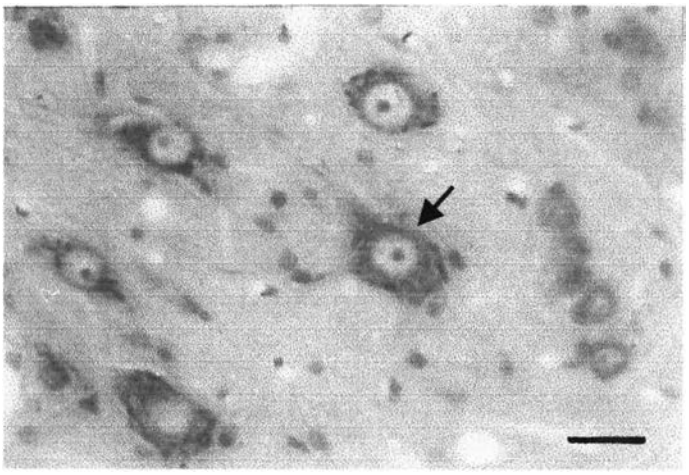
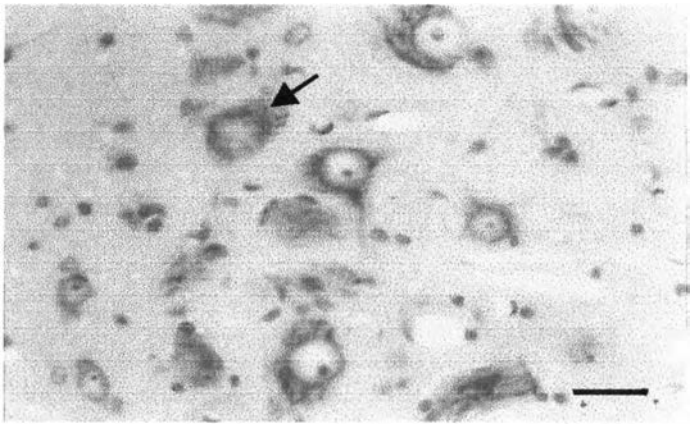
CHAPTER 4
RESULTS

1. Sensory and Motor neurons differentiate after axotomy and axotomy with ligation

The differentiation of motor neurons appear normal after axotomy and axotomy with ligation at several survival time 1, 2, 4, 6, 8, 16 and 32 weeks (Figure12) but otherwise sensory neurons in C7, C8, and T1 dorsal root ganglia were chromotolytic cells with eccentrically positioned nuclei at all postoperative times (Figure13). Chromatolysis was found more frequently at middle to later time periods. The most conspicuous changes in the initial stages are those affecting the nucleus and the Nissl granules. The nucleus is displaced to opposite the axon hillock; its membrane may show indentations, and the nucleolus enlarges. The Nissl granules lose their compact arrangement, breaking into fine dust-like particles, which are dispersed, through the cytoplasm that becomes diffusely and weakly basophilic. This change commences in the vicinity of the nucleus and gradually extends to the periphery.

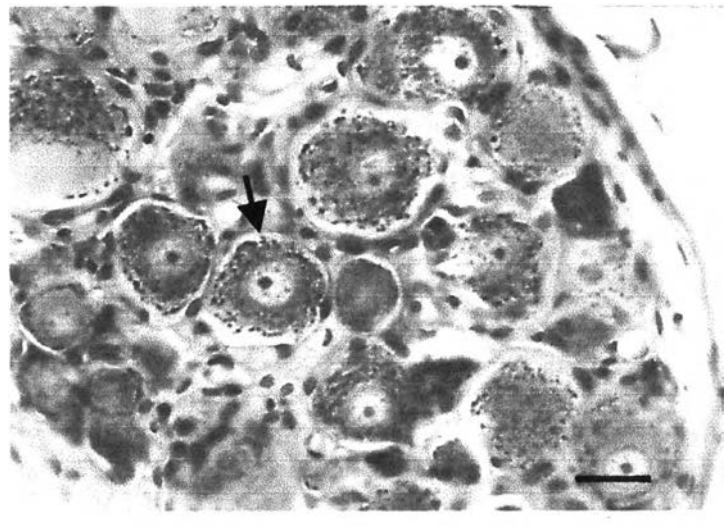


(A)

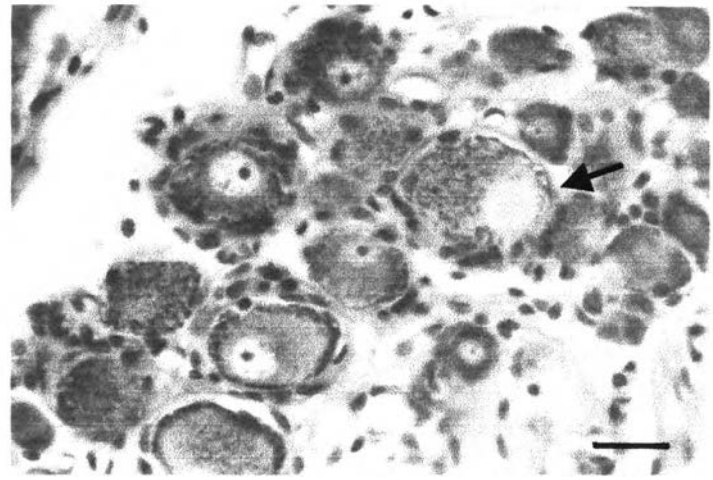


(B)

