Comparative Medicine
Copyright 2004
by the American Association for Laboratory Animal Science

Vol 54, No 2
April 2004
Pages 202-208

Neonatal Anesthesia for Studies of Hamster Parental Behavior when Infanticidal Aggression Is a Possibility

Emily T. Vella, PhD, Christopher C. D. Evans, Siân Williams, and Katherine E. Wynne-Edwards, PhD*

Experiments involving investigation of the neuroendocrine basis for paternal care in rodents risk activation of aggressive behavior toward pups. To minimize pain and suffering during tests of parental responsiveness requiring retrieval of a displaced pup to its nest, a method of anesthetizing the pup was developed in Djungarian hamsters, *Phodopus campbelli*. A surgical plane of anesthesia, as measured by criteria, such as respiratory depression, loss of the pedal reflex, and failure to increase respiratory rate or to vocalize in response to handling, was achieved by use of intraperitoneal administration of a combination of ketamine and xylazine. Both parents (tested separately) expressed normal behavior toward anesthetized pups. In random order, a saline-injected or anesthetized pup was displaced from its nest in the home cage. There were no differences in pick-up or retrieval rates between saline and anesthetized pups for either parent. A third test using an unmanipulated pup confirmed that parental behavior was not reduced toward an anesthetized pup. However, if anesthetized pups were tested first among littermates, retrieval by males was less likely. This method will, therefore, underestimate retrieval behavior in males, but not females. Adult male hamsters that had never been parents also expressed expected behavior by attacking the pup in 45% of cases. This method provides an efficient and effective means of protecting pups while allowing adults to express a wide range of parental and infanticidal behaviors. It also has application in behavioral screening of transgenic strains toward unrelated young.

Infanticidal behavior directed toward unrelated neonatal pups is common in male rodent species (4, 6, 25). Even rodent species with extensive paternal behavior repertoires, including retrieval, licking, and grooming of pups, and huddling on the litter in the nest (e.g., Djungarian hamster [*Phodopus campbelli*], Mongolian gerbil [*Meriones unguiculatus*], prairie vole [*Microtus ochrogaster*], and California mouse [*Peromyscus californicus*] (5, 9, 17, 19, 22, 23), exhibit pup-directed aggression at some developmental stages (10, 18, 24, 25). There also is evidence suggesting that the biological mechanisms that inhibit infanticidal behavior have a similar neuroendocrine basis as do those facilitating parental behavior (4, 9, 11, 31, 38, 39). For example, adult male mice are infanticidal and do not exhibit paternal behavior, yet the male progesterone receptor knock-out mouse (PRKO) does not show pup-directed aggression. Instead, the PRKO male retains aggression in response to a same-sex intruder, loses pup-directed aggression, and expresses an elaborate paternal behavior repertoire, including pup retrieval (35).

Interest in the biological mechanisms responsible for male parental behavior has increased in recent years with the publication of studies suggesting that men becoming fathers undergo hormonal changes that are temporally related to the impending birth (1, 2, 16, 37). Neuroendocrine mechanisms in the paternal brain might also be more amenable to experimental study than those in the maternal brain because they occur in the absence of the potentially confounding neuroendocrinology of pregnancy, parturition, and lactation (43, 44). Thus, these types of studies have the potential to yield new insight into the biological mechanisms underlying affiliative behavior of adults toward infants.

To move these studies from correlation between hormones and behavior to an understanding of causal relationships, research involving pharmacologic manipulation of the hormones of males followed by detailed assessment of the males' interactions with neonatal pups may be helpful. Expected results will include reduction in paternal responsiveness in normally attentive males and responsive behaviors from males that would normally behave aggressively toward a pup. Although the researcher can be close by and intervene immediately if a pup is attacked, the attack often is so sudden that the experimenter does not have time to remove the pup before injury occurs (32). Neonatal rats can experience pain (9), and might have a lower pain threshold than that of adult rats (28). Thus, using live pups during paternal responsiveness tests with manipulated adults carries risk of pain and suffering for the pup(s).

Male Djungarian hamsters, *Phodopus campbelli*, have an exceptional array of paternal behaviors that includes active 'midwifery' during the birth. Males mechanically assist the delivery, clear the airway, and consume the placenta (22, 23). Males also experience hormonal changes that, as the birth approaches and passes (33), might affect paternal behavior (44). Thus, our interest was in development of a method to protect neonatal pups during manipulative tests of hormone-induced behavior relationships in adult males.

In a 1989 experiment by Perrigo and co-workers (32), CF-1

Received: 9/05/03. Revision requested: 11/05/03. Accepted: 1/06/04.
Department of Biology, Queen's University, Kingston, ON, Canada K7L 3N6.
**Corresponding author.*

mouse pups were protected from experiencing pain by enclosing individual pups in small wire mesh cages. Adult males would pick up and return the cages containing pups to their nest to the same extent as they would return unprotected pups. However, using different strains of mice, Elwood and co-workers (14) in 1990 failed to observe a similar response, especially if protected pups were tested first. During preliminary testing of this method, using three-day-old Djungarian hamsters, the dam did not retrieve the caged pup, and the behavior of an unrelated adult male that had previously attacked an unrelated pup was indistinguishable from the behavior of the dam. Even ultrasonic vocalizations did not allow researchers to discriminate the intentions of adults during a behavioral test. As an alternative, we report promising results using neonatal anesthesia.

