Appendices

Appendix 1: Reporting Abnormalities in Experimental Animals

Report Checklist

- Describe the species, strain, age, weight, and sex.
- Describe any abnormal clinical signs: size, position, coloration, etc.
- Isolate the cage.
- Assess the animal's environment.
- Check the feeder and water bottle (or watering system).
- Check the inside of the cage and the litter for abnormal fecal material or urine.
- Observe the behavior and attitude of the animal in its home cage.
- Open the cage and proceed with the examination of the animal.
- Note the animal's general appearance, fur and color of the skin, any abnormal movements, thinness or obesity, discharges or excessive secretions, etc.
- Note the reaction of the animal when handled (aggressiveness, hypotonia, dehydration).
- Examine the animal's back, abdomen, and body extremities.
- Check for any misalignment or overgrowth of incisors.
- Check the animal's records for any previous clinical observations, history, surgery, etc.

Be familiar with the characteristics of the particular species and strain being examined, since important differences do exist (alopecia, obesity). In any case, consult with the principal investigator and/or veterinarian in charge.

B. Ferry et al., *Stereotaxic Neurosurgery in Laboratory Rodent,*
DOI 10.1007/978-2-8178-0472-9, © Springer-Verlag France 2014

Appendix 2A: Typical Follow-Up Record of Stereotaxic Surgery

Name of operator :	Protocol or project number / code :
Animal id number / code :	Species / strain / sex :
Study / experimental number :	N° cage :
Lot number :	Date of reception :
Room number :	Date of surgery :

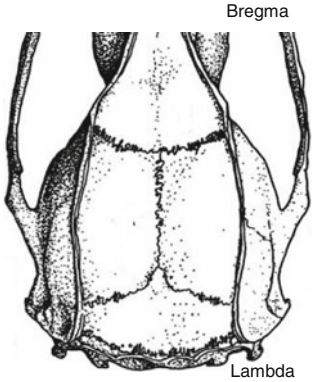
Type of surgical operation: Pre-operatory body weight:

Anesthetic		Date and time of administration	Analgesic		Date and time of administration
Type	*Dose*		*Type*	*Dose*	

Record of stereotaxic coordinates:

Target area / region :			
Atlas coordinates:	AP: - Y	ML: ± X	DV: - z
Coordinates used :	AP	ML	DV
Bregma	A	B	C
Lambda	U	B	C
Calculation of target coordinates	A - Y	B ± X	V - Y
Connector or cannula (e;g. 1 mm above the target)			V – Y - 1
Strereotaxic apparatus station / n°			
A, B, C, U *are arbitraryunits used here as an example*; V is the DV coordinate measured above the target			

Visual localization:

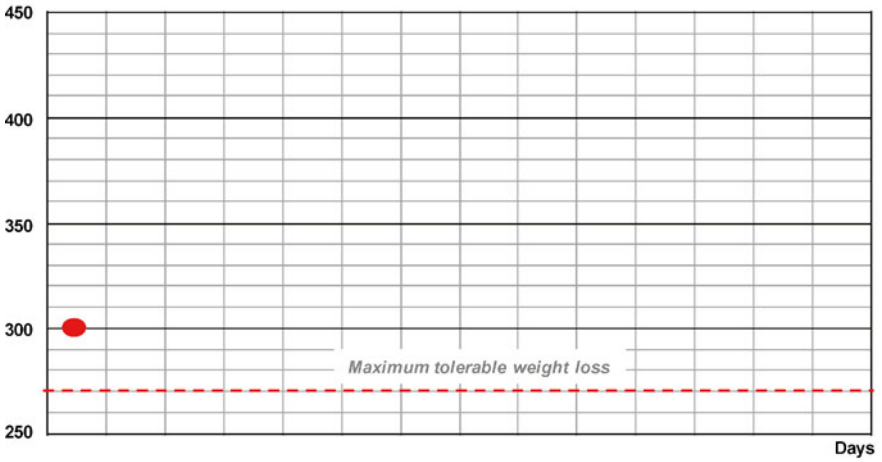


This surgical follow-up form should be retained by the research investigator in his/her laboratory notebook. A part of the form should be reserved for use by animal care staff to record any additional treatments that may eventually be carried out.