Figure 12 Photomicrographs of histological section stained with cresyl violet of spinal cord showing motor neurons from a normal spinal cord (arrow) *(A)*, and motor neurons from spinal cord 1 week after axotomy (arrow) *(B)*.(Magnification 500X, bar represent 20 μm)



(A)

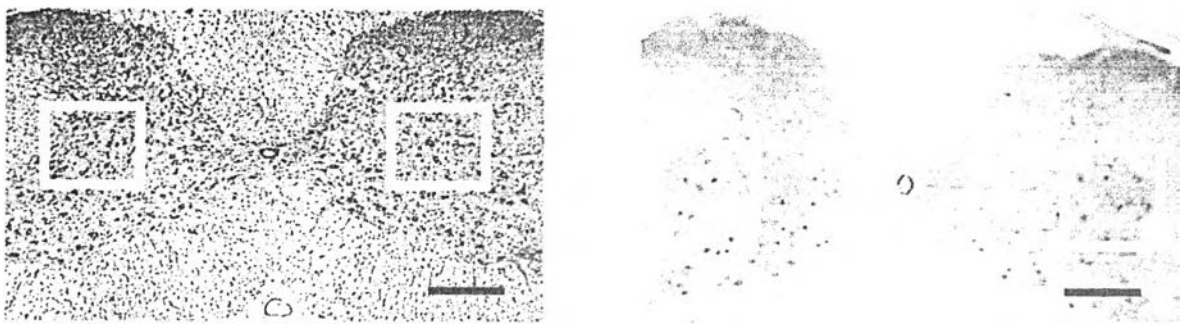


(B)

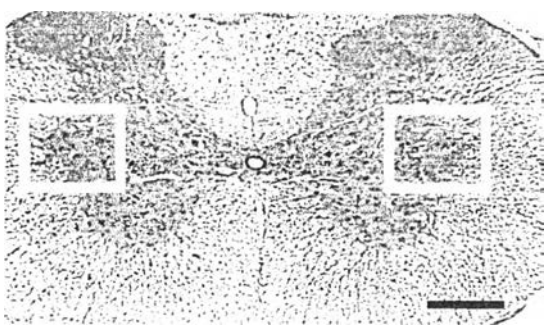
Figure 13 Photomicrographs of histological section stained with cresyl violet of the dorsal root ganglion *(A)*, Nerve cell bodies form a normal dorsal root ganglion (arrow) and *(B)*, a ganglion 1 weeks after axotomy of its spinal nerve (arrow). (Magnifications 500X, bar represent 20 μm)

2. Identification of median and ulnar motor neurons

The location of the median and the ulnar motor neurons in spinal cord was identified. Motor neurons that projected into the median and ulnar nerve are located within the spinal segments C7, C8 and T1 of spinal cord. This localization was confirmed in this study using the retrograde transport of Fluorogold. The enumeration was quite straight forward as they were clustered in the lateral region of ventral horn (Figure 14,15).



(A) (B)



(C)

Figure 14 Photomicrographs of histological section stained with cresyl violet of spinal cord. *(A)*, The lateral region where ulnar and median motor neurons of spinal cord at level of C7 normally reside, or are lost after axotomy, is outlined by the arrows. *(B)*, The lateral region where ulnar and median motor neurons of spinal cord at level of C8 normally reside, or are lost after axotomy, is outlined by the arrows. *(C)*, The lateral region where ulnar and median motor neurons of spinal cord at level of T1 normally reside, or are lost after axotomy, is outlined by the arrows. (Magnifications 500X, bar represents 20 μm)

