Comparison of the properties of neural stem cells of the hippocampus in the tree shrew and rat *in vitro*

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Abstract. Neural stem cells (NSCs) are characterized by the ability of self-renewal and capacity to proliferate and produce new nervous tissue. NSCs are capable of differentiating to three lineages of neural cells, including neurons, oligodendrocytes and astrocytes. Furthermore, hippocampal NSCs transplantation can improve the neurological deficits associated with expression of cytokines. Therefore, to compare the properties of NSCs of tree shrews and rats *in vitro*, NSCs from tree shrews (tsNSCs) and rats f(rNSCs) were isolated. Nestin was used as a marker to identify the cultured NSCs. Neuronal nuclei protein and glial fibrillary acidic protein (GFAP) were utilized to demonstrate the differentiation of NSCs towards neurons and astrocytes, respectively, *in vitro*. Furthermore, the expression of neurotrophin 3 (NT3), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and transforming growth factor (TGF)β1 was also investigated in tsNSCs and rNSCs. The expression of all of the aforementioned proteins was detected using immunofluorescence methods. The results demonstrated that, after 5 days of culture, the average number of neurospheres in the cultured tsNSCs was significantly lower compared with rNSCs (P=0.0031). Additionally, compared with the rNSCs, tsNSCs exhibited an enhanced differentiation ability towards neurons. Furthermore, the expression of NT3 in the tsNSCs was higher compared with rNSCs (P<0.01), while the expression of BDNF was lower (P=0.045). However, no significant differences were observed in the expression level of GDNF and TGFβ1 between rNSCs and tsNSCs. Therefore, these results indicate that tsNSCs exhibit specific characteristics that are different from rNSCs, which provides novel information for the understanding of NSCs obtained from tree shrews. Overall, the results of the current study provide evidence to support the increased application of tree shrews as models for diseases of the central nervous system.

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Introduction

Neural stem cells (NSCs) are a type of stem cell that possess self-renewal, self-replication and multi-differentiation properties. Under certain conditions, NSCs may be induced to differentiate into neurons, astrocytes and oligodendrocytes (1-3). It has been demonstrated that NSCs have important roles in the replacement, recovery, and neurotrophy and immunoregulation (4). NSCs promote the recovery of animals with motion, sensory and cognitive dysfunction to a certain extent (5-8). Therefore, NSCs may have wide applications in clinical practice.

Several studies have demonstrated that NSCs are resident in various areas of the rat brain, including the hippocampus, cerebral hemisphere, hindbrain, spinal cord, cerebral ventricle area in the lateral ventricles, subventricle area and the cerebral cortex (9,10). For the source of cultured NSCs *in vitro*, previous reports have established methods for the separation and culture of NSCs derived from the hippocampus of embryos in rats, mice, crab-eating macaques and humans; the cultured NSCs were cultured successfully *in vitro* and the cultured NSCs were induced to differentiate into neurons and glial cells (11-14). However, regarding research on central nervous system (CNS) diseases, rats and mice are rodents, and there are substantial differences between rodent and primate models. Crab-eating macaques have certain disadvantages, including high cost, difficult to breed and the use of fewer animals is permitted. Furthermore, ethical issues are associated with the use of human embryos. Therefore, it is necessary to identify more suitable experimental animals as a source of NSCs in *in vitro* models.

Tree shrews exhibit various characteristics that are similar to humans, including their biological features, metabolism,

physiology, biochemistry and genome. Therefore, tree shrews are considered as a type of novel experimental animal model that may partially replace the primate models (15,16). Due to the developed brain of the tree shrew, it is primarily employed for studies concerning the nervous system and the preparation of models of nervous system diseases (17-19). The use of the tree shrew as an animal model has attracted increasing attention and researches have already obtained useful results (20-23). However, little information exists concerning the differences between the stem cell origins of tree shrew NSCs (tsNSCs) and rat NSCs (rNSCs) Determining whether tsNSCs have identical or different properties to rNSCs is crucial for the application of tsNSCs.

In the present study, the features of NSCs derived from rats and tree shrews were compared. Furthermore, the expression of certain growth factors was also compared, with the aim of increasing the understanding of the biological characteristics of tsNSCs and improving their application in research.

Materials and methods

Animals and ethical statement. Pregnant (E16) Sprague-Dawley rats (4 months old, n=3) and pregnant (E38) tree shrews (8 months old, n=3) of clean grade, weighing 170 g, were used in the present study. All animals were provided by the Animal Experimental Center of Kunming Medical University (Kunming, China). Animal care and all experimental protocols were approved by the guidelines of the Institutional Medical Experimental Animal Care Committee of Sichuan University, West China Hospital, (Chengdu, China). Guidelines for Laboratory Animal Care and Safety from the National Institutes of Health were also followed (24). The animals were bred in separate cages in a temperature (20±5˚C), CO_2 (0.03%) and humidity (40-60%)-controlled room with a 12 h light/dark cycle and free access to pellet chow and water.