Appendix 2B: Typical Postsurgical Follow-Up Report with Daily Observations

Animal identification number :

Monitoring of body weight (in grams), e.g. 300 g on the day of surgery



Date	Behavior	Food / Drinking water	Signs of pain, discomfort	Treatment	Observations

Remarks :

Any animal euthanized or found dead should be retained for autopsy by the principal investigator or veterinarian.

Appendix 3: General Criteria for Deciding on Euthanasia

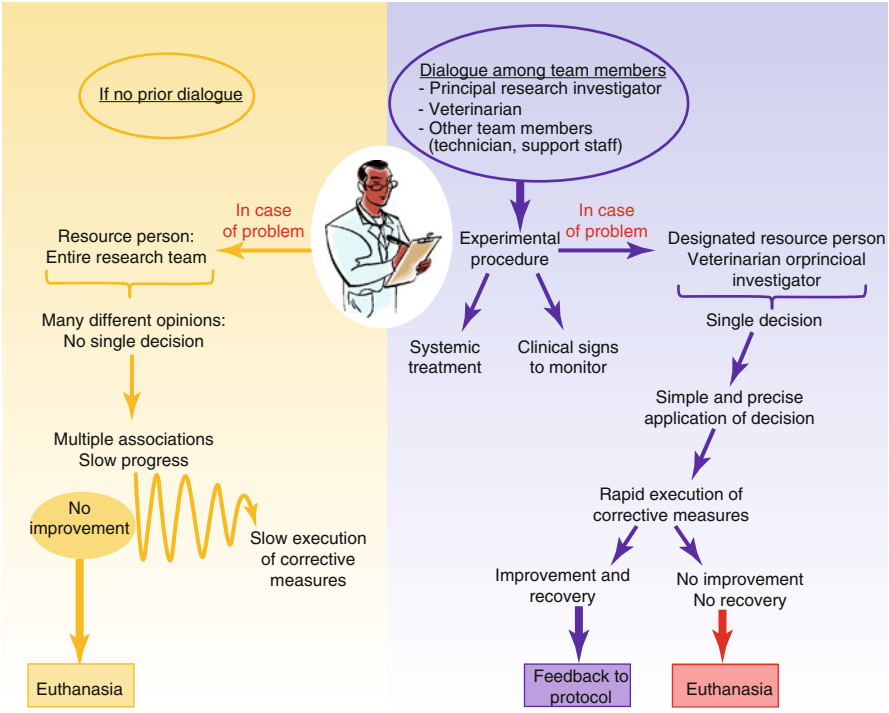
During the course of an experiment, an endpoint is reached when, considering the objectives of the study, any pain and/or distress of the animal needs to be minimized, reduced, or stopped. Measures must be taken to terminate any procedure that produces suffering, either by appropriate interventions and treatments of the animal or by its humane euthanasia. In practice, the decision to euthanize requires the use of an evaluation tool for assessing pain or discomfort, based on different but easily observable criteria.

Examples of Criteria

- Anorexia and weight loss
- Self-injurious behavior, alopecia (loss of hair), and skin abrasions
- Altered state of consciousness: lethargy or apathy
- Modified, irregular, difficult, or noisy breathing; coughing, rattling, wheezing, or whistling
- Abnormal color/appearance of the mucous membranes: anemia, jaundice, or congestion
- Development of spontaneous tumors affecting the animal's normal behavior and habits
- Abnormal neurological signs: inclination of the head, tremors, spasticity, convulsions, stereotypies, paresis, paraplegia, and paralysis
- Changes in locomotor function affecting drinking and food intake

These criteria are evaluated quantitatively and regularly, in order to score the condition of the animal relative to a preestablished and quantifiable threshold score defined by consensus as the endpoint. All available means (analgesic or other) are used to help the animal regain a state of health and behavioral well-being that will allow its comfort and survival. However, if the threshold is reached or exceeded, the animal must be euthanized.