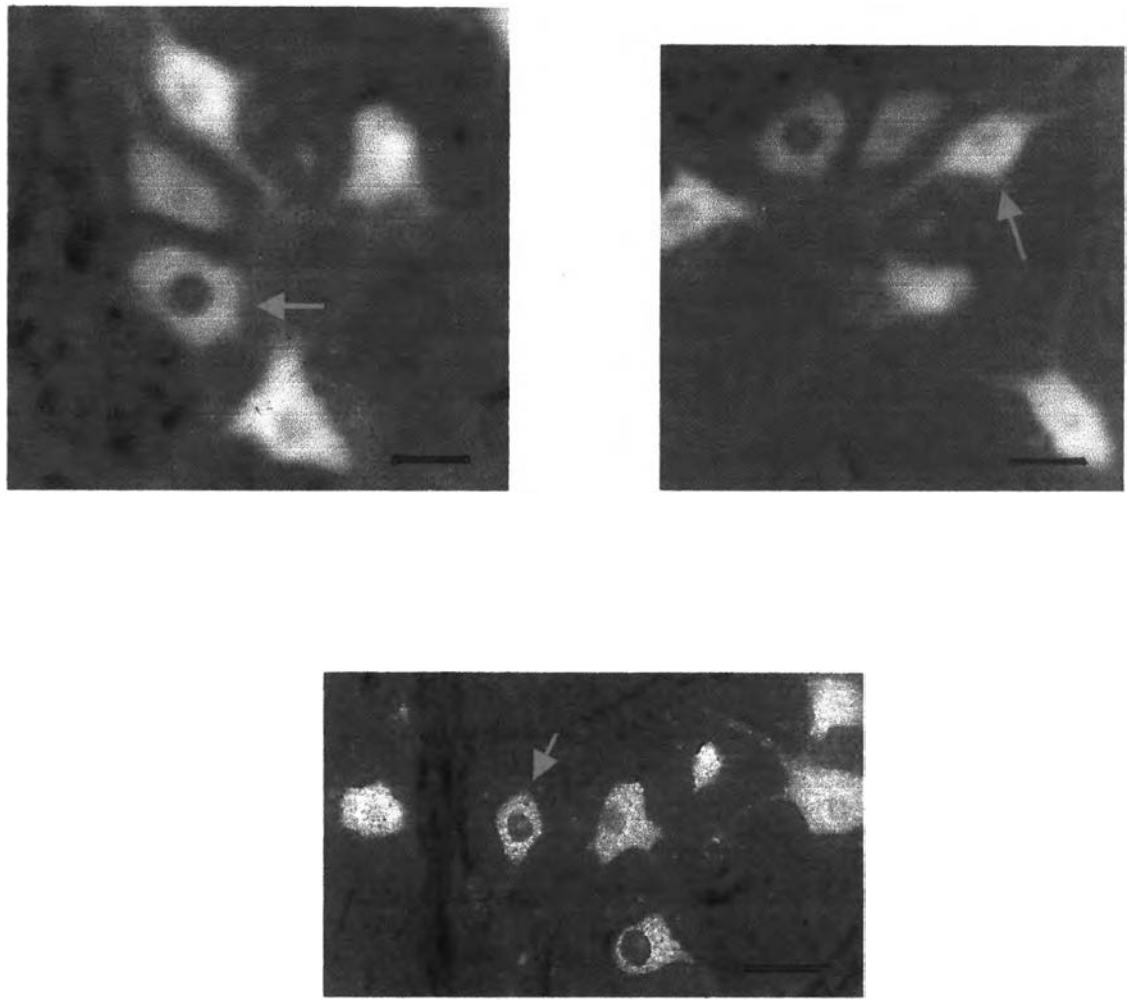


Figure 15 Photomicrograph of Fluorogold-labled motor neurons with in the spinal cord of an adult rat. Retrograde Fluorogold into the median and ulnar nerve labeled these control motor neurons. At higher magnification, the cell bodies and processes are extensively filled with Fluorogold (arrow). Fluorescence microscope pictures at 680X. Scale bar = 15 μm

3. Time courses of adult rat sensory and motor neuronal loss following axotomy

In this experiment, the aim was to determine the rate and extent of sensory and motor neuronal loss in the ipisilateral C7, C8 and T1 spinal ganglia following axotomy.

In the ipisilateral C7 dorsal root ganglion, one week after injury, there was a 14% cell loss; the mean valve of the cell loss was steady at 14% by week 2; 15% by week 4; 15% by week 6; 15% by week 8; 15% by week 16 and 16% by week 32 (Figure 16).

In C8 dorsal root ganglion, the neuron loss was steadily similar to C7 DRG. One week after axotomy, there was a 12% cell loss on the lesioned side of DRG; 12% by 2 weeks; 13% by 4 weeks; 15% by 6 weeks; 16% by 8 weeks; 18% by 16 weeks and 19% by 32 weeks (Figure 17).

In T1 DRG; the neuronal loss increased steadily, one week after axotomy there was a 12% cell loss on the lesion side of DRG; 14% by 2 weeks; 15% by 4 weeks; 15% by 6 weeks; 16% by 8 weeks; 19% by 16 weeks; and 20% by 32 weeks (Figure 18).

In C7 motoneuron; one week following axotomy, a small neuronal loss was found at approximate 10%. No apparent neuronal loss was detected 2, 4, 6, 8, 16 and 32 weeks after axotomy. Similar results have been obtained following axotomy in adult rats (Figure 19).

In C8 motoneuron; the neuronal loss is similar to the results of C7 motoneuron. One week following axotomy, there was a small neuronal loss 10%. No apparent neuronal loss was detected 2, 4, 6, 8, 16 and 32 weeks after axotomy. Similar results have been obtained following axotomy in adult rats (Figure 20).

In T1 motoneuron; the neuronal loss is similar to the results of C7 motoneuron. One week following axotomy , a small neuronal loss 8%. No apparent neuronal loss was detected 2, 4, 6, 8, 16 and 32 weeks after axotomy. Similar results have been obtained following axotomy in adult rats (Figure 21).

