Surgical procedures in neonatal rats
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These notes provide a detailed description of the surgical preparation of rats two weeks of age and older for electrophysiologic recordings. They have been adapted from procedures used routinely in our laboratory for recording from adult rats. The procedures for young rats are probably readily adaptable for use in adult mice.

The explicit goal is to minimize procedural differences between young and adult animals (see Simons and Carvell, 1989) Anesthesia was induced with isoflurane and maintained with halothane in 50/50 N_2/O_2 mixture. For P13-15 and P20-22 pups, initial halothane concentration was 2% which was then reduced to 1.5% after approximately 3 minutes to avoid respiratory depression. For P27-29 and P65 animals, initial halothane concentration was 2.5% reduced to 1.8% after 3-5 minutes. Depth of anesthesia during surgery was monitored using respiratory rate and absence of foot or tail withdrawal in response to pinch. In P13-15 and P20-22 pups, respiratory rate of approximately 60 breaths per minute was found to correspond to adequate anesthetic control with minimal risk of lethal overdose. Hypothermia, especially in younger animals, was prevented by warming the rats with a 20W halogen lamp. In our experience, the lamp should be positioned no closer than 8 inches from the body to avoid halothane-related malignant hyperthermia.

Three preparatory surgical procedures - external jugular vein catheterization, tracheotomy, and femoral artery catheterization - were performed. For drug delivery during the experiment, the external jugular vein was cannulated according to the "through needle technique" described by Harms and Ojeda (1974). We use 7 cm piece of silastic tubing with an inside diameter of 0.30 mm and an outside diameter of 0.64 mm (Dow Corning, Midland, MI). The catheter was tunneled subcutaneously to the nape of the animals neck and kept patent during surgery with 0.02 mL bolus injections of 5% dextrose in 0.9% NaCl solution at 10-15 minute intervals.

In preparation for artificial ventilation, a tracheotomy was performed using a 25 mm piece of polyethylene tubing (PE90 for P13-15 and P20-22 animals, PE160 for P27-29 animals, and PE240 for P65+ rats) (Fisher Scientific, Pittsburgh, PA). The longitudinal neck muscles (sternohyoid and longus colli) were dissected along the midline through a small skin incision 2-3 mm rostral to the manubrium, and the trachea was visualized. To avoid impinging the phrenic nerves, inferior thyroid vessels and the recurrent nerve of the vagus were dissected away from the trachea bilaterally and used as lateral boundaries for passing a piece of 6-0 silk suture under the trachea. The trachea was incised transversely 2-3 tracheal rings caudal to the thyroid isthmus but 1 tracheal ring rostral to the underlying suture. The tracheotomy tube was inserted 8 mm into the trachea and secured in place using the suture. The wound margins were approximated using the distal ends of the suture.

For continuous blood pressure monitoring during the experiment, the left femoral artery was catheterized using PE10 tubing (inside diameter 0.28 mm, outside diameter 0.61 mm; Dow Corning, Midland, MI) with the cannula tip stretched to approximately 1/3 of its original

diameter and beveled at 45°. The femoral neurovascular bundle was visualized through a T-type incision using the lateral edge of the abdominal wall and the knee as the transverse and longitudinal landmarks, respectively. The nerves of the anterior division of the femoral nerve and the saphenous nerve were preserved by dissecting them away from their adjacent vasculature. The muscular and the superficial epigastric branches of the femoral artery and vein were ligated, and the vascular bundle was then mobilized proximally as far as the abdominal wall and distally to the level of the great saphenous artery and vein. Two pieces of 6-0 silk suture were passed under the vascular bundle - the proximal one close to the abdominal wall and the distal one at the level of the great saphenous vessels. With tension applied to the vascular bundle using the distal suture, a small spatula (~3-mm wide) was passed under the vessels and gently elevated in order to occlude blood flow. The artery was punctured with a 30 gauge needle angled proximally at 45°, and the arterial lumen was dilated with microdissecting forceps. The catheter was inserted by angling the 45° bevel parallel to the flat surface of the spatula, which provides a firm support during this procedure. The cannula was inserted ≈10 mm past the abdominal wall and the spatula was removed. Arterial blood should flow into the cannula upon application of slight negative pressure on the syringe. The proximal and distal sutures were tied; two additional sutures were used to secure the cannulated artery and adherent vein to the underlying muscles, taking care to preserve the saphenous nerve. The catheter was looped around the leg for tension relief, and wound edges were approximated. During the rest of the surgical preparation, the patency of the catheter was maintained using 0.02 mL bolus injections of 5% dextrose in 0.9% NaCl solution containing 1 USP unit/mL of heparin sodium (American Pharmaceutical Partners, Inc., Los Angeles, CA) at 30-45 minute intervals.

