Journal of Surgery
Deacon RMJ. J Surg 11: 11699
www.doi.org/10.29011/2575-9760.11699
www.gavinpublishers.com
Review Article

OPEN ACCESS
GAVIN PUBLISHERS

Stereotaxic Surgery in Rodents

Robert MJ Deacon*

***Corresponding Author:** Robert MJ Deacon, Email: xm27dor@gmail.com

Citation: Deacon RMJ (2026) Stereotaxic Surgery in Rodents. J Surg 11: 11699 DOI: 10.29011/2575-9760.11699

Received Date: 22 September 2026; **Accepted Date:** 30 September 2026; **Published Date:** 02 October 2026

In earlier times, brain lesions for research in animals were made by mechanical techniques such as aspiration, micro-knife cuts, or electrolysis. However, Jarrard (1989) [1] developed the more specific cytotoxic technique, in which only nerve cell bodies are destroyed, leaving any passing axons from other structures (fibres of passage) intact. Jarrard initially used ibotenic acid as the cytotoxin, but this is very expensive and NMDA (N-methyl-D-aspartic acid), an excitatory amino acid, performs equally well. The ionotropic NMDA subclass of glutamate receptors is hyper-stimulated, excess calcium ions enter the cell and rapidly destroy it. A stereotaxic apparatus allows the surgeon to fix the head of the animal in a rigid position (Figures 1,2). Then, using reference base points, he/she can insert lesioning devices or implant electrodes into the (invisible) target brain tissue using precise xyz coordinates. A stereotaxic frame or apparatus is an extremely well-engineered product and must be treated with great care. Joints made loose by constant wear or bad handling can render the instrument virtually useless. The smaller the target brain or region, the more important this is.



Figure 1: Typical equipment necessary to perform a rodent brain lesion. Few instruments are necessary, but those you select must be of the highest quality. Weiss make very good instruments, and you can peruse their catalogues online, which go back as far as the American Civil War.

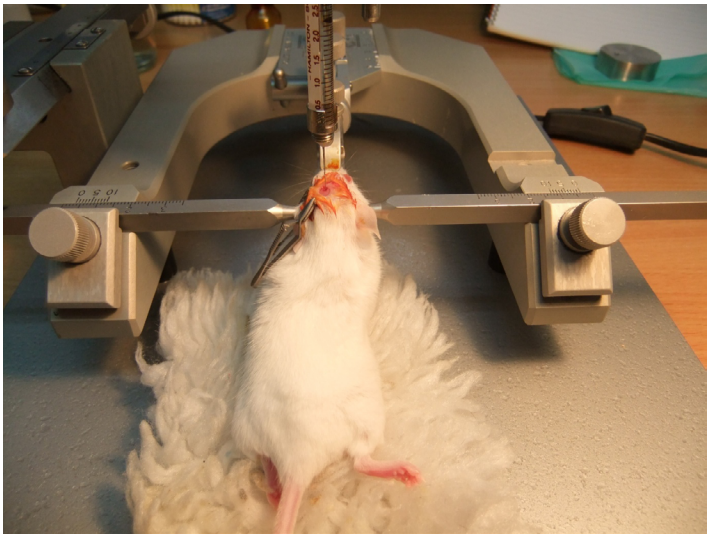


Figure 2: An albino mouse, deeply anaesthetised, mounted in a stereotaxic apparatus. Note the ear bars and incisor (upper front teeth) bar and nose clamp holding the head firmly in position. It is crucial that the head is held rigidly and securely, without exerting excessive pressure that might cause unwanted tissue damage. A microinjector syringe projects through a hole drilled in the left cranium ready for injection of 0.1 µl of a solution of the cytotoxin NMDA, which will lesion an area of the dorsal hippocampus, 1.9 mm below the skull surface.

