

Journal of Visualized Experiments

A Middle Cerebral Artery Occlusion Technique for Inducing Post-Stroke Depression in Rats --Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58875R5
Full Title:	A Middle Cerebral Artery Occlusion Technique for Inducing Post-Stroke Depression in Rats
Keywords:	Ischemic Stroke, Post-Stroke Depression, Rat Model, Middle Cerebral Artery Occlusion, Infarct Volume, Brain Edema, Porsolt's Forced-Swim Test, Sucrose Preference Test.
Corresponding Author:	Honore Nkafor Shiyntum Oles' Honchar Dnipro National University Dnipro, UKRAINE
Corresponding Author's Institution:	Oles' Honchar Dnipro National University
Corresponding Author E-Mail:	hnkafor@yahoo.com
Order of Authors:	Ruslan Kuts Israel Melamed Honore Nkafor Shiyntum Dmitri Frank Julia Grinshpun Alexander Zlotnik Evgeni Brotfain Michael Dubilet Dmitry Natanel Matthew Boyko
Additional Information:	
Question	Response
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US\$2,400)
Please indicate the city, state/province, and country where this article will be filmed. Please do not use abbreviations.	Soroka, Negev, Beersheba, Israel

Ben-Gurion University of the Negev Beersheba, Israel.
P.O. Box 151, Beer-Sheva, 98105, Israel.
Tel: +972-8-6479881,
Fax: +972-8-6403795
E-mail: matthewboykoresearch@gmail.com
July 31, 2018

Editorial Board,
JoVE

Dear Aaron Berard,

Please find attached the protocol "*A Modified Middle Cerebral Artery Occlusion Technique for Inducing Stress-free PSD in Rodents*," that we wish to have published with JoVE. This study narrates the reproduction of a modified middle cerebral artery occlusion technique that eradicates unwanted side injuries upon inducing the intended depressive mood (PSD).

As explained in the manuscript, this method could as well help in deciphering the mechanism of action of the factors involved in triggering depression and in assessing future PSD therapies. Results obtained show that, despite mitigating side injuries, this modified technique, like the original technique, was able to properly induce PSD, as seen in comparison with Sham-operated rats. It is therefore, our wish that this finding be made available to the public.

All authors listed contributed immensely to the day to day experimental procedure, discussion and evaluation, and write up of the manual. We have continually been in communication with Aaron Berard as we sought to come up with a protocol that is comprehensible to all.

We hope that you will find our protocol worthy of publication with JoVE.

Yours Faithfully,
Matthew Boyko

TITLE:

A Middle Cerebral Artery Occlusion Technique for Inducing Poststroke Depression in Rats

AUTHORS & AFFILIATIONS:

Ruslan Kuts^{1,*}, Israel Melamed^{2,*}, Honore N. Shiyntum³, Dmitri Frank¹, Julia Grinshpun¹, Alexander Zlotnik¹, Evgeni Brotfain¹, Michael Dubilet¹, Dmitry Natanel¹, Matthew Boyko¹

¹Division of Anesthesiology and Critical Care, Soroka Medical Center, Ben-Gurion University of the Negev, Beersheba, Israel

²Department of Neurosurgery, Soroka Medical Center, Ben-Gurion University of the Negev, Beersheba, Israel

³Department of Biophysics and Biochemistry, Oles Honchar Dnipro National University, Dnipropetrovsk, Ukraine

*These authors contributed equally.

Corresponding author:

Matthew Boyko (matthewboykoresearch@gmail.com)

Tel: + 97286479881

Email addresses of co-authors:

Ruslan Kuts (ruslanKo@clalit.org.il)

Israel Melamed (IsraelMe@clalit.org.il)

Honore N. Shiyntum (hnkafor@yahoo.com)

Dmitri Frank (berkut84@mail.ru)

Julia Grinshpun (juliag7648@gmail.com)

Alexander Zlotnik (AleksZl@clalit.org.il)

Evgeni Brotfain (bem1975@gmail.com)

Michael Dubilet (Michaeldu@clalit.co.il)

Dmitry Natanel (dmitry.natanel@gmail.com)

Matthew Boyko (matewboyko@gmail.com)

KEYWORDS:

Ischemic stroke, poststroke depression, rat model, middle cerebral artery occlusion, infarct volume, brain edema, Porsolt forced swim test, sucrose preference test

SUMMARY:

Here we present a protocol to induce poststroke depression in rats by occluding the middle cerebral artery via the internal carotid artery. We use the Porsolt forced swim test and the sucrose preference test to confirm and evaluate induced depressive moods.

ABSTRACT:

Poststroke depression (PSD) is the most recurrent of all psychiatric complications resulting from an ischemic stroke. A greater majority (about 60%) of all ischemic stroke patients suffer from

PSD, a disorder considered to be an ischemic stroke-related precursor for increased death and degradation in health. The pathophysiology of PSD is still obscure. To study the mechanism of development and occurrence of PSD further, and to find out a therapy, we attempted to develop a new protocol that requires occluding the middle cerebral artery (MCA) via the internal carotid artery (ICA) in rats. This protocol describes a model of PSD induced in rats through the middle cerebral artery occlusion (MCAO). Also used in the experiment are the Porsolt forced swim test and the sucrose preference test to confirm and evaluate the depressive mood of the rats under investigation. Rather than inserting the catheter through the external carotid artery (ECA), as stipulated for the original procedure, this MCAO technique has the monofilament passing directly through the ICA. This MCAO technique was developed a few years ago and leads to a reduction in mortality and variability. It is generally accepted that the criteria used are preferred in the selection of biological models. The data obtained with this protocol show that this model of MCAO could be a way of inducing PSD in rats and could potentially lead to the understanding of the pathophysiology and the future development of new drugs and other neuroprotective agents.

INTRODUCTION:

Stroke is fourth on the list of death-perpetrating diseases in the United States^{1–3}, while it causes the majority of disabilities in adults in developed countries⁴; this makes stroke a leading contender among the world's most significant health issues. Normalcy in stroke-surviving patients is rare, with about 15%–40% of survival victims suffering permanent disability, 20% requiring institutional care 3 months after stroke onset⁵, and about a third of 6-month survivals needing others to help them live through each day⁶. Stroke reportedly also accounts for the rising national health expenditures⁷. Estimates from the American Heart Association has stroke-related costs in the United States at over \$50 billion in 2010⁸.

Not only does stroke cause individuals' long-term damages, but some survivors tend to suffer emotional and behavioral disorders, such as dementia, fatigue, anxiety, depression, delirium, and aggression^{9–14}. The most recurrent psychological sequel after a stroke is poststroke depression (PSD), diagnosed in about 40%–50% of survivals^{15–17}. Stroke-induced depression results in increased morbidity and mortality^{18–22}. The pathophysiology of PSD is not known completely, but it is apparently caused by multiple factors and is linked to disability, cognitive impairment, and lesion site²³.