Appendix 4: Taking Care of Pain: From Observation to Decision to Execution



To ensure and facilitate a proper postoperative follow-up, a simple decisional tree should be established at the outset of the experimental protocol, through dialogue between active and accessory participants in the study. This dialogue will help to define a procedure that addresses any eventual clinical signs to be aware of, the systemic treatments to use if necessary, and the contact person (principal investigator and/or veterinarian) in case of problems. This flowchart is intended to provide a quick and single unambiguous decision and a simple and precise action plan of corrective measures. If problems are common, it may be necessary to modify the experimental protocol, provide operators with additional training in surgical or animal care procedures, or increase the postoperative recovery time of the animal.

Appendix 5: Checklist for Surgical Procedure Setup

THE DAY BEFORE SURGERY	Stereotaxic base and frame	Verniers
		Electrode holders
	Magnifying lenses and optics	Light bulbs, other sources of illumination
		optics
	Artificial respirator, anesthetic vaporizer	Anesthetics fluid levels
		Tubing and connections
	Surgical intruments	Instrument boxes
		Sterilization of instruments
	Stock of consumable items	Drugs
		Sutures and compresses
	Verification of working order and cleanliness of equipment required for the procedure	

- *Preparation of rooms:* cleaning of Faraday cages, magnifying lenses, and bench tops and preparation of surgical instrument trays, wash bottles of alcohol, chlorhexidine or Hibitane®, hair clippers and combs, anesthesia tray, syringes and needles, and injection vials.
- *Verification of operating room equipment and materials*: instrument tray checklists; instrument cases, trays, or holding cups; surgical drapes, sterile sponges, and swabs; dental cement preparation trays; and organic waste bins, kidney dishes, and containers for sharp objects (e.g., disposable scalpel blades, syringe, or suture needles).

The Day of Operation

1H before	Clinical examination of the animal	Observation of behavior	
		Weigh-in	
NON-STERILE	**SURGICAL GLOVES AND GROWNS**		
	Analgesia and anesthesia	Injection of Rimadyl®(NSAI)	
		Sedation	
		Induction of anesthesia	
	Disinfection of the paws, tail and operatory field	Shaving	
		Cleaning of the surgical zone (chlorhexidine SCRUB (Hibitane®, Iodine antiseptics Betadine®)	
		Alcohol wash	
	Anesthesia	Injection or intubation	
	Positioning the animal	Heating blanket or pad, temperature probe	
		Ear bars, nose clamp, incisor bar	
STERILE	**SURGICAL GLOVES AND GOWNS**		
	Placing the sterile drapes	Opening of drape box	Surgical assistant
	Surgical instrument tray	Opening of surgical instrument boxes, blades, compresses	Surgical assistant
		Fill disinfectant cups	Surgical assistant
	Aseptic operating zone	Fill irrigation cups	Surgical assistant
	Placement of waterproof surgical barrier drape		
	Incision		
	Dissection		
	Hemostasis		
	Hydrogen peroxide wash	Disposal of used compresses	
	Checking the positioning of the animal	Respecting the chain of asepsis	
	Taking coordinates of the stereotaxic reference points		
	Drilling		
	PROTECTION OF SURGICAL WOUND WITH COMPRESSES + STERILE PHYSIOLOGICAL SALINE SOLUTION		
NON-STERILE	Technical procedure: verification of electrodes, connectors, surgical screws, dental cement, adhesive resin…		
	DISINFECTION WITH CHLORHEXIDINE SPRAY		
	CHANGE OF SURGICAL GLOVES		
STERILE	**ACUTE ELECTROPHYSIOLOGY**	**CHRONIC ELECTROPHYSIOLOGY**	**ACUTE MICRODIALYSIS** / **CHRONIC MICRODIALYSIS**