Before a stereotaxic cytotoxic lesion is attempted, a reference point on the skull surface of the animal must be selected and determined. Many researchers work from the intersection of the sagittal and bregma sutures on the anterior skull surface but this can be difficult to see in some mouse strains, particularly the C57BL/6J which is frequently used in neuroscience studies. Bregma may be more accurate for anterior brain regions especially for mice of varying weight/age but stereotaxic zero (the point midway between the earbars which are inserted into the ear canals) serves well for the closely adjacent hippocampus (as long as the mouse is accurately positioned in the ear bars), and it has even been used for medial prefrontal and orbitofrontal cortex lesions (Deacon et al. 2003; Finger et al. 2011) [2,3]. If work is planned using stereotaxic zero as a reference landmark, first fix the left ear bar at zero minus half the diameter of the injector needle and adjust the carrier arm until the needle just touches the central tip of the ear bar. Note this lateral reading, also the anterior-posterior reading.

Make sure all instruments and anything that might come into contact with the exposed tissues of the mouse is sterile. Observe good sterile technique throughout the operation. Autoclaving can be used for heat-resistant objects like most metal surgical instruments, alternatively a proprietary cold sterilant can be employed. Use 70% ethanol with a very small amount of tincture of iodine added to sterilise the microinjector as the glues used in its construction are heat-sensitive, and there is a risk of cold sterilants, which naturally contain solutes, causing blockages or damage to the fine tubing or syringe itself. Create a sterile area next to the stereotaxic frame where instruments etc. can be placed. A heated glass bead steriliser is useful to maintain sterility of instruments during the operation; alternatively, use cold sterilant liquid. Observe recommended exposure durations for all sterilising procedures.

Anaesthetise the mouse. Isoflurane is a recommended inhalation anaesthetic although almost any injectable or inhalation anaesthetic will suffice. Take care when using ketamine or other NMDA receptor antagonists, although the high local concentration of NMDA at the microinjector tip will probably adequately compete with the relatively lower systemic concentration of any circulating anaesthetic with competitive NMDA receptor antagonistic properties. The author and others have successfully used Zoletil, which is a 50:50 mixture of tiletamine (a competitive NMDA receptor antagonist, dissociative anaesthetic), and zolazepam, a pyrazolodiazepinone structurally related to the benzodiazepine minor tranquillisers, either alone, in common shrews (Sorex araneus) in Russia, or mixed with xylazine (an α2 noradrenergic agonist). Contrary to a report in the literature, Zoletil has minimal cardiorespiratory depressant actions in mice, at least in the C57BL/6 strain. Indeed, in 2014 110 stereotaxic surgeries on this strain, (sham, rostral or caudal hippocampal lesions) with not a single case of morbidity or mortality. Recovery may be slow (2-3 h) but this may not be detrimental following cytotoxic removal of hippocampal tissue, which frequently results in lethal seizures, especially with isoflurane anaesthesia; the zolazepam has anticonvulsant properties.

Avertin (an old trade name) anaesthetic, a mix of tribromoethanol and tertiary amyl alcohol, has also been used in stereotaxic surgery. Reports of paralytic ileus following Avertin administration may have resulted from it becoming degraded into toxic and irritant compounds if not stored correctly (in a fridge or freezer and away from sunlight). Stored correctly or made up fresh, it appears to be suitable for rats and mice, although the duration of action is a little short (approximately 20-30 minutes). As with any intraperitoneally administered compound, it must, of course, be injected correctly, neither too deeply into the intestines or too shallowly, intradermally or subcutaneously.

Fix the mouse in the stereotaxic frame. (A Kopf small animal frame, model no. 960, is envisaged in the present description; details may vary on other devices). This is the only part of the operation that requires real manual skill, and practise is needed before it can be done routinely. The incisor bar is moved clear of the mouse's nose; it is of no assistance (in fact an encumbrance)