The rat model of focal brain ischemia, created by MCAO, is the most widespread animal model of stroke^{24–27}. In demonstrating the induction of PSD in rats by occluding the MCA via the ICA, techniques that minimize mortality and variability in the MCAO model are employed²⁸.

The primary objective of this protocol is to outline the steps for inducing PSD in rats by occluding the MCA via the ICA, a modified model of MCAO, which reduces mortality and the outcome of variability²⁸. Specific aims include performing neurological and histological examinations (determining the neurological severity score [NSS], the volume of the infarction zone, and brain edema) to verify the efficacy of MCAO and using behavioral tests to examine the influence of this MCAO procedure on the development of emotional disorders, mainly PSD.

PROTOCOL:

The Animal Care Committee of the Ben-Gurion University of the Negev, Israel approved all treatment and testing procedures used in this protocol.

1. Preparation of rats for the experimental procedure

NOTE: Select adult male Sprague-Dawley rats weighing 300–350 g.

1.1. House the rats, four per cage, in a vivarium at 22 °C and 40% humidity, with a reversed 12 h light/dark cycle (lights off at 8:00 a.m.) and unlimited access to food and water.

1.2. Randomly assign the rats to two groups, namely an MCAO group ($N = 24$) and a Sham group ($N = 19$).

2. Preparation of rats for surgery

2.1. To prepare the rats for the modified MCAO procedure, anesthetize each rat for 30 min with a mixture of isoflurane (4% for induction, 2% for surgery, and 1.3% for maintenance) in 24% oxygen (2 L/min) in an induction chamber and allow it to breathe spontaneously^{29–30}.

NOTE: Consider the anesthesia sufficient for surgery when the tail reflex of the rat ceases to occur.

2.2. Maintain the core body temperature at 37 °C with a heating plate.

2.3. Measure the body temperature of all rats through a probe placed in the rat's rectum before beginning the surgical procedure.

2.4. Keep the body temperature constant (37 °C) for all rats to minimize any hypothermic effect on the neurological outcome and any neurological injury.

2.5. Apply artificial tears ointment to both of each animal's eyes while they lie on the prep table.

2.6. Shave the neck of each rat and disinfect the skin with 70% alcohol chlorhexidine (70% alcohol and 0.5% chlorhexidine gluconate).

2.7. Cover the rats with sterilized surgical drapes.

3. Surgery (the MCAO technique)

NOTE: Perform surgery as described by Boyko et al.²⁸ and use the instruments provided by McGarry et al.²⁹ and Uluç et al.³⁰.

3.1. Perform a ventral midline incision and dissect the superficial fascia.

3.2. Carefully perform a sharp and blunt dissection within the three triangle-shaped muscle (the sternohyoid, the digastric, and the sternomastoid muscle) to identify the carotid arteries (the ECA, the ICA, and the common carotid artery [CCA]).

3.3. Carefully dissect and expose the right CCA and the ICA.

3.4. Separate the right CCA and ICA from the vagus nerve.

NOTE: Upon identifying the carotid arteries as stipulated in step 3.2, the ICA would be recognized as one-half of the CCA branches alongside the ECA.

3.5. Insert a catheter (heat-blunted or silicone-coated 4-0 nylon) monofilament directly through the ICA, ~18.5–19 mm from the bifurcation point of the right CCA into the circle of Willis until reaching a mild resistance, to occlude MCA.

NOTE: The heat-blunted filament and the silicone-coated 4-0 nylon play the same role and are the more preferred monofilaments in recent times, given that they provide better occlusion than the plain nylon thread^{28,30}.

3.6. Tie a 4-0 silk suture around the ICA just above the right CCA (pterygopalatine artery) bifurcation to block the ICA proximal to the filament insertion point permanently and distal to it temporarily.

3.7. Tie the ICA around the intraluminal thread to fasten the silk suture to prevent bleeding.

3.8. Subject the sham-operated rats to the same surgical procedure as the MCAO rats but insert a nylon thread instead²⁸.

NOTE: The nylon thread, like the silicon-coated nylon, occludes the ICA, but it is not as effective as the latter.

3.9. Close the wound on the rat's neck after occluding the MCA, turn off the anesthesia, and place the rat in an incubator under observation until it wakes up.

NOTE: The need for the proximal ligation is to occlude the ICA, and that of the additional distal ligation is to reduce the bleeding around the filament and secure it in place. Also, the rat should wake up a few minutes after its wound has been closed.

4. Postsurgical recovery

4.1. Administer 5 mL of 0.9% saline solution to each rat intraperitoneally, immediately after surgery, to prevent dehydration.

4.2. Give diluted dipyrone (0.5 g dissolved in 400 mL of drinking water) to rats showing symptoms of pain during the first 3 days.

4.3. Sacrifice any rats with seizures (seizures are caused by the increased intracranial pressure from cerebral edema or cerebral hemorrhage²⁸).

5. Neurological severity score³¹

NOTE: This procedure is performed by two observers who do not take part in the surgical proceedings; they test the neurological deficits and grade motor deficits on a cumulative score of 0–4³². The evaluation of this score may be performed at different time intervals; in this investigation, it was performed 50 min, 24 h, 7, 15, and 30 days postsurgery. Find below the steps for evaluating the NSS. Although not a necessity in this situation, this score is required to ascertain stroke in rodents in order to administer treatment.

5.1. Place the rat on a ceramic floor and allow it to move about freely for 1 min.

5.2. Gently pull the rat backward by the tail and grade as follows.

5.2.1. Grade the rat with score 0 for no neurological deficit.

5.2.2. Grade the rat with score 1 for forelimb flexion.

5.2.3. Grade the rat with score 2 for contralateral weak forelimb grip.

5.2.4. Grade the rat with score 3 for circling to the paretic side when pulled by the tail.

5.2.5. Grade the rat with score 4 for spontaneous circling³².

NOTE: If more than one of the reactions are observed, preference is given to the action with a higher score.

6. Determination of the infarct volume (histologic examination)

6.1. Measurement of the infarct volume

NOTE: Perform this procedure as described previously^{33–34}. Measure brain infarct volume using 2,3,5-triphenyltetrazolium chloride (TTC) staining 24 h after reperfusion.

6.1.1. Euthanize five rats from each group, 24 h after the last NSS, by exposing them to an isoflurane overdose in an induction chamber.

6.1.2. Decapitate the rats and quickly isolate their brains using small scissors and forceps.

221 6.1.3. Wash the isolated brains in 0.9% saline.

222
223 6.1.4. Examine bleeding points on the brains to exclude the mice that underwent subarachnoid
224 hemorrhage at the circle of Willis.

