Standard operating procedures (SOP) in experimental stroke research: SOP for middle cerebral artery occlusion in the mouse

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Thus far, the translation of promising results from preclinical stroke research into effective clinical therapy has not met with success ***(1)***. Among the numerous possible reasons for this failure, quality problems in some of the basic research or preclinical studies have to be considered. False-positive results, inflated effect sizes, and marginal reproducibility may have overestimated or even affected the potential of novel stroke therapeutics ***(2)***. Systematic reviews have found quantitative evidence that low study quality may have introduced a bias into preclinical stroke research ***(3,4,5)***. As opposed to many other causes of the 'translational roadblock', study quality is fully under the control of the researcher, and thus a prime target for improvement. Increasingly, funding bodies and review boards overseeing animal experiments are taking a proactive stance, and demand auditable measures of quality control in preclinical research ***(6)***. The Stroke Therapy Academic Industry Roundtable (STAIR) recently updated its recommendations for the evaluation of preclinical data on neuroprotective drugs ***(7)*** to include good laboratory practice (GLP) issues ***(8)***.

Monitoring, auditing, and standard operating procedures (SOPs) are key elements of quality control in randomized clinical trials (RCTs). It has been proposed that experimental stroke research adapt some of the tools used in clinical stroke research. In particular, stroke laboratories should set up and publish their SOPs (e.g., on their institutional websites), and guarantee that their studies adhere to these standards ***(9)***. This is all the more important, as a certain portion of their experiments, evaluations, etc. are not performed by professionals, but rather by students in training who are unaware of these issues.

A standard operating procedure (SOP) is a set of instructions with the force of a directive covering those features of operations that lend themselves to a definite or standardized procedure without loss of effectiveness. The primary purpose of an SOP in experimental stroke research is to guide and standardize working procedures in order to ensure data reliability and integrity. It is crucial that researchers, students and technicians read and follow

the SOPs. If this is not the case, SOPs will not only fail to achieve their goal; they will also engender a false sense of security. Failures are often due to technical shortcomings in the SOPs themselves. SOPs should be written by the user, as they must convey a clear instruction. The user must not only understand the instruction but also be prepared to carry it out.

We in the following introduce for the first time an SOP in experimental stroke research. Write down what you do, do what is written down!

SOP for middle cerebral artery occlusion (MCAO)

Title	**Middle cerebral artery occlusion (MCAO) in the mouse (intraluminal suture)**
Date	28.07.09
Name of Author	Vincent Prinz, Janett König, Shengbo Ji, Ute Lindauer, Andre Rex, Ulrich Dirnagl
Purpose	Experimental induction of focal cerebral ischemia after occlusion of the middle cerebral artery
Scope and Applicability	Applies to a procedure in a standard lab equipped and certified for in vivo experimentation in rodents (including anesthesia with volatile anesthetics). Experimental procedures require approval by the relevant committees (in Germany: *Tierversuchsgenehmigung, LAGeSo*)
Introduction	Experimental focal ischemia is most commonly studied after permanent or transient occlusion of the middle cerebral artery (MCA) in rodents. Proximal MCA occlusion can be induced by an intraluminal suture (so-called filament model) or with a vascular clip and causes injury to cortex and deeper brain structures (striatum). Distal MCA occlusion (the so-called 'Brint' or 'Tamura'-models) is usually produced by placing a vascular clip on a pial vessel or by cautery. Distal occlusion typically spares the striatum and primarily involves the neocortex. Pannecrosis develops in the territory supplied by the respective artery with glial and endothelial cell death. If recirculation is established early (2 hrs or less) outcome is better (transient MCA occlusion). In some ways, the reperfused brain imitates restoration of blood flow after spontaneous lysis of a