in obtaining a correct ear bar placement. The left ear bar should be fixed in its near-final position (for most mice this is 3.5 mm, i.e. the distance between the two ear bar tips, when inserted correctly, is 7 mm.). Support the mouse under the lower jaw, taking care not to obstruct the airway. It may be advisable, especially if you are inexperienced, to have the animal only lightly anaesthetised at this stage, to minimise any risk of killing it by pressing on, and occluding, the airway. Look at the head from directly above: is it in the correct position – as near as possible to its final attitude when correctly mounted, pointing straight ahead, not inclined to the left or right. Keeping the head level, gradually raise it until the left ear bar can be inserted into the external auditory meatus (ear canal). With the left hand squeezed tight between the left ear bar and the base of the frame, immobilising the palm region and thereby facilitating precise movement of the fingers and the ability to maintain a set position once achieved, the position of the mouse can be adjusted exactly. Maintain slight pressure of the mouse's head against the left ear bar. Now move to the right ear bar, which should be loosely held in its socket (permitting at most about 1 mm of lateral movement at the tip) and introduce it into the right meatus. Gently push it in until the head is held firmly and loosely tighten the retaining screw until the bar is just held firm.

Then gently rotate the mouse, in the pitching plane, by the tail, up and down on the ear bars a few times to centre the head, adjust both ear bars successively until the head is exactly central and the bars as tight as possible, then rotate the mouse a few more times. The ideal tightness of the ear bars is when the head just remains in the same position when rotated in the pitching plane; take great care not to damage the ear canals with excessive pressure. Finally fix the head by inserting and tightening the incisor bar. This is done by depressing the lower jaw and tongue of the mouse, opening the jaws wide (use a coarse pair of forceps or a small L-shaped rod made of approximately 2 mm diameter stainless steel. This can be made from a section cut from a bicycle spoke, the vertical, longer limb being 6 cm and the short, horizontal limb 1 cm long). The incisor bar, with its restraining screw tightened to allow at most 2 mm vertical movement at the tip, is now moved until it just touches the upper incisors. Gently ease the bar towards the mouse; the upper incisors should be guided up the bar and then glide along the upper bar surface; while keeping steady gentle pressure down by depressing the lower jaw, the upper incisors should drop into the hole in the incisor bar. Now release the lower jaw and use the forceps/bar to push down on the mouse's nose, ensuring the upper incisors correctly enter the hole in the bar. Very carefully move the incisor bar in a direction away from the mouse until a small amount of resistance is felt, then tighten the incisor bar restraining screw. Do not exert any more than very gentle forward pressure, or the mouse may slip out of the ear bars. This generally results in bleeding, damaged ears and the necessity to immediately kill the animal. The head should now be rigidly fixed, and with a microsyringe attached to the carrier arm of the stereotaxic frame, which can be moved in xyz coordinates by turning the adjusting screws, precise quantities of NMDA solution can be delivered to a target site within the brain.

Although this description may seem excessively long and detailed, it is necessary to describe a sequence of procedures that rapidly become assimilated into a brain schema that will eventually become virtually automatic.

Shrews (Sorex auraneus) have tiny ear canals, less than 1 mm in diameter. Shrew-size ear bars can be made by glueing 26 ga hypodermic tubing to the reversed ends of the normal Kopf ear bars. The author devised a platform surmounted by a selected number of microscope slides to position the ear canals close to these bars in the vertical plane. The snout of the shrew was further elevated by a coin. Thus, the head of the anaesthetised shrew could be slid into the correct vertical plane. This makeshift arrangement made it possible to insert these tiny bars into the equally small ear canals. A similar sliding platform arrangement might be useful when inserting ear bars into mouse, or even rat ears, if necessary, for stereotaxic beginners.

General Procedure

It is essential to keep the animal warm during surgery. Use a heating pad under the animal; the ones that are thermostatically controlled by a rectal thermometer are best. Or use an angle poise lamp over the mouse; this is also useful for seeing the microinjector scale and operative site. NB take care not to position the lamp too close to the mouse. Core body temperature will not reflect the temperature of the extremities, particularly the ears and feet which are not protected by fur. These may appear normal in appearance during surgery, but the damage will be apparent several hours later, even resulting in the virtual loss of the fragile ears.