Sample harvesting. Pregnant (E16) Sprague-Dawley rats and pregnant tree shrews (E38) were sacrificed after being anesthetized by intraperitoneal injection of 3.6% chloral hydrate (1 ml/100 g). Following rinsing in 75% ethanol for 3 min, the embryonic rat and tree shrews were removed under sterile conditions and kept in a culture dish containing Hank's balanced salt solution (Thermo Fisher Scientific, Inc., Waltham, MA, USA) on ice. The skulls were dissected and the brain hemisphere was removed, subsequently, the brain tissues were placed into pre-cooled PBS. Under an anatomic microscope, the meninges, olfactory bulb, cerebellum and brain stem were attentively removed and the hippocampal tissues were exposed and harvested. The samples were washed twice with pre-cooled PBS and placed into centrifuge tubes (25).

Preparation of single cell suspension. Hippocampal tissues were sheared into 1 mm^3 sized tissue blocks. Trypsin (0.25%; 1:250; EMD Millipore, Billerica, MA, USA) was used to digest the tissue block at room temperature for 20 min. The digested tissue solution was collected and placed in a 15-ml centrifuge tube and Dulbecco's modified Eagle's medium/F12 (DMEM/F12; 1:1; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal calf serum (Thermo Fisher Scientific, Inc.) was added to stop the digestion. Centrifugation was subsequently performed at 560 x g (4˚C) for 5 min. The supernatant was discarded and the cell suspension was harvested using DMEM/F12 culture media (1:1; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 2% B-27 (Gibco; Thermo Fisher Scientific, Inc.), 20 ng/ml basic fibroblast growth factor (bFGF; R&D Systems, Inc., Minneapolis, MN, USA), 20 ng/ml epidermal growth factor (EGF; R&D Systems, Inc.), 2 mmol/l glutamine (Gibco; Thermo Fisher Scientific, Inc.), 10,000 U/l penicillin and 10 mg/l streptomycin.

Cell inoculation. Cell density was determined in the cell suspension and the density was adjusted to $5x10^5$/ml. The cells were inoculated onto the culture plates or bottles and kept in an incubator containing 5% CO_2 at 37˚C. Half of the culture medium was replaced every other day.

NSC passage. At 7 days post-culture, the diameter of neurospheres was commonly ~100 μm and subculturing was performed. In detail, NSCs were digested using 0.25% trypsin (1:250, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at 37˚C for 10 min and DMEM/F12 (1:1; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing serum was used to stop the digestion. NSC suspension was collected into a 15 ml centrifuge tube. Subsequently, centrifugation at 560 x g (4˚C) was performed for 5 min. The supernatant was discarded. The cell suspension was resuspended in DMEM/F12 (1:1; Gibco; Thermo Fisher Scientific, Inc.) containing 2% B-27 (Gibco; Thermo Fisher Scientific, Inc.), 20 ng/ml bFGF (R&D Systems, Inc.), 20 ng/ml EGF (R&D Systems, Inc.), 2 mmol/l glutamine (Gibco; Thermo Fisher Scientific, Inc.), 10,000 U/l penicillin and 10 mg/l streptomycin. The cellular density was adjusted to $1.5\text{-}2.5x10^6$/ml and inoculated into a culture bottle (25 ml in volume), which was gently swayed for even distribution. The cells were incubated in an incubator at 37˚C.

Morphological observation. During the primary and secondary culture of cells derived from the hippocampus of rats and tree shrews, inverted phase contrast microscopy (Leica Microsystems GmbH, Wetzlar, Germany) was employed to observe and record the morphology and growth of NSCs. Prior to observation, cells were fixed with 4% paraformaldehyde for 20 min at room temperature.

Identification and differentiation of NSCs in vitro and the detection of neurotrophic factors by immunofluorescence. In order to confirm the cultured NSCs and detect the expression of neurotrophin 3 (NT3), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and transforming growth factor (TGF) β1, NSCs were cultured to the third generation, then immunofluorescence staining of nestin (a maker of NSCs) and neurotrophic factors (NT3, BDNF, GDNF and TGFβ1) was performed. For detecting the differentiation of NSCs, immunofluorescence staining of neuronal nuclei protein (NeuN; a neuronal marker) and glial fibrillary acidic protein (GFAP; an astrocyte marker) was performed to identify the characteristics of NSCs following serum induction (DMEM supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.)) for 48 h. The antibodies used were showed in Table I. Briefly, after discarding the culture medium of the third passage of NSCs, 0.25% trypsin

Table I. Primary antibody details.