For access to the brain region of interest, the scalp was incised to the level of the aponeurosis, and a steel post was fixed to the right parietal bone using cyanoacrylate glue and dental acrylic. Care was taken to extend the dental acrylic over occipital and frontal bones in order to minimize movement due to unfused skull sutures. The steel post was used to hold the animal's head without pressure points throughout the rest of the experiment. The skull overlying the appropriate region was carefully thinned by drilling, and a craniotomy was made. In immature rats, bregma's position in relation to underlying brain structures differs between animals; therefore bregma was found to be less as a landmark in developing animals than the position of the surface vasculature. In general, brain nuclei tend to shift posteriorly and laterally during development. An atlas by Sherwood and Timiras (1970) provides an excellent resource.

The animal was then transferred to a vibration isolation table, the arterial line containing the heparinized saline was connected to a pressure monitor (BP-1 WPI, Inc., Sarasota, FL), and the venous line to two continuous infusion pumps (Razel, Inc., Stamford, CT). Paralysis was induced with a bolus dose of pancuronium bromide (0.8 mg/kg, slowly!), and artificial ventilation with 50/50 O_2/N_2 mixture was initiated using a mouse ventilator MiniVent for P13-15 and P20-22 animals and Inspira volume-controlled ventilator for P27-29 and P65 rats (both ventilators from Harvard Apparatus, Cambridge, MA). Initial ventilator settings are summarized in Table 1. Halothane was discontinued, and for the rest of the experiment, the animal was maintained on continuous infusion of pancuronium bromide (1.6-2.0 mg/kg/hr) and a potent synthetic opioid, fentanyl (≈10 μg/kg/hr) in 5%/0.9% dextrose/NaCl solution (0.5 ml/hr for P13-15 and P20-22, 1.0 ml/hr for P27-29 and P65+). The rats' body temperature was maintained at 37°C by means of a servo-controlled heating blanket (Harvard Apparatus, Cambridge, MA) and a

20W halogen lamp. We found that the extra heat provided by the halogen lamp (powered by a DC source) was required in the small animals.

To assess the rat's physiological condition during the recording experiment, we continuously monitored mean arterial blood pressure (MAP), pulse rate, tracheal airway pressure waveform including peak end inspiratory pressure (PEIP), capillary perfusion of glabrous skin and pupillary reflexes. As in other mammals, MAP increases during development (e.g. 40-50 mm Hg for P14, 110-125 mm Hg for adult) making the use of an absolute MAP value impractical as an objective criterion of the adequacy of the animal's physiological state. In preliminary studies, we found that blood pressure is depressed by 15-30 mm Hg under halothane. Therefore, if MAP failed to rise upon discontinuation of halothane, or if it fell below the halothane value during the recording session, the experiment was terminated. Preliminary studies also indicated that increases in PEIP above 15 cm H_2O were associated with significant damage to lung tissue in young animals. The ranges for physiologically relevant parameters in each age group are shown in Table 1.

Table 1.

Age/Parameter	P13-15	P20-22	P27-29
Weight, g	29-35	42-58	80-90
BP_H, mm Hg	25-30	40-45	50-58
BP_F, mm Hg	40-48	65-75	75-90
V_T, µl	175-320	340-520	450-540, or to desired PEIP
RR, breaths/min	120	111-120	100-110
PEIP, cm H_2O	9-10	9-10	10-12

Legend: BP_H = mean arterial pressure under halothane anesthesia; BP_F = mean arterial pressure under fentanyl-induced, opioid narcosis; V_T = tidal volume; RR = respiratory rate; PEIP = peak end inspiratory pressure.

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