225
226 6.1.5. Place each brain on a clean glass slide on a -20 °C ice pack and then place them in a -20 °C
227 fridge for 5 min to make the brain easier to slice.

228
229 6.1.6. Take the glass slide with the brain on it out of the -20 °C fridge, put it back on the -20 °C ice
230 pack, and dissect the frontal pole and the cerebellum with blade and forceps.

231
232 6.1.7. Slice the brain sections horizontally into 2 mm thickness with a blade to produce six slices.

233
234 6.1.8. Prepare a 0.05% TTC solution by adding 1.25 g of TTC powder to 500 mL of normal saline
235 solution prior to sacrifice, transfer the solution to a 24-well plate (1 mL per well) covered in foil,
236 and store it at 4 °C.

237
238 NOTE: TTC and tissue stained with TTC are light sensitive.

239
240 6.1.9. Using forceps, transfer the brain slices to the 24-well plate containing the TTC solution (one
241 slice per well) and stretch out the slices in the solution.

242
243 6.1.10. Incubate the plate content at 37 °C in a shallow water bath for 30 min.

244
245 6.1.11. Aspirate the TTC solution from the plate with a pipette, wash them with a brainwashing
246 fluid, and incubate at room temperature for 30 min.

247
248 6.1.12. Place the slices in the order in which they were cut on a laboratory glass and examine the
249 segments with a scanner.

250
251 6.1.13. Analyze the infarct size as a percentage of the whole brain slice, using ImageJ analysis
252 software, based on visual identification.

253 254 **6.2. Analysis of the infarct volume³⁵**

255
256 6.2.1. Quantify the infarct volume by using a standard image analysis software (ImageJ) and
257 analyze the infarct brain volume as a percentage of the whole brain size³³.

258
259 6.2.2. Place the brain slices on glass microscope slides and scan them with an optical scanner at
260 a high resolution (1,600 x 1,600 dpi) for an adequate analysis.

261
262 6.2.3. Crop the images and standardize the scale for all images, using the metric ruler included in
263 the scanned image.

6.2.4. Measure the area of marked pallor in six consecutive 2 mm coronal sections using a Wand (tracing) tool and freehand selection on the ImageJ 1.37v software³⁶.

6.2.5. Calculate the indirect infarct volume using the following formula³⁷.

$$\text{Corrected Infarct Size} = \text{Infarct Size} \times \frac{\text{Contralateral Hemisphere Size}}{\text{Ipsilateral Hemisphere Size}}$$

7. Measurement of brain edema³⁸

NOTE: Brain edema was measured 24 h after the last MCAO.

7.1. Assess the magnitude of the edema of the right hemisphere by using the summation of coronally sliced areas to calculate the volumes of the right and left hemispheres in arbitrary units (pixels).

NOTE: Use the ImageJ 1.37v software for this calculation, after optical scanning (resolution: 1,600 x 1,600 dpi). Select the area of interest and use the **Measure** function from the **Analysis** menu. Macros were used in our investigation.

7.2. Express the brain edema area as a percentage of the standard areas in the unaffected contralateral hemisphere.

7.3. Calculate the degree of swelling using the equation developed previously³⁹.

$$\text{The Extent of Edema} = \frac{\text{Volume of Right Hemisphere} - \text{Volume of Left Hemisphere}}{\text{Volume of Left Hemisphere}}$$

8. Behavioral paradigms

8.1. Perform behavioral tests between days 30 and 33 after surgery in a closed, quiet, and light-controlled room.

8.2. Assign investigators blinded to all experimental procedures to videotape all behavioral tests with a commercially available program.

8.3. Sucrose preference test^{40–41}

8.3.1. Place the rats in individual cages in the same room as where they are housed during the dark cycle.

8.3.2. For the next 24 h, place one bottle of 100 mL of 1% (w/v) sucrose solution in each cage with the rat to be tested and allow for the adaptation of the rat.

8.3.3. After 24 h, deprive the rats of food and water for 12 h by removing the bottles.

8.3.4. Then after 12 h, place two bottles in each cage for 4 h, one containing 100 mL of tap water and another containing 100 mL of sucrose solution (1% [w/v]).

8.3.5. Record the amount of sucrose solution and water consumed by rats in milliliters. Calculate the affinity to sucrose preference as follows.

$$\text{Sucrose Preference} = \frac{\text{Sucrose Intake (g)}}{\text{Sucrose Intake (g)} + \text{Water Intake (g)}} \times 100$$

8.4. Porsolt forced swim test⁴²

NOTE: The Porsolt forced swim test was carried out as described in a previous protocol published by Zeldetz et al.⁴⁰ and Boyko et al.⁴². The principle of this test states that, when rats are forced to swim in a restricted area from whence, they cannot escape, they eventually become immobile, ceasing any attempts to escape the water^{43–44}. This test was carried out in a different room during the dark cycle.

8.4.1. Place each rat in a vertical plexiglass cylinder (height: 100 cm; diameter: 40 cm) containing 80 cm of water at 25 °C for 15 min for habituation.

8.4.2. Take the rat out and allow it to dry for 15 min in a heated enclosure (32 °C).

8.4.3. Return the rat to its home (original) cage.

8.4.4. Repeat step 8.4.1 24 h later, this time for the investigation, for 5 min.

8.4.5. Videotape the 5 min test and calculate the total duration of immobility during that period.

REPRESENTATIVE RESULTS:

Histological findings (**Table 1**) revealed a statistically significant infarct volume as a percentage of total brain ($p < 0.0001$) post-MCAO when compared to animals in the sham-control group. Also reported was a statistically significant brain edema when the evaluation from the experimental group ($p < 0.0003$) was put side by side with that of the sham-control group.

The NSS scores obtained, as represented in **Table 2**, show lower neurological performances in the experimental group (MCAO) compared to higher figures for the sham-control group following Mann-Whitney tests: $p < 0.001$ after 50 min, $p < 0.05$ after 24 h, and $p < 0.05$ after 7 days.

Findings from the sucrose preference evaluations revealed that MCAO rats also consumed a significantly less amount of sucrose ($p < 0.0001$, **Figure 2A**) and had a longer immobility duration ($p < 0.0001$, **Figure 2B**) compared to the sham-operated rats.

FIGURE LEGENDS:

Figure 1: Graphic demonstration of the protocol timeline. The various tests run on rats at different times are shown on the scheme: MCAO = middle cerebral artery occlusion at the beginning of the experiment; NSS = neurological severity score, 50 min, 24 h, and 7 and 30 days post-MCAO; and behavioral tests (sucrose preference and Porsolt forced swim tests) from days 30 to 33 post-MCAO. This figure has been modified from Ifergane et al.⁴⁵.