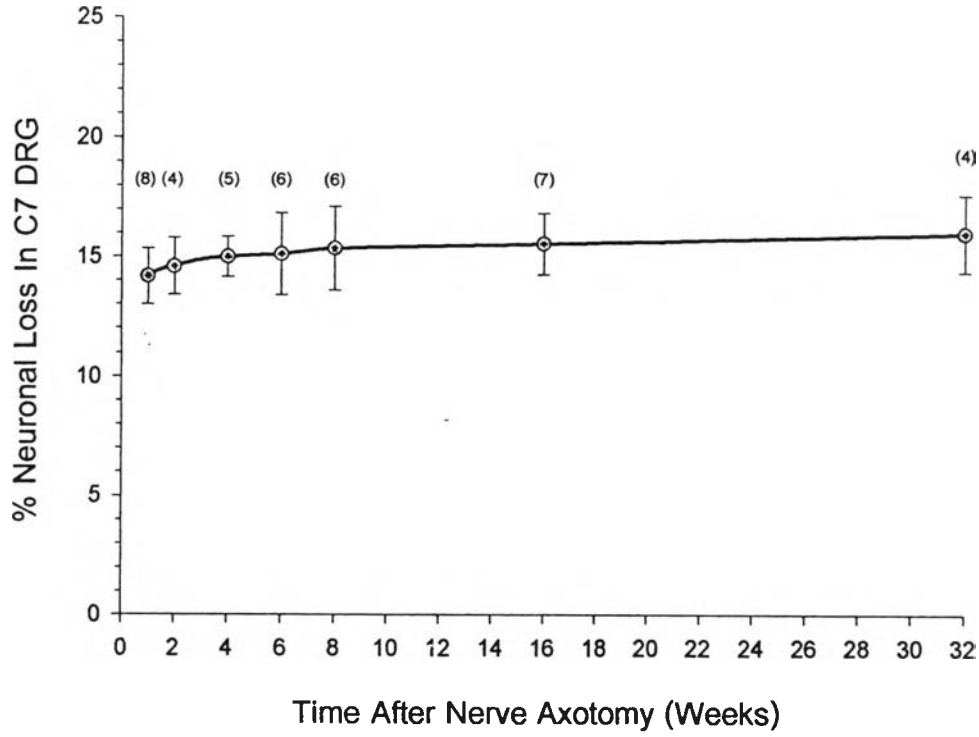


Figure 16 The loss of sensory neurons in C7 at several survival times after median and ulnar nerve transection in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group.*$p \leq 0.05$ using ANOVA and post-hoc (Duncan) test

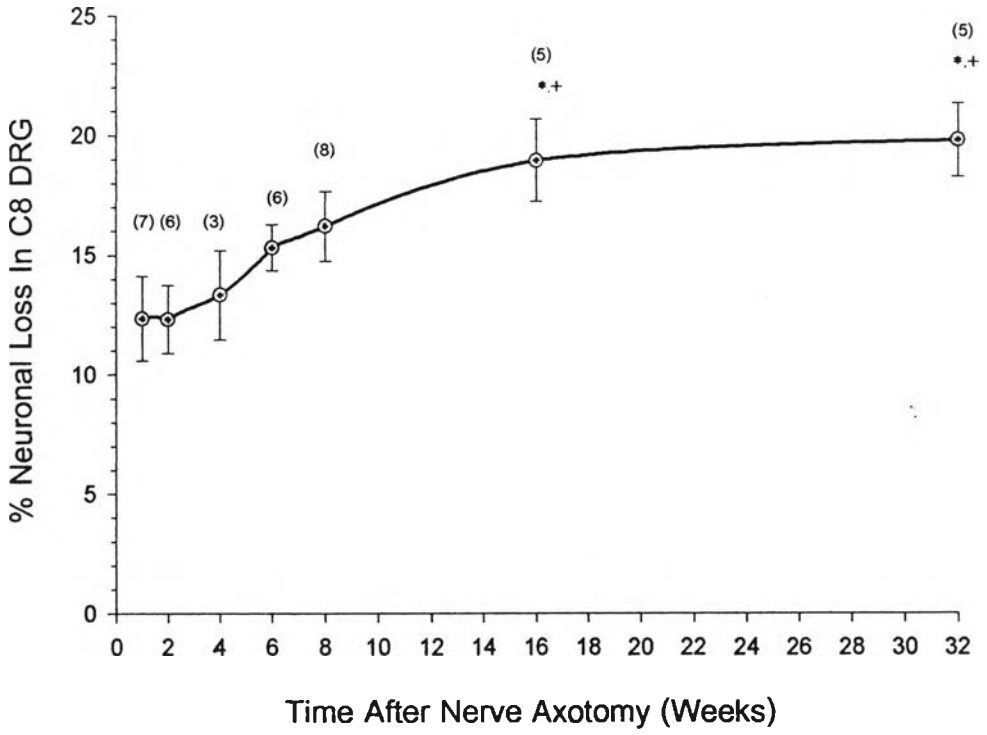


Figure 17 The loss of sensory neurons in C8 at several survival times after median and ulnar nerve transection in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group. *$p \leq 0.05$ versus 1 week after axotomy group, $^{+}p \leq 0.05$ versus 2 weeks after axotomy group, ANOVA and post-hoc (Duncan) test.