Primary antibody	Company	Concentration	Catalog number
BDNF	Boster	1:50	MGC34632
NT-3	Abcam	1:50	ab65804
GDNF	ZhongShanJinQiao	1:100	EIA-1067
TGFβ1	Abcam	1:100	ab92486
GFAP	ZhongShanJinQiao	1:50	ZA-0117
NeuN	Abcam	1:100	ab177487
Nestin	Abcam	1:50	ab92391

All primary antibodies were rabbit antibodies. BDNF, brain-derived neurotrophic factor; NT3, neurotrophin 3; GDNF, glial cell-derived neurotrophic factor; TGF, transforming growth factor; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei protein.

(1:250, Sigma-Aldrich; Merck KGaA) was added. Inverted phase contrast microscopy (Leica Microsystems GmbH, Wetzlar, Germany) was employed to observe the morphology of the NSCs. The majority of the cells were round in shape with cytoplasmic retraction and were loosened and floating. An appropriate amount of culture medium containing 10% fetal bovine serum was added to stop the digestion. The cell suspension was gently blended for even distribution using pap dropper. Subsequently, the cell suspension was transferred to 6-well plates and dropped onto sterile cover slips. The 6-well plates containing $1.2x10^6$ per well NSCs were incubated in an incubator at 37˚C for 4 h. After 5 days of culture, tsNSCs and rNSCs were fixed with 4% paraformaldehyde for 30 min at room temperature, rinsed with 0.01 M PBS and incubated with 3% goat serum (Gibco; Thermo Fisher Scientific, Inc.) for 30 min at 37˚C to quench non-specific binding. Subsequently, cells were incubated overnight at 4˚C with primary antibodies (Table I). As for the control group, the primary antibody was substituted with 0.01 M PBS. The glass slides were washed with 0.01 M PBS three times, each for 2 min, which was followed by incubation with cyanine 3-labeled anti-rabbit secondary antibody (1:200; cat. no. 111-165-003; Jackson Laboratory, Ben Harbor, ME, USA) at 37˚C for 30 min. Sections were observed under a fluorescent microscope. DAPI was used to counterstain the nuclei and the images were acquired using a Leica AF6000 cell station (Leica Microsystems GmbH). Finally, the proportion of positive cells was quantified using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). Each detection involved the preparation of six plates (6-well plates) of cells and each well was put into one sterile cover slip. Three random fields were selected per slide and evaluated by three investigators blinded to the experimental information, and the mean proportion of positive cells for each detection was calculated.

Counting the number of neurospheres. To investigate the expansion rates, tsNSCs and rNSCs were seeded in 6-well plates ($1.2 x10^6$ per well) and cultured at 37˚C for up to 72 h. Neurospheres formed within 2-3 days *in vitro*. The aforementioned culture medium: DMEM/F12 supplemented with 2% B-27, 20 ng/ml bFGF, 20 ng/ml EGF;, 2 mmol/l glutamin, 10,000 U/l penicillin and 10 mg/l streptomycin, was changed every 2 days. Numbers of neurospheres of tsNSCs and rNSCs were counted after culturing for 72 h. The images were captured with a Leica AF6000 cell station (Leica Microsystems GmbH). The number of neurospheres was quantified by using Image-Pro Plus 6.0 software (Media Cybernetics, Inc.). Five fields of vision were randomly selected per well and evaluated by three blinded investigators, and the mean number of neurospheres per well was calculated.

Statistical analysis. SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Experimental data are presented as the mean + standard deviation and were analyzed by Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Growth of NSCs. Following the inoculation of tsNSCs and rNSCs, cells were well distributed under the microscope, cells commonly exhibited a round shape with a transparent cytoplasm. At 1 day after inoculation, the majority of cells were single celled and only a few exhibited a proliferative growth style. At this time, 2 or 4 cells connecting together was observed, and the cells were in a good growth state with good refraction and a transparent cytoplasm (Fig. 1). At day 3 of culture, the number of cell spheres increased and the size of cell spheres was uneven. Certain cells proliferated and formed embryonic spheres consisting of tens to several tens of cells. The cytoplasm of all the cell spheres was transparent. Occasionally, individual cells exhibited an adherent growth style with outgrowing processes (Fig. 1). At day 5 of culture, the volume of the cell spheres was enlarged in addition to the number of cell spheres. At this time, the majority of cells exhibited a suspended growth style, with regular morphology and strong refraction, without process outgrowth (Fig. 1).