Figure 2: Sucrose preference test performed from days 30 to 33 with post-MCAO ($n = 16$) and sham-control rats ($n = 14$). Percentage (%) of sucrose preference. MCAO rats consumed less sucrose ($p < 0.0001$) than the sham-control rats, with a significant difference in sucrose consumption between the two groups shown in the figure. MCAO = middle cerebral artery occlusion. All data represent the group mean \pm SEM. This figure has been modified from Ifergane et al.⁴⁵.

Figure 3: Porsolt forced swim test performed from days 30 to 33 post-MCAO ($n = 16$) and post-sham procedure ($n = 14$). Immobility duration (in seconds). The immobility time in the forced swim test was significantly longer in the MCAO group than in the sham group ($p < 0.0002$). MCAO = middle cerebral artery occlusion. All data represent the group mean \pm SEM. This figure has been modified from Ifergane et al.⁴⁵.

Table 1: Histological findings for infarct volume and brain edema. MCAO = middle cerebral artery occlusion ($n = 5$); sham ($n = 5$).

Table 2: Neurological severity score (NSS) for MCAO and sham-operated rats. MCAO = middle cerebral artery occlusion ($n = 16$); sham ($n = 14$). This table is taken from Ifergane et al.⁴⁵.

DISCUSSION :

One of the ways in which the MCAO technique presented here could be deemed safer than the original MCAO model is illustrated by the fact that the ECA and its branches, including the occipital artery, the terminal lingual, and the maxillary artery, are not compromised when occluding the MCA via the ICA. The original MCAO model's offset of the ECA (and its branches), by distally dissecting and coagulating them⁴⁶, causes impaired mastication, owing to a compromise to the vascular supply to masticating muscles⁴⁷. The destruction of the muscle could eventually cause the release of additional computational fluid dynamics. Contrary to the original MCAO technique in which access to the MCA occurs through the ECA, the technique described here is modified for stroke, with no occurrence of blood flow interruption in the ECA and its tributaries.

The most important arguments for preferring the novel MCAO model to the original MCAO rest in its ability to reduce variability in brain edema, infarct volume, and weight changes, significantly, as well as decrease MCAO-related mortality. Mortality in MCAO procedures is an important factor⁴⁸; a 20% mortality rate is deemed reasonable^{49–50}. The mortality rates (20% for original MCAO, 12.5% for novel MCAO, and 0% for control) in the current investigation were all

within range of the acceptable rate, but the novel MCAO technique fared better than the original MCAO.

Rats undergoing the novel MCAO procedure lost less weight right after surgery and gained more weight by the end of the investigation than rats subjected to the original MCAO technique²⁸. More weight loss and less weight gain in rats subjected to the original MCAO could result from ligating the ECA during surgery, which causes distal hypoperfusion in the facial, lingual, and maxillary arteries, as well as ischemia-related damages to the muscles that support mastication. If mastication is impaired, oral intake lessens, which, when coupled with catabolism, could account for weight loss, and in the long run, for a poor neurological outcome, morbidity, and death⁴³. Access to the MCA via the ICA does not require interfering with the ECA and its branches. Thus, no impairments are triggered during the novel MCAO procedure, and no weight, morbidity, or mortality problems are experienced by rats undergoing the procedure.

The tests used to assess underlying depressive factors, such as depressive behavior, anhedonia, immobility, and learning and memory impairment, are standard procedures applied in animal models of depression^{51–52}. A behavioral test, like the Porsolt forced swim test, is commonly affected by unusual motor abilities following MCAO. Here, this test was applied from days 30 to 33 after the surgical procedure to ascertain that the induction of PSD in the modified MCAO rats did not overly affect their motor abilities. According to the results, rats from the experimental group showed a comparable total escape behavior to that of sham-control rats. MCAO rats had significantly more escape failures, a significantly raised duration of immobility, and a reduced preference for sucrose when compared with sham-operated animals. This would suggest that this MCAO technique is a capable alternative to the original MCAO method.

The difference between inclusion and exclusion of rats in the MCAO procedure for a broader experimental evaluation depends very much on the outcome of the operation. With the MCAO-related PSD-inducing model, some unwanted side-effects of MCAO could be curtailed, leaving room for the inclusion of smaller, and possibly fragile rats in the MCAO procedure. The animal model of MCAO presented here provides a scenario for lessening unintended outcomes after MCAO-induced PSD because it has the potential to reduce variability in weight changes, brain edema, and infarct volume, as well as MCAO-related deaths. This technique could potentially serve as a tool for assessing future PSD therapies and provide preclinical data on the efficacy of therapeutic substances, as well as monitor other factors modifying modalities on the clinical outcome of stroke and PSD.

ACKNOWLEDGMENTS:

None

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Miniño, A.M., Murphy, S.L., Xu, J., Kochanek, K.D. Deaths: final data for 2008. *National Vital Statistics Reports*. **59** (10), 1-126 (2011).
2. Roger, V.L. et al. Executive summary: Heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation*. **125** (1), 188-197 (2012).
3. Towfighi, A., Saver, J.L. Stroke declines from third to fourth leading cause of death in the United States: Historical perspective and challenges ahead. *Stroke*. **42** (8), 2351-2355 (2011).
4. Guo, J.M., Liu, A.J., Su, D.F. Genetics of stroke. *Acta Pharmacologica Sinica*. **31** (9), 1055-1064 (2010).
5. Lloyd-Jones, D. et al. Heart disease and stroke statistics – 2010 update: A report from the American Heart Association. *Circulation*. **121** (7), e46-e215 (2010).
6. Warlow, C.P. Epidemiology of stroke. *Lancet*. **352** (Suppl 3), SIII1-SIII4 (1998).
7. Demaerschalk, B.M., Hwang, H.M., Leung, G. US cost burden of ischemic stroke: A systematic literature review. *The American Journal of Managed Care*. **16** (7), 525-533 (2010).
8. Heidenreich, P.A. et al. Forecasting the future of cardiovascular disease in the United States: A policy statement from the American Heart Association. *Circulation*. **123** (8), 933-944 (2011).
9. de Groot, M.H., Phillips, S.J., Eskes, G.A. Fatigue associated with stroke and other neurologic conditions: Implications for stroke rehabilitation. *Archives of Physical Medicine and Rehabilitation*. **84** (11), 1714-1720 (2003).
10. Kim, J., Choi, S., Kwon, S.U., Seo, Y.S. Inability to control anger or aggression after stroke. *Neurology*. **58** (7), 1106-1108 (2002).
11. Leys, D., Hénon, H., Mackowiak-Cordollani, M.S., Pasquier, F. Poststroke dementia. *Lancet Neurology*. **4** (11), 752-759 (2005).
12. McManus, J., Pathansali, R., Stewart, R., Macdonald, A., Jackson, S. Delirium post-stroke. *Age and Ageing*. **36** (6), 613-618 (2007).
13. Robinson, R.G. Poststroke depression: Prevalence, diagnosis, treatment, and disease progression. *Biological Psychiatry*. **54** (3), 376-387 (2003).
14. Tang, W. et al. Emotional incontinence and executive function in ischemic stroke: A case-controlled study. *Journal of the International Neuropsychological Society*. **15** (1), 62-68 (2010).
15. Astrom, M., Adolfsson, R., Asplund, K. Major depression in stroke patients: A 3-year longitudinal study. *Stroke*. **24** (7), 976-982 (1993).
16. Eastwood, M.R., Rifat, S.L., Nobbs, H., Ruderman, J. Mood disorder following cerebrovascular accident. *The British Journal of Psychiatry*. **154**, 195-200 (1989).
17. Robinson, R.G., Bolduc, P.L., Price, T.R. Two-year longitudinal study of poststroke mood disorders: Diagnosis and outcome at one and two years. *Stroke*. **18** (5), 837-843 (1987).
18. Kauhanen, M. et al. Poststroke depression correlates with cognitive impairment and neurological deficits. *Stroke*. **30** (9), 1875-1880 (1999).
19. Morris, P.L., Robinson, R.G., Andrzejewski, P., Samuels, J., Price, T.R. Association of depression with 10-year poststroke mortality. *The American Journal of Psychiatry*. **150** (1), 124-129 (1993).
20. Paolucci, S. et al. Post-stroke depression, antidepressant treatment and rehabilitation results. A case-control study. *Cerebrovascular Diseases*. **12** (3), 264-271 (2001).
21. Schwartz, J.A. et al. Depression in stroke rehabilitation. *Biological Psychiatry*. **33** (10), 694-699 (1993).