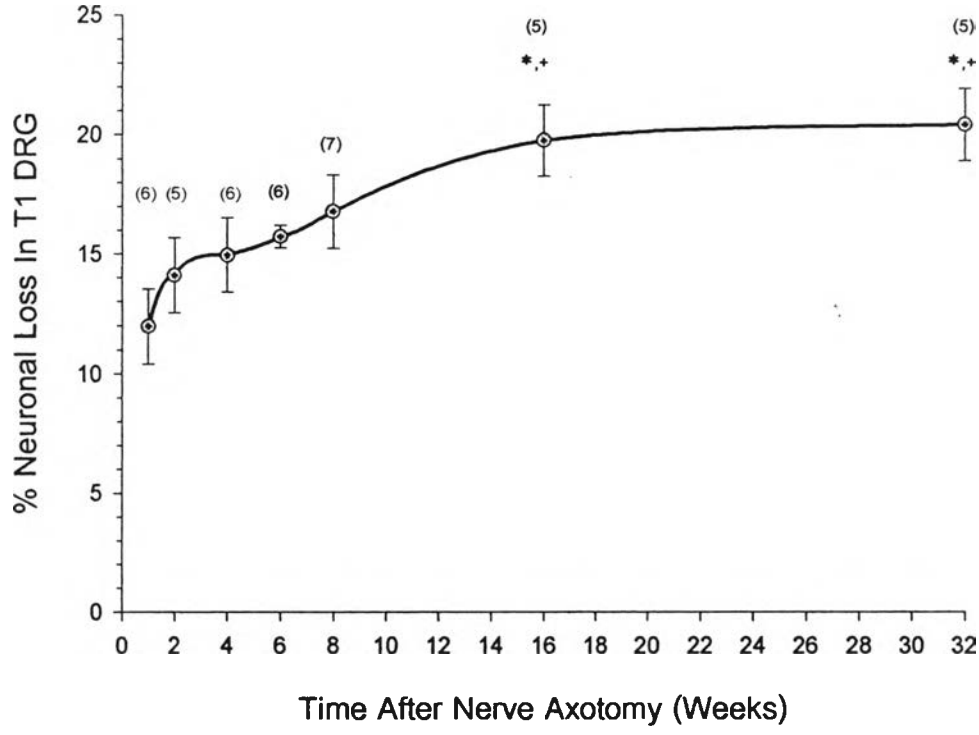


Figure 18 The loss of sensory neurons in T1 at several survival times after median and ulnar nerve transection in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group. *$p \leq 0.05$ versus 1 week after axotomy group, $^{+}p \leq 0.05$ versus 2 weeks after axotomy group, ANOVA and post-hoc (Duncan) test.

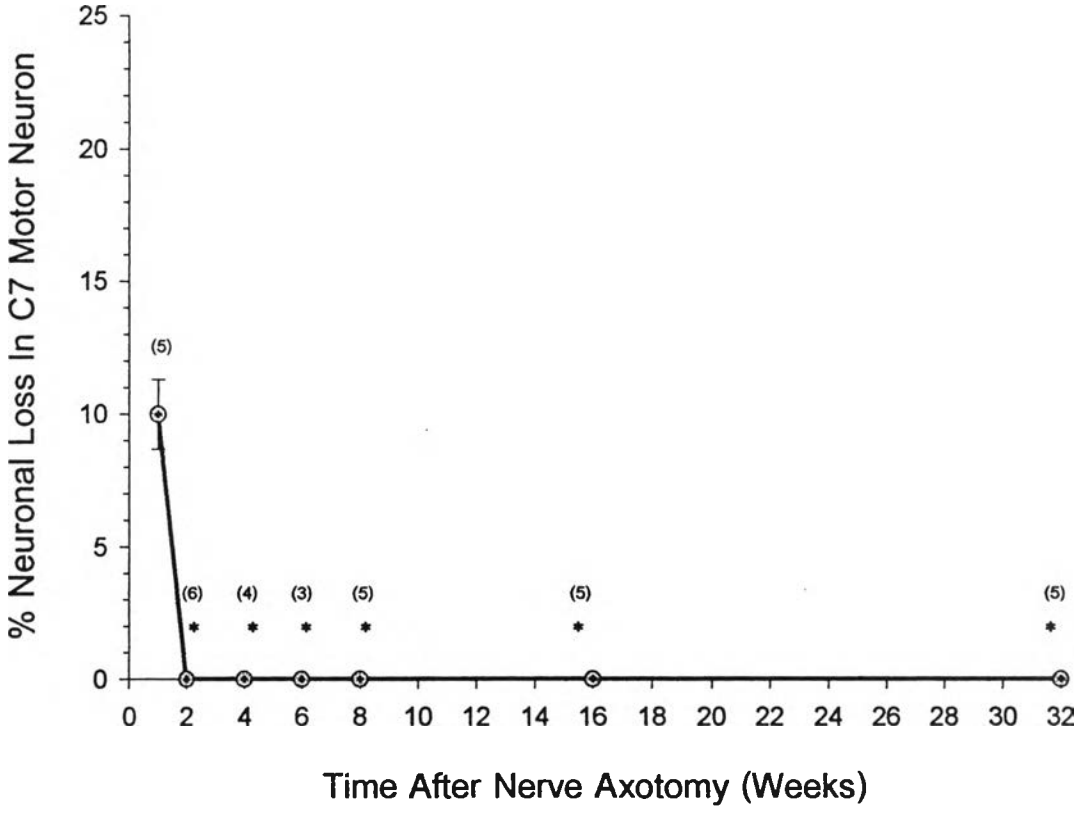


Figure 19 Time course of motor neuronal loss in the C7 region for several survival time after median and ulnar nerve transection in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group. *$p \leq 0.05$ versus 1 week after axotomy, ANOVA and post-hoc (Duncan) test.