Materials and Methods

Animal husbandry. Djungarian hamsters were the outbred descendants of wild-caught individuals from Tuva, Siberia. They have been maintained in a large breeding population at Queen's University since 1990 (41, 42). Populations are seronegative for standard viral pathogens (pneumonia virus of mice, reovirus, Sendai virus, lymphocytic choriomeningitis virus). Adult hamsters weighing 20 to 40 g were housed in polycarbonate caging ($27 \times 21 \times 14$ cm, Nalge Nunc International, Rochester, N.Y.) at an ambient temperature of 18.0 ± 1.0°C to reflect burrow temperatures in the wild (42), a photoperiod of 14:10-h (light:dark; (with 00:00 h corresponding to the middle of the dark phase), and ad libitum access to food (Purina 5001 Rodent Chow, St. Louis, Mo.) and water. Research reported here was conducted in accordance with the guidelines of the Canadian Council on Animal Care approved by Queen's University as protocol 2001-041.

From weaning until pairing, animals were housed in same-sex sibling groups of one to three individuals. As adults, virgin males and females, with no prior alloparental experience with sibling litters, were paired. After 18 days, cages were checked daily for birth, and the day of birth was considered day zero of lactation (L0). Parents were together until birth of offspring, and were separated for the trials only.

Neonatal anesthesia. Delivery of inhalant anesthetic agent was rejected because it would require a nose cone during the behavioral testing and would introduce novel odor to the testing environment. In contrast, injected anesthesia was expected to sustain a surgical plane throughout behavioral testing. Ketamine hydrochloride-xylazine combination was chosen as the anesthetic because of its efficacy in *Phodopus* sp. (8, 27, 36). Intraperitoneal injection was chosen to avoid the technical challenges of intramuscular or subcutaneous delivery in neonates.

A paired design with two pups (n = 12 pairs) from the same litter was used. Three-day-old pups (approx. 3.0 g) were removed from the cage at the same time and placed on a paper towel under a desk lamp to keep them warm. Temperature under the incandescent bulb ranged from 25 to 28°C, which approximates nest temperature of 27°C under a huddle of three-day-old pups (29). One pup was anesthetized by use of a single intraperitoneal (i.p.) injection from a 10-µl Hamilton 901N, 26s, syringe containing 5 µl of ketamine hydrochloride (20 mg/ml; Rogarsetic [40 mg/kg of body weight], an N-methyl-D-aspartate [NMDA] receptor antagonist, STP Inc., Toronto, Ont., Canada) and 5 µl of of xylazine (2.0 mg/ml; Rompun [4 mg/kg], an analgesic and muscle relaxant, Bayer, Toronto, Ont., Canada). Ketamine and xylazine were combined in the syringe, and stock anesthetic was diluted, using sterile 0.9% physiologic saline. The control pup was given a 10-µl volume of 0.9% saline. The paper towel absorbed any blood found at the injection site. Ketamine anesthesia has multiple developmental effects on the brain (20), and the neonatal hamster brain is developing rapidly at three days of age. Thus, all pups that received the anesthetic treatment were killed before they recovered from anesthesia. Unmanipulated and saline-injected pups were returned to their litter.

Response to anesthesia. Both pups were observed for a period of 35 min after delivery of the injection. Degree of spontaneous movement (none, some, vigorous) and spontaneous audible vocalization (yes or no) were recorded for each pup at zero, one, two, four, five, 10, 15, 20, 25, 30, and 35 min after injection. Resting respiratory rate (breaths/5 s) also was recorded visually (on the basis of movement of the rib cage) at all time points except one and four minutes. Immediately after these measurements, the pup was picked up in a gloved hand, and the toes of the right hind limb were gently pinched between the thumb and forefinger as the limb was drawn out to full extension. The extent of the pedal reflex was scored (weak, strong). Immediately following the reflex test, the audible vocalization response (yes or no) and the respiratory rate were recorded. Responses of the pup following handling were calculated as the difference between the measure after handling and the measure before handling within an individual.

Parental adult behavior test. For this test to be useful in studies of parental behavior, it was essential that the male and female parent would respond to their anesthetized pup in the same way they responded to an unmanipulated pup from their litter. Retrieval of a pup that is experimentally displaced from the nest is the traditional paradigm (3, 34) used to assess parental responsiveness in rodent species, and has been used in previous research with Djungarian hamsters (23, 33). Thus, adult female (n = 15) and male (n = 20) dwarf hamsters, between 90 and 120 days of age at pairing, were tested for their response to anesthetized and saline-treated pups from their first litter, using a standard pup displacement test. To control for the possible effects of injection on pup stimuli, six females and six males also were tested, using unmanipulated pups.

During the last hours of the light phase (1600 to 1900 h) on day 3 (L3) after the birth of their first litter (L0), one adult and one pup were removed from the family group. The adult was placed in a cage out of sight of the home cage, but within potential olfactory and auditory range. The pup was held outside the nest for seven to 10 min before testing to allow the anesthetic to take effect and to control for duration of absence from the huddled litter. The adult remaining in the home cage was tested, using either the anesthetized or the saline-injected pup. Testing order was recorded and randomly determined. The waiting interval and behavior test were then repeated for the same adult with the second category of pup (anesthetized or saline-injected). The subset of females and males that were tested, using unmanipulated pups, were given those tests as a third and final test on the same day.

After displacing a single pup to the furthest corner from the natal nest, adult behavior was assessed by continuous focal animal observation for five minutes. Dependent measures were presence or absence and latency (seconds) for contact (sniff, lick, or touch) with the displaced pup, presence or absence and latency (seconds) to pick up the displaced pup, presence or absence and

latency (seconds) to retrieve the displaced pup to the nest, and duration (seconds) on the nest with the rest of the litter. A value of 300 sec was assigned as the latency if the behavior was not seen during the test.