RECOVERY FROM ANESTHESIA

The animal is maintained on the heating blanket, and then placed in heated holding cage or box until it awakens.

or

EUTHANASIA		
Deep anesthesia	Perfusion	Dissection of tissue samples for histological examinations

OPERATING BLOC CLEAN-UP	**KEEP GLOVES ON, PUT ON SURGICAL GOWN**	
	Gather up contaminated items	Organic wastes
		Sharp object waste containers
	Management of surgical equipment	Soak surgical instruments and related equipment
		Drain briefly, wipe and let dry
	CLEAN AND DISINFECT BENCHES, STEREOTAXIC STAND AND OPERATING MICROSCOPE USING CHLOREXIDINE SPRAY OR TOWELETTES	
	Disposal of wastes	Collect reusable surgical linens for washing
		Empty the waste bins
	REMOVE GLOVES AND GOWN THEN CLOSE THE OPERATORY BLOCK	

THE DAY AFTER THE OPERATION	Arrange the clean dry instruments in their boxes
	Close the box with sterilization indicator tape, record date and operator name, sterilize for 1.5H at 180°C
	Refill the compresses boxes
	Package the boxes of surgical linens
	close the box with sterilization indicator tape, record date and operator name, sterilize for 1.5H at 180°C
	Restock the instrument trays
	Check the stock of injectables (anesthetics, analgesics)
	Store away clean equipment and materials

Appendix 6: Ethics in Animal Experimentation

Since ancient times, medical practitioners have used animals to satisfy their intellectual curiosity about structural and functional anatomy. It was not until the early seventeenth century, however, that British physiologists began to be concerned about the welfare of experimental animals. Since then, considerable efforts have been made to replace animals with alternative models for teaching the life sciences, for example, by using cell cultures derived from skin or tumors, lower vertebrates (bacteria, yeast, shellfish, and insects), or computer simulations. Over time these alternative techniques have allowed a reduction in the number of animals used in research and teaching. These methods have their limitations; however, the complexity of an entire intact living organizing cannot be reproduced in the laboratory by a simple layer of single uniform cells, by bacteria, or by a computer model. Thus, when it can be shown that there is no alternative method available to achieve the objectives of research having an established scientific or medical need, recourse is given to the ethical use of animals. Ethics define and clarify the concepts that characterize good or bad practices and recommend corrective measures accordingly. Whenever the use of animals in research is discussed, ethical issues and animal rights must obligatorily be taken into account.

The Principles of the "3Rs"

After the First World War, certain groups interested in the welfare of animals used for scientific purposes came into being, including the Universities Federation for Animal Welfare (UFAW). The UFAW commissioned William Russell, a philosopher, and Rex Burch, a microbiologist, to write a manual, *The Principles of Humane Experimental Technique (1959)*. This seminal work first introduced the notion of the "3Rs," which became a focus point for more than 2000 scientists, veterinarians, animal care technicians, students, community representatives, and animal welfare associations who contributed to the ethical review system and supervision of the Canadian Council on Animal Care (CCAC) established in Canada since 1968. These three principles are "replacement," "reduction," and "refinement."

Replacement often means the use of an inanimate system as an alternative (e.g., mannequins, computer models or programs, multimedia teaching aids such as videos). It can also mean the replacement of sentient animals (usually vertebrates) with less sentient animals (usually invertebrates such as worms and bacteria). It also includes the use of cell and tissue cultures. The cells must come from somewhere and often this means animals.

Reduction means a decrease in the number of animals used previously with no loss of useful information. This can be achieved by reducing the number of variables through good experimental design, by using genetically homogeneous animals, or by ensuring that the conditions of the experiment are rigorously controlled.

When animals cannot be replaced, their number should be limited to the minimum number required to achieve statistically significant results. Often this can be determined a priori by a statistical "power analysis" to estimate the requisite sample size (minimum number of animals per experimental group). Protocols should not unnecessarily repeat previously validated studies.

