SUPPLEMENTAL DATA

Justification of anesthesia in the experiment:

The experiment was designed based on many previous publications in journals and textbooks. All the procedures and justifications were reviewed and approved by the institutional animal care committee and the institutional veterinarian, as required by the regulations. Justifications are described in the following:

1. The anesthesia method is the method used by the research group from Washington University in St Louis (see **Reference # 23** of our present manuscript**: Body and brain temperature coupling: the critical role of cerebral blood flow.** ***J Comp Physiol B.*** **2009;179:701-710**). In this Reference paper, authors compared 3 commonly used anesthesia methods (including chloral hydrate, isoflurane, and chloralose) for their influences on brain and body temperature as well as cerebral blood flow. Their results demonstrated that both chloral hydrate (CH) and isoflurane (IF) have less influence of brain temperature and cerebral blood flow, compared with alfa-chloralose. In addition, no significant differences were seen between CH and IF.
2. Another recent study observed that CH did not significantly alter heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular pressure (+dp/dt), cell shorting amplitude and survival rate, compared to the other anesthetic agents. (**A comparison of the effects of ketamine, CH and pentobarbital sodium anesthesia on isolated rat hearts and cardiomyocytes.** ***J Cardiovasc Med (Hagerstown).*** **2011 Oct;12(10):732-735**.)
3. The anesthesia methods used in this study are based on the methods described in Reference 23; Ketamine/xylazine is the most commonly used injectable anesthetic cocktail. For rat, its duration of effect is about 30 min. From the beginning of preparation till the end of MCAO occlusion, it takes about 15-20 min. After occlusion, animals need to be sedated for another 90 min and then for reperfusion; therefore, CH was co-administered to extend the duration of the anesthesia effect. Isoflurane inhalation can be performed by a dedicated gas anesthesia instrument (Matrx®, Orchard Park, New York) which can serve for 3 animals simultaneously. However, during the whole procedure, gas anesthesia in not practical since the diseased rat model cannot tolerate for 90 min continuous anesthesia inhalation, according to our and others experiences.

4. During the stem cell transplantation procedure, the animal should be placed in a stereotactic instrument for about 40 min, and the mouth of the animal needs to be hooked by a head holder, which made it impossible to use gas anesthesia. Therefore, ketamine/xylazine is used for induction of anesthesia, and chloral hydrate was used to extend the duration of anesthesia effect.
5. Although CH used in rodents has been reduced in the States in recent years due to many reasons, it has been used as an anesthetic in many species – according to the textbook of anesthesia/analgesia by the American College of Laboratory Animal Medicine (***Anesthesia and Analgesia in Laboratory Animals,* 2nd Edition, 2008, pp 266**), CH is still used with adequate justifications in some institutions in the US. Many recent publications, especially on rat brain studies, applied CH as a sole anesthesia agent. i.e., ***PNAS.* 2011; 108(12) 5087-5092; *J Clin Invest.* 2008; 118(7): 2482-2495; *J Comp Physiol B.* 2009; 179(6): 701-710; *Neuropharmacology.* 2008;55(7):1191-1198; *Am J Nephrol.* 2010;32(4):347-55**. In addition, CH is accepted by the in vivo imaging community and is listed as an injectable anesthetic agent in an online reference: http://www.invivoimagingcommunity.com/lab_animal_anesthesia.

In addition, before, during and after procedures, the animals were observed and pain control was performed in the first 24 hours. All the animals in this study remained alert, with normal appetite and demeanor after recovery from all the experimental procedures. Before and after the MCAO procedure, body weights between control and treatment groups were compared. No statistically significant difference was found in animals in this study. All the procedures and justifications were reviewed and approved by the institutional animal care committee and the institutional veterinarian, as required by the regulations.

Induction of Middle Cerebral Artery Occlusion

Animal preparation and induction of anesthesia for the MCAO procedure and stem cell transplantation followed the institutional guidelines and the advice of veterinary staff. Briefly, after initial induction of anesthesia with a ketamine–xylazine mixture (60 mg/kg ketamine plus 10 mg/kg xylazine, i.p.) (*23*), the rat was shaved, transported to the surgery table in a dedicated surgery room, and fixed on the platform with a warm pad (Model 69001, RWD Life Science) to maintain the body

