This SOP replaces:	Version: C
Reason for Change:	Adjustments in response to Vet Office review
Related SOPs:	SOP-LTK-TRT-13 Isoflurane anesthesia SOP-LTK-TRT-18 Injection anesthesia SOP-LTK-RES-5 Scoring and withdrawal criteria of i.c. tumors SOP-LTK-RES-6 Bioluminescent in vivo imaging SOP-LTK-TRT-43 Buprenorphine analgesia SOP-LTK-TRT-19 Tail bleeding SOP-LTK-Res-41 Scoring post-application of binder proteins
Indication of Use:	Implantation of intracranial (i.c.) (tumor-) cell suspensions, application of biologically active (cytokines, small molecules, chemical drugs, peptides, antibodies, viral particles) and inactive (tracers) substances
Aim of SOP:	This procedure describes how to perform stereotactic surgery for the intracranial (i.c.) application of liquids containing biologically active or inactive compounds, tumor cell suspensions, lymphocytes, viral particles or tracer substances into anatomical structures at defined three dimensional coordinates
Distribution:	1. Server 2. Animal facility 3. Group vom Berg
Attachments:	

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by: Michal Beffinger	by: Johannes vom Berg

Responsible Persons: Any person with Module 1 and registered on a particular animal permit

Safety:

1. General rules for working with sharp tools (scalpels, syringes, scissors) have to be followed.
2. Only in the case of viral particles or chemotherapeutic agents additional biosafety rules have to be obeyed
3. Follow the rules of the animal house

Material to be used:

Surgical tools: scalpel and forceps

Betadine® Iodine solution

Sterile cotton swabs

Dental cement / high-viscosity acrylamide glue

0.4 mm nylon / metal clamps / tissue glue (Indermil®, Henkel®)

Small animal stereotactic frame (e.g. Stoelting or DKI)

A suitable syringe (for tumor cells a blunt needle, 26 gauge, gas tight, Hamilton, Reno, NV)

Electrical heating mat, small animal electrical hair trimmer

Vit A eye ointment / Humigel

Principle of Method:

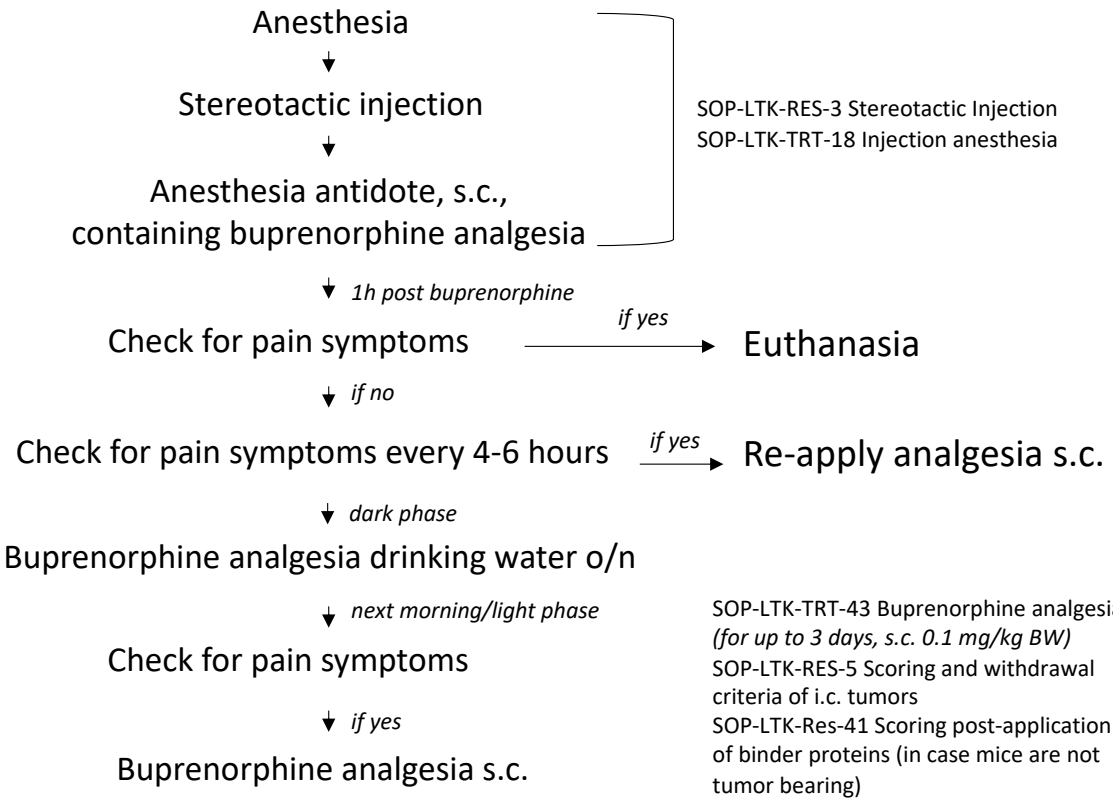
By fixing the head in all three dimensions, exact anatomical regions within the brain can be reached by using manipulator arms. Stereotactic coordinates for specific structures can be found in stereotactic atlases, such as "The Mouse Brain in Stereotaxic Coordinates" by George Paxinos, Keith B. J. Franklin

