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Research Report

Effects of spinal cord injury on the rat estrous cycle

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ABSTRACT

Approximately 3000 women of childbearing age are afflicted with spinal cord injuries each year and many experience temporary amenorrhea immediately following injury. In the present study, the effect of mid-thoracic spinal contusions on the rat estrous cycle was examined. The sixteen rats used for this study all had 4-day cycles (proestrus, estrus, metestrus, diestrus), as determined during the 2 weeks prior to injury. Following contusion at the T8 spinal level (made using the Infinite Horizon impactor device), seven of the animals (44%) experienced a temporary interruption in the progression of the estrous cycle (mean of 9.4 days delay), which was not correlated with impact force or total damage at the lesion epicenter. The presence of a delay was, however, correlated with damage/sparing of ventromedial white matter at the lesion epicenter. The results indicate that the rat's hormonal status is an experimental variable that is present during the acute phase following spinal cord injury. The temporary nature of the cycle delay may reflect compensatory mechanisms related to the dual innervation (spinal and vagal nerve supply) of the ovaries.

1. Introduction

There are approximately 11,000 new cases of spinal cord injuries (SCI) each year in the United States. According to the National SCI Statistical Center at the University of Alabama in Birmingham (www.spinalcord.uab.edu—updated June 2005), the trend has been a slight increase in the prevalence of SCI's occurring among women (20.4% prevalence since the year 2000). While SCI has a major impact on fertility in young males (who are disproportionately afflicted with SCI), fertility among women is not considered a major problem. There are, however, some critical issues and risks surrounding pregnancy and childbirth, which include urinary tract infections, premature delivery, deep venous thrombosis, pressure ulcers, constipation and autonomic dysreflexia (Berard, 1989; Burns and Jackson, 2001; Linsenmeyer, 2000). The ability for some SCI women to have children is an issue that gets raised during rehabilitation following injury (Axel, 1982), since some SCI women continue to menstruate following injury while others don't.

A survey of 132 articles published in Journal of Neurotrauma between April 2002 and May 2003 indicates that the majority of those using rats to model SCI in their studies use females, and none of the experiments in these articles controlled for or followed the estrous cycle (unpublished observations). There is evidence in the traumatic brain injury and stroke literature as well as a few studies in the SCI literature that progesterone and estrogen could be neuroprotective and thus impact experimental outcome/functional recovery (Alkayed et al., 2000; Jiang et al., 1996; Labombarda et al., 2002; Liao et al., 2001, 2002; Roof et al., 1993, 1994, 1996; Roof and Hall, 2000; Thomas et al., 1999; Wilson et al., 2000; Wright et al., 2001). Thus, a possible issue surrounding experimental studies in animals concerns the

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impact of potential hormonal variations following acute injury on secondary injury and recovery processes. In the present study, the estrous cycle was followed both pre- and post a commonly used clinically relevant contusion injury. The cycling results are consistent with those observed in humans. The presence/absence of an interruption of the estrous cycle is discussed relative to such variables as the impact force, area of damage at the lesion epicenter, and the stage of the cycle at the time of injury.

2. Results

2.1. Effects of SCI on the progression of the estrous cycle

The estrous cycle of nine (56%) of the sixteen 4-day cycling animals was unaffected by the contusion injury (4 day post-injury cycles). Since not all animals experienced cycle delays, surgical controls (such as for the spinal cord exposure) were not necessary. For 7 animals (44%), a mean delay of 9.4 ± 1.4 days in their estrous cycle was observed post-contusion injury, which ranged from 6 to 17 days. Throughout this delay period, the cell populations observed within the vaginal smears contained many leukocytes, some cornified cells and some nucleated cells (654, 27, and 32, respectively—averages calculated from 4 animals chosen at random). No significant correlations were found between the length of cycle delay and the amount of each of the various cell types contained within the smears during the delay. In the animals that stopped cycling for a short period, the length of their cycle once it resumed again continued as it was before surgery; i.e., animals that had 4-day cycles prior to the injury had 4-day cycles after their cycles resumed post-injury. A comparison of the cell populations within each of the four stages (proestrus, estrus, metestrus and diestrus) obtained for 4 complete cycles both pre-injury and post-resumption of their cycle is presented in Table 1. Examples of smears obtained from normal animals can be seen in our previously published paper (Hubscher et al., 2005). No significant differences were found in the three cell populations present in vaginal smears for each estrous stage pre-injury versus after their cycles resumed. Note that no relationship was found between the presence/absence of a cycle delay and the stage of estrous on the day of the spinal contusion injury. Both the delayed and non-delayed groups had animals with lesions that had been done in each of the various stages of estrous.

Table 1 – Comparison of cell populations within vaginal smears pre-SCI (lightly shaded lines) and after resumption of cycling post-injury (darkly shaded lines)

	Leukocytes	Cornified cells	Nucleated cells
Proestrus (n = 24)	24.4 ± 9.9	42.0 ± 17.2	75.6 ± 30.8
Proestrus (n = 36)	18.9 ± 7.7	37.8 ± 15.4	49.0 ± 20.0
Estrus (n = 24)	3.2 ± 1.3	81.7 ± 33.3	31.5 ± 12.9
Estrus (n = 30)	1.2 ± 0.5	134.3 ± 54.8	60.3 ± 24.6
Metestrus (n = 16)	1133.2 ± 308.4	11.8 ± 3.2	103.6 ± 32.3
Metestrus (n = 36)	1343.4 ± 548.3	20.8 ± 8.5	64.7 ± 26.4
Diestrus (n = 42)	273 ± 111.5	14.2 ± 5.8	67.2 ± 27.4
Diestrus (n = 53)	274.9 ± 112.2	21.9 ± 8.9	75.7 ± 30.9

Pre-injury values are based upon data from six normal cycling animals as observed in a 200 μm^2 area (at 200× magnification) at the densest part of the smear. No significant data were found (t test; $P > 0.05$) pre-injury versus post-injury (darkly shaded values, from four contused animals, represent smear data obtained after the cycle resumed post-delay).
n = total number of stages observed in sample.

2.2. Impact force

Data specific to the injury was collected and analyzed for ten of the sixteen animals (not all injury parameters were recorded for the initial six animals). The actual force used to produce the contusion injuries at the T8 spinal level ranged from 198 to 279 kdyne with a mean of 227.2 ± 9.4 kdyne. Examples showing the displacement of the spinal cord by the impactor are illustrated in Fig. 1A. As shown, peak displacement of the spinal cord was not an indicator of the presence or the absence of a delay that an animal would experience. The actual force of the impact as measured by the IH impactor device was also not an adequate predictor of the delay of length of interruption an animal would experience (Fig. 1B).

2.3. Area of damage at lesion epicenter

Sections obtained from each spinal cord lesion were stained at the level of the lesion using the Klüver–Barrera method and the epicenter of injury was subsequently found. In the epicenter, lesion areas ranged from 0.52 mm^2 to 1.42 mm^2. Extent of damage/sparing at the lesion epicenter was compared across animals. Upon first appearance under the light microscope, no obvious trends were observed in terms of size or extent of the injury for animals with versus animals without a cycle delay. Quantification of total spinal cord damage did not show a relationship between percentage of area damaged (mm^2) at the epicenter and cycle delay (see Fig. 2A). Calculation of the correlation coefficient did not reveal any correlation between these variables.

Upon closer inspection, there did appear to be one location, the ventromedial white matter, which was consistently damaged bilaterally in those animals with cycle delays. Quantification of damage to ventromedial white matter showed that animals with less than 80% of ventromedial white matter damage had no delays in the cycle, whereas those with greater than 80% damage had delays. A positive correlation was found between the percentage of the lesion and cycle delay, as shown in Fig. 2B. An image taken at one of the lesion epicenters showing severe ventromedial white matter damage bilaterally (from one of the animals with a cycle delay) is inserted in Fig. 2B.

3. Discussion

Spinal cord contusions at T8, given to rats having normal 4 day estrous cycles, resulted in an interruption of the cycle in some (44%), but not all of the animals. The cycle delay