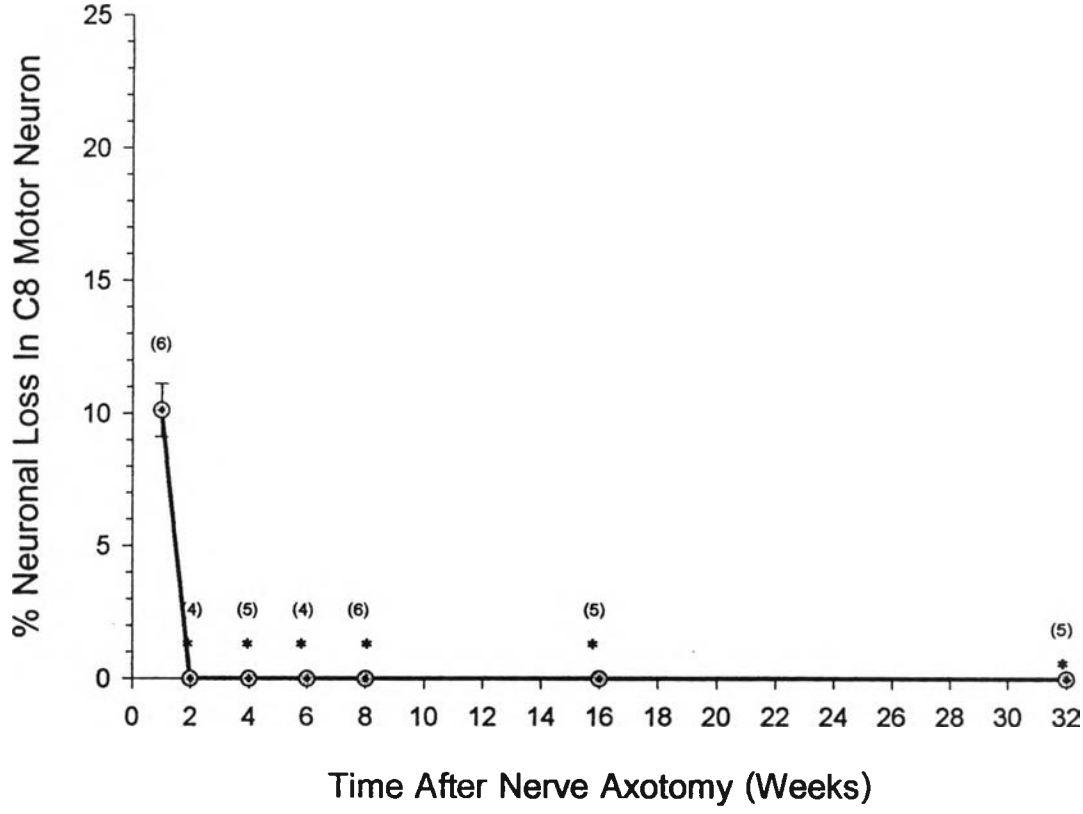


Figure 20 Time course of motor neuronal loss in the C8 region for several survival time after median and ulnar nerve transection in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group. *$p \leq 0.05$ versus 1 week after axotomy ANOVA and post-hoc (Duncan) test.

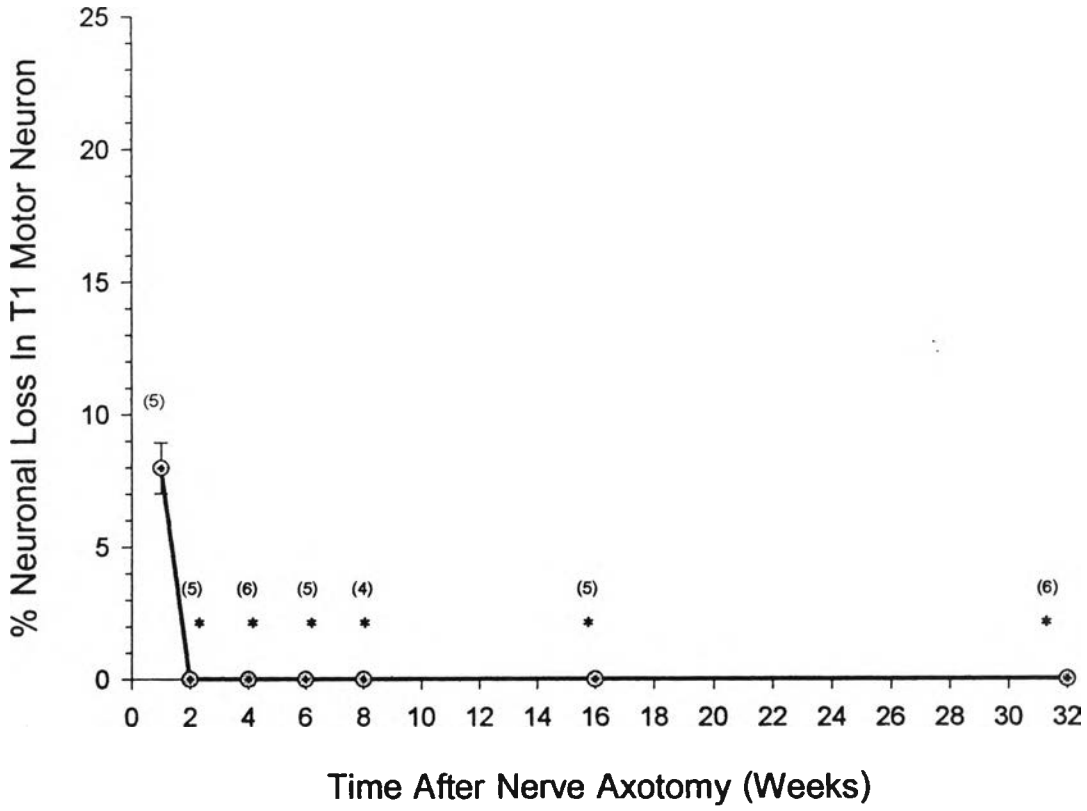


Figure 21 Time course of motor neuron loss in the T1 region for several survival time after median and ulnar nerve transection in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group. *$p \leq 0.05$ versus 1 week after axotomy ANOVA and post-hoc (Duncan) test.

