

Procedure Type: Custom - Surgical Procedure

Procedure Title: Stereotaxic Compound Administration in Rats

Species: Rat

Pain/Distress Category: D

General Instructions:

Please make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

General compound administration guidelines: Doses will comply with ACUC Guidelines for "Dosing Techniques and Limits" (<http://www.acuc.berkeley.edu/guidelines/dosing.pdf>). If doses will not comply with ACUC Guidelines, insert variation with justification below in the section: "How does this procedure fit into or address your overall research goals?"

General Information about Stereotaxic Compound Delivery: Stereotaxic surgery is an invaluable tool in systems neuroscience. It can be used for the injection of anatomical tracers or neural stem cells, as well as for gene delivery and direct drug delivery to the CNS, bypassing the blood-brain barrier. This procedure can be used to introduce stable genetic manipulations at different stages of postnatal life to specific brain regions in the rat. Since the brain is contained within a closed skull container, delivering large volumes to a particular area of the brain can increase intracranial pressure and may lead to irreversible damage of the brain parenchyma, therefore maximum dose volumes must be closely adhered to. Stereotaxic coordinates for specific brain regions are determined from either empirical data or a brain atlas (e.g., *The Mouse Brain in Stereotaxic Coordinates* by G. Paxinos and B.J. Franklin, Academic Press, 2005). The coordinates are given as three-dimensional (x, y, and z) distances in mm from bregma, which is the intersection of the coronal and sagittal sutures on the surface of the skull, with x representing the medial-to-lateral plane, y representing the rostral-to-caudal plane, and z representing the dorsal-to-ventral plane. Please see "Steps for positioning a mouse in a stereotaxic apparatus" for a reference figure, at the OLAC web site: <https://www.olac.berkeley.edu/pi-resources/pre-approved-procedures>. To obtain targeting coordinates for a specific injection region, subtract the atlas coordinates from the position of the animal's bregma in the stereotaxic apparatus (Cetin A., Komai S, Eliava M, Seeburg PH, & Oster P. Stereotaxic gene delivery in the rodent brain. *Nat. Protoc.* 1, 3166-73 (2006)).

Important Note: Most gene delivery involves the use of viral vectors (e.g., adenovirus, lentivirus, rhabdovirus, etc.) which must be handled under BSL2 conditions. Their use also requires an approved Biohazard Use Authorization (BUA). You will need to complete the "Are You Using" section of the protocol form for Biohazardous Agents and provide details about the safe handling of the agent and the animals receiving the agent. If any of the other compounds to be administered via this route are hazardous (biohazard, radiological, toxic, hESCs, nanomaterial) you must also list them in the "Are You Using?" section, as well as provide a list of all compounds that will be administered via this route, and any known toxicities.

Note: The stereotaxic apparatus and hand-held drill and drill bits should be disinfected with an appropriate disinfectant prior to use; all instruments should be autoclaved; a hot bead sterilizer can be used to sterilize instruments between animals.

Below is a table of common stereotaxic coordinates in an adult rat:

Targeted region: Rostral(+) & Caudal (-)(mm)

- *Subthalamic nucleus -1.9
- *Dorsal hippocampus, CA1 -2.1
- *Basolateral amygdala -1.5
- *Lateral ventricle +0.5
- *Nucleus accumbens, core +1.1

Targeted region: Lateral (mm):

- *Subthalamic nucleus 1.6
- *Dorsal hippocampus, CA1 2.0
- *Basolateral amygdala 2.75
- *Lateral ventricle 0.75
- *Nucleus accumbens, core 1.2

Targeted region: Ventral (mm)

- *Subthalamic nucleus 4.4
- *Dorsal hippocampus, CA1 1.4
- *Basolateral amygdala 4.75
- *Lateral ventricle 2.5
- *Nucleus accumbens, core 4.5

Procedure Description Tab:

Procedure Description:

1. Anesthetize rat per regimen outlined in the Anesthetic Regimen tab. Record weight on blue postsurgical cage card. Administer analgesia per Peri procedure Care/Analgesics tab.
2. Place ophthalmic ointment in both eyes.
3. Remove hair from the dorsal surface of the rat's skull, with electric clippers or approved depilatory cream. Remove at least a 2 mm border of hair from planned surgical margin.
4. Place rat on pre-warmed circulating water heating pad or feedback-controlled electric heating pad, covered with clean absorbent material to reduce hypothermia.
5. Place the rat in the stereotaxic apparatus:
 - a. To symmetrically fix the position of the animal's head in the apparatus, the intra aural positioning studs will be gently positioned into the animal's ear canal.
 - b. The second ear stud will be slowly positioned into the opposite ear to complete fixation; only blunted style ear studs will be used for survival procedures.
 - c. To insert the incisor adapter, small forceps will be used to pull down the animal's lower jaw and the incisor adapter will be moved slowly into the mouth until the incisors fit in the opening; the adapter is fixed in place by gently pulling back.

d. The incisor screw is adjusted to make the head level so that bregma and lamda are equal; the head is leveled horizontally in the caudal-to-rostral direction.

e. If using a nose clamp, it is gently positioned by resting on top of the nose using very low pressure. Note: You can omit the nose clamp altogether, using only the ear studs and incisor adapter for fixation.

5. Aseptically prepare the shaved skin according to the ACUC Guidelines for Surgical Procedures (http://www.acuc.berkeley.edu/guidelines/surgical_procedures.pdf). Briefly, the surgical site is scrubbed with a surgical disinfectant, such as dilute chlorhexidine or betadine scrub. Scrubbing starts at the center of the surgical site and radiates outward with disinfectants subsequently wiped off with alcohol or sterile water. Repeat at least three times. Assure at least 3-5 minutes of contact time with skin before surgical incision.

6. Lidocaine or bupivacaine is injected at the incision site as a local anesthetic, per Peri procedure Care/Analgesics tab.

7. Drape planned surgical site with a sterile gauze, paper, cloth, or clear plastic adhesive drape.

8. Before making an incision, verify a surgical plane of anesthesia (i.e., toe-pinch to verify absence of a withdrawal reflex and a regular respiratory rate).

9. Using sterile tips technique, a single midline incision is made (~12-15mm long) on top of the skull in a rostrocaudal direction with small surgical scissors or a scalpel blade; the subcutaneous and muscle tissue are separated and the skin gently retracted using sterile forceps or hemostats to visualize the structures below.

10. The bregma and lamda areas are gently scraped clean using a small bone scraper, spatula, or cotton swab; the skull is kept moist with sterile saline applied with a sterile cotton swab.

11. The position of the x and y coordinates of bregma are measured and the coordinates of the target injection area subtracted.

13. A small craniotomy (~0.5-1mm) is made over the target injection site using a hand-held drill (e.g., Dremel) and a sterile steel burr (typically 1-2mm) with slight downward pressure. The exposed dura is kept moistened with sterile saline throughout.

14. A sterile injection micropipette or 30 gauge Hamilton syringe that has been pre-loaded with compound (details of which are provided in the Other Agents Utilized tab) is placed into the holder of the stereotaxic arm and the tip brought to bregma; the x, y, and z coordinates that will be used for the injection are calculated.

15. The micropipette/syringe is lowered to the desired z coordinate in the brain, gently penetrating the dura, and the injection is begun (approximately 2.5 mm). The injection is done with slow, steady pressure (at an approximate rate of about 75 nl per minute), total volume not to exceed 1-5 μ l. Note: If using an injection pump, the flow rate is commonly set for 0.0005 ml/min.

16. The micropipette/syringe is withdrawn slowly after waiting for 2-3 minutes to avoid backflow of injectate to the surface (approximately 0.5 mm/min).