Dependent measures listed previously also were reduced to a parental responsiveness score for each adult on the basis of time penalties and behavioral bonuses (23). Time penalties were based on the return of the displaced pup to the nest. Each male received a score from 0 (pup back in the nest within 50 sec) to –6 (pup still displaced at the end of the test) points. Although pup motility was limited on L3, some pups might return to the nest unaided. In those instances, the adult received the time penalty as if he or she had carried the pup to the nest. Behavioral bonuses were awarded for contacting the displaced pup (1 point), picking up the displaced pup (1 point), and retrieving the displaced pup (4 points). Retrieval is given substantial weight (4 points) because failures in maternal behavior typically involve loss of the retrieve response, but maintenance of the contact-and-pick up responses (26). Thus, possible bonus scores were 1, 2, and 6 points. A maximum score of +6 points, therefore, indicated that the adult contacted, carried, and retrieved the displaced pup within 50 sec. A minimum score of –6 points indicated that the adult did not touch the pup during the test (23).

Naïve adult behavior test. For this method to be useful in behavioral studies when there is risk of infanticide, it also was essential that an unrelated adult male could show aggressive behavior when presented with an anesthetized pup. Eleven naïve, adult males, between 58 and 62 days old, were given behavioral tests using an anesthetized pup. All males were housed individually for 24 h before testing to establish a home cage nest. In all other respects, the test was the same as that given to parental adults. After 10 min to ensure anesthesia induction, one unrelated pup was placed in the furthest corner of the home cage from the naïve adult male. All dependent measures from the parental adult test were recorded, with the addition of presence or absence of, and latency to, an attack on the pup.

Statistical analysis. Paired *t*-tests were used for within-subject comparisons, and *t*-tests were used for differences between subjects. When comparisons were based on presence/absence nominal scores, χ^2-test results were reported. A critical *P* value of 0.05 was applied in all cases, and all dependent measures were tested against a two-tailed null hypothesis.

Results

Responses of saline-injected pups to repeated measurements. Throughout the 35 min of behavioral testing, three-day-old Djungarian hamster pups that had received an injection of saline remained behaviorally stable and consistent in their responses to handling (Fig. 1A-1C). They retained spontaneous movement, their pedal reflex was always strong, and they vocalized in response to handling. For example, 11 of 12 pups vocalized in response to handling at one or more time points; over all tests with the 12 animals, 90 of 132 (68%) handling tests induced an audible vocalization response (Fig. 1C). Mean ± SEM spontaneous respiratory rate for an individual saline-injected pup was 12.1 ± 1.2 (n = 12) breaths per five seconds, all repeated measures for one pup averaged before comparison (Fig. 2A). Respiratory rate also reliably increased in response to handling (paired t = 2.84, df = 11, $P < 0.02$; average increase = 1.12 ± 1.36 breaths/5 s; all repeated measures for one pup averaged before comparison, Fig. 2B).

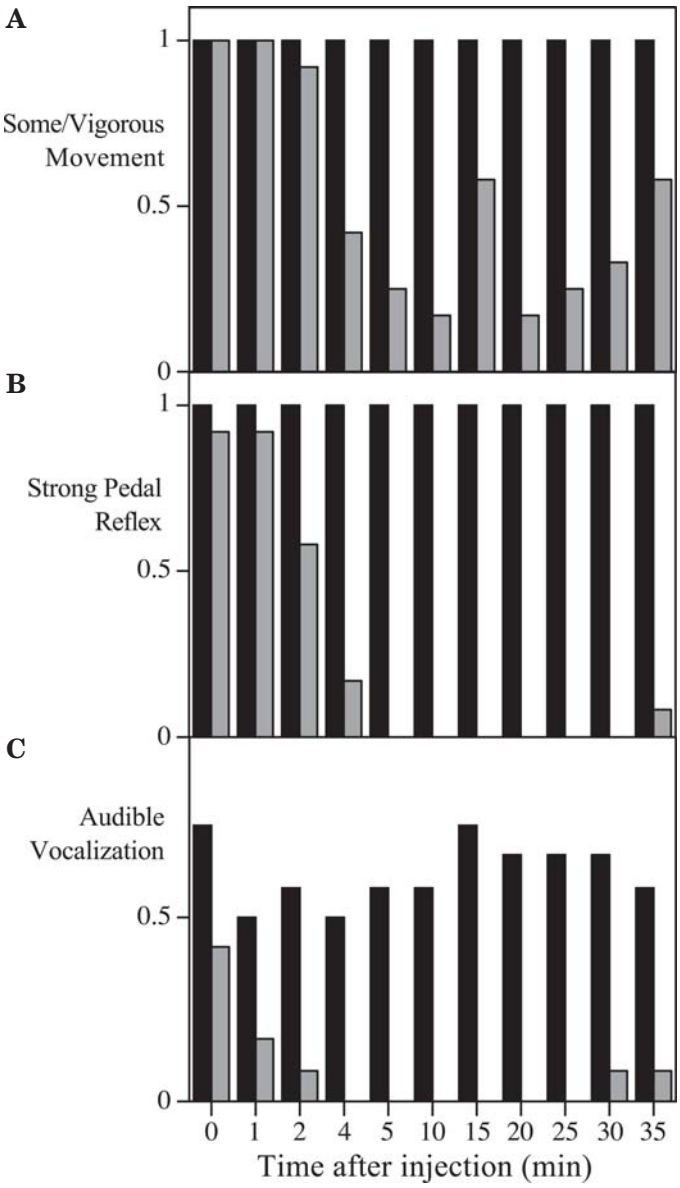

Figure 1. Physiologic and behavioral responses of three-day old Djungarian hamster pups to repeated handling, with or without anesthesia, over a 35-min interval. A total of 12 pairs of saline-injected (black bars) and anesthetized (gray bars) pups were tested. (A) Proportion of saline-injected and anesthetized pups spontaneously showing some or vigorous movement at each time point. (B) Proportion of saline-injected and anesthetized pups with a strong pedal reflex. (C) Proportion of saline-injected and anesthetized pups producing an audible vocalization in response to handling.