Identification of NSCs. As revealed by immunofluorescence staining of nestin, primary cultured neurospheres from tree shrews and rats exhibited positive nestin staining. The cytoplasm of neurospheres exhibited a clear red color (Fig. 2), indicating strong positive nestin expression. DAPI staining stained the nuclei of NSCs blue (Fig. 2). Merged synthetic images demonstrated that nestin (red) and DAPI (blue) were co-localized in the cultured cells (Fig. 2). These results

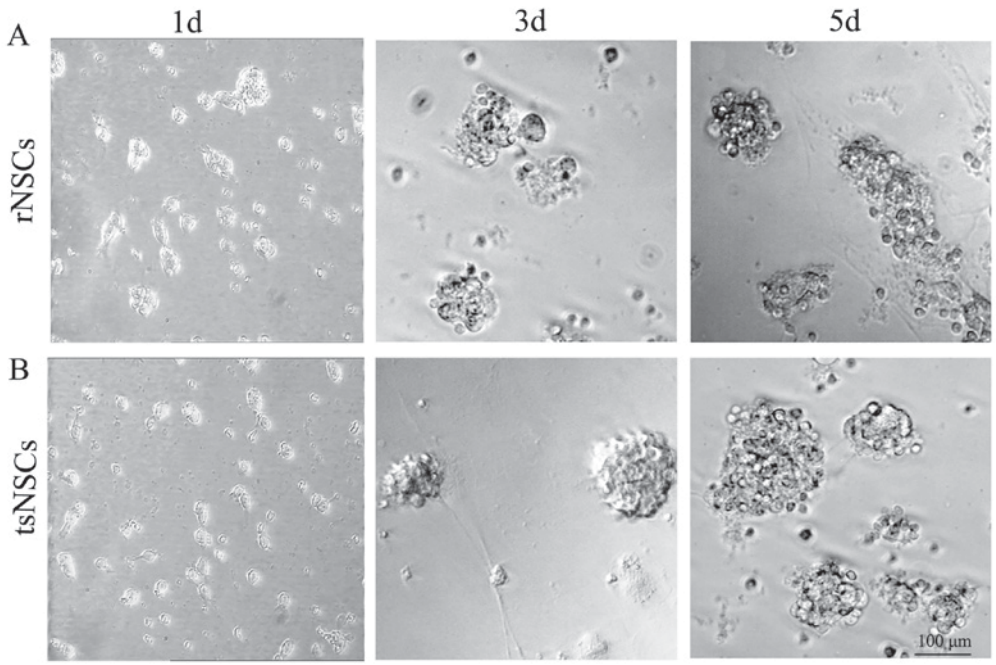

Figure 1. Morphology of NSCs *in vitro* following culture for 1, 3 and 5 days. (A) Primary cultures of (A) rNSCs and (B) tsNSCs at 1, 3 and 5 days of culture. Scale bar=100 μm, applies to all images. NSCs, neural stem cells; rNSCs, rat NSCs; tsNSCs, tree shrew NSCs; d, days.