	thromboembolic clot in humans, even though reperfusion after clot lysis is certainly more complex than an on-off phenomenon as modeled by placement and retraction of an intravascular filament. This SOP describes a mouse model of proximal MCAO, which may be permanent or transient.
References	Carmichael, S.T. (2005) Rodent models of focal stroke: size, mechanism, and purpose *NeuroRx*. **2(3)**, 396-409. Review. Dirnagl, U. (2006) Bench to bedside: The quest for quality in experimental stroke research *J Cereb Blood Flow Metab* **26,** 1465-78. Dirnagl, U., Iadecola, C., Moskowitz, M.A. (1999) Pathobiology of ischaemic stroke: an integrated view *Trends Neurosci.* 22(9), 391-7. Shah, Z.A., Namiranian, K., Klaus, J., Kibler, K., Dore, S. (2006) Use of an optimized transient occlusion of the middle cerebral artery protocol for the mouse stroke model *J Stroke Cerebrovasc Dis.* **15,** 133-8.
Materials and Supplies	• filament USP 4/0 or 6/0 Suprama • 8.0 nylon filament for coating with Xantopren M and Activator NF Optosil • Xantopren M Mucosa Haereus Kulzer • Activator NF Optosil Xantropren • surgical needle and thread for suture
Instrumentation	• dissecting microscope (max. x 40) • temperature feedback controlled heating plate • forceps (Dumont 0,05mm x 0,02 mm) • scissors

	• microvascular clip • vascular scissors • heated recovery cage
Cautions	Maintain a body temperature of 36.5 +/- 0.5 °C also after reperfusion (for 2 hours). See also Appendix below. Ensure proper pain relief in the perioperative and postoperative period, e.g. by topical lidocaine ointment and tilidine. Surgial procedures should be carried out under almost sterile conditions. (sterile surgical instruments and materials, clean gown, gloves, etc.). See also Appendix below.
Personnel Qualifications	*In general:* Surgeons need: • general supervision and instruction, • the appropriate certification according to FELASA (A/B), • official registration. *Also required in Germany:* • official registration (*Personenanzeige*) • in Berlin: *Landesamt für Gesundheit und Soziales LAGeSo,* http://www.berlin.de/lageso/ *For internal use:* • If you have questions contact Andre Rex (andre.rex@charite.de) • http://141.42.165.178/expneurointern/anweisungen/einweisischaemie.html

	• In the initial training phase, review our video demonstration of the MCAO procedure ***New surgeons must have passed practical qualification test (see Appendix below)***
Names of SOP Reviewers	Ulrich Dirnagl (ulrich.dirnagl@charite.de) Berlin, 28.7.2009
Protocol	1. Mice are anaesthetized for induction with 1.5% Isoflurane and maintained in 1.0% Isoflurane with 2/3 N_2O and 1/3 O_2 using a vaporizer. 2. A midline neck incision is made and the soft tissues are pulled apart. 3. The left common carotid artery (LCCA) is carefully dissected free from the surrounding nerves (without harming the vagus nerve) and a knot is made using 6.0/7.0 string. 4. Then the left external carotid artery (LECA) is separated and a second knot is made. 5. Next, the left internal carotid artery (LICA) is isolated and a knot is prepared with a 6.0 filament. 6. After obtaining good view of the left middle cerebral artery (LMCA) and the left pterygopalatine artery (LPA), both arteries are clipped. 7. A small hole is cut in the LCCA before it bifurcates to the LECA and the LICA. A monofilament made of 8.0 nylon coated with silicon hardener mixture (see below) is then introduced into the artery. 8. The clipped arteries are opened while the filament is inserted into the LICA to occlude LMCA. 9. The third knot on the LICA is closed to fix the filament in position.

10. The mice receive saline 0.5 ml subcutaneously as volume replenishment.
11. After X min/hours occlusion, the third knot is opened and the filament withdrawn (if reperfusion is intended)
12. All animals receive a second volume replenishment as described above.
13. The remaining filament is cut and the skin is adapted with a surgical suture
14. The body temperature of the mice during surgery is maintained at 36.5°C ± 0.5°C using a heating plate.