479 22. Williams, L.S., Ghose, S.S., Swindle, R.W. Depression and other mental health diagnoses
480 increase mortality risk after ischemic stroke. *The American Journal of Psychiatry*. **161** (6), 1090-
481 1095 (2004).

482 23. Whyte, E., Mulsant, B. Post-stroke depression: Epidemiology, pathophysiology, and biological
483 treatment. *Biological Psychiatry*. **52**, 253-264 (2002).

484 24. Belayev, L., Alonso, O.F., Busto, R., Zhao, W., Ginsberg, M.D. Middle cerebral artery occlusion
485 in the rat by intraluminal suture. Neurological and pathological evaluation of an improved model.
486 *Stroke*. **27** (9), 1616-1623 (1996).

487 25. Longa, E.Z., Weinstein, P.R., Carlson, S., Cummins, R. Reversible middle cerebral artery
488 occlusion without craniectomy in rats. *Stroke*. **20** (1), 84-91 (1989).

489 26. Spratt, N.J. et al. Modification of the method of thread manufacture improves stroke
490 induction rate and reduces mortality after thread-occlusion of the middle cerebral artery in
491 young or aged rats. *Journal of Neuroscience Methods*. **155** (2), 285-290 (2006).

492 27. Yu, F., Sugawara, T., Chan, P.H. Treatment with dihydroethidium reduces infarct size after
493 transient focal cerebral ischemia in mice. *Brain Research*. **978** (1-2), 223-227 (2003).

494 28. Boyko, M. et al. An experimental model of focal ischemia using an internal carotid artery
495 approach. *Journal of Neuroscience Methods*. **193** (2), 246-253 (2010).

496 29. McGarry, B.L., Jokivarsi, K.T., Knight, M.J., Grohn, O.H.J., Kauppinen, R.A. A Magnetic
497 Resonance Imaging Protocol for Stroke Onset Time Estimation in Permanent Cerebral Ischemia.
498 *Journal of Visualized Experiments*. (127), e55277 (2017).

499 30. Uluç, K., Miranpuri, A., Kujoth, G.C., Aktüre, E., Başkaya, M.K. Focal Cerebral Ischemia Model
500 by Endovascular Suture Occlusion of the Middle Cerebral Artery in the Rat. *Journal of Visualized*
501 *Experiments*. (48), e1978 (2011).

502 31. Boyko, M. et al. Morphological and neurobehavioral parallels in the rat model of stroke.
503 *Behavioural Brain Research*. **223** (1), 17-23 (2011).

504 32. Menzies, S.A., Hoff, J.T., Betz, A.L. Middle cerebral artery occlusion in rats: a neurological and
505 pathological evaluation of a reproducible model. *Neurosurgery*. **31** (1), 100-107 (1992).

506 33. Boyko, M. et al. Cell-free DNA - A marker to predict ischemic brain damage in a rat stroke
507 experimental model. *Journal of Neurosurgical Anesthesiology*. **23** (2), 222-228 (2011).

508 34. Zheng, Y. et al. Experimental Models to Study the Neuroprotection of Acidic Postconditioning
509 Against Cerebral Ischemia. *Journal of Visualized Experiments*. (125), e55931 (2017).

510 35. Poinsette, K. et al. Quantification of neurovascular protection following repetitive hypoxic
511 preconditioning and transient middle cerebral artery occlusion in mice. *Journal of Visualized*
512 *Experiments*. (99), e52675 (2015).

513 36. ImageJ. <https://imagej.nih.gov/ij/>.

514 37. Liu, S., Zhen, G., Meloni, B.P., Campbell, K., Winn, H.R. Rodent stroke model guidelines for
515 preclinical stroke trials. *Journal of Experimental Stroke & Translational Medicine*. **2** (2), 227
516 (2009).

517 38. Boyko, M. et al. Pyruvate's blood glutamate scavenging activity contributes to the spectrum
518 of its neuroprotective mechanisms in a rat model of stroke. *European Journal of Neuroscience*.
519 **34** (9), 1432-1441 (2011).

520 39. Kaplan, B. et al. Temporal thresholds for neocortical infarction in rats subjected to reversible
521 focal cerebral ischemia. *Stroke*. **22** (8), 1032-1039 (1991).

522 40. Zeldetz, V. et al. A New Method for Inducing a Depression-Like Behavior in Rats. *Journal of*
523 *Visualized Experiments*. (132), e57137 (2018).

524 41. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders,*
525 *Fourth Edition*. American Psychiatric Association. Washington, DC (2000).

526 42. Boyko, M. et al. Establishment of an animal model of depression contagion. *Behavioural Brain*
527 *Research*. **281**, 358-363 (2015).

528 43. Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M. Behavioral despair in rats: a new model sensitive
529 to antidepressant treatments. *European Journal of Pharmacology*. **47** (4), 379-391 (1978).

530 44. Boyko, M. et al. The neuro-behavioral profile in rats after subarachnoid hemorrhage. *Brain*
531 *Research*. **1491**, 109-116 (2013).

532 45. Ifergane, G. et al. Biological and Behavioral Patterns of Post-Stroke Depression in Rats.
533 *Canadian Journal of Neurological Sciences*. **45** (4), 451-461 (2018).