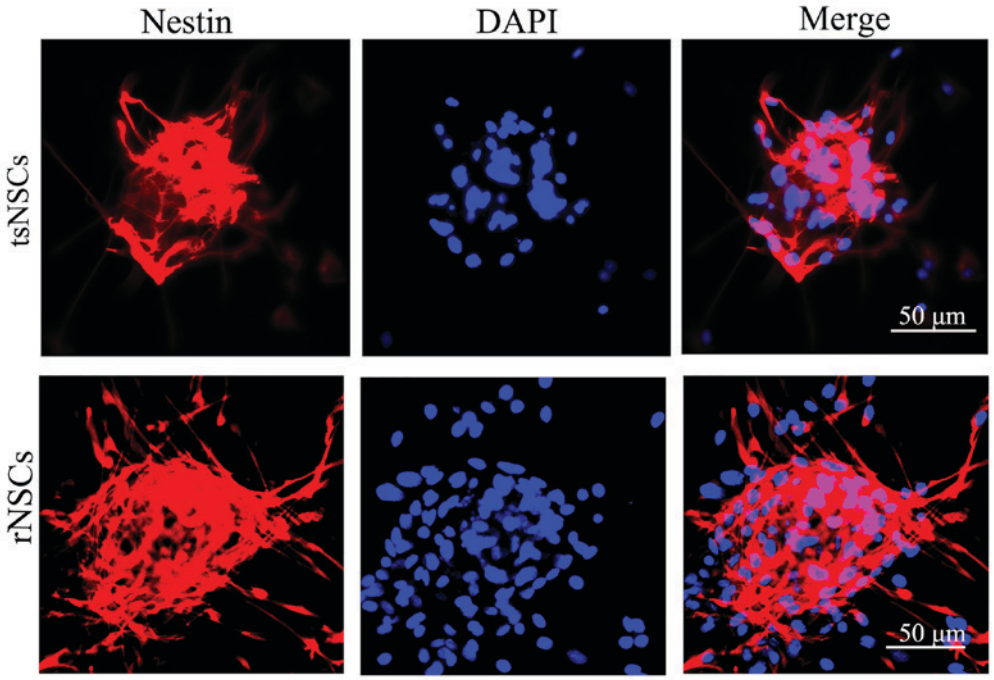

Figure 2. *In vitro* culture and identification of NSCs. Nestin staining (red fluorescence) was performed on tsNSCs and rNSCs on third passage cells. DAPI (blue fluorescence) was employed to stain nuclei. Scale bar=50 μm, applies to all images. NSCs, neural stem cells; tsNSCs, tree shrew NSCs; rNSCs, rat NSCs.

indicate that the separation and culture tsNSCs and rNSCs was successful in the present study, which allowed subsequent experiments to be performed.

Measurement of NSC proliferation. At 5 days following inoculation *in vitro*, tsNSCs and rNSCs were present as cell colonies with round shapes (Fig. 3A). In order to detect the proliferation ability of rNSCs and tsNSCs, quantitative analysis of the average number of neurospheres demonstrated that the number of neurospheres in the cultured tsNSCs was significantly decreased compared with rNSCs (P=0.0031; Fig. 3B).

Detection of differentiation ability in NSCs cultured in vitro. At 5 days after induction by serum, certain NSCs exhibited radiate protuberance, and adherent and suspension culture was observed (Fig. 4A). Following immunofluorescence staining,

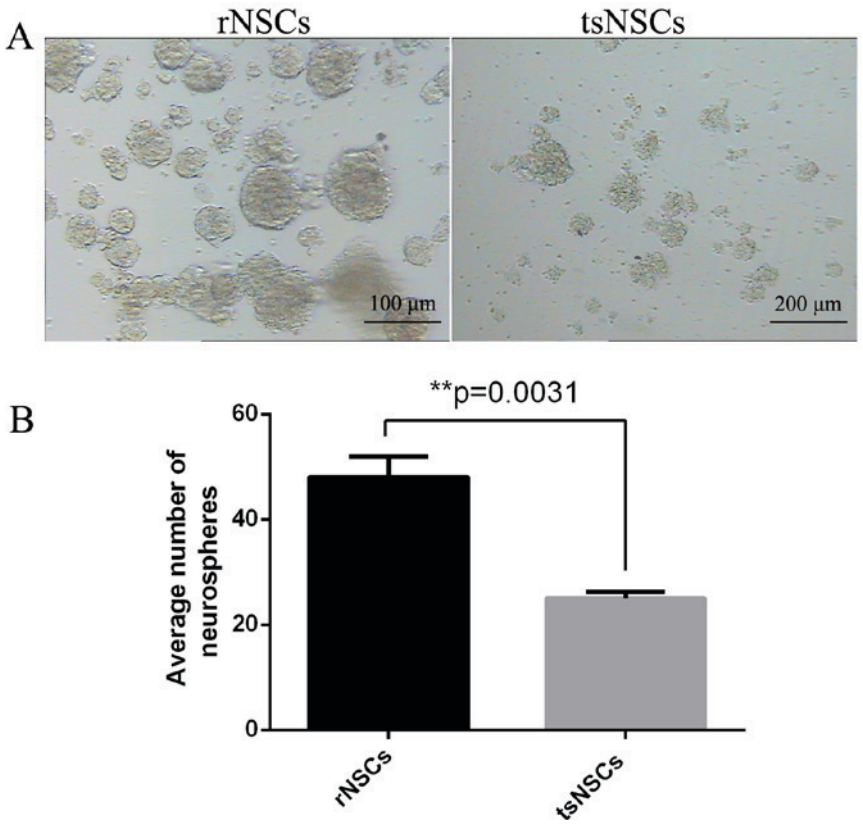

Figure 3. Comparison of neurosphere number in tsNSCs and rNSCs at 5 days following inoculation. (A) Representative images demonstrating the formation of neurospheres at 5 days following inoculation in the rNSC and tsNSC groups. (B) Quantitative analysis of the average number of neurospheres in the rNSC and tsNSC groups. Data are presented as the mean + standard deviation. **P<0.01, as indicated. NSCs, neural stem cells; tsNSCs, tree shew NSCs; rNSCs, rat NSCs.