Responses of anesthetized pups over repeated measurements. The physiologic and behavioral tests indicated that this anesthetic regimen of ketamine and xylazine induced more than 30 min of surgical anesthesia in three-day-old Djungarian hamster pups (Fig. 1). The strong pedal reflex was completely extinguished within five minutes of anesthetic administration (Fig. 1B), and no anesthetized pup vocalized spontaneously, or vocalized in response to handling, between five and 30 min after anesthetic administration (Fig. 1C). At time 0, there were no differences in respiratory rate or respiratory response to han-

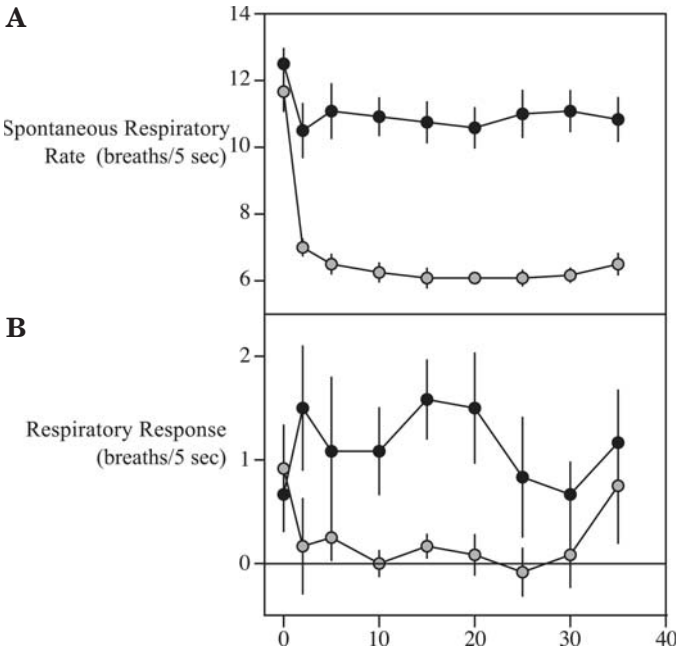

Figure 2. Respiratory rate (A) and respiratory response (B) to handling in saline-injected and anesthetized three-day-old pups. (A) Spontaneous respiratory rate (breaths/5 s [mean ± SEM]) in 12 saline-injected (black symbols) and 12 anesthetized (gray symbols) pups at each point in time. Respiratory rate in the two groups at the time of injection is not different. Between five and 30 min after injection, spontaneous respiration is suppressed in anesthetized pups. (B) Respiratory response (change in respiratory rate from spontaneous to after handling; breaths/5 s) in saline-injected and anesthetized pups. Saline-injected pups have a significant, positive respiratory response, whereas anesthetized pups do not respond to handling between five and 30 min after injection.