Refinement means a change or improvement in some aspect of the experiment that results in a reduction or replacement of animals or in a reduction of any pain, stress, or distress that animals may experience. The establishment of early endpoints for intervention in a study that has the potential to cause pain or distress is an example of refinement. The use and development of techniques and noninvasive technologies can contribute to refinement, improving the quality of experimental results by minimizing the discomfort of the animal (better procedures in anesthesia and analgesia, magnetic resonance imaging, telemetry for remote recording of temperature, blood pressure and heart rate, etc.).

- A fourth "R" has been defined by the University of Sherbrooke (Canada) as the "responsibility" of the individual investigator with respect to the use of animals for research purposes, i.e., credibility and scientific justification for using experimental animals as well as empathy, compassion, sensitivity, and attention to their physical and psychological well-being.

Appendix 7: Nonsteroidal and Opioid Analgesics and Dosages

Molecule	Rat	Mouse (hamster, gerbil)
Paracetamol (acetaminophen)	200 mg/kg p.o. 24 h	120 mg/kg p.o. 24 h
Carprofen	5 mg/kg IM, SC or p.o. 24 h	5 mg/kg SC 12 h
Flunixin	2.5 mg/kg IM or SC 12 h	2.5 mg/kg IM or SC 12 h
Ketoprofen	2.5 mg/kg IM, SC or p.o. 24 h	
Meloxicam	1–2 mg/kg SC or p.o. 12 h	2.5 mg/kg IM, SC or p.o. 24 h
Buprenorphine	0.01–0.05 mg/kg SC or IV 8 to 12 h 0.1–0.25 mg/kg p.o. 8 to 12 h	0.01–0.05 mg/kg SC 8 to 12 h
Butorphanol	1–2 mg/kg IM or SC 2 to 4 h	1–2 mg/kg IM or SC 2 to 4 h
Morphine	2–5 mg/kg IM or SC 4 h	2–5 mg/kg SC 4 h
Nalbuphine	1–2 mg/kg IM 4 h	2–4 mg/kg IM 4 h
Pentazocine	5–10 mg/kg IM or SC 3 to 4 h	5–10 mg/kg IM or SC 3 to 4 h
Pethidine (meperidine)	10–20 mg/kg IM or SC 2 to 3 h	10–20 mg/kg IM or SC 2 to 3 h
Naloxone antagonist	0.01–1 mg/kg IM, SC or IV	

IM intramuscular, *SC* subcutaneus, *IV* intravenous, *p.o.* per os

Appendix 8: Anesthetics and Dosages

	MOUSE	RAT	DURATION ANESTHESIA / RECOVERY TIME	ANTAGONISTS OR RESPIRATORY STIMULMANTS	
Ketamine (Imalgène®, kétamine-Virbac, Ketanest, Ketaset, Ketalar) + Acepromazine (acetylpromazine; Calmivet, Vétranquil)	100 mg/kg 2.5 mg/kg IM, IP	75 mg/kg 2.5 mg/kg IM, IP	20 - 30 min / 2 h		Doxapram® (Dopram): 5-10 mg/kg IM, IV or IP respiratory stimulant, effective for all types of anesthesia including inhalational
Ketamine (Imalgène®) + Diazepam (Valium®)	100 mg/kg 5 mg/kg IP	75 mg/kg 5 mg/kg IP	20 min / 2 h		
Ketamine (Imalgène®) + Xylazine (Rompun®)	50 to 90 mg/kg + 5 to10 mg/kg IM, IP	90 mg/kg + 10 mg/kg IM, IP	20 - 30 min / 2 to 4 h	Yohimbine, non specific antagonist 0.2 mg/kg IV ou 0.5 mg/kg ; not recommended IM	
Ketamine (Imalgène®) + Medetomidine (Domitor®)	75 mg/kg + 0.5 mg/kg IP	75 mg/kg + 0.5 mg/kg IP	20 - 30 min / 2 to 4 h	Atipamezole, specific antagonist 1 mg/Kg IM, IP, SC ou IV	
Urethane	-	1000 mg/kg IP	6 - 8 h / WITHOUT RECOVERY		
Metomidate (Marinil, Methoxynol) + Fentanyl (Sublimaze, Actiq, Duragesic, Fentora…)	60 mg/kg 0.06 mg/kg SC	-	30 min / 1 h	Reverse opioid-induced respiratory depression and analgesia Naloxone (Narcan, Nalone, Narcanti): 0.01 – 0.1 mg/kg IV, IP or IM	
Fentanyl + Fluanison (Hypnorm®)	0.01 ml/30g IP	0.4 ml/kg IM, IP	20 min / 60 min +		
Fluanisone (Hypnorm®) + Diazepam (Valium®)	0.3 ml/Kg 5 mg/kg IP	0.3 ml/kg 2.5 mg/kg IP	45 - 60 min / 3 - 4 h +	Reverses respiratory depression and maintains analgesia Nalbuphine : 1 - 2 mg/kg/ 3h IM	
Fluanisone (Hypnorm®) + Midazolam (Hypnovel®)	10 ml/kg IP	2.7 ml/kg IP	45 - 60 min		
	Mixture: 1 part fluanisone + 1 part midazolam (5mg/ml) + 2 parts water		3 - 4 h +		