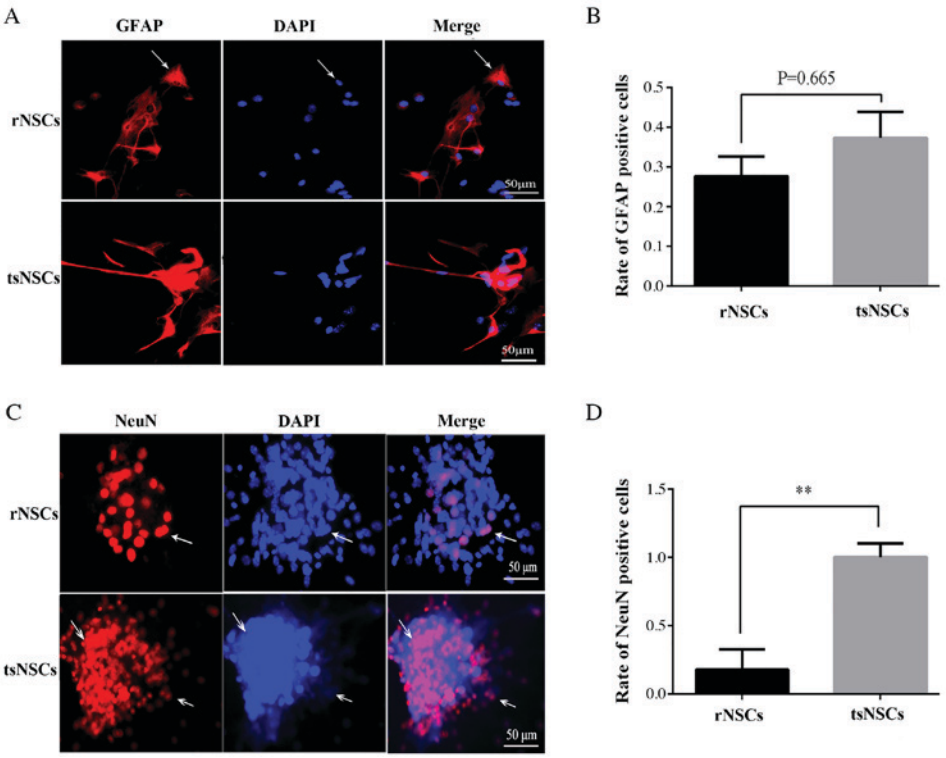

Figure 4. Comparison of the differentiation of tsNSCs and rNSCs into neurons and astrocytes. (A) Immunofluorescence staining of GFAP (red fluorescence) and nuclear staining with DAPI (blue fluorescence) in rNSCs and tsNSCs. Merged images are also presented. (B) Quantitative analysis of the proportion of GFAP positive cells. (C) Immunofluorescence staining of NeuN (red fluorescence) and nuclear staining with DAPI (blue fluorescence) in rNSCs and tsNSCs. Merged images are also presented. Examples of positive cells are indicated by white arrows. Scale bar, 50 μm. (D) Quantitative analysis of the proportion of GFAP positive cells. Data are presented as the mean + standard deviation. Scale bar=50 μm, applies to all images. **P<0.01, as indicated. NSCs, neural stem cells; tsNSCs, tree shrew NSCs; rNSCs, rat NSCs; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei protein.