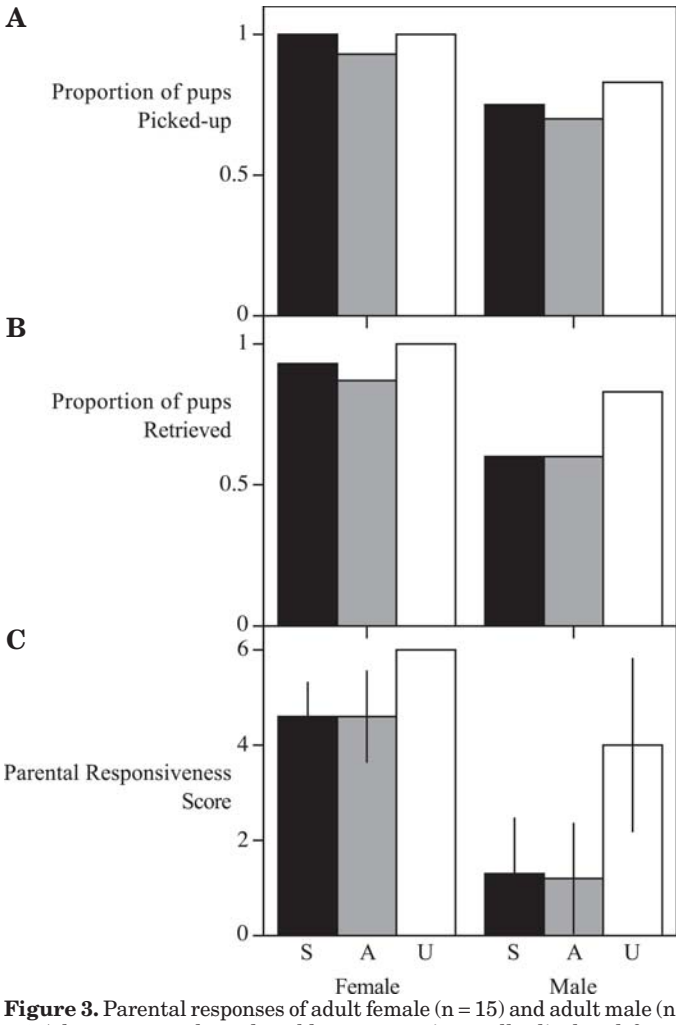

Figure 3. Parental responses of adult female (n = 15) and adult male (n = 20) hamsters to three-day-old pups experimentally displaced from the nest. Females tested with saline-injected pups (black bars) were more likely than males to pick up and retrieve the displaced pup, yielding higher parental responsiveness scores. Females and males treated anesthetized (gray bars) pups similarly to the way they treated saline-injected pups. A subset of six female and six male parents was tested a third time with an unmanipulated pup (open bars). There was no within-female or within-male differential in behavior toward the unmanipulated pup and the saline-injected pup.

dling between saline-injected and anesthetized pups (rate: paired $t = 1.60$, $df = 11$, $P = 0.14$; response: paired $t = 0.18$, $df = 11$, $P = 0.86$; Fig. 2). Within two minutes after anesthetic injection, respiratory rate was significantly decreased, relative to that of the matched pup receiving saline injection (paired $t = 8.44$, $df = 11$, $P = 0.0001$). Over the six samples, from five through 30 min after injection, respiratory rate before handling averaged only 6.19 ± 0.11 breaths/5 s (n = 12, Fig. 2). Over the same interval, there was no evidence of a respiratory response to handling in anesthetized pups (0.08 ± 0.09, paired $t = 0.92$, $df = 11$, $P = 0.38$, Fig. 2B).

Parental responses to pup displacement. As expected, parental responsiveness was higher in female parents than in male parents, although male parents were very responsive to a displaced pup (Fig. 3). All female parents picked up the saline-injected pup, whereas 15 of 20 male parents picked up the saline-injected pup (Pearson χ^2 test = 4.38, $df = 1$, $P = 0.04$; Fig. 3A). Results were similar for retrieval of the displaced, saline-injected pup, with 14 of 15 females retrieving the pup, compared with 12 of 20 males (Pearson χ^2 test = 4.99, $df = 1$, $P = 0.03$; Fig. 3B). As a result of these behavioral differences, the parental responsiveness score of female parents (4.60 ± 0.71 points) was significantly higher than the parental responsiveness score of male (1.30 ± 1.16 points, Fig. 3C) parents ($t = 2.23$, $df = 33$, $P = 0.03$).

Female and male parents responded similarly to the saline-injected and anesthetized pups. No adult parent showed aggression toward a pup during a behavioral test. Female parents picked up 14 of 15 anesthetized pups, and retrieved 13 of 15 of those pups to the nest. These responses were not significantly different from maternal responses to saline-injected pups (pick-up Pearson $\chi^2 = 1.03$, $df = 1$, $P = 0.31$; retrieve Pearson $\chi^2 = 0.37$, $df = 1$, $P = 0.54$). However, there was an effect of testing order. If the anesthetized pup was tested first, it was retrieved more quickly ($t = 2.17$, $df = 13$, $P < 0.05$). However, the behavioral repertoire was not changed. Parental responsiveness score was not increased ($t = 2.08$, $df = 13$, $P = 0.06$) relative to testing the anesthetized pup second.

Male parents picked up 14 of 20 anesthetized pups, and retrieved 12 of 20 of those pups to the nest. These responses were not significantly different from paternal responses to saline-injected pups (pick-up Pearson $\chi^2 = 0.13$, $df = 1$, $P = 0.72$; retrieve Pearson $\chi^2 = 0$, $df = 1$, $P = 1$). Unlike that in females, there was no effect of testing order on latency to retrieve the pup ($t = 0.49$, $df = 18$, $P = 0.63$). However, retrieval by the male parent was more likely if anesthetized pups were tested second (Pearson $\chi^2 = 7.5$, $df = 1$, $P < 0.01$). Parental responsiveness score was not affected by testing

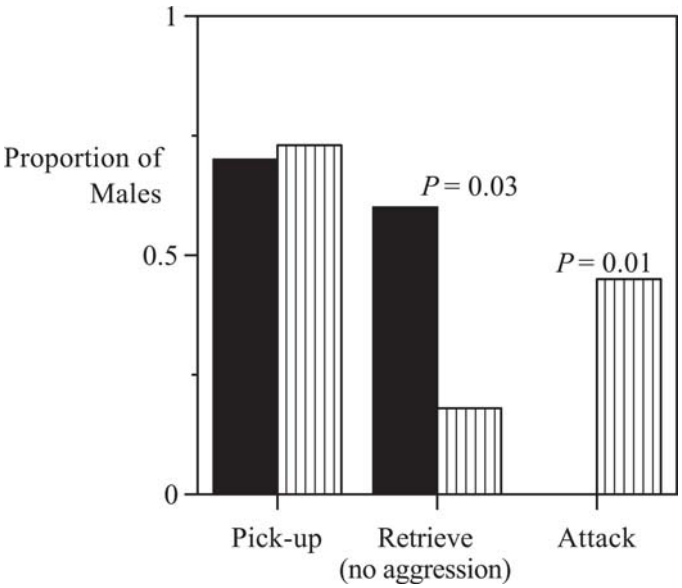

Figure 4. Contrasting behavior of parental male hamsters and naïve male hamsters presented with a similarly anesthetized pup. A significantly higher proportion of parental males (solid bars) picked up and retrieved the displaced pup, whereas a significantly higher proportion of naïve males (hatched bars) attacked the pup. Three naïve males that attacked the pup after retrieving it were not included in the proportion of naïve males that retrieved the pup.

order ($t = 0.76$, $df = 18$, $P = 0.46$).

