# *Spinal Anesthesia in Infant Rats:*
# *Development of a Model, Preliminary Observations, and Assessment of Neurologic Outcomes*

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## *INTRODUCTION:*

Spinal anesthesia is commonly performed in infants undergoing inguinal procedures as an alternative to general anesthesia. Recent concerns about potential neurodevelopmental toxicities of general anesthetics were initiated by laboratory observations involving infant rats [1]. In previous laboratory studies, prolonged general anesthetic exposure produced apoptotic neurodegeneration in the brains of infant, but not adolescent, rats[1], while very brief exposures did not [2]. Other infant rat models examined peripheral nerve blockade and age-related differences in local anesthetic systemic toxicity [3,4]. We hypothesized that spinal anesthesia in infant rats was technically feasible and that doses could be identified that were effective (producing lower extremity sensory and motor blockade), safe (absence of upper extremity blockade, respiratory distress, cyanosis, or mortality) and without any potential motor or neurodevelopmental effects.

## *METHODS:*

Laboratory protocols were approved by the Institutional Animal Care and Use Committee. Sprague-Dawley rats (Charles River Laboratories), postnatal days 7 (P7), 14 (P14) and 21 (P21), were cared for according to standard practice. To achieve brief immobility and to minimize procedural stress, the spinal injections were performed following brief (< 2 minute) inhalation of 5% isoflurane that had been added to oxygen. One group of the P7 pups was kept awake during the spinal injections to examine the effect, versus not, of brief exposure to isoflurane. Spinal anesthesia was performed in a prone position using customized Hamilton® syringes and needles at the L5-L6 or L4-L5 interspace. Intrathecal placement was confirmed by observation of a tail flick. Bupivacaine 0.75% hyperbaric solution or saline control was used in all spinal injection cases, with dosing varying by adjustment of the injection volume. The P7 rat pups that were exposed to the general anesthesia, of 1% isoflurane in oxygen, were kept in a temperature controlled chamber for either 1 or 6 hours. These animals served as the positive control group for the brain and spinal cord apoptotic cell counts.

Behavioral testing was blinded. Sensory testing was adapted from those methods described previously [3]; paws were removed from the modified unilateral hotplate at 12 seconds to avoid tissue injury or hyperalgesia. A subgroup of each age group which underwent spinal injections of either 3.75 mg/kg or saline was left in cages for a week before being euthanized and perfused. Lumbar spinal cords sections from these animals were extracted for histopathological examination by an expert who was blind to the treatment. A second subgroup of animals was overdosed with pentobarbital 10 min after spinal injection or at the end of 1 hour of general anesthesia. Cardiac blood was collected for blood gas analysis. A third subgroup of P7 pups was euthanized 6 hours following intraspinal injection or at the beginning of general anesthesia. Brains and spinal cords were extracted for Caspase-3 immunohistochemistry staining. The fourth subgroup of P7 pups was treated with spinal bupivacaine, saline, or 1% isoflurane at 1 or 6 hours. These pups were kept in cages until age of P30 and examined for motor function using a rotarod. Data were analyzed using ANOVA with Bonferroni-corrected post-hoc t-tests.

## *RESULTS:*

Following a learning curve, spinal anesthesia could be achieved with high success rates in all age groups studied. Saline control injections were benign, or produced no signs of sensory or motor impairment, in all treatment groups. Preliminary experiments with bupivacaine doses < 2 mg/kg showed signs of incomplete block. Dosing with 3.7 mg/kg or 7.5 mg/kg produced complete block of the lower extremities at 10 minutes in all animals, mid-thoracic levels of sensory block (Figure 1). None of the animals dosed with 3.7 mg/kg showed signs of block to cervical levels, cyanosis, or distress. However dosing with 7.5 mg/kg produced a slightly higher percentage of animals at all ages with cervical levels of sensory and motor blockade or transient cyanosis. Sensory block regressed gradually from 30 minutes onwards, and at all age groups, returned to baseline latencies by 40 minutes (Figure 1). Sufficient and safe block of lower extremities could be achieved by spinal injection of bupivacaine to awake P7 pups (data not shown). No animals in this series of experiments exhibited persistent gait disturbances, hyperalgesia, spontaneous guarding of a limb, or self-mutilation.

No histopathology lesions were observed at the lumbar level of the spinal cords following intrathecal injections of either bupivacaine 3.75 mg/kg or saline at any age group (data not shown). Blood gas analysis of rats that underwent spinal injections of saline, bupivacaine 3.75 or 7.5 mg/kg or 1 hour of general anesthesia of P7 similarly looked benign (data not shown).