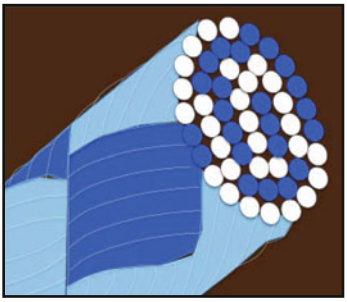
IM intramuscular, *SC* subcutaneus, *IV* intravenous

Appendix 9: Overview of Suturing Materials

While the sterility of suturing materials is a paramount prerequisite, a good tolerance or biocompatibility is of equal importance. The presence of a ligature causes an inflammatory reaction of the body, provoking an inflammatory granuloma that is particularly susceptible to infection. The intensity of the reaction depends upon the material (natural or synthetic) that constitutes the suture thread, its structure (monofilament or braided), and its capillarity. The breakdown process (hydrolysis or proteolysis) and time required for suture resorption are also contributing factors. Resorption time is a decisive factor in the choice of the thread, since the ligature needs to remain effective during the entire healing process. To prevent diffusion of liquids into the spaces between the filaments of the thread, it is preferable to use a non-capillary material such as a monofilament thread. Braided (multifilament) threads that are coated to reduce capillarity and drag can also be used. The strength of the thread must be able to resist tensile forces when the ligature is made, to last until the end of the wound healing process, and, despite a 40–50 % loss of this characteristic at the level of the knot, to allow for doing an additional knot.

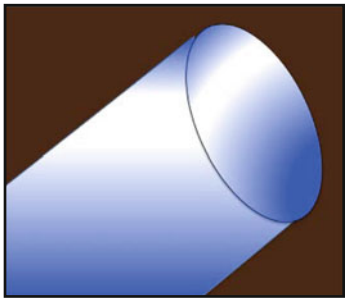
Types of Suture Thread

Braided (multifilament)



Capillarity (or wicking action)
Higher risk of infection
More drag
Lower tensile strength
Easier to grip
Secured knots hold well

Monofilament



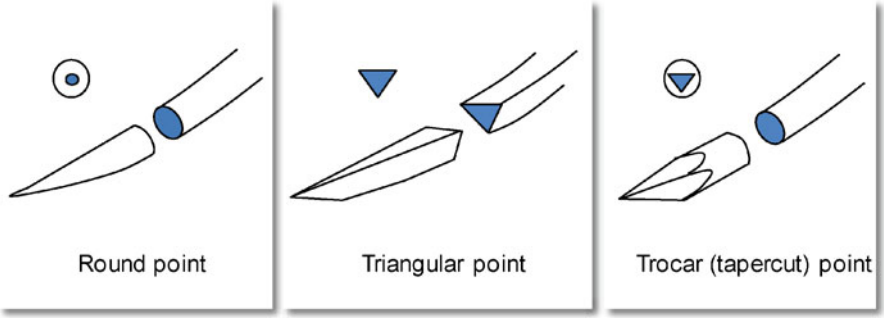
No capillarity
Lower risk of infection
Less drag
Greater tensile strength
Easier to grip
Greater memory effect of material*
Knots do not hold well