Shave the planned incision site. Sterilise the scalp with a suitable sterilant such as povidone iodine (Betadine) or a chlorhexidine-based scrub. Make a small anterior-posterior (A-P) incision in the scalp, beginning from just posterior to an imaginary line between the back of the eyes, which should be over the bregma suture, extending about 4 mm back to the lambda suture where the skull starts to curve down. Infuse a little local anaesthetic solution (a drop or two of 2-5% Xylocaine (lidocaine) or better (longer duration) Marcaine (bupivacaine) between the skull and overlying skin. Renew this every 30 min throughout the procedure and 5 min before final suturing. Calculate the stereotaxic coordinates from either stereotaxic zero or bregma. The A-P and lateral (L) position of bregma is determined by lowering the injector until it is just over and aligned with the intersection of the central and bregma sutures. The vertical (V) coordinate can be derived later from the

surface of the brain over the injection site, alternatively from the surface of the skull, either directly over the injection site or at bregma. Lower the microinjector until it just touches the skull; by gently pushing the injector from side to side with e.g. a needle while lowering it, the exact point when the tip first scrapes the skull can be precisely determined. Likewise, the vertical position of lambda (the posterior skull landmark) can be determined. The best and most recent mouse brain atlas (Paxinos & Franklin 2019) uses a level head, i.e. bregma and lambda are in the same horizontal plane. Adjust the incisor bar to achieve a level head; -2 mm is approximately correct for the C57BL/6 mouse strain.

Use the mouse brain atlas to initially calculate coordinates, then check these by pilot experiments; mice will differ slightly according to strain, sex and age. One way to mark injection sites in these pilots is by injecting a small amount of dye (say 0.05 ml of cresyl violet or Evan's blue solution). Then the brain is perfused, via the intra-cardiac route, with saline (20 ml or more to obtain a clear perfusate) followed by formalin-saline (20-40 ml), and sectioned prior to microscopical examination. These preliminary determinations of the best injection sites would then be followed by second-stage full lesion pilots with injections of cytotoxin followed by recovery before perfusion, sectioning and examination. (Normally gliosis at the lesion site will be evident after a few days, but many lesions are easier to evaluate after weeks or even months because the lesioned tissue largely disappears). To make a microinjection, first set the stereotaxic coordinates, then lower the microsyringe until just over the skull. Memorise this position, raise the microinjector, then, using an 009-012 gauge dental burr, drill a tiny pilot mark at this point. Check, adjust if necessary, and proceed to drill the full hole in the skull. Lower the microinjector to check the hole is correctly positioned. The dura may need to be cut, e.g. with a 26 ga needle tip. Alternatively, an area of skull may be removed rather than making individual holes.

Microsyringes must be treated with great care. In particular, if filled directly with a solution, the plunger may become stuck due to evaporation of the solvent. Therefore, it is safer to initially partially fill the syringe with distilled/deionised water. Check all air bubbles are removed and the water can be ejected freely, i.e. the microinjector is not blocked. Then lower the plunger so that most but not all water is expelled, dry the injector tip, then withdraw the plunger a small amount (0.1 – 0.2 ml) so as to create a small air bubble in the syringe. Then fill it with the dye or cytotoxin. The small air bubble will serve as a buffer between the water protecting the syringe and the cytotoxin. More importantly, it will allow you to be sure that the solution has been actually injected. For example, the microinjector needle might become blocked on its descent into the brain. Then, when the plunger was depressed, the solution could be forced back around the piston and not overcome the block. During a good microinjection, you should be able to watch both the upper and lower meniscus of the air bubble move smoothly together down the tube of the microsyringe. If only the upper meniscus moves, there must be an obstruction.

To make the lesion, simply fill the syringe with cytotoxin, carefully wipe the injector to remove any external NMDA solution, lower it through the hole you have made in the skull, then slowly inject it. Minimal tissue damage is caused at a rate of 0.1 ml/min. Wait for the toxin to diffuse into the surrounding tissue for at least 1 min. Slowly withdraw the syringe by a small amount (0.2-0.5 mm depending on site). Leave for 3 min, then slowly withdraw the microinjector completely from the brain. Repeat this process for each injection site. NMDA is a very effective cytotoxin and much cheaper than ibotenic acid which can also be used. Other substances include quinolinic and kainic acid; toxins can differ in their selectivity. NB for unknown reasons which cannot be entirely typographical, the concentrations reported in some publications may vary by several orders of magnitude from that expected; double-check! Most researchers use 10 mg/ml NMDA, dissolved in phosphate-buffered saline. Typical injection volumes range from 0.05 to 0.2 ml per site. If the structure to be lesioned is large, it may be good practise to lower the injector tip beyond the target site then withdraw it to the target before injecting, so the solution can better diffuse into the ventral tissue.