Cleaved caspase-3 staining showed no increase in apoptosis in brain or spinal cord in rats receiving spinal anesthesia as compared to controls or 1 hr exposure to 1% isoflurane, but was significantly less than those with 6 hr exposure to 1% isoflurane (Figure 2).

Groups of rats at 30 days of age who had undergone exposure at P7 to spinal bupivacaine, spinal saline, isoflurane for 1 hour, or isoflurane for 6 hours, respectively, were tested for motor performance using the Rota Rod apparatus. No group differences were found from this test (data not shown).

## *DISCUSSION:*.

Regional anesthetic techniques are routinely utilized as alternatives to general anesthesia in appropriate surgical procedures in pediatric patients. However, the effect of spinal anesthesia on the developing central nervous system has not been previously investigated. Infant animal studies have the theoretical potential to detect age-specific toxicities and thereby prevent harm to human infants. This study demonstrates that spinal anesthesia with bupivacaine can be safely administered to neonatal rats resulting in a motor block and thermal anesthesia of the lower extremities. Spinal anesthesia appears technically feasible in infant rats, and a preliminary bupivacaine dose range of 3.75-7.5 mg/kg was identified that produced thoracic-level blockade without cervical motor blockade or visible signs of respiratory difficulty. LD50s for peripheral extravascular dosing of bupivacaine in rats of different ages range from 30 – 90 mg/kg [4]. Thus, deaths occurring at doses above 7.5 mg/kg were likely due to respiratory and cardiovascular effects of high spinal anesthesia.

## *CONCLUSIONS:*

An infant animal model was developed to examine neurodevelopmental effects of spinal anesthesia. Under the conditions shown here, spinal anesthesia seems benign in terms of effects on brain and spinal cord neurotoxicity and long-term neurobehavioral consequences.

An ongoing human randomized controlled trial of infant inguinal hernia repairs under general versus spinal anesthesia is expected to provide better information about neurodevelopmental consequences in humans.

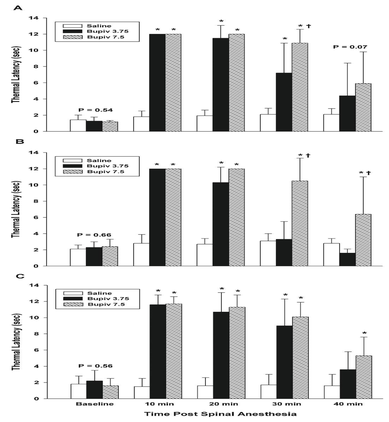


**Figure 1.**

**Intensity and Duration of Sensory Blockade From Spinal Anesthesia.** Lower extremity blockade, assessed by withdrawal latency to a modified hotplate test [3, 4] in rats at baseline (BL) and at times shown post-block, for ages: P7 (A), P14 (B), and P21(C),,using bupivacaine in lower dose (3.7 mg/kg), higher dose (7.5 mg/kg), or saline. n=7-9 rat pups for all treatment groups.* P<0.05 compare to saline. † P<0.05 compare to low dose.

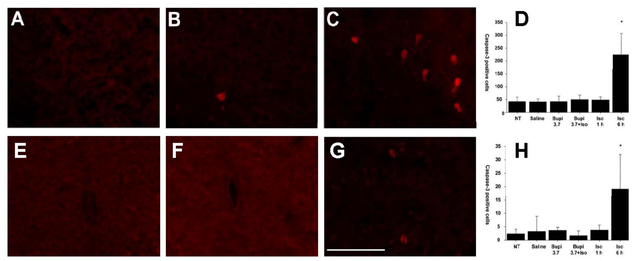


**Figure 2.**

**Caspase-3 activation in the brain and spinal cord of P7 rat pups.** Representative photomicrographs illustrate examples of cleaved caspase-3 immunocytochemical labeling in the cortex and spinal cord. Minimal labeling was found in brain (A,B) and spinal cord (E,F) sections from groups treated with spinal normal saline (A,E), spinal bupivacaine (B, F), or isoflurane 1 hour (not shown), while markedly increased labeled was seen in both brain (C) and spinal cord (G) in rats exposed 6 hours of isoflurane. Summary data on cleaved caspase-3 positive cells are shown for brain (D) and spinal cord (H). Data are presented as mean ± standard deviation, * p < 0.05 compared to other cohorts. Scale bar 100μm.

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