534 46. Longa, E.Z., Weinstein, P.R., Carlson, S., Cummins, R. Reversible middle cerebral artery
535 occlusion without craniectomy in rats. *Stroke*. **20** (1), 84-91 (1989).

536 47. Dittmar, M., Spruss, T., Schuierer, G., Horn, M. External carotid artery territory ischemia
537 impairs outcome in the endovascular filament model of middle cerebral artery occlusion in rats.
538 *Stroke*. **34** (9), 2252-2257 (2003).

539 48. Ryan, C.L. et al. An improved post-operative care protocol allows detection of long-term
540 functional deficits following MCAo surgery in rats. *Journal of Neuroscience Methods*. **154** (1-2),
541 30-37 (2006).

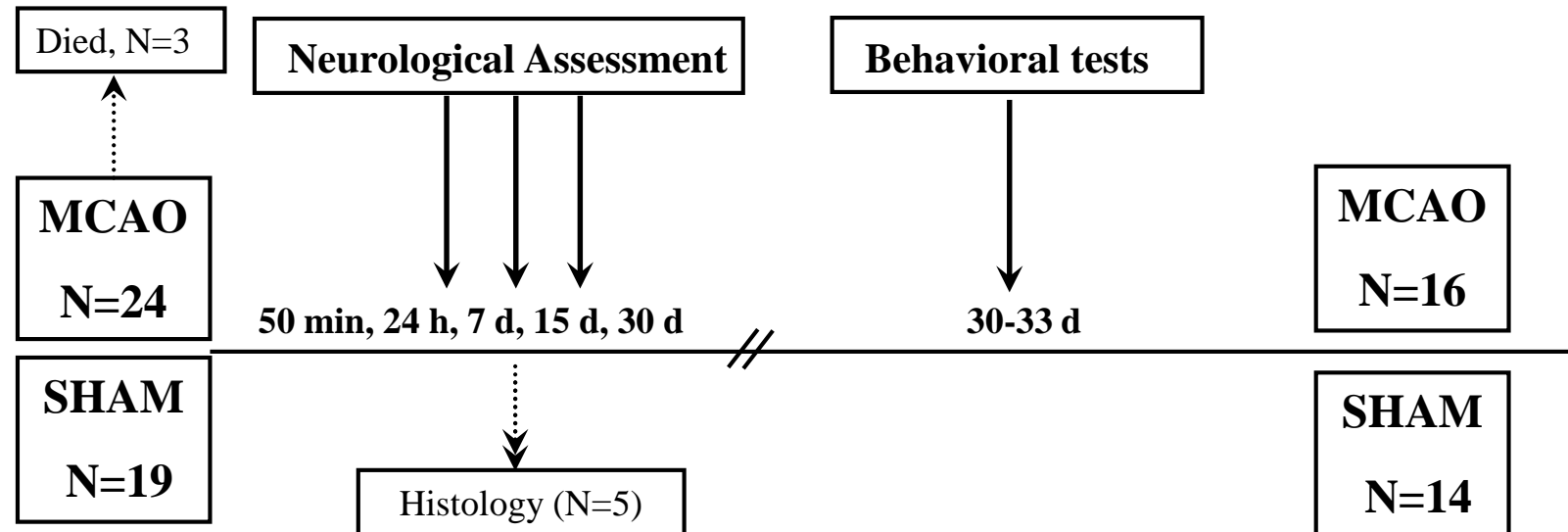
542 49. Aspey, B.S., Cohen, S., Patel, Y., Terruli, M., Harrison, M.J. Middle cerebral artery occlusion in
543 the rat: consistent protocol for a model of stroke. *Neuropathology and Applied Neurobiology*. **24**
544 (6), 487-497 (1998).

545 50. Spratt, N.J. et al. Modification of the method of thread manufacture improves stroke
546 induction rate and reduces mortality after thread-occlusion of the middle cerebral artery in
547 young or aged rats. *Journal of Neuroscience Methods*. **155** (2), 285-290 (2006).

548 51. Cryan, J.F., Markou, A., Lucki, I. Assessing antidepressant activity in rodents: recent
549 developments and future needs. *Trends in Pharmacological Sciences*. **23** (5), 238-245 (2002).

550 52. Nestler, E.J. et al. Preclinical models: status of basic research in depression. *Biological*
551 *Psychiatry*. **52** (6), 503-528 (2002).

Figure 1



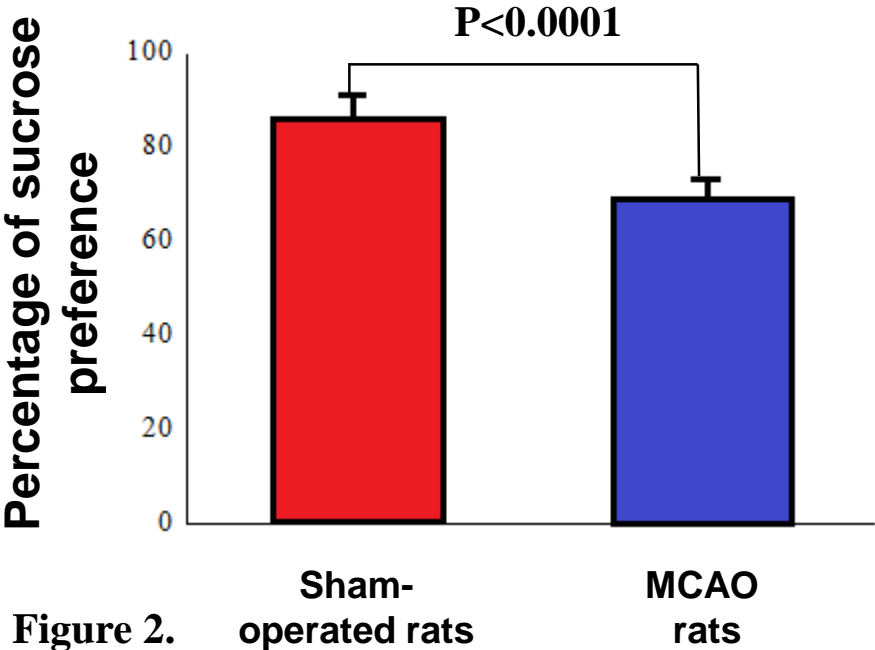


Figure 2.