Parental responsiveness to saline-injected pups was so high that there was no evidence for differential treatment of unmanipulated pups, even though those tests were always third in order (Fig. 3). All females and five of six males picked up and retrieved the displaced, unmanipulated pup. Time to retrieve the saline-injected and unmanipulated pups was not significantly different for females (86.67 ± 45.09 and 32.67 ± 3.26 sec, respectively; paired $t = 1.27$, $df = 5$, $P = 0.26$) and males (72.50 ± 45.84 and 69.17 ± 46.79 sec, respectively; paired $t = 0.43$, $df = 5$, $P = 0.69$). Likewise, in the subset of six animals tested with saline and with unmanipulated pups, parental responsiveness scores did not change between the saline-injected and unmanipulated pup for females (4.0 ± 1.63 and 6 ± 0 points, respectively; paired $t = 1.22$, $df = 5$, $P = 0.28$), or males (4.0 ± 1.81 points for each; paired $t = 0$, $df = 5$, $P = 1.0$).

Naïve male responses to an anesthetized pup. All naïve males contacted the anesthetized pup, and the latency to first contact was not different from that for parental males with anesthetized pups ($t = 0.56$, $df = 29$, $P = 0.58$). Eight naïve males picked up the pup (Fig. 4). As anticipated by the context for this study, 45% (5/11) of naïve adult males showed aggression toward the anesthetized pup during the five-minute test. Consistent with descriptions of infanticidal behavior in other rodent species (13, 21), aggression toward the pup consisted of a bite that broke the skin, and was often directed toward the head or neck. This difference between male parents and naïve males was significant (0/10 versus 5/11, respectively; Pearson $\chi^2 = 5.97$, $df = 1$, $P = 0.01$). For those five pups, the average time to attack was 117.6 ± 30.93 (range, 56 to 225) sec. In addition, 45% of naïve males retrieved the pup. However, three of the naïve males that attacked the pup also retrieved the pup before the attack. Thus, only two of 11 naïve males retrieved the anesthetized pup and did not follow that retrieval with aggression during the test. This was significantly lower than the proportion of parental males that retrieved the anesthetized pup without showing aggression (12/20 vs 2/11, respectively; Pearson $\chi^2 = 5.01$, $df = 1$, $P = 0.03$).

Discussion

All of the physiologic and behavioral parameters used to quantify the effectiveness of the ketamine-xylazine anesthetic combination indicated that dwarf hamster pups reached a surgical depth of anesthesia. One of the earliest indicators that an anesthetic is taking effect is the slowing and eventual cessation of movement (15). Reduced movement, relative to that in saline injected pups, was observed within two minutes of injection and continued through the 30-min period. Complete cessation of minor movements was not expected because ketamine is a known cataleptic that induces spastic movements in anesthetized animals, even when they are co-treated with an analgesic, such as xylazine (15). In addition, the strong pedal reflex was never seen beyond four minutes after injection or for the ensuing 30 min (15, 30, 40).

Further support for the surgical depth of anesthesia was the cessation of audible vocalizations in response to handling. As expected (28), saline-injected pups reliably emitted audible squeaks in response to handling, whereas anesthetized pups did not produce any audible vocalizations. The absence of this distress response suggests that the handling stimulus was not perceived. The spontaneous respiratory rate of anesthetized pups was reduced by almost 50%, relative to that of the matched saline-injected pups, and those pups did not show the increased respiratory rate in response to handling that occurred in the saline-injected control pups. The handling challenge intended to simulate the physical contact of an adult pup with a neonate (either aggressive or parental contact), therefore, induced expected physiologic and behavioral responses in saline-injected, but not anesthetized pups. Thus, we conclude that a surgical plane of anesthesia was induced in the three-day old pups.

Of particular importance, our results also indicated that use of the anesthetic did not eliminate normal expression of parental behavior toward displaced pups. Females picked up all but one of the 36 displaced pups, with no evidence that anesthetized pups were avoided. After pick up, 92% of displaced pups were retrieved to the nest area. This response was so rapid that the parental responsiveness scores were high. Average scores > +4, on the scale from –6 through +6 points, indicated that females not only retrieved displaced pups, but did so early in the five-minute test period, often within the first 50 sec. As expected (7, 25), parental responsiveness of males was lower than that of females, with 74% of displaced pups being picked up and 63% of displaced pups being retrieved to the nest. However, like the female parents, there was no evidence that male parents treated their own pups differently if they were anesthetized. This proportion of males retrieving pups was lower than the 86 and 78% of pups retrieved in earlier studies (23, 33); however, in those earlier studies, twice as long (10 min) a duration was allowed for the behavior test.

There was, however, evidence suggesting that testing order affected parental behavior. Specifically, female parents retrieved the anesthetized pup more rapidly if it was the first stimulus pup offered than if it was the second stimulus pup. Also, male parents were more likely to retrieve the anesthetized pup if it was the second, rather than the first stimulus of the day. This is qualitatively

similar to the finding that mice were less responsive to a displaced, caged pup if that pup was tested first (14), and suggests that male parents might require a stimulus of greater strength to elicit retrieval behavior the first time. If so, retrieval behavior by male parents will be underestimated by use of this method, whereas the retrieval behavior of female parents will be assessed accurately. Thus, using this method, sample sizes for males will need to be larger than sample sizes for females to detect differences between experimental groups.

