## ISOFLURANE ANESTHESIA OF WILD-CAUGHT GOLIATH BIRDEATER SPIDERS (*THERAPHOSA BLONDI*) AND CHILEAN ROSE SPIDERS (*GRAMMOSTOLA ROSEA*)

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Abstract: Anesthesia is used in theraphosid spiders to facilitate medical procedures (e.g., physical examination, sample collection, surgery); however, most information on this subject is anecdotal. This study was conducted to systematically determine the anesthetic parameters of wild-caught, subadult goliath birdeater spiders (*Theraphosa blondi*) (n = 11) and Chilean rose spiders (*Grammostola rosea*) (n = 12). Each spider was placed in a 3-L gas anesthetic chamber and subjected to an induction of 5% isoflurane at a rate of 1 L/min oxygen. Anesthetic depth was monitored by evaluating the righting reflex every 5 min. Animals were recovered in 100% oxygen. Induction, recovery, and overall anesthetic times were determined. After an 8-wk washout period, the procedure was repeated. For both species, median induction time was 10 min. Median recovery time was 30 min for *T. blondi* and 12.5 min for *G. rosea*.

Key words: anesthesia, Grammostola rosea, isoflurane, spider, Theraphosidae, Theraphosa blondi.

## **BRIEF COMMUNICATION**

Invertebrates are increasingly gaining attention as pets and exhibit animals, and thus are more commonly presented to the veterinarian for medical attention. Knowledge of the health care of these animals is often based on anecdotal information rather than systematic analysis. The performance of common medical procedures, such as anesthesia, would benefit from more detailed analysis. The importance of this information cannot be overstated, as anesthesia in theraphosid spiders is required to facilitate other procedures, such as physical examination, collection of diagnostic samples (e.g., hemolymph), and surgery. An experimental study is presented to evaluate isoflurane anesthesia in goliath birdeater spiders (Theraphosa blondi) and Chilean rose spiders (Grammostola rosea). Eleven (six male and five female) T. blondi were obtained from an invertebrate importer in Florida (LASCO, Naples, Florida 34119, USA). Twelve G. rosea of unknown gender were obtained from a vendor at a reptile show in Mandeville, Louisiana. All the animals in this study were wild-caught subadults and were clinically normal based on physical exams. The spiders were housed in rectangular, 5.7-L plastic storage containers. A 50:50 mixture of potting soil and vermiculite was used for the substrate for the *T. blondi*. Bed-A-Beast ground coconut hull (Pet-Tech Products LLC, Van Nuys, California 91406, USA) was used as the substrate for the *G. rosea*. The spiders were provided a 12hr light cycle. The temperature and humidity in the enclosures were approximately 23.9°C (75°F) and 75%, respectively.

After a 72-hr acclimation period, each spider was placed into a square, 3-L plastic storage container that was modified into a gas anesthetic chamber. The container was customized by drilling a hole in one side and inserting an endotracheal tube adapter. Each spider was anesthetized with 5% isoflurane (Isoflo, Abbott Laboratories, North Chicago, Illinois 60064, USA) at a flow rate of 1 L/min oxygen (event 1). Each spider was monitored at intervals of 5 min for the presence of a righting reflex and reaction to tactile stimuli. Righting reflex was scored on an ordinal scale: 0 = normal, 1 =attempted but unsuccessful, and 2 = absent. Once each spider had lost its righting reflex, the isoflurane was discontinued and the spider removed from the anesthetic chamber. The spider was quickly weighed and an intracardiac hemolymph sample taken.7 Additionally, 0.5 ml of 0.9% saline (Hospira Inc., Lake Forest, Illinois 60045, USA) was administered into the opistho-

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soma of each *G. rosea.* These procedures were conducted for a separate study<sup>8</sup> and required less than 1 min to perform. Each spider was returned to the anesthetic chamber for recovery in 100% oxygen and monitored at 5-min intervals until it recovered. Recovery was defined as the return of a normal righting reflex. The temperature for each anesthetic event was between 22.2-22.8°C (72–73°F).

The spiders were held under the conditions described earlier for an additional 8 wk. The spiders had constant access to chlorinated tap water and were fed adult crickets (*Acheta domesticus*) weekly. The crickets had access to a high-calcium cricket food and water source (High Calcium Cricket Diet and Cricket Quencher Calcium, Fluker Farms, Port Allen, Louisiana 70767, USA) until being offered to the spiders. The anesthesia protocol described above was then repeated (event 2). The temperature for each anesthetic event was between 22.2–22.8°C (72–73°F).

Times for induction (defined as minutes until righting reflex was lost) and recovery (defined as minutes from end of induction until righting reflex returned to normal) were calculated. The distribution of both induction and both recovery times was evaluated for normality using the Shapiro-Wilk test, skewness, and kurtosis. The Wilcoxon signed ranks test was used to determine if there were differences between the induction times and the recovery times. The median, 10-90% percentiles, and minimum-maximum are reported for the anesthetic events. For each anesthetic event for each species, a Friedman two-way analysis of variance by ranks was used to evaluate for differences between interval righting reflex scores. When a significant difference was found, the Wilcoxon signed ranks test was used to compare each interval score to the initial score in order to determine the overall anesthetic time. Significance testing was set at P $\leq$  0.05. The statistical analysis was performed using a commercial software package (SPSS 15.0, SPSS Inc., Chicago, Illinois 60606, USA).