Site	AP(+)	L(±)	V(-)	Volume (µl)	Initially (mm) past target	Withdraw (mm) 1 min after injection
1	2.1	1.2	1.9	0.1	0	0.2
2	1.5	1.7	1.9	0.15	0	0.2
3	1	2.2	2	0.1	0.3	0.2
4	0.7	2.8	4	0.2	0.2	1

Table 1: Stereotaxic coordinates for mouse (C57BL/6 strain) hippocampal lesions. Values are mm from (AP) the interaural line; (L) the midline; (V) the surface of the skull at bregma. NB values have been changed slightly since the references cited below as part of a process of continual refinement.

Finally suture the scalp incision. To inhibit mice from pulling out the stitches, place a tiny drop of veterinary super-glue (cyanocrylate adhesive) on each knot. Give 0.2 ml sterile saline subcutaneously or intraperitoneally per hour of operation. Remove the mouse from the stereotaxic frame (having identified it by ear-marking or an electronic tag/chip if desired) (Deacon 2006) [4,5] and keep it in a warm place until it recovers enough to move. (It is essential to keep it warm during the operation too; this may be achieved by homeothermic heating pads or simpler options such as wrapping it in insulating material such as plastic bubble-wrap and directing an angle-poise lamp over it). The proprietary fluffy material "Vetbed" is ideal for insulating the mouse from the cold base of the stereotaxic frame.

To control seizures from hippocampal lesions, give 10 mg/kg chlordiazepoxide HCl intraperitoneally approximately halfway through the operation, or as necessary (indications of NMDA toxicity: respiration rate increases, vibrissae start to twitch) and as necessary after the operation. This will prolong recovery time, but mice can easily die from seizures. Remember that the chlordiazepoxide will also potentiate the anaesthetic during the operation. It may be best to do the larger injection volume sites at the start of the operation to minimise post-operative complications from NMDA toxicity. Finally replace the mouse in its home cage, preferably with plenty of insulating bedding (make it into a nest shape as they will have lost the ability to do this). Glucose (5-10%) added to the drinking water will encourage rehydration and provide a calorie boost for the first day or two. Weigh the mice every day until weights have recovered towards pre-operative levels. Leave at least 7 days and preferably longer before beginning behavioural tests.

Habenula Lesions

These are performed similarly to the above but of course different coordinates are used, viz. AP + 0.22 and 0.29 cm, L ± 0.04 cm, V – 0.3 cm.

Microinjector Manufacture

Fitting a 34 ga stainless steel tube into a 26 ga outer support tube into a commercial microlitre syringe:

1) Basic syringe: ESGE 5ml removable needle model Superflex 001150. This is supplied with a 26 ga needle, but this is too small internally to take a 34 ga tube. Also, the butt of the needle, where it fits into the syringe barrel, is 0.32 mm o.d., whereas the main shaft, distal to the brass collar, is 0.47 mm o.d. (26 ga) (Hamiltons are 26 ga the whole length). Remove the syringe needle, and the white plastic washer, push a 26 ga tube, tapered to about 0.32 mm (can be done on an abrasive wheel with a light touch and a steady hand, or better by holding the tube in a pin chuck and tapering it down on a very fine abrasive stone) if fitting into an ESGE syringe, through the latter and into the syringe barrel to make a tight fit. Fill the junction with Araldite Rapid cement to make a liquid-tight seal. Insert a 34 ga tube just into the 26 ga, and apply a tiny amount of Araldite to the former. Slide/twist the 34 ga into the 26 ga the required amount.