Sham-
operated rats

MCAO
rats

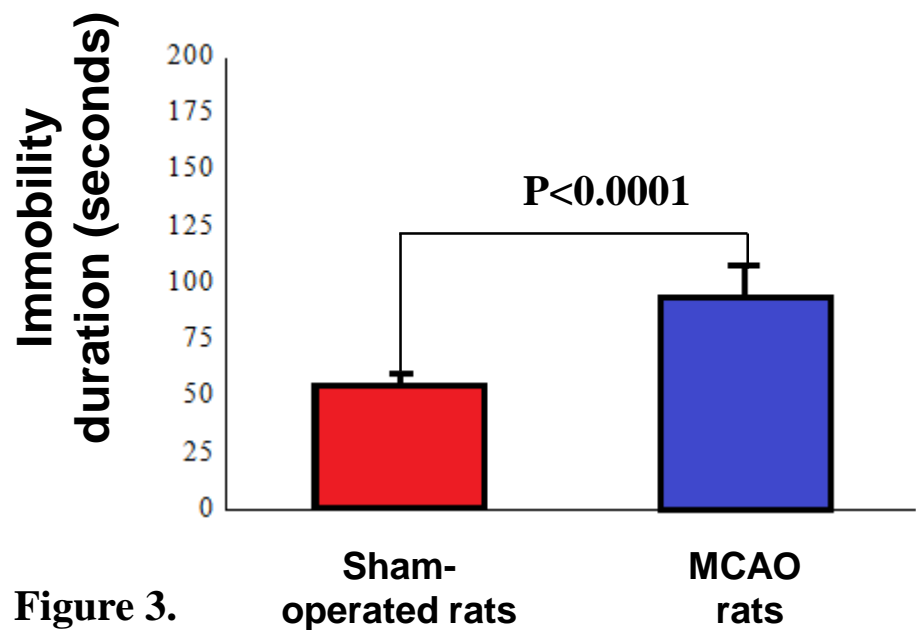


Figure 3.

Table 1 - Histological findings for Infarct Volume and Brain Edema		
Histological Findings	Middle cerebral artery occlusion group	Sham-operated group
Total brain (Infarct volume)	8.8% ± 6.5	0.3% ± 0.1
Brain Edema	10.2% ± 4.6	2.6% ± 1.2
All data represent group mean ± S.E.M		

Table 2 - Neurological Severity Score (NSS) for MCAO and sham-operated rats

Neurological Severity Score	Middle cerebral artery occlusion group	Sham-operated group
50 min post-surgery	2.75 ± 0.14	0.0 ± 0.0
24 hours post-surgery	3.2 ± 0.15	0.0 ± 0.0
7 days post-surgery	0.91 ± 0.2	0.0 ± 0.0

All data represent group mean ± S.E.M

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Absorbent pad	-	-	-
Black lusterless perspex box	-	-	(120 cm × 60 cm × 60 cm), divided into a 25% central zone and the su
	Techniplas		
Bottles	t	ACBT0262SU	150 ml bottles filled with 100 ml of water and 100 ml 1%(w/v) sucrose
Electric Shock	Heat Syster -	-	-
Horizon-XL	Mennen Medical Ltd		
Imaging System	Kodak	-	For imaging and quantification
Monofilament	-	-	-
Paper towels	Pharmacy	-	Dry towels used for keeping rats dry after immersing them in water
Pexiglass cylinder	-	-	a 100 cm tall and 40 cm in diameter cylinder used for carrying out the
Purina Chow	Purina	5001	Rodent laboratory chow given to rats, mice and hamster is a life-cycle
	Techniplas		
Rat Cages	t	2000P	Conventional housing for rodents. Was used for housing rats through
Scanner	Canon Canc	4200F	-
	ETHO-		
	VISION		
Video Camera	(Noldus)	-	Digital video camera for high definition recording of rat behavior und

rounding border zone

a solution

: forced swim test

: nutrition that has been used in biomedical research for over 5 decades. Provided to rats ad libitum in this experiment

out the experiment

er open field test

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:

A Modified Middle Cerebral Artery Occlusion Technique for Inducing Stress-free PSD in Rodents

Author(s):

R. Kuts#, I. Melamed#, H. N. Shiyntum, D. Frank, J. Grinshpun, A. Zlotnik, E. Brotfain, M. Dubilet, D. Natanel, M. Boyko

Item 1: The Author elects to have the Materials be made available (as described at <http://www.jove.com/publish>) via:

☒ Standard Access

☐ Open Access

Item 2: Please select one of the following items:

☒ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: **"Agreement"** means this Article and Video License Agreement; **"Article"** means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; **"Author"** means the author who is a signatory to this Agreement; **"Collective Work"** means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; **"CRC License"** means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>; **"Derivative Work"** means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; **"Institution"** means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; **"JoVE"** means MyJoVE Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; **"Materials"** means the Article and / or the Video; **"Parties"** means the Author and JoVE; **"Video"** means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion

of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

ARTICLE AND VIDEO LICENSE AGREEMENT

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum

rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to

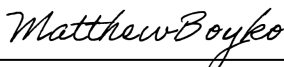
the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication of the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to be one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:	Matthew Boyko	
Department:	Division of Anesthesiology and Critical Care, Soroka Medical Center	
Institution:	Ben-Gurion University of the Negev	
Title:	Head, Research Lab., Department of Anesthesiology and Critical Care, Soroka University Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev	
Signature:		Date: 30.07.2018

Please submit a **signed** and **dated** copy of this license by one of the following three methods:

1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140

Dear Dr. Bajaj,

We have read and have, to the best of our ability, responded to every comment made by you and the reviewers. We hope these are enough to move forward. That notwithstanding, we are open to provide further material whenever necessary.

Yours faithfully,
Matthew Boyko, Ph.D.

Editorial comments:

1. The editor has formatted the manuscript to match the journal's style. Please retain the same.

Done!

2. Once all the comments of the reviewers are addressed, please ensure that the protocol is no more than 2.75 pages and the highlight is no more than 2.75 pages including headings and spacings.

Done!

3. Please proofread the manuscript well before submitting.

Done!

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Now this is acceptable to print.

Thank you!

Reviewer #2:

Manuscript Summary:

The authors have incorporated all suggested changes. A few minor points remain to be considered.

Minor Concerns:

2.2 This should be first (2.1)

Thank you for the remark. 2.2 is now 2.1.

2.5 Ointment application is best to be done on prep table

Thank you for the correction. This has been rectified.

2.7 Bupivacaine is not necessary since the animals are anaesthetized.

Thank you for the correction. This step has been removed from the protocol.

2.8 The pack of the surgery should be already sterilized: cover mice with sterilized surgical drape.

Thank you for calling our attention to that. It has been rectified.

3.7 Cage or incubator?

Thank you for that correction. Incubator it is.

5 Describe the criteria for NSS scores 0-4.

Instead of elaborating on this description, we have provided a reference for the detailed explanation of the procedure: "Ref 32. Menzies, S.A., Hoff, J.T. & Betz, A.L. (1992) Middle cerebral artery occlusion in rats: a neurological and pathological evaluation of a reproducible model. Neurosurgery, 31, 100–106; discussion 106–107."

6.1.3 Wash brains in saline 0.9% not water.

Thank you for the correction. Water has been replaced with 0.9% saline.