Usefulness of this method also depends on the normal expression of aggressive behavior toward anesthetized pups. Direct comparison of behavioral responses of infanticidal males to control and anesthetized pups was precluded by the ethical context of this study. Nevertheless, it was possible to show that adult male hamsters that were unrelated to the pup showed aggressive behavior. Forty-five percent of naïve males attacked the anesthetized pup within five minutes, in contrast to the complete absence of aggressive behavior during the 36 female and 46 male parental tests. In addition, naïve males also retrieved the pup in 45% of cases. Retrieval was then followed by attack. Thus, these results support the need to allow the behavior test to proceed to attack behavior before inferring the presence or absence of pup-directed aggression. Without allowing the attack behavior, naïve male retrieval behavior might have been inappropriately classified as "parental".

We conclude that non-parental males will attack anesthetized pups, whereas parental males will not, and we conclude that behavior tests involving only anesthetized pups will successfully discriminate parental from infanticidal behavior without risking pain or suffering for the pup. This method of neonatal anesthesia with ketamine hydrochloride and xylazine provides an efficient and effective alternative to previously developed methods of protecting pups during tests of parental behavior when infanticidal aggression is a possibility. The method also has the potential to minimize the risk of pain and suffering during behavioral screening of transgenic strains where the reactions of adults to pups are not known.

Acknowledgments

This research was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) and the Canadian Institutes of Health (CIHR) research to author KEW-E. Author ETV was supported by an Advisory Research Council postdoctoral fellowship from Queen's University. Author CCDE held an undergraduate summer research award (USRA) from NSERC. Brenda Nielsen contributed to the design of this study during her NSERC USRA tenure.

References

1. **Berg, S. J. and K. E. Wynne-Edwards.** 2001. Changes in testosterone, cortisol, and estradiol levels in men becoming fathers. Mayo Clin. Proc. **76:**582-592.
2. **Berg, S. J. and K. E. Wynne-Edwards.** 2002. Salivary hormone concentrations in mothers and fathers becoming parents are not correlated. Horm. Behav. **42:**424-436.
3. **Bridges, R. S. and P. M. Ronsheim.** 1990. Prolactin (PRL) regulation of maternal behavior in rats; bromocriptine treatment delays and PRL promotes the rapid onset of behavior. Endocrinology **126:**837-848.
4. **Brown, R. E.** 1986. Social and hormonal factors influencing infanticide and its suppression in adult male Long-Evans rats (*Rattus norvegicus*). J. Comp. Psychol. **100:**155-161.
5. **Brown, R. E., T. Murdoch, P. R. Murphy, and W. H. Moger.** 1995. Hormonal responses of male gerbils to stimuli from their mate and pups. Horm. Behav. **29:**474-491.
6. **Cassady, St. Clair C.** 1996. Infanticide and parental care. Anim. Behav. **52:**219-220.
7. **Dewsbury, D. A.** 1985. Paternal behavior in rodents. Am. Zool. **25:**841-852.
8. **Edwards, H. E. and K. E. Wynne-Edwards.** 1994. Spontaneous termination of an induced pseudopregnancy in the Djungarian hamster, *Phodopus campbelli*. Horm. Behav. **28:**165-180.
9. **Elwood, R. W.** 1977. Changes in the responses of male and female gerbils (*Meriones unguiculatus*) towards test pups during the pregnancy of the female. Anim Behav **25:**46-51.
10. **Elwood, R. W.** 1980. The development, inhibition, and disinhibition of pup-cannibalism in the Mongolian gerbil. Anim. Behav. **28:**1188-1194.
11. **Elwood, R. W.** 1986. What makes male mice paternal? Behav. Neural Biol. **46:**54-63.
12. **Elwood, R. W. and H. F. Kennedy.** 1990. The relationship between infanticide and pregnancy block in mice. Behav. Neural Biol. **53:**277-283.
13. **Elwood, R. W.** 1991. Ethical implications of studies on infanticide and maternal aggression in rodents. Anim. Behav. **42:**841-849.
14. **Elwood, R. W., A. A. Nesbitt, and H. F. Kennedy.** 1990. Maternal aggression in response to the risk of infanticide by male mice, *Mus domesticus*. Anim. Behav. **40:**1080-1086.
15. **Flecknell, P. A.** 1987. Laboratory animal anesthesia. Academic Press, Toronto, Ontario.
16. **Fleming, A. S., C. Corter, J. Stallings, and M. Steiner.** 2002. Testosterone and prolactin are associated with emotional responses to infant cries in new fathers. Horm. Behav. **42:**399-413.
17. **Gubernick, D. J., and J. R. Alberts.** 1989. Postpartum maintenance of paternal behaviour in the biparental California mouse, *Peromyscus californicus*. Anim. Behav. **37:**656-664.
18. **Gubernick, D. J., K. A. Schneider, and L. A. Jeannotte.** 1994. Individual differences in the mechanisms underlying the onset and maintenance of paternal behavior and the inhibition of infanticide in the monogamous biparental California mouse, *Peromyscus californicus*. Behav. Ecol. Sociobiol. **34:**225-231.
19. **Gubernick, D. J., J. T. Winslow, P. Jensen, L. Jeanotte, and J. Bowen.** 1995. Oxytocin changes in males over the reproductive cycle in the monogamous, biparental California mouse, *Peromyscus californicus*. Horm. Behav. **29:**59-73.
20. **Hayashi, H., P. Dikkes, and S. G. Soriano.** 2002. Repeated administration of ketamine may lead to neuronal degeneration in the developing rat brain. Paediatr. Anaesth. **12:**770-774.
21. **Jakubowski, M. and J. Terkel.** 1985. Incidence of pup killing and parental behavior in virgin female and male rats (*Rattus norvegicus*): differences between Wistar and Sprague-Dawley stocks. J. Comp. Psychol. **99:**93-97.
22. **Jones, J. S. and K. E. Wynne-Edwards.** 2000. Paternal hamsters mechanically assist the delivery, consume amniotic fluid and placenta, remove fetal membranes, and provide parental care during the birth process. Horm. Behav. **37:**116-125.
23. **Jones, J. S. and K. E. Wynne-Edwards.** 2001. Paternal behaviour in biparental hamsters does not require contact with the pregnant female. Anim. Behav. **62:**453-464.
24. **Lonstein, J. S. and G. J. De Vries.** 2000. Influence of gonadal hormones on the development of parental behavior in adult virgin prairie voles (*Microtus ochrogaster*). Behav. Brain Res. **114:**79-87.
25. **Lonstein, J. S. and G. J. De Vries.** 2000. Sex differences in the parental behavior of rodents. Neurosci. Biobehav. Rev. **24:**669-686.
26. **Lucas, B. K., C. J. Ormandy, N. Binart, R. S. Bridges, and P. A. Kelly.** 1998. Null mutation of the prolactin receptor gene produces a defect in maternal behavior. Endocrinology **139:**4102-4107.
27. **McInroy, J. K. E., D. G. Brousmiche, and K. E. Wynne-Edwards.** 2000. Fathers, fat, and maternal energetics in a biparental hamster: paternal presence determines the outcome of a current reproductive effort and adipose tissue limits subsequent reproductive effort. Horm. Behav. **37:**399-409.
28. **Narsinghani, U. and M. B. B. S. Anad.** 2000. Developmental neurobiology of pain in neonatal rats. Lab. Animal **29:**27-39.