**The folded loops of thread, formed by packaging and storage (typically in a blister pack), tend to retain this shape during use and may cause accidental knotting or snagging when the working length of thread is not properly handled.*

Several features are used to describe a needle: its size, i.e., the length measured from the eye or the swaged (crimped insertion of thread) end of the needle to its point, the shape of the body, its curvature, and the form of the point.

The size is given in millimeters for conventional surgical sutures and in micrometers for microsurgical sutures. The majority of needles used today are swaged (crimped) onto a thread of the same diameter, the nature and length of which are specified on the package. Reusable and sterilizable needles can still be obtained from suppliers such as Unimed (Switzerland). The shape of its body and the form of its tip (point) define the needle. In cross section, the body is typically rounded, rectangular, or triangular. Points can be round, triangular, or trocar (tapercut, with three sharp cutting edges). The needle may combine identical or different body shapes and points, multiplying the assortment of needle types available.

Examples of Suture Needles Tips



Suture selection is dependent on the anatomic site, surgeon's preference, and the required suture characteristics. The choice depends on the tissue, septic constraints, strength requirement, and durability in situ. Absorbable sutures can cause more inflammation than non-resorbable types. On the other hand, with animals that are difficult to handle or sensitive to stress, it is best to avoid having to remove the stitches after the skin has healed. In this case, a rapidly resorbable thread is preferable for skin suturing. The main consideration in needle selection is to minimize trauma. The length, diameter, and curvature of the needle influence the surgeon's ability to place a suture. A triangular point is preferable for thick skin and muscle tissue. The curvature of the needle is chosen according to the type of suture envisaged. For example, for a scalp suture, needle curvatures between 1/3, 3/8, and ½ are typically used.

Appendix 10: Procedure for Cresyl Violet Staining

Cresyl violet is a basic histological dye that stains purple the basophilic (acidic) cellular components DNA and RNA.

Once the tissue sections have been mounted on glass slides and thoroughly dried, prepare a solution of the dye by dissolving 5 g of cresyl violet acetate (Sigma, product n° 1791) in a mixture of 600 ml distilled water, 60 ml of 1 M sodium acetate (8.16 g sodium acetate in 60 ml of distilled water), and 340 ml of 1 M acetic acid (20.4 ml of concentrated acetic acid in 320 ml of distilled water). After stirring the solution for 48 h, filter. When the solution is ready, proceed with staining. This takes place in three stages:

- *Staining of Slides*
  - Soak the slides (rinse) in distilled water for 2 min.
  - Soak the slides in cresyl violet solution (preheated to 55 °C) for 9 min, checking the coloration of the tissue sections regularly since this amount of time may vary as a function of the staining power of the dye solution.
- *Dehydration of Slides: Soaking Steps*
  - 2 min in three successive baths of distilled water.
  - 2 min in 70 % ethanol.
  - 2 min in 95 % ethanol.
  - 30 s in an acid alcohol mixture (10 ml of concentrated acetic acid in 200 ml of 9 % ethanol).
  - 2 min in 95 % ethanol.
  - 2 min in two successive baths of 100 % ethanol.
  - 5 min in a solution of LMR© (mixture of purified paraffin and plastic polymers, used for protecting the tissue sections for several years against wrinkling and loss of transparency). LMR© replaces toluene and xylene that have typically been used at this step in the past.
- *Cover Slipping*
  When the stained slides are completely dry, mount the glass cover slips using Eukitt© (Sigma). Dry the slides for 2 h in a 37 °C oven or at room temperature for 24 h before microscopic observation.