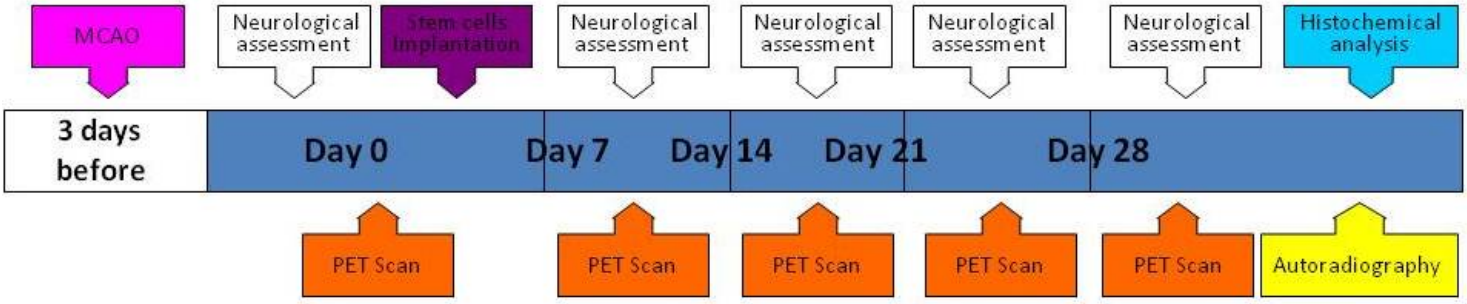
temperature throughout the procedure. Aseptic technique principle was strictly followed, and sterilized instruments were used in the survival operative procedures. Once the rat head was secure, the skin was cleaned three times using Iodophor followed by 70% alcohol. The animal received an intra-peritoneal (IP) injection of 10% chloral hydrate (350 mg/kg) (*23*). Focal brain ischemia was induced by the intraluminal suture technique. The midline skin incision was made in the neck with subsequent exploration of the left common carotid artery, the external carotid artery, and the internal carotid artery. MCAO was achieved by advancing a 3-0 monofilament nylon suture with a rounded tip to the left internal carotid artery via the external carotid artery until slight resistance was felt. After 90 min of MCAO, reperfusion was performed by withdrawal of the suture until the tip cleared the internal carotid artery, and the wound was closed. The animals were allowed to recover until awake and were returned to their cages. Rats were treated for pain for the first 24 hours after operation, by receiving an injection of buprenorphine (0.05 mg/kg, SC) every 8-12 hours (*24*). All rats were observed during the next postoperative days, and pain medication was given as necessary. The surgical wound was checked every day for signs of bleeding and draining. When wound care was required, the rat was anesthetized in an anesthesia induction chamber (2% isoflurane and O_2 mixture; Matrx®, Orchard Park, New York), the wound was cleaned and a new layer of antibiotic ointment was applied. The rats were housed three per cage in environmentally enriched conditions under a 12-h light/dark cycle throughout the experiments and were allowed free access to food and water.

Immunohistochemical staining

After the last microPET scan, 18 animals (6 per group) were kept anesthetized with 4% isoflurane and injected with 10% chloral hydrate (350 mg/kg), then transported immediately to a dedicated experimental table in the same room, transcardially perfused with saline followed by 200 mL of 4% paraformaldehyde in phosphate buffer. By the end of transcardial perfusion, death of animal could be confirmed. The brains were dissected and post-fixed in paraformaldehyde for 6-8 h and then dehydrated with 30% sucrose in 0.1 M PBS for 2 days. The brains were frozen on powdered dry ice, and then coronal cryostat sections (20 μm) were processed with a cryostat (Leica) at 20°C throughout the entire ischemic damaged brain. For immunohistochemical staining, the sections were permeabilized with 0.04% Triton X-100 and blocked with 10% normal goat serum and 0.5% bovine serum albumin in PBS for 1 h and then treated overnight at 4°C with primary antibodies:

rabbit polyclonal antibody against glial fibrillary acidic protein (GFAP) (1:300 dilution; Abcam); mouse monoclonal antibody against neuronal nuclei (NeuN) (1:200 dilution; clone: A60, Millipore); or rabbit polyclonal antibody against von Willebrand factor (vWF) (1:200 dilution; DAKO). The sections were washed three times in PBS for 10 min each and incubated in biotinylated secondary antiserum (Vectastain ABC kit, Vector Lab) for 30 min at room temperature. The sections were rinsed and incubated in ABC reagents (Vectastain ABC kit, Vector Lab) for 30 min and then washed thoroughly and incubated in 0.05% diaminobenzidine (DAB) and 0.03% H_2O_2 for 3–5 min until a brown reaction product was observed.

Immunohistochemical studies were performed to determine whether transplanted stem cells can induce neurogenesis and angiogenesis. NeuN was used as a mature neuronal marker, GFAP as the mature astrocyte marker, and vWF as the endothelial cell marker. We counted the number of cells positively stained with NeuN or vWF and calculated the mean values and SDs. The number of positively stained cells with NeuN or vWF in three different microscopic fields (X200 magnification) was calculated. To evaluate the average integral optical density (IOD) of GFAP, the hot spots of the section were selected with use of a microscope (X100 magnification). Individual counts were then made in X200 fields (BX60, Olympus). Sections (three microscopic fields per slice, one slice/animal) were digitized with the use of a camera. The IOD of GFAP was obtained by using Image-ProPlus 5.0 software (Media Cybernetics).



Supplemental Figure 1. Schematic of experimental workflow.

Supplemental TABLE 1. Statistical analysis

	Variables	Analysis method	P value
Figure 1	mNSS (pre-transplantation: treated vs control)	ANOVA	0.779
	mNSS (post-transplantation: treated vs control)	ANOVA	<0.05
Figure 2B	L/N ratio (treated vs control)	ANOVA	<0.001
	L/N ratio (ESCs vs iPSCs)	ANOVA	<0.05
	L/N ratio (ESCs vs iPSCs, 3w)	ANOVA	0.97
Figure 4	NeuN (iPSCs vs control)	ANOVA	<0.05
	NeuN (iPSCs vs ESCs)	ANOVA	0.195
	vWF (iPSCs vs control)	ANOVA	0.022
	vWF (ESCs vs control)	ANOVA	0.046
	vWF (iPSCs vs ESCs)	ANOVA	0.67
	GFAP (iPSCs vs ESCs)	ANOVA	0.001
	GFAP (iPSCs vs control)	ANOVA	0.001
	GFAP (ESCs vs control)	ANOVA	0.023
Data not presented in this manuscript	Body weight (Treatment vs control)	t-test	0.085