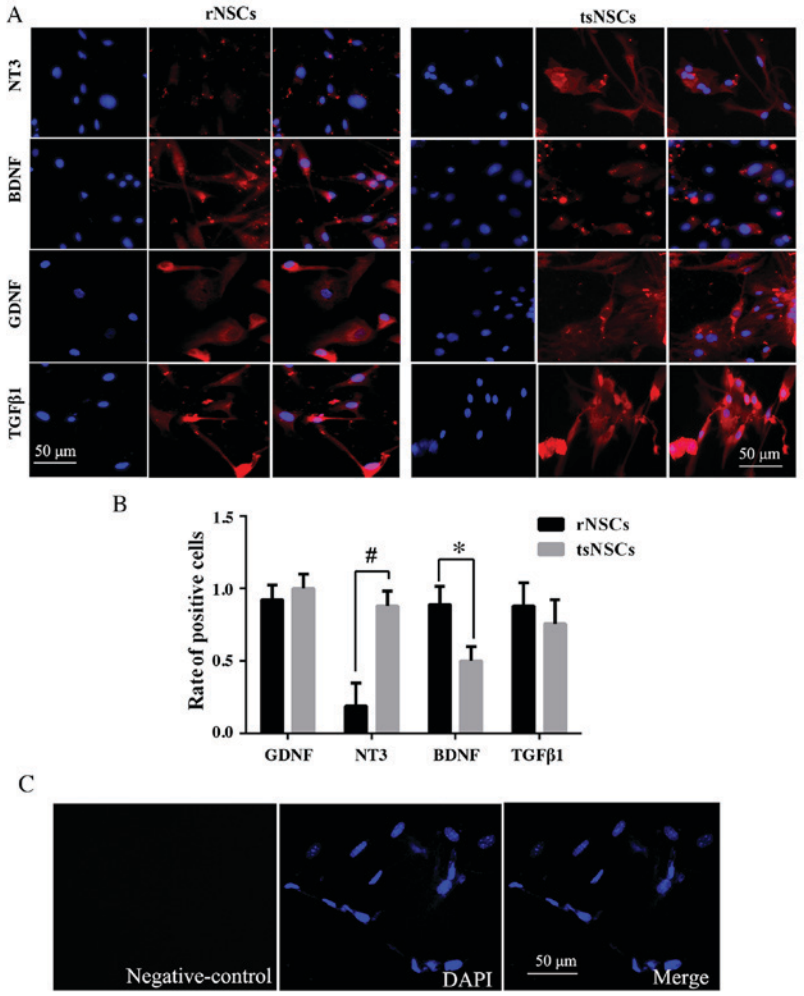

Figure 5. Expression of neurotrophic factors in tsNSCs and rNSCs. (A) Immunofluorescence staining of NT3, BDNF, GDNF and TGFβ1 in rNSCs and tsNSCs. Additionally, the left images are for DAPI staining, the middle images are for staining with NT3/BDNF/GDNF/TGFβ1 and the right images are for merged DAPI and NT3/BDNF/GDNF/TGFβ1 staining. (B) Quantitative analysis of the proportion of NT3, BDNF, GDNF and TGFβ1 positive rNSCs and tsNSCs. (C) Negative control for immunofluorescence staining, where PBS was employed instead of a primary antibody. Data are presented as the mean + standard deviation. Scale bar=50 μm, applies to all images. *P<0.05 and $^{\#}$P<0.01, as indicated. NSCs, neural stem cells; tsNSCs, tree shrew NSCs; rNSCs, rat NSCs; NT3, neurotrophin 3; BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor; TGF, transforming growth factor.

compared with rNSCs, tsNSCs exhibited stronger GFAP positive immunoreactivity, however, the proportion of GFAP positive tsNSCs was not significantly different compared with rNSCs (P=0.665; Fig. 4A and B). NeuN immunofluorescence staining demonstrated that tsNSCs exhibited stronger NeuN positive reactivity compared with rNSCs (Fig. 4C). In addition, quantitative analysis demonstrated that the proportion of NeuN tsNSCs was markedly higher compared with rNSCs (P=0.0002; Fig. 4D).

Positive expression of NT3, BDNF, GDNF and TGFβ1 in tsNSCs and rNSCs. Immunofluorescence staining was also performed to detect the expression of NT3, BDNF, GDNF and TGFβ1 in rNSCs and tsNSCs. Compared with rNSCs, tsNSCs expressed stronger NT3 positive immunoreactivity, and the proportion of NT3 positive tsNSCs was markedly higher compared with rNSCs (P<0.01; Fig. 5A and B). In addition, the BDNF positive immunoreactivity in rNSCs was stronger compared with tsNSCs, and quantitative analysis showed that the proportion of BDNF positive tsNSCs was markedly lower compared with rNSCs (P=0.045; Fig. 5A and B). Compared with rNSCs, GDNF positive immunoreactivity in tsNSCs was higher. However, quantitative analysis of the proportion of GDNF positive cells indicated no significant difference between the two groups (P=0.173; Fig. 5A and B). Furthermore, according to immunofluorescence staining analysis, the proportion of TGFβ1 tsNSCs was not significantly different compared with rNSCs (P=0.26; Fig. 5A and B). The negative control exhibited no positive immunoreactivity (Fig. 5C).

Discussion

Two primary conclusions were drawn based on the results of the present study. The first is that the ability of tsNSCs to differentiate into neurons was stronger compared with rNSCs. In addition, the level of NT3 expression in tsNSCs was significantly higher compared with rNSCs, and the level of BDNF expression was lower in tsNSCs.

