Figure 2A). There was some overlap of microglial staining (FITC-conjugated microglial marker B4) with transplanted cell staining, suggesting the presence of some immune response (Figure 2B and C). Higher magnification confirmed the presence of intact PKH-26-positive cells, indicating MSC survival throughout the experiment (Figure 2D-F). We did not find any PKH-26 labeled cells in other brain areas, such as the subventricular zone or the cortex.

3.3. Co-culture of astrocytes and MSCs: Effect on transcripts associated with glutamate processing and oxidative stress

Co-culture of astrocytes with MSCs under PCP incubation was associated with a significant upregulation (by 1.5-fold) of *GFAP* expression ($p<0.01$), which served as a marker of

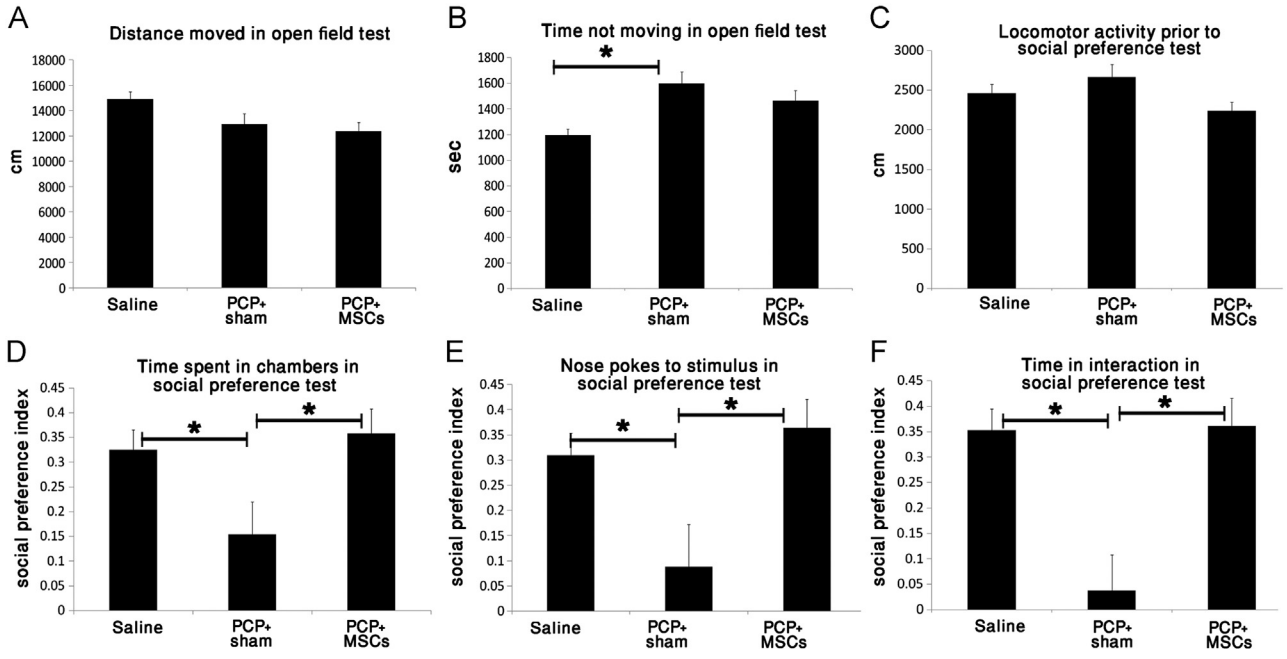

Figure 1 Behavioral tests: Open field test. (A) Total distance moved over 60 min in the open field arena. (B) Total time not moving in the open field arena. Social preference test. (C) Total distance moved in the 10 min prior to the exploration of the lateral compartments harboring the social and inanimate stimuli. (D-F) Preference index calculated in 10 min at which the test mouse was free to explore all chambers. (D) Preference index of time spent in chamber. (E) Preference index of frequency of nose pokes to the social stimulus. (F) Preference index in social exploration. Results are displayed as mean + SEM. Preference index was (S−NS)/(S+NS) when S is the time spent in the vicinity of the social stimulus and NS, the time in the vicinity of the non-social stimulus. In each experimental group, $n=14$ ($^{*}p<0.01$).

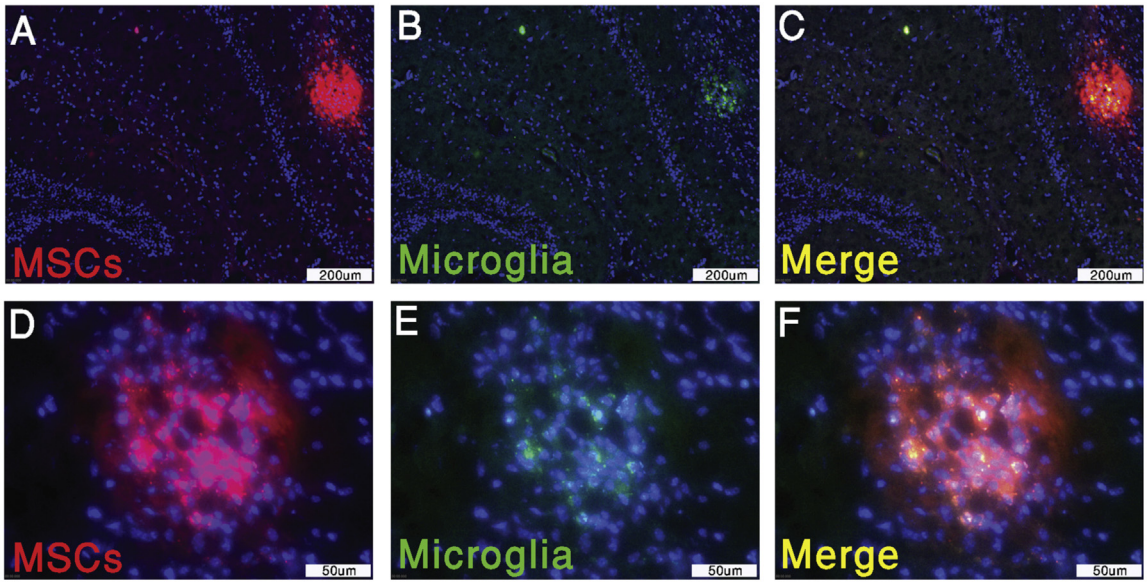

Figure 2 Immunohistochemistry of hippocampal brain slices. (A) Red cells are human MSCs pre-labeled with PKH-26. (B) Green signal indicates microglia stained with FITC-conjugated IB4. (C) Merged photo of PKH-26 labeled MSCs and microglia staining. (D–F) Higher magnification of transplanted MSCs in the hippocampus of mice.

astrocyte activation (Letournel-Boulland et al., 1994). PCP by itself did not induce any change in *GFAP* expression (Figure 3A).

On analysis by real-time PCR, PCP significantly reduced the expression levels of genes that contribute to glutamate transport/clearance (*EAAT1 and EAAT2*) and glutamate metabolism (glutamine synthetase) and significantly increased the transcription levels of *Nrf2* and *DJ-1/PARK7*, which control cellular mechanisms against oxidative stress. The addition of MSCs almost completely inhibited both these effects ($p<0.01$ for both, Figure 3B–D and Figure 3E and F).

3.4. Effect of MSC transplantation on proteins associated with glutamate processing and apoptosis in the brain of PCP treated mice

Western blot analysis was performed to determine if the effect of MSC co-culture with astrocytes in vitro under exposure to PCP is replicated in the brains of PCP-treated mice after MSC transplantation. Protein extracts from the hippocampi of the PCP-treated sham mice showed a decrease (by 60%) in glutamine synthetase levels (Figure 4A), which did not reach statistical significance ($p=0.087$). This reduction was partially inhibited in the PCP-treated mice after MSC transplantation. Cleaved caspase 3 levels were analyzed to determine the global effect of MSC transplantation on apoptosis following PCP insult in the hippocampus (Figure 4B). MSC transplantation was associated with reduced the levels of active caspase 3 compared with the control group and with the PCP-treated sham group ($p=0.127$).

3.5. Effect of MSC transplantation on hippocampal BDNF levels in PCP treated mice

Since we had previously found that MSCs transplantation to the cortex of PCP-treated mice resulted in increased cortical brain-derived neurotrophic factor (BDNF) levels (Barzilay et al., 2011), we measured total BDNF levels in protein extracts from the hippocampi of the mice using ELISA. We did not find significant difference between groups in the hippocampal levels of BDNF ($p=0.397$), as levels in saline treated mice were 32.18 ± 1.8, in sham operated PCP-treated mice 29.22 ± 1.59 and in MSCs transplanted PCP treated mice 32.66 ± 2.35 pg/ug protein [(mean $\pm$ SEM), df (2,9)].

4. Discussion

PCP was reported to protrude neuron-glia interaction through impairing key astrocyte functions such as glutamate clearance. Transgenic mice harboring mutants of genes involved in glutamate processing display behavioral and biochemichal endophenotypes associated with schizophrenia (Gaisler-Salomon et al., 2009; Karlsson et al., 2009). Therefore genes involved in the glutamate–glutamine cycle may serve as possible targets for intervention in rodent models of schizophrenia. To determine if the benefit of MSCs is a product of their influence on astrocyte function in glutamate processing, we used a co-culture system to evaluate the effect of MSCs on astrocytes exposed to PCP in vitro. We observed a significant transcriptional restoration of the genes related to glutamate processing that were affected by PCP, such as those encoding glutamate transporters and glutamine synthetase. These results suggest that, at least in vitro, MSCs counteract the interference of PCP with the function of astrocytes in maintaining glutamate homeostasis.

Therapy with MSCs has been widely applied in animal models of neurodegenerative diseases (Sadan et al., 2009b). Various mechanisms have been postulated to underlie their neurotrophic effect (Boucherie and Hermans, 2009; Horwitz and Dominici, 2008; Prockop, 2009a; Sadan et al., 2009b), including in situ enhancement of the host's capacity to attenuate oxidative stress (Lanza et al., 2009; Valle-Prieto

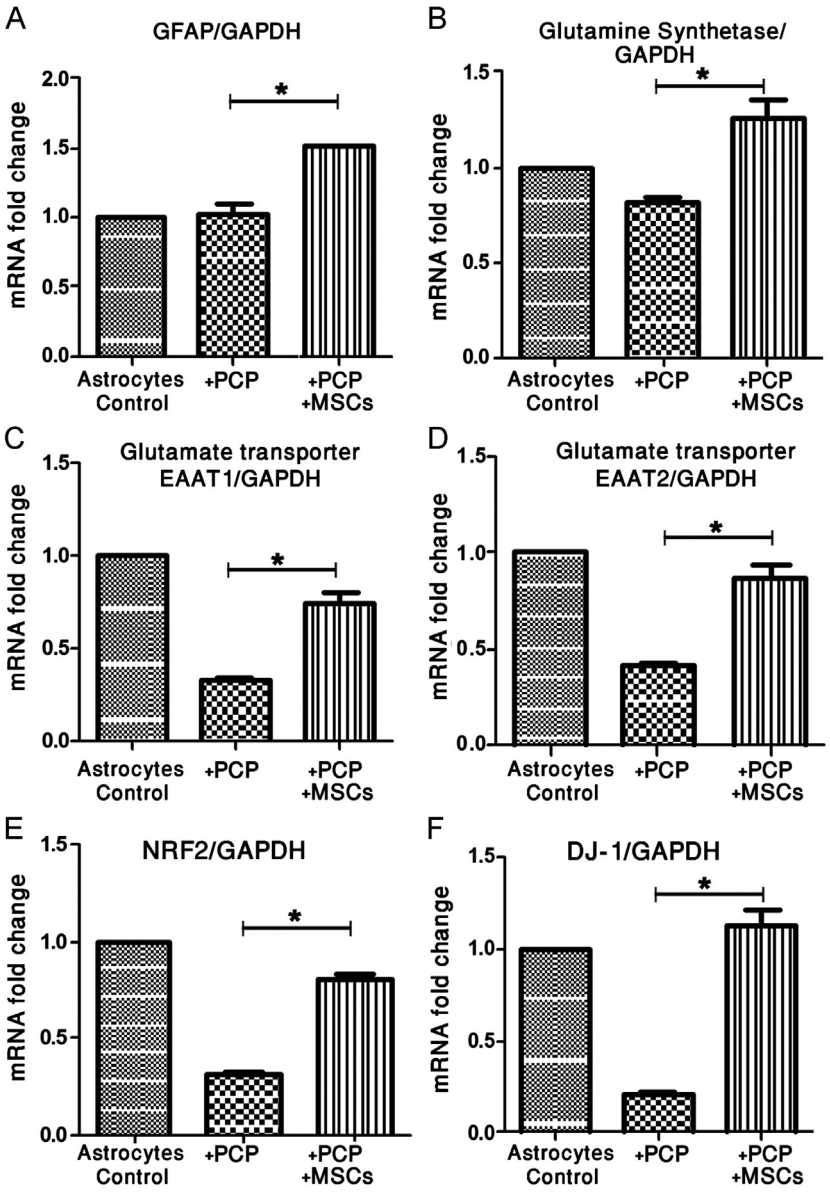

Figure 3 Quantitative real-time PCR analysis of transcripts associated with glial activation (GFAP), glutamate metabolism (glutamine synthetase), glutamate clearance (EAAT1 and EAAT2), and oxidative stress (NRF2 and DJ1) expression in astrocytes following exposure to PCP and co-culture with MSCs. For each gene, level of expression was compared to untreated astrocytes without PCP and without co-culture with MSCs. Expression was normalized to GAPDH; calculations were done using the $\Delta\Delta$ct method. Results are displayed as mean $\pm$ SEM ($^{*}p<0.01$).

and Conget, 2010; Yust-Katz et al., 2011). Schizophrenia is primarily a disease of neurodevelopmental origin (Lewis and Levitt, 2002), but accumulating data show that key processes, often associated with neurodegenerative diseases, such as oxidative stress and mitochondrial dysfunction, play a pivotal pathophysiologic role (Bitanihirwe and Woo, 2011; Csernansky, 2007; Fatemi and Folsom, 2009; Ng et al., 2008; Rezin et al., 2009).Interestingly, according to recent studies, impaired antioxidant capacity may be involved in the still-obscure neural mechanism responsible for first-episode psychosis and schizophrenia onset (Mico et al., 2011; Raffa et al., 2011). This is supported by laboratory findings showing that impaired antioxidant capacity may well be representative of an animal model for schizophrenia-like behaviors (Castagne et al., 2004; Kulak et al., 2012). Furthermore, PCP-induced neuronal degeneration in the rat cortex was reported to be attenuated by prior administration of antioxidants (Rajdev et al., 1998). In the present study, following PCP insult, MSC co-culture with astrocytes restored the expression levels of genes encoding two key transcription factors involved in sensing, triggering, and modulating the antioxidant response: *Nrf2* (de Vries et al., 2008) and *DJ-1/PARK7* (Gan et al., 2010). Therefore, we suggest that MSCs may be used as a tool to enhance the host response against oxidative stress. By restoring the normal antioxidant capacity of the host astrocytes, MSCs provide better support for neurons in pathological environments