29. **Newkirk, K. D., B. L. W. Cheung, S. J. Scribner, and K. E. Wynne-Edwards.** 1995. Maternal constraints prolong pup dependence in *Phodopus campbelli*, but not *P. sungorus*. Physiol. Behav. **63:**435-443.
30. **Olfert, E. D., B. M. Cross, and A. A. McWilliam.** 1993. Canadian Council on Animal Care: guide to the care and use of experimental animals. Bradda Printing Services, Ottawa, Ontario.
31. **Perrigo, G., L. Belvin, and F. S. Vom Saal.** 1991. Individual variation in the neural timing of infanticide and parental behavior in male house mice. Physiol. Behav. **50:**287-296.
32. **Perrigo, G., W. C. Bryant, L. Belvin, and F. S. Vom Saal.** 1989. The use of live pups in a humane, injury-free test for infanticidal behaviour in male mice. Anim. Behav. **38:**897-899.
33. **Reburn, C. J. and K. E. Wynne-Edwards.** 1999. Hormonal changes in males of a naturally biparental and a uniparental mammal. Horm. Behav. **35:**163-176.
34. **Rosenblatt, J. S., A. Olufowobi, and H. I. Siegel.** 1998. Effects of pregnancy hormones on maternal responsiveness, responsiveness to estrogen stimulation of maternal behavior, and the lordosis response to estrogen stimulation. Horm. Behav. **33:**104-114.
35. **Schneider, J. S., M. K. Stone, K. E. Wynne-Edwards, T. H. Horton, J. P. Lydon, B. O'Malley, and J. E. Levine.** 2003. Progesterone receptors mediate male aggression towards infants. Proc. Natl. Acad. Sci. USA **100:**2951-2956.
36. **Scribner, S. J. and K. E. Wynne-Edwards.** 1994. Disruption of body temperature and behavior rhythms during reproduction in dwarf hamsters (*Phodopus*). Physiol. Behav. **55:**361-369.
37. **Storey, A. E., C. J. Walsh, R. Quinton R, and K. E. Wynne-Edwards.** 2000. Hormonal correlates of paternal responsiveness in new and expectant fathers. Evol. Human Behav. **21:**79-95.
38. **Vom Saal, F. S.** 1983. Variation in infanticide and parental behavior in male mice due to prior intra-uterine proximity to female fetuses: elimination by prenatal stress. Physiol. Behav. **30:**675-681.
39. **Vom Saal, F. S.** 1985. Time-contingent change in infanticide and parental behavior induced by ejaculation in male mice. Physiol. Behav. **34:**7-15.
40. **Warren, R. G.** 1983. Mosby's fundamentals of animal health technology: small animal anaesthesia. C.V. Mosby, St. Louis.
41. **Wynne-Edwards, K. E.** 1995. Biparental care in Djungarian but not Siberian dwarf hamsters (*Phodopus*). Anim. Behav. **50:**1571-1585.
42. **Wynne-Edwards, K. E.** 1998. Evolution of parental care in *Phodopus*: conflict between adaptations for survival and adaptations for rapid reproduction. Am. Zool. **38:**238-250.
43. **Wynne-Edwards, K. E.** 2001. Hormonal changes in mammalian fathers. Horm. Behav. **40:**139-145.
44. **Wynne-Edwards, K. E. and C. J. Reburn.** 2000. Behavioural endocrinology of mammalian fatherhood. Trends Ecol. Evol. **15:**464-468.