2) Basic syringe: Hamilton 5ml removable needle model 75RN (Fisher Scientific, Loughborough, UK). The plunger diameter is 0.33 mm (10 ml Hamilton is 0.50 mm)

This is supplied with a 26 ga needle, but this is too small internally to take a 34 ga tube. Therefore remove the supplied needle and fit a length (e.g. 25 mm) of 26 ga tube, Araldite it into the base of the Hamilton, then Araldite/superglue the 34 ga into this 26 ga. NB Before applying glue to the base of the Hamilton + 26 ga tube, ensure that the small white washer in the former, into which the 26 ga fits, is pushed firmly in, otherwise a dead space and leaks will be created.

Probably a better way as you do less damage to the microsyringe

Alternatively, link a 26 ga (with a 34 ga tube glued in) to the supplied 26 ga needle, cut to size, with a "sleeve" of 21 ga tubing, although again some dead space will be created. Minimise the dead space by proceeding as follows: first glue 34 ga into 26 ga. With 26 threaded on to 34 and about 8 mm 34 protruding, glue on to 34 then pull back into the 26 almost flush, turning as you go to form a seal all the way round, and if necessary wiping off any excess glue before it comes almost flush. When set, glue a 21

on, then when that is set, finally thread on to the 26 ga needle of the Hamilton.The tip of the 34 ga microinjector will penetrate the brain tissue more effectively if it is sharpened (bevelled all round). This can be easily achieved by holding the 34 ga in a pin chuck (before mounting it into the 26 ga) and stroking on a fine oilstone away (to lessen the risk of blocking the fine tube) from the end, while gently rotating it. The tip can be viewed under a low power microscope and improved until satisfactory.

A larger diameter microinjector can be made from a (widely commercially available) 30 ga needle cut from an insulin syringe. This has the advantage of being less liable to suffer from blockages, which are sometimes a problem when using 34 ga. There will, however, be slightly more mechanical tissue damage. How to cut stainless steel hypodermic tubing to obtain a cleanly severed end: holding the tube with a pair of pliers, with a small ("mouse tail") triangular file make a small abrasion on the tube wall (it shouldn't go all the way through). Keeping the same steady hold with the pliers, grasp the tube on the other side of the cut, close to the cut, with another small pair of pliers; wiggle back and forth towards and away from the file mark and the metal will fatigue and break. Finish off by smoothing the edges with a file or a sharpening stone.

Euthanasia

Generally, an animal is killed at the beginning of an experiment (for biochemical and physiological research), or in behavioural experiments, typically, at the end to verify the extent of the brain lesion etc. which the experimenter performed. Killing, or giving assent (to a technician) to kill an animal, is not a decision without emotion. This is natural, and most researchers become used to it as a necessary evil of their work. If you have to do it yourself, become perfect at several different techniques. Cervical dislocation: Pin the animals head with your fingers to a solid surface such as a bench. Holding the tail, pull up and away. Concussion: Holding the animal by the tail, strike the dorsal surface of the skull hard on a solid surface Carbon dioxide: Place the animal(s) in a euthanasia chamber and slowly increase the CO2 concentration until all respiration has ceased. Leave for 10-30 min to confirm death by rigor mortis. Lethal injection: Administer a large overdose of an anaesthetic such as Euthatal (pentobarbitone).

Confirmation of death: Rigor mortis or prolonged cessation of respiration.

References

1. Jarrard LE (1989) On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. J Neurosci Methods 29: 251-259.
2. Deacon RMJ (2006) Housing, husbandry and handling of rodents for behavioral experiments. Nat. Protocols 1: 936-946.
3. Deacon RMJ, Penny C, Rawlins JNP (2003) Effects of medial prefrontal cortex cytotoxic lesions in mice. Behavioural Brain Research 139: 139-155.
4. Finger BC, Deacon RMJ, Burns P, Campbell TG (2011) The effects of lesions of the hippocampus or orbitofrontal cortex on an operant box based intertemporal choice task in mice. Journal of Neuroscience, Psychology, and Economics 4: 217-234.
5. Paxinos G, Franklin KBJ (2019) The Mouse Brain in Stereotaxic Coordinates, 5th Edition. Academic Press/Elsevier 2019.