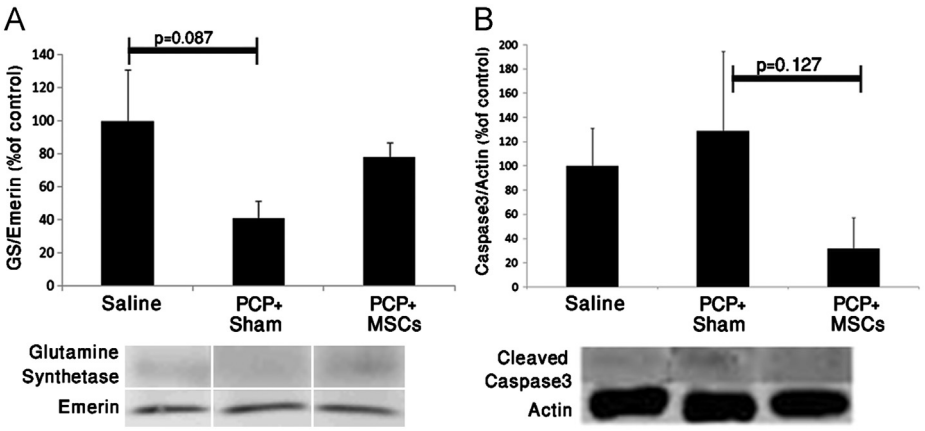

Figure 4 Western blot for (A) glutamine synthetase and (B) cleaved caspase 3 in the hippocampus of mice treated with PCP and transplanted with MSCs. The intensity of the bands was standardized to Emerin or to actin. The results are presented as a percentage of the levels of expression measured in the hippocampus of untreated mice. Results are displayed as mean ± SEM. The results represent a mean of 4 repeats.

In this study, we aimed to transplant the MSCs to the hippocampus, adjacent to the dentate gyrus, which is known to have high rates of neurogenesis and synaptic plasticity, processes that were previously reported to be enhanced by MSCs (Bao et al., 2011; Kan et al., 2010; Tfilin et al., 2010). An earlier study by our group showed that transplantation of MSCs into the cortex of mice subchronically treated with PCP increased cortical levels of BDNF and protected against the social deficits induced by the PCP insult (Barzilay et al., 2011). In the current study, we report a similar behavioral benefit of MSCs transplantation to the hippocampus, not accompanied by a parallel significant increase in hippocampal BDNF levels, suggesting that other molecular mechanisms may underlie the behavioral effect. In vitro, we demonstrated that MSCs had a positive effect on expression of genes involved in maintenance of appropriate astrocyte functioning following exposure to the deleterious effect of PCP.

Taken together, our data show for the first time that stem-cell-based regenerative strategies may be effective in animal models of PCP-induced schizophrenia. Specifically, we show that this strategy is useful in preventing the social deficit, which represents an endophenotype related to the debilitating negative symptoms of the disorder. Moreover, our finding that MSCs at least partially exert their trophic effect through positive modulation of astrocyte gene expression patterns suggests that MSCs intervene in astrocyte glutamate processing and antioxidant response. These neurotoxic processes (glutamate dysregulation and oxidative stress) are likely involved in the pathophysiology of PCP-induced schizophrenia-like behavior. Thus, MSC transplantation might be used in the future to counteract these processes, and MSCs might be considered a candidate for cell-based treatment of schizophrenia.

In conclusion, our study suggests that cellular transplantation of MSCs into the hippocmapus may restore crucial astrocytic functions that are involved in regulating the tone of glutamatergic neurotransmission and protect against apoptotic mechanisms and oxidative stress in a pharmacological mouse model relevant to schizophrenia.

Role of the funding source

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Contributors

RB designed the study, wrote the protocol, handled the animals, undertook the statistical analysis and wrote the manuscript. JG conducted in vitro studies involving gene expression analysis in glia cells and participated in manuscript preparation. OS handled the stem cell culture and transplantation. TBZ was responsible for tissue processing and immunohistochemical analysis and established the primary astrocyte culture for the in vitro study. ZB handled the animals including daily injections of phencyclidine undertook all behavioral studies. NH established astrocyte primary culture and mesenchymal stem cell cultures and conducted the co-culture experiment. MT conducted all western blots. IG, AW and DO all participated in the study design, writing of protocols and preparation of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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