For sham operations the filament is inserted to occlude LMCA and withdrawn immediately to allow instant reperfusion (8.). The subsequent operation is identical to the animals undergoing cerebral ischemia (9.-14.).

A video is available which demonstrates the above described procedure.

Monofilament construction:

- 8.0 nylon filament is cut into pieces of 11 mm length under the microscope
- the filament tip must be coated over a distance of 8 mm completely and evenly with a hardener mixture of Xantopren M Mucosa and Activator NF Optosil

Appendix to SOP:

1. Entry qualification experiment for mouse MCAO surgeons

2. Randomized selection of animals from cage and concealment of treatment allocation

   2 a. Pharmacological study

   2 b. Genetically manipulated animals

3. Temperature control

4. Outcome assessment

5. Physiological parameters

6. Quasi-sterile surgery

1. Entry qualification experiment for mouse MCAO surgeons:

New surgeons need to demonstrate in a series of experiments that in 20 animals (10/10) they reach a certain infarct volume within a given standard deviation. Half of the animals will receive saline, the other half treatment.

As positive control we propose FK506 (1mg/kg/BW i.p., directly after induction of ischemia) (Macleod et al. 2005)

MK801 is not suited, as treatment allocation cannot be blinded because of its psychotropic effects. In addition, MK801 may protect against damage after MCAO, but may also increase it (Henrich-Noack et al. 2008).

References:

Macleod, M.R., O'Collins, T., Horky, L.L., Howells, D.W., Donnan, G.A. (2005) Systematic review and metaanalysis of the efficacy of FK506 in experimental stroke *J Cereb Blood Flow Metab.* **25**(6), 713-21.

Henrich-Noack, P., Baldauf, K., Reiser, G., Reymann, K.G . (2008) Pattern of time-dependent reduction of histologically determined infarct volume after focal ischaemia in mice *Neurosci Lett.* **432**(2), 141-5.

Positive control Standard:

- 60min MCAO, C57 Bl6 mouse, male, 6-10weeks old, approx. 20g;
- Mean infarct volume (measured after 24 h, histological or TTC) should be between 90-130mm^3;
- SD: <40 % of the mean;
- Effect of FK506 (1mg/kg/BW i.p., directly after induction of ischemia): 20-40 % infarct volume reduction.

2. Randomized selection of animals from cage and concealment of treatment allocation

2 a. Pharmacological study:

Animals in cage are marked with bar/dot code at the beginning of the procedure.

Computer program (random number generator) selects animal, and assigns it to concealed treatment arm ('A', 'B', etc.).

Stock solution or pharmaceutical ready for application is prepared by assistant, and randomly assigned code ('A', 'B', etc.).

2 b. Genetically manipulated animals:

Animals in both cages (e.g. knockout / wildtype) are marked with bar/dot code at the beginning of the procedure. Computer program (random number generator) selects animal and assigns it to concealed experimental arm ('A', 'B', etc.). Blinded intervention whenever possible.

3. Temperature control:

The body temperature of mice during surgery is maintained at 36.5°C ± 0.5°C using a temperature controlled heating plate. Maintain a body temperature of 36.5 +/- 0.5 °C also after reperfusion (for 2 hours) using a heated recovery box.

4. Outcome assessment

Infarct volume should be evaluated blinded. Functional outcome (*more than Bederson Score!)* should be assessed as well. Mortality and exclusion of animals have to be reported, including specific causes for exclusion.

Exclusion criteria:

- no stroke,
- Problems during induction of MCAo (excessive bleeding, prolonged operation time ≥ 15 min, thread placement).

CAVE:

Especially in genetically manipulated animals, be aware of vascular alterations, which might directly affect stroke outcome

5. Physiological parameters:

MABP, HR, blood gases, CBF should be measured in selected animals.