In the current study, as revealed by *in vitro* culture, the proliferation of tsNSCs was substantially slower compared

with rNSCs. Concerning NSC culture, Pan *et al* (26) reported that NSCs from embryonic rats exhibited a higher number of NSCs and a markedly stronger proliferative ability compared with those from neonatal rats, therefore, they were more suitable for clinical therapy for nerve regeneration and recovery. Tian (27) demonstrated that goat embryonic stem cell (ESC)-like cells were similar to mice ESCs in colony morphology, however differences existed in the *in vitro* mode of passage. Additionally, these two cell types formed cell colonies only in the form of cell aggregates and the cloning efficiency may be substantially lower. In the present study, embryonic rats and tree shrews were employed as animal models to examine the proliferation ability of NSCs. A nestin antibody was used to demonstrate that the *in vitro* culture models of tsNSCs and rNSCs were successfully established. Comparison of the morphology of these two types of NSCs revealed that both formed clonal cell clumps that exhibited a suspended growth style with similar morphology, as they exhibited a round shape with strong refraction and clear boundaries. Both cell types grew into neurospheres, however, compared with rNSCs, tsNSCs grew and proliferated relatively slower. These results indicated that from rodents to primates, the proliferation ability of NSCs reduces.

Using immunofluorescence staining, the present study demonstrated that tsNSCs and rNSCs, induced by serum, differentiated into neurons and astrocytes. This indicates that both types of NSCs exhibit multi-differentiation properties. However, tsNSCs exhibited a stronger ability to differentiate into neurons compared with rNSCs. Previously, certain studies reported that low concentrations of serum promoted the differentiation of NSCs derived from neonatal rats into neurons, while high concentrations facilitated the differentiation of NSCs into neural glial cells, such as GFAP positive cells (28,29). Additionally, the presence of neurotrophic factors in culture medium was reported to be important in the differentiation of bone marrow mesenchymal stem cells into neural stem cell-like cells (28-31). Furthermore, one study demonstrated that hypoxia promoted the differentiation of NSCs into neurons by activating the Notch signaling pathway (30). The results of the above studies indicate that rNSCs may be induced to differentiate into neurons and astrocytes under certain conditions. In the present study, the results demonstrated that the ability of tsNSCs to differentiate into glial cells was similar to rNSCs, however, tsNSCs exhibited a stronger ability to differentiate into neurons. To the best of our knowledge, there are few previous reports that have investigated the differences in the differentiation potentials between tsNSCs and rNSCs.

Further immunofluorescence experiments demonstrated that tsNSCs expressed a lower level of BDNF and a higher level of NT3 compared with rNSCs, while no significant differences were observed for GDNF and TGFβ1 expression between the two groups. BDNF is widely distributed in various brain areas, including the hippocampus, thalamus, amygdala and cortical layer, and has essential roles in the survival, growth and development of neurons (32,33). Researchers demonstrated that the expression level of BDNF in adult tree shrews was substantially higher compared with the expression in fetal and neonatal tree shrews (33,34). However, few studies have compared BDNF levels between rat and tree shrews. Based on the established functions of BDNF (32,33) and the stronger proliferation ability of rNSCs in the current study, we hypothesize that this increased proliferation ability may be associated with the higher expression of BDNF in rNSCs compared with tsNSCs. Notably, the NT3 expression level was higher in tsNSCs compared with rNSCs in the current study. It has been demonstrated that NT3 is associated with the differentiation of neurons in the CNS and peripheral nervous system, and the higher intelligence level of tree shrews compared with rats (34-36). The results of the present study indicate that the higher expression of NT3 in tsNSCs may be associated with the increased ability of tsNSCs to differentiate into neurons compared with rNSCs. Therefore, higher NT3 and lower BDNF expression in tsNSCs may contribute to the increased ability to differentiate into neurons and weaker proliferation ability in tsNSCs compared with rNSCs, which was observed in the present study.

Concerning the application of tree shrews in research, researchers have reported that tree shrews were useful and easier to work with compared with rats in a hepatitis B virus injection study (37). Furthermore, tree shrews were considered to be a good animal for depression studies, and they may be widely applied for the pathological and physiological investigation of depression (38,39). Therefore, tree shrews may be a suitable alternative for rodents and may partially replace experiments on monkeys in the preparation of animal brain disease models.

In conclusion, the results of the current study demonstrated that, compared with rNSCs, tsNSCs exhibited a weaker proliferative ability, however, their ability to differentiate into neurons was much stronger. These results provide valuable evidence for the increased use of tree shrews as models for CNS diseases in humans.

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Competing interests

The authors declare that they have no competing interests.

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