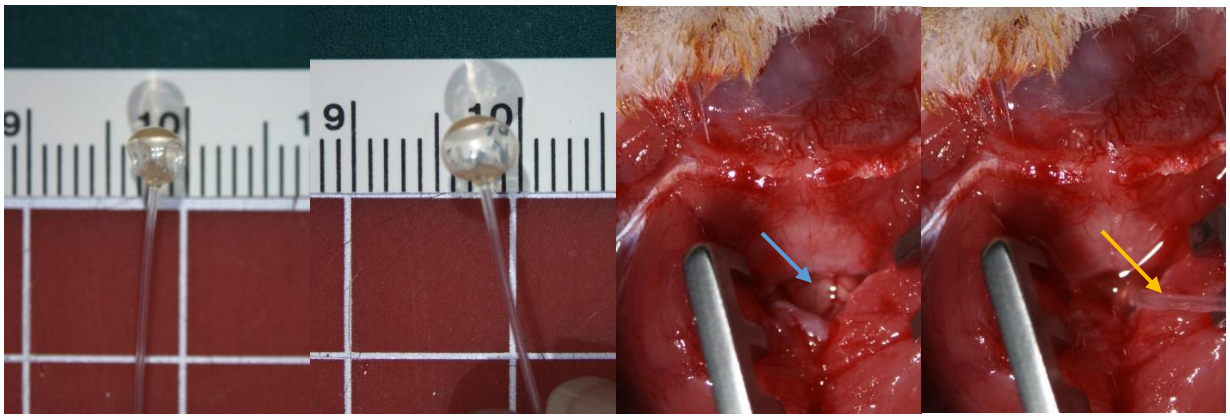
Progress Report
ASRF grant LG2014-3, *Developing an animal model of the upper cervical subluxation*

1. With regard to progress against key milestones and the extent to which the objectives of the approved proposal have been met

Specific Objectives:

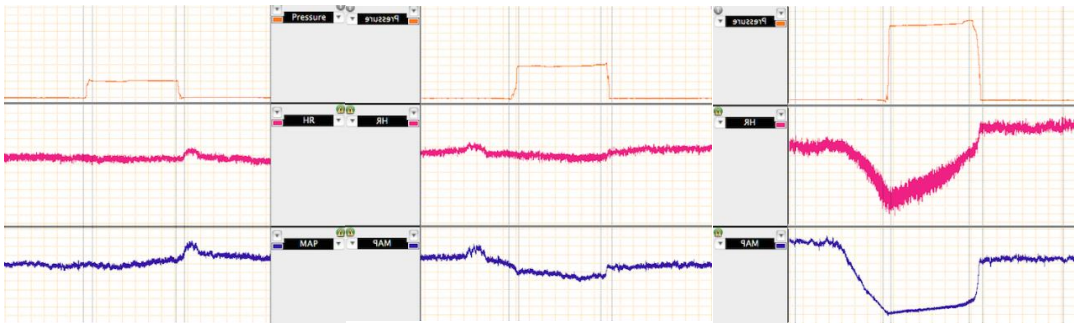
i) We have piloted a minimally-invasive, acute surgical model of non-destructive cervical spinal cord compression in the rat; production of the balloon catheters has become routine, and the balloons perform in a reliable fashion. The surgical approach has also become routine and we have had no surgical failures.

In more detail, and as illustrated in the photographs below, it is now possible to produce almost perfectly spherical balloons which inflate at physiological pressures. On the far left is a balloon at 0 mm Hg internal pressure. The small divisions on the scale in the photograph mark off millimeters. Second from the left is the same balloon at 40 mm Hg internal pressure and at a diameter sufficient to compress the rat spinal cord at the C1 level. Third from the left is a photograph of the surgical approach with the blue arrow indicating the spinal cord exposed through the reflected atlanto-occipital membrane. The animal's occiput begins just above the head of the arrow. On the right is a photograph of the catheter (orange arrow) running into the spinal canal and attached to the balloon pictured in the two photographs on the left.



ii) Using the acute model, we have identified, in each animal, the threshold (in terms of pressure) for attenuation of descending sympathetic tone within the spinal cord and we have demonstrated recovery, in every case, of sympathetic function following the removal of pressure. This is illustrated in the screen captures below, taken from the same animal in which balloon pressure was raised from 0 mmHg to 20 mm Hg (left most panel), 40 mm Hg (centre panel) and 90 mm Hg (right most panel). In each screen capture the orange tracing shows balloon pressure raised from 0 mm Hg and held at the elevated level for approximately 2 minutes. The red tracings show heart rate and the purple tracings show mean arterial pressure. In all experiments so far, at

20 mm Hg there is no response or a small response which may be an increase or decrease in heart rate and mean arterial pressure. At 40 mm Hg, there is a clear but moderate response of decreased heart rate and blood pressure. When the balloon is inflated to the individual animal's mean arterial pressure, in this case 90 mm Hg, there is a profound decrease in heart rate and blood pressure, and in some trials we have cut short the period of compression in order to prevent cardiac arrest. The changes in measures of heart rate and blood pressure are matched by changes in sympathetic tone as calculated from heart rate variability.



iii) We have demonstrated the non-destructive nature of the experimental compression via post-mortem examination of fixed and excised spinal cord tissue, looking for both disruption of normal tissue boundaries and histological evidence of microscopic tissue disruption.

In more detail, at the end of each trial the animals are more deeply anesthetized and perfused with formalin to fix the spinal cords with the balloon in place. Then the spinal cord beneath the balloon is removed for histological examination. We have used a variety of staining methods. In the instance below, the spinal cord was treated with silver stain which brings out very clearly the boundaries between white and gray matter, and shows that anatomical boundaries have not been disrupted.

2. Regarding the percentage of work complete and an estimated completion date, we have completed the first of three series of experiments, and have begun the second series of experiments. Consequently, approximately 35% of the study has been completed. This places us at approximately 40 weeks from project completion i.e. July 2017.

3. Regarding the purchase of equipment including the location and working order of this equipment (if applicable), no new equipment has been purchased since all required equipment is on hand and in working order. As per the attached financial statement, $1,561.96 CAD has been spend on consumables, including animals, since the inception of the project.

4. Regarding funding and/or other resources provided by other funding organisations towards meeting the aims of the approved proposal, no other organization has contributed to this project.

5. Regarding funding and/or other resources provided by any other institution other than the grant recipient towards meeting the aims of the approved proposal, none has been received.

6. No publications, presentations or posters have yet arisen from this project.

Of interest to your board, a requirement of our institution's certification by the Canadian Council on Animal Care is that the Animal Care Committee conduct post approval monitoring annually on all approved protocols. An inspection was conducted on September 27, 2016 during an actual experiment and the Animal Care Committee has not requested any changes/improvements in our protocol.

In summary, the proposed work is proceeding without technical difficulties and we are routinely obtaining consistent and useable data. Consequently, we anticipate that the project will be completed as originally proposed. We deeply appreciate the kind support of the Australian Spinal Research Foundation and believe that this project will turn out to have been a very productive investment.