8.4.1 Height is not 1000 cm (10 m); please correct. Also correct in Table of Materials.

Thank you for the correction. This has been corrected in the manuscript and table of materials.

I. 381: Change "model is illustrated in the fact that the ECA and its branches, the occipital artery" to: model is illustrated by the fact that the ECA and its branches, including the occipital artery

Thank you for the correction. This change has been effected, and the manuscript as a whole has been proofread for grammatical errors.

Table 1 and 2: include the N value; which values were significantly different from sham?

The N values for MCAO and Sham have been added to the legends of both tables.

Reviewer #4:

Manuscript Summary:

This manuscript provides procedures for a new surgical technique for inducing MCAO. The strengths of this technique, such as decreased mortality and lower variability in weight, edema,

and infarct volume have been previously established. The authors seek to document the procedure and validate it by demonstrating that it produces a significant deficit in forced swim and sucrose preference tests, which are common models of PSD. This contribution establishes that the technique can be used to assess PSD, but does not establish that it produces PSD with any higher efficacy or advantage over the traditional model. This manuscript is much improved from its former version. It still needs some work, including an improvement in the language and grammar. However, it makes a significant contribution to the literature, and the new procedure is ideally transmitted via videotaped demonstration.

Major Concerns:

First concern: The introduction states that "The data obtained with this protocol show that this model of MCAO could be a more effective way of inducing PSD in rats and could potentially lead to a better understanding of the pathophysiology and the future development of new drugs and other neuroprotective agents." This is simply not true - it is neither supported by the data nor by previous data. If there is no comparison MCAO group utilizing the traditional MCAO procedure with which the requisite comparison can be made, the claim simply cannot be made that this procedure "more effectively" produces PSD in the model.

I suggest the following: in abstracts, introduction, and discussion, de-emphasize PSD as the major advantage of the method since there is no evidence that there is any such advantage. Instead, summarize advantages that have previously been established in other studies that did compare to traditional MCAO (I think it was implied that mortality, variability of infarct volume and weight, etc. may be such variables). Then, instead of stating that "The primary objective of this protocol is to outline the steps for inducing PSD in rats by occluding the MCA via the ICA" State that the objective is to validate that this new MCAO method (which can be utilized along with any endpoint, not just PSD), has some impact (NOT a better, worse or similar impact compared to other procedures) on an important behavioral paradigm: PSD.

This would require some minor restructuring mainly of the introduction, abstracts, and a bit in the discussion.

Thank you for your observation. However, we have removed the word more, giving the entire manuscript the intended meaning. As can be seen from the manuscript, we do not make comparisons between different models that cause the development of PSD. The purpose of the work remains unchanged. "The primary objective of this protocol is to outline the steps for inducing PSD in rats by occluding the MCA via the ICA". The changes you suggested require a fundamental review of the article, which will include: change of the main goal that comes with changes to the methods, results, discussion, and conclusions. In other words, the article should be radically changed, and it will have other objectives, methods, results, discussion, and perhaps even a title. This means complete rewriting.

Second concern: Reference in methods to striatum and cortex has been removed, and this improves the clarity of the methods greatly. However, the striatum and cortex are still mentioned in results, which is confusing since there is no method to match those results. These procedures should either be clarified or the results removed.

We agree with this assertion. We might have just forgotten to redo the results last time. We have now removed the words 'striatum' and 'cortex' from the protocol and table 1. The protocol will contain only the value of the total brain, which is convenient since we measured total brain only.

Minor Concerns:

The measurement methods for the two hemisphere sizes should be specified. On line 269, the formula makes sense only so long as the ipsilateral hemisphere is measured in entirety (not just the healthy tissue), but I could not verify that this is what they did.

Thank you for this observation. In our protocol, we applied the method previously described in an article published with JoVE (Reference 35 "Poinsatte, K. et al. Quantification of neurovascular protection following repetitive hypoxic preconditioning and transient middle cerebral artery occlusion in mice. Journal of visualized experiments. (99), e52675 (2015)."

That said, this equation will undercorrect the infarct size since the swelling in and around the stroke will be higher than in healthy tissue. Even if this remains the best method for assessing edema, it will systematically produce an inaccuracy, which is a point that should be mentioned so that measurements can be properly qualified.

We used the conventional and the best method for measuring the cerebral edema described in the guidelines in our investigation and have provided a reference to back that up: "ref 37. Liu, S., Zhen, G., Meloni, B.P., Campbell, K., Winn, H.R. Rodent stroke model guidelines for preclinical stroke trials. Journal of Experimental Stroke & Translational Medicine. 2 (2), 227 (2009)."

Figure 3 legend - the authors should not refer to learned helplessness if they're not measuring learned helplessness directly. The phenomenon may factor into the swim test, but since they never establish a scenario in which kicking would be efficacious for escape, they're not directly assessing it.

Thank you for this correction. The figure legend has been rewritten to remove 'learned helplessness.'

Lines 53, 80, and 84 - the term "variability" is used without mentioning what measure shows higher variability in this model compared to the traditional one. Those variables should be listed and references cited of course.

Line 53 is in the abstract where it is usually not customary to provide citations. The necessary reference to this term is given in line 80.

Line 80 has a reference at the end of the phrase (reference 28), and

Line 84 - the term "variability" was used without citation to avoid repetition given that ref 28 had been cited earlier in line 80. Nevertheless, at the request of the reviewer, we have again added this citation to line 84.

Dear Author,

4 adapted figures and 1 table from: Ifergane, G., Boyko, M., Frank, D., Shiyntum, H., Grinshpun, J., Kuts, R., Geva, A., Kaplan, Z., Zeldetz, V., Cohen, H. (2018). Biological and Behavioral Patterns of Post-Stroke Depression in Rats. *Canadian Journal of Neurological Sciences/Journal Canadien Des Sciences Neurologiques*, 45(4), 451-461 © 2018 The Canadian Journal of Neurological Sciences Inc., published by Cambridge University Press

Thank you for your email enquiry.

You may include four adapted figures and one table from the above journal article in your forthcoming work *A Middle Cerebral Artery Occlusion Technique for Inducing Post-Stroke Depression in Rats*, however we request that you kindly give full copyright acknowledgement to the original source. Please be aware that this non-exclusive permission is granted, free of charge, for this specific use, on the understanding that you have checked that we do not acknowledge another source for this material. This permission does not include the use of copyright material owned by any party other than you as author. Consent to use any such material must be sought by you from the copyright owner concerned.

Further information for our authors can be found on our website at the following link:

<http://www.cambridge.org/about-us/rights-permissions/permissions/permissions-requests-our-authors/>

Kind regards,

Permissions Sales Team
Cambridge University Press
University Printing House
Shaftesbury Road
Cambridge CB2 8BS, UK

<http://www.cambridge.org/about-us/rights-permissions/permissions/>