6. Quasi-sterile surgery

Prior to surgery, the surgeon has to scrub his hands. It is advisable to wear clean gown, and non-sterile gloves at all times the animal is being handled. The surgeon has to wear a

clean gown, cap and mask during surgery. Surgical gloves ought to be worn. If gloves cannot be used, a surgical hand scrub from tips to elbows must precede every operation. The necessary components of aseptic techniques in rodents include also sterile instruments, and separate surgical and animal prep areas. The use of glass bead sterilizer for resterilization of instruments during for repetitive procedures is recommended.

- All instruments used must be sterilized prior to each group of surgeries.
- Instruments must be kept on sterile non-porous drapes during use.
- Instruments must be cleaned of blood and debris by brushing or wiping with sterile water or saline and sterile gauze sponges between surgeries.
- If contamination has occurred, instruments must be placed in 70% ethanol or a glass bead sterilizer for the appropriate period of time for the method used to be effective (or the instrument pack replaced by a new sterile instrument pack) between animals.
- If 70% ethanol is used, instruments must be rinsed with sterile water or saline before being used on the next animal.
- Surgical gloves and blades should be changed after contamination.
- Following surgery all instruments must be thoroughly cleaned and rinsed.

7. Postoperative care:

The animals must be checked daily after surgery for signs of discomfort. The mice could show some weight loss post-surgery. The mice receive mashed food in a petri-dish placed on the floor to encourage eating. The food is replaced daily for seven days.

References:

1. Endres, M., Engelhardt, B., Koistinaho, J., Lindvall, O., Meairs, S., Mohr, J.P., Planas, A., Rothwell, N., Schwaninger, M., Schwab, M.E,. Vivien, D., Wieloch, T., Dirnagl, U. (2008) Improving outcome after stroke: overcoming the translational roadblock *Cerebrovasc Dis*. **25**(3), 268-78.

2. Dirnagl, U., Macleod, M.R. (2009) Stroke research at a road block: The streets from adversity should be paved with meta-analysis and good laboratory practice *Br J Pharmcol* (in press).

3. Bath, P.M.W., Gray, L.J., Bath, A.J.G., Buchan, A., Miyata, T., Green, A.R., on behalf of the NXY-059 Efficacy Meta-analysis in individual Animals with Stroke (NEMAS) investigators (2009) Effects of NXY-059 in experimental stroke: an individual animal meta-analysis *Br J Pharmacol* (in press).

4. Crossley, N.A., Sena, E., Goehler, J., Horn, J., van der Worp, B., Bath, P.M., Macleod, M., Dirnagl, U. (2008) Empirical evidence of bias in the design of experimental stroke studies: a meta-epidemiological approach *Stroke* **39**(3), 929-34.

5. Macleod, M.R., van der Worp, H.B., Sena, E.S., Howells, D.W., Dirnagl, U., Donnan, G.A. (2008) Evidence for the efficacy of NXY-059 in experimental focal cerebral ischaemia is confounded by study quality *Stroke* **39,** 2824-9.

6. Macleod, M.R., Fisher, M., O'Collins, V., Sena, E.S., Dirnagl, U., Bath, P.M., Buchan, A., van der Worp, H.B., Traystman, R., Minematsu, K., Donnan, G.A., Howells, D.W. (2009) Good Laboratory Practice: Preventing Introduction of Bias at the Bench *Stroke* **40,** e50-2, published as reprint in *J Cereb Blood Flow Metab* **29**, 221-3.

7. Stroke therapy academic industry roundtable (Fisher, M., Chair) (1999) Recommendations for standards regarding preclinical neuroprotective and restorative drug development *Stroke* **30,** 2752-8.

8. Fisher, M., Feuerstein, G., Howells, D.G., Hurn, P.D., Kent, TA., Savitz, S.I., Lo, E. (2009) Update of the Stroke Therapy Academic Industry Roundtable (STAIR) preclinical recommendations **Stroke** [Epub ahead of print].

9. Dirnagl, U. (2006) Bench to bedside: The quest for quality in experimental stroke research *J Cereb Blood Flow Metab* **26,** 1465-78.