17. The injection site is cleaned with a saline-moistened cotton swab.

18. The skin is closed in a simple interrupted pattern with 5-0 to 7-0 sutures or wound clips, and topical antibiotic (e.g., triple antibiotic ointment) is applied to the area. Sutures will be removed by the surgeon if not removed by the rat 10-14 days postoperatively.

Potential Adverse Events: Excessive bleeding, damage to tympanic membrane, respiratory distress, respiratory arrest, increased intracranial pressure, subdural hematoma, brain damage, or infection.

How does this procedure fit into or address your overall research goals?

[Insert protocol-specific rationale here]

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

While negative clinical effects from this procedure are not expected, excessive bleeding, damage to tympanic membrane, respiratory distress, respiratory arrest, increased intracranial pressure, subdural hematoma, brain damage, or infection may occur.

Describe post procedure monitoring that will be performed.

Rats will be monitored until they are fully awake (e.g., upright and ambulatory), before returning any animal to their housing room. Rats will be monitored following surgery for general appearance, activity level, weight loss, signs of infection, or dehiscence at the surgical site.

Rats that have difficulty accessing food and water, appear dehydrated, and/or are anorexic, will be provided with moistened food on the cage floor. Nutritional supplements (e.g., NutriCal) may be provided to help stimulate appetite.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

1. If anesthetic depth cannot be properly controlled during the surgery or imaging, the animal will be euthanized. 2. After surgery, lack of eating and drinking within 48 hours post-surgery, despite hydration and analgesia, will trigger euthanasia. 3. Individual rats that exhibit persistent bleeding, abnormal posture and/or movement, or signs of infection will be removed from the study and euthanized. 4. If the animal reaches 10% weight loss, OLAC vet staff will be contacted for further advice. Animals will be euthanized as per ACUC Guidelines on Humane Endpoints in Animal Studies.

Although contact with responsible lab members will be attempted prior to euthanasia, animals in severe distress may require euthanasia before lab members can be notified.

Anesthetic Regimen Tab:

Parameters that will be monitored during anesthesia (check all that apply):

- ✓ Respiratory Rate
- ✓ Pedal Reflex
- ✓ Other (Describe): Skin color or mucous membrane color.

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC [Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals](#).

Stable respiratory rate, lack of pedal reflex, and skin color or mucous membrane color will be monitored during anesthesia and recorded initially, as well as every fifteen minutes if necessary, until the animal has fully recovered. Anesthetic records will be kept in the lab notebook.

Anesthetic Agents:

Agent Name: Isoflurane

Dosage (in mg/kg if possible) and volume: Induce 3-4%; Maintain 1-2%

Route: Inhalation (IN)

Describe timing, frequency and duration of administration: Rats are initially anesthetized with 3-4% isoflurane by placing them in an induction chamber. Once anesthetized, the rat is then transferred to a nose cone respirator connected to a precision vaporizer that delivers 1-2% isoflurane for maintenance.

Agent Name: Ketamine hydrochloride

Dosage (in mg/kg if possible) and volume: 80-100 mg/kg

Route: Intraperitoneal (IP)

Describe timing, frequency and duration of administration: Administered once in conjunction with Xylazine for induction of anesthesia prior to procedure. If more anesthetic is required, add isoflurane to effect (recommended) or re-dose with one-third initial dose of ketamine alone.

Agent Name: Xylazine

Dosage (in mg/kg if possible) and volume: 5-10 mg/kg

Route: Intraperitoneal (IP)

Describe timing, frequency and duration of administration: Administered once in conjunction with Ketamine for induction of anesthesia prior to procedure.

Other premedications not already listed above:

Agent Name: Ocular Lubricant

Dosage (in mg/kg if possible) and volume: N/A

Route: Topical (Topical)

Describe timing, frequency and duration of administration: A thin strip of ointment is applied to each eye upon induction of anesthesia.