Procedure Description:

1. Anesthetise the mouse using injection anesthesia (**SOP-LTK-TRT-18 Injection anesthesia**)
2. Shave the head of the animal by using an electric hair trimmer for small animals
3. Disinfect skin of the head with Betadine® Iodine solution, cover eyes with eye ointment
4. Using a scalpel, make a 1 cm skin incision along the midline
5. Fix mouse on a suitable stereotactic head holder
6. Find the intersection of the coronal and sagittal sutures (bregma), place the drill over bregma, drill a hole using a sterotactic drill at the desired XY coordinates (for the implantation of tumors in the striatum 1.5 mm lateral and 1 mm anterior to Bregma) until you reach the dura mater.
7. exchange the drill for a suitable syringe (for tumor cells a blunt needle, 26 gauge, gas tight, Hamilton, Reno, NV) and move it to the desired XY coordinates. Slowly lower the syringe into the burr hole to the desired Z coordinates (For the implantation of tumors in the striatum, lower it 4mm and retract it 1mm. Inject 2 µl of solution (cells in DMEM or PBS) over a 2 min period. Apply biologically active or inactive compounds or cells at maximal volume of 5 µl and a maximal infusion rate of 1 µl/min. Retract the needle, wash the site of surgery with 0.9% NaCl (sterile) and suture the skin with a 0.4 Nylon (alternative: use metal clamps instead of Nylon or surgical glue such as Indermil® Henkel).
8. Apply analgesia and antidote in one mix (**SOP-LTK-TRT-18 Injection anesthesia**)
9. Move animal into wake up cage (a regular cage placed on a 37°C electrical heating mat, covered with a surgical cloth), only put fully awake animals back to the housing cage.

10. Check for postoperative complications and signs of pain after 1 h, apply analgesia (**SOP-LTK-TRT-43 Buprenorphine analgesia)** s.c. after 4 hours and repeat every 4-6 hours during the light phase of the light cycle. During the dark phase of the light cycle (within 24 hours post-surgery) administer Buprenorphine via drinking water (**SOP-LTK-TRT-43 Buprenorphine analgesia)**. Check the next day and re-apply analgesia if necessary. Repeat this cycle of checks every 4-6h during the day plus the drinking water in the night until you do not detect pain signs anymore; for up to 3 days. Monitor mice according to **SOP-LTK- RES-5 Scoring and withdrawal criteria of i.c. tumors** or **SOP-LTK-Res-41 Scoring post-application of binder proteins** (if no tumors are implanted)

Pain Management:



Documentation:
Lab book, Surgery Protocol and Score sheet according to **SOP-LTK-RES-5 Scoring and withdrawal criteria of i.c. tumors**

The mice have to be placed in the respective experiment and project in iRATS. The actual severity has to be recorded in iRATS at the end of the experiment for each mouse.

Problem management:
Report any adverse event to your supervisor, In case there is arterial bleeding (strong and pulsating bright-red bleeding), euthanize the animal by an overdose of injection anesthesia immediately (5x times the regular dose)

Literatur:
Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection. **Vom Berg** J, Vrohlings M, Haller S, Haimovici A, Kulig P, Sledzinska A, Weller M, Becher B. J Exp Med. 2013 Dec 16;210(13):2803-11. doi: 10.1084/jem.20130678. Epub 2013 Nov 25

The Mouse Brain in Stereotaxic Coordinates by George Paxinos, Keith B. J. Franklin