The induction and recovery times for each species did not exhibit a Gaussian distribution (P < 0.0001). The data for both induction times (T. blondi: Z = -1.00, P = 0.32; G. rosea: Z = -0.45, P = 0.66) and both recovery times (T. blondi: Z = -0.28, P = 0.78; G. rosea: Z = -0.49, P = 0.62) were not significantly different in the two species. The mean values for induction and recovery were calculated for each spider, and the descriptive statistics for these parameters are

listed in Table 1. A significant difference between interval scores was found for each anesthetic event for *T. blondi* (event 1:  $\chi^2 = 122.13$ , *P* < 0.0001; event 2:  $\chi^2 = 94.70$ , *P* < 0.0001) and *G. rosea* (event 1:  $\chi^2 = 27.9$ , *P* < 0.0001; event 2:  $\chi^2$ = 168.51, *P* < 0.0001). For *T. blondi*, a significant difference was found until 70 min for event 1 (Z = -1.89, *P* = 0.06) and 50 min for event 2 (Z = -1.732, *P* = 0.08). For *G. rosea*, a significant difference was found until 25 min for event 1 (Z = -1.89, *P* = 0.06) and 30 min for event 2 (Z = -1.00, *P* = 0.3).

All the spiders survived the anesthetic events without apparent complications or detrimental effects. Based on the results presented here, isoflurane appears to be a safe and effective anesthetic for immobilizing theraphosid spiders. Anecdotal protocols for isoflurane anesthesia of theraphosid spiders indicate that a concentration of 3–10% should be used, and that induction can be accomplished within 10-15 min.<sup>1,3,4</sup> Only one study appears to have been published in which isoflurane anesthesia was systematically monitored in a theraphosid spider species.<sup>2</sup> Sixteen G. rosea were anesthetized in a chamber with 5% isoflurane in oxygen, and time to loss of righting reflex (mean  $\pm$  SD: 3.97  $\pm$  2.57 min) and time to recovery (mean  $\pm$  SD: 8.10  $\pm$  4.65 min) were measured. The result for induction time was less than that found in this study. This difference is most likely due to the 3-min monitoring interval that was used, compared to the 5-min interval used in this study. The result for recovery time is similar to what was found in this study for G. *rosea* when the measures of dispersion of the data are taken into account. Also, the collection of a hemolymph sample and administration of fluids in our study did not appear to have an adverse effect on the spiders' ability to cope with anesthesia.

The difference in overall anesthetic times between event 1 and event 2 for *T. blondi* is an interesting finding. Although the induction times for the two events were similar, there were two spiders that had prolonged recoveries during event 1 (e.g., 95 min and 90 min). The difference in the overall anesthetic times is most likely due to these outliers. It is also possible that subclinical changes in the hydration, nutrition, or health status of the animals over the course of the study could have affected these results.

Isoflurane is a member of the modern halogenated anesthetic agents, a group that also includes enflurane, desflurane, and sevoflurane.<sup>6</sup> In mammals, the mechanism of action of

|                   | Median | 10–90% Quantiles | Minimum-maximum |
|-------------------|--------|------------------|-----------------|
| Theraphosa blondi |        |                  |                 |
| Induction         | 10     | 10–15            | 10-20           |
| Recovery          | 30     | 12.5-52.5        | 12.5-72.5       |
| Grammostola rosea |        |                  |                 |
| Induction         | 10     | 10-25.5          | 10-30           |
| Recovery          | 12.5   | 8.25–47          | 7.5-57.5        |

**Table 1.** Descriptive parameters for induction and recovery during isoflurane anesthesia of goliath birdeater spiders (*Theraphosa blondi*) and Chilean rose spiders (*Grammostola rosea*). All values are expressed in minutes.

isoflurane is thought to be enhancement of inhibitory postsynaptic activity and inhibition of excitatory synaptic activity via action on ion channels.<sup>6</sup> The biodegradation of isoflurane in humans is 0.2%, and elimination is accomplished solely via the lungs.<sup>5</sup>

The mechanism of action and method of elimination of isoflurane in theraphosid spiders are likely to be similar to that of mammals. However, the primitive nature of the respiratory and nervous systems of theraphosid spiders may allow for physiologic differences. Despite this, the clinical effects of isoflurane on both vertebrate and invertebrate taxa appear to be similar.

In this study, the righting reflex was found to be the only reliable method of monitoring the anesthetic depth of the spiders. Other methods that were tried, but quickly abandoned, included stroking the carapace and opisthosoma with a cotton-tipped applicator and prodding the distal limb with the tip of a hypodermic needle. These methods were discontinued because it was difficult to stimulate a consistent response for these techniques in nonanesthetized spiders. A crystal Doppler was also used in an attempt to assess heart rate, but was not found to be useful. Respirations could not be measured because there is no visible movement of the abdomen. Future efforts should be directed at developing better methods of anesthetic monitoring, such as tracking physiologic parameters (e.g., heart and respiratory rates).

The use of a chamber for delivery of anesthetic gasses to invertebrates is a common practice. However, this does present a risk of exposure to the anesthetic gasses by personnel in the vicinity of the chamber. Development of more direct administration of inhalant anesthetic agents to the book lungs could ameliorate this situation. It would also allow for better control of the concentration of the anesthetic gas that is delivered. Future studies should investigate the use of other anesthetic agents in theraphosid spiders, including other inhalant (e.g., sevoflurane) or intravenous (e.g., propofol) agents.

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