Peri Procedure Care/Analgesics Tab:

Pre-emptive Agents (analgesics given prior to/during procedure) – Select those that apply:

Agent Name: Buprenorphine

Dosage (in mg/kg if possible) and volume: 0.05mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: Buprenorphine will be administered once upon induction of anesthesia. Best if administered 30 – 60 minutes prior to incision, especially with isoflurane.

Agent Name: Meloxicam

Dosage (in mg/kg if possible) and volume: 2 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or meloxicam) is administered once prior to recovery from anesthesia.

Agent Name: Carprofen

Dosage (in mg/kg if possible) and volume: 5 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or meloxicam) is administered once prior to recovery from anesthesia.

Agent Name: Bupivacaine (local block)

Dosage (in mg/kg if possible) and volume: 1-2 mg/kg local infiltration

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: A local anesthetic (e.g., lidocaine or bupivacaine) will be infused once subcutaneously at the planned surgical site prior to incising the skin.

Agent Name: Lidocaine (local block)

Dosage (in mg/kg if possible) and volume: 2-4 mg/kg local infiltration

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: A local anesthetic (e.g., lidocaine or bupivacaine) will be infused once subcutaneously at the planned surgical site prior to incising the skin.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Stable respiratory rate, lack of pedal reflex, and skin color or mucous membrane color will be monitored.

Antibiotics or anti-Microbials:

Agent Name: Bacitracin/Neomycin/Polymyxin

Dosage (in mg/kg if possible) and volume: NA

Route: Topical (Topical)

Describe timing, frequency and duration of administration: Triple antibiotic ointment will be applied to the surgical margin once daily for 3 days post-operatively.

Post-procedure Analgesics:

Agent Name: Carprofen

Dosage (in mg/kg if possible) and volume: 5 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or meloxicam) is administered 24 hours post-operatively. Animals will be checked 48 hours post-operatively for signs of pain or distress as outlined in post procedure monitoring. If animals exhibit any signs of pain or distress, another dose will be administered and the OLAC veterinary staff consulted.

Agent Name: Meloxicam

Dosage (in mg/kg if possible) and volume: 2 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or meloxicam) is administered 24 hours post-operatively. Animals will be checked 48 hours post-operatively for signs of pain or distress as outlined in post procedure monitoring. If animals exhibit any signs of pain or distress, another dose will be administered and the OLAC veterinary staff consulted.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.):

Rats will be monitored following surgery for general appearance, activity level, weight loss, signs of infection, or dehiscence at the surgical site.

Monitoring Duration:

Rats will be monitored every 15 minutes until upright and ambulatory. Once rats are fully recovered from anesthesia, they are placed back into a clean cage and returned to the housing room.

Monitoring Frequency:

Rats will be monitored every 15 minutes until fully awake. Animals will be monitored daily for the first week after surgery or more frequently if indicated by the compound being infused (e.g., Body Condition Scoring index, body weight) while implanted, and wound clips/sutures removed in 7-14 days.

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be consulted or the mouse will be euthanized.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Rats will be allowed to recover in a paper-lined cage on top of a heating pad.

Rats that have difficulty accessing food and water, appear dehydrated, and/or are anorexic, will be provided with moistened food on the cage floor. Nutritional supplements (e.g., NutriCal) may be provided to help stimulate appetite.

Describe record keeping/documentation methods for post-procedure monitoring:

A surgical record must be kept for each surgical patient (e.g., blue post-surgical cage card, anesthetic record, surgical notes in the lab notebook) per ACUC Guidelines on Recordkeeping for Surgical Procedures on Laboratory Animals. Rats will be weighed prior to surgery, as well as daily for 7 days

post-operatively. Weights and observations will be recorded on the blue post-surgical cage card during the immediate post-op period, and the lab notebook thereafter.

Other Agents Utilized Tab:

Agent Name: Add Agents to be administered here.

Dosage (in mg/kg if possible) and volume:

Route: Other - Intracranial

Describe timing, frequency and duration of administration: