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Date: 27.07.2022	Stereotactic Injection on EAE animals	LTK-RES-86-A-EN Version: A
This SOP replaces: Version: None		
Reason for Change: None		
Related SOPs: <ul style="list-style-type: none"> SOP-LTK-TRT-52-EN Injection anesthesia for stereotactic injections on EAE animals SOP-LTK-RES-1-EN EAE Scoring SOP-LTK-TRT-50-EN Carprofen analgesia on EAE animals 		
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: <ul style="list-style-type: none"> 1. Server 2. Animal facility 3. Group Buch Attachments:		
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Responsible Persons: Any person with Module 1 and registered on the animal permit where this procedure is part of.


- 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

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Procedure Description:

1. Anesthetise the mouse using injection anesthesia (**SOP-LTK-TRT-52-EN Injection anesthesia for stereotactic injections on EAE animals**)
2. Shave the head of the animal by using an electric hair trimmer for small animals
3. Disinfect skin of the head with 2 component disinfection (Betadine® Iodine solution), cover eyes with eye ointment
4. Fix mouse on a suitable stereotactic head holder
5. Using a fresh scalpel, make a 1 cm skin incision along the midline
6. Find the intersection of the coronal and sagittal sutures (bregma), place the drill over bregma, drill a hole using a stereotactic drill at the desired XY coordinates (e.g. 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 (e.g. 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. 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 according to **SOP-LTK-TRT-50-EN Carprofen analgesia on EAE animals**
9. Apply antidote (**SOP-LTK-TRT-52-EN Injection anesthesia for stereotactic injections on EAE animals**)
10. Move animal into wake-up cage (a cage placed on a 37°C electrical heating mat, covered with a surgical cloth), only put fully awake animals back to the housing cage.
11. Further monitoring and interventions according to **SOP-LTK-RES-1-EN EAE Scoring, Attachment B.**

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Documentation:

Lab book, Surgery Protocol and Score sheet according to **SOP-LTK-RES-1-EN EAE Scoring, Attachment B**

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:

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