4. Time courses of adult rat sensory and motor neuronal loss following ligation with axotomy

In this experiment, the aim was to determine the rate and extent of sensory and motor neuronal loss in the ipisilateral C7, C8 and T1 spinal ganglia following axotomy with ligation and compared with axotomy only.

In C7 dorsal root ganglion, one week after axotomy and ligation injury, there was 14% cell loss on the lesion side of DRG ; 14% by 2 weeks; 15% by 4 weeks; 16% by 6 weeks; 17% by 8 weeks; 18% by 16 weeks and 18% by 32 weeks (Figure 22).

In C8 dorsal root ganglion, the neuron loss increased steadily, one week after axotomy and ligation there was a 13% cell loss on the lesion side of DRG; 15% by 2 weeks; 16% by 4 weeks; 16% by 6 weeks; 17% by 8 weeks; 20% by 16 weeks and 23% by 32 weeks (Figure 23).

In T1 dorsal root ganglion, one week after axotomy and ligation injury, there was 14% cell loss on the lesion side of DRG; 15% by 2 weeks; 16% by 4 weeks; 16% by 6 weeks; 16% by 8 weeks; 19% by 16 weeks and 20% by 32 weeks (Figure 24).

In C7 motoneuron; one week following axotomy, there was a small neuronal loss of approximate 10%. No apparent neuronal loss was detected 2, 4, 6, 8, 16 and 32 weeks after axotomy. Similar results have been obtained following axotomy in adult rats (Figure 25).

In C8 motoneuron; the neuronal loss is similar results of C7 motoneuron. One week following axotomy, there was a small neuronal loss 12%. No apparent neuronal loss was detected 2, 4, 6, 8, 16 and 32 weeks after axotomy. Similar results have been obtained following axotomy in adult rats (Figure 26).

In T1 motoneuron; the neuronal loss is similar results of C7 motoneuron. One week following axotomy, there was a small neuronal loss 8%. No apparent neuronal loss was detected 2, 4, 6, 8, 16 and 32 weeks after axotomy. Similar results have been obtained following axotomy in adult rats (Figure 27).

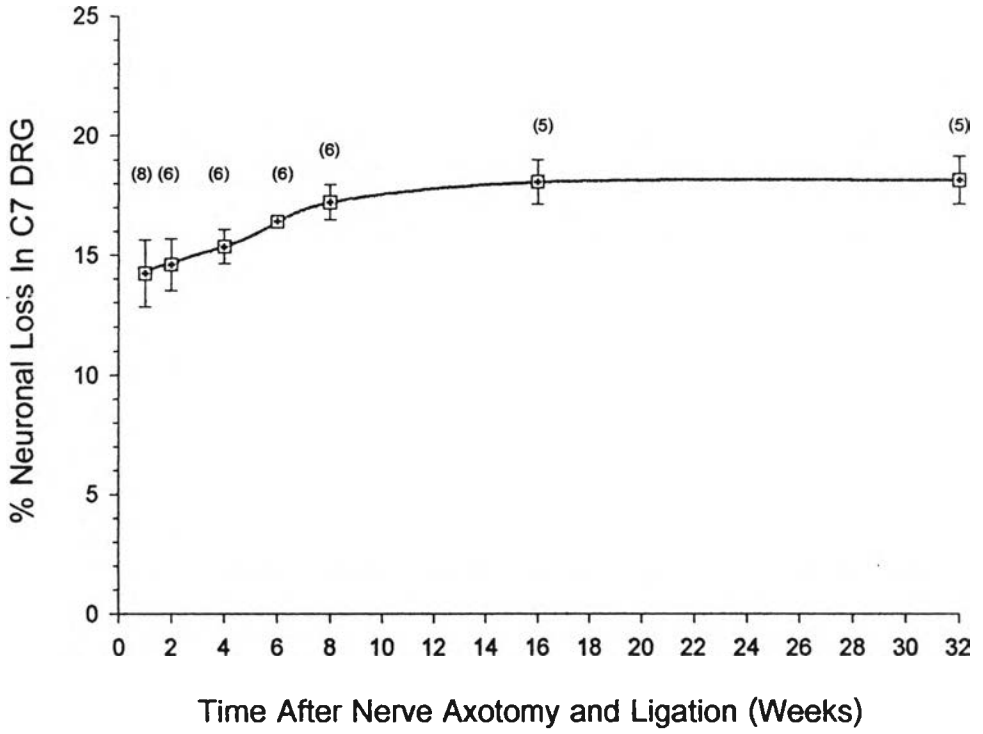


Figure 22 The loss of sensory neurons in the C7 DRG region for several survival times after median and ulnar nerve axotomy with ligation in adult rats. Data are expressed as percentage (mean ±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group. *$p \leq 0.05$ using ANOVA and post-hoc (Duncan) test.

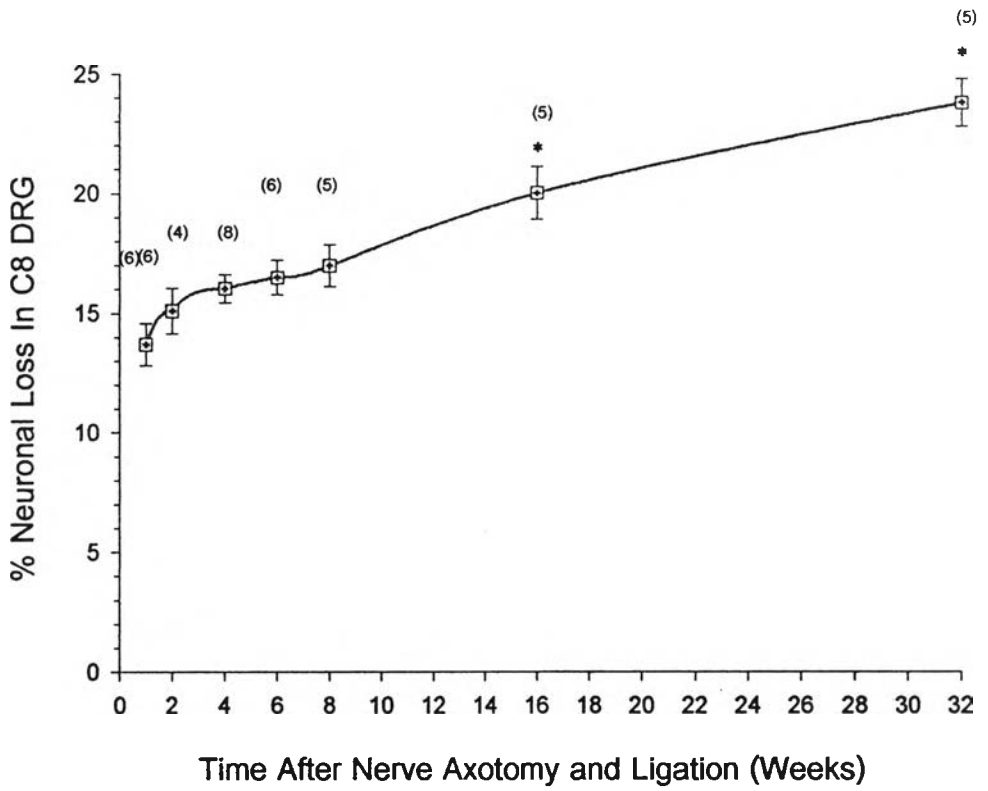


Figure 23 The loss of sensory neurons in the C8 DRG region for several survival times after median and ulnar nerve axotomy with ligation in adult rats. Data are expressed as percentage (mean ±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examined in each group. *$p \leq 0.05$ versus 1 week after axotomy with ligation group, ANOVA and post-hoc (Duncan) test.

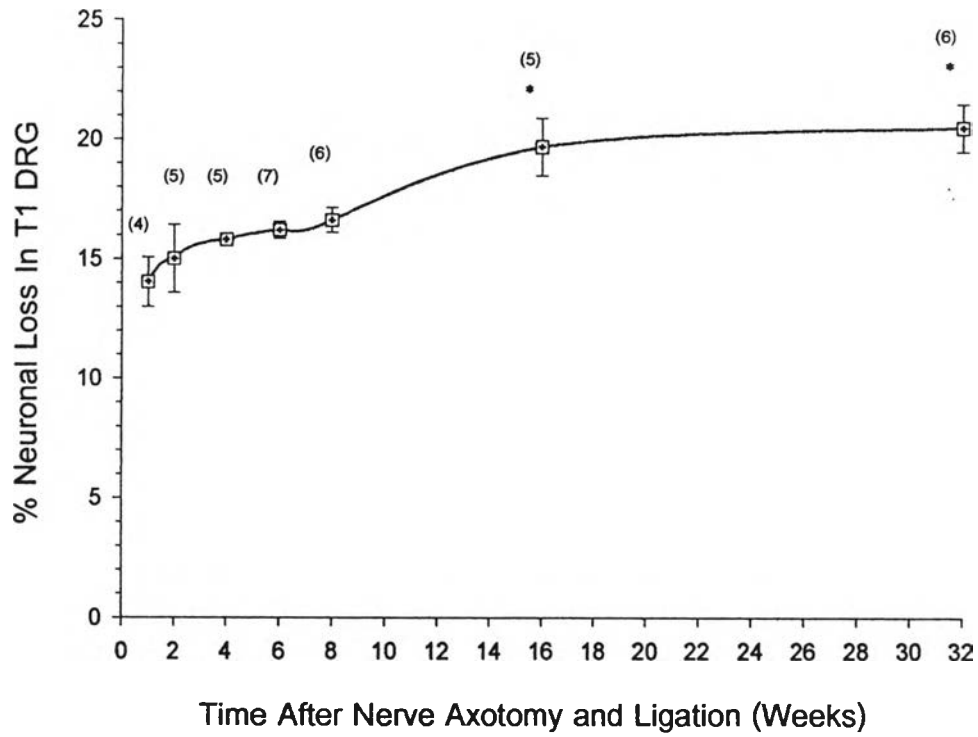


Figure 24 The loss of sensory neurons in the T1 DRG region for several survival times after median and ulnar nerve axotomy and ligation in adult rats. Data are expressed as percentage (mean ±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examine in each group.*$p \leq 0.05$ versus 1 week after axotomy with ligation group, ANOVA and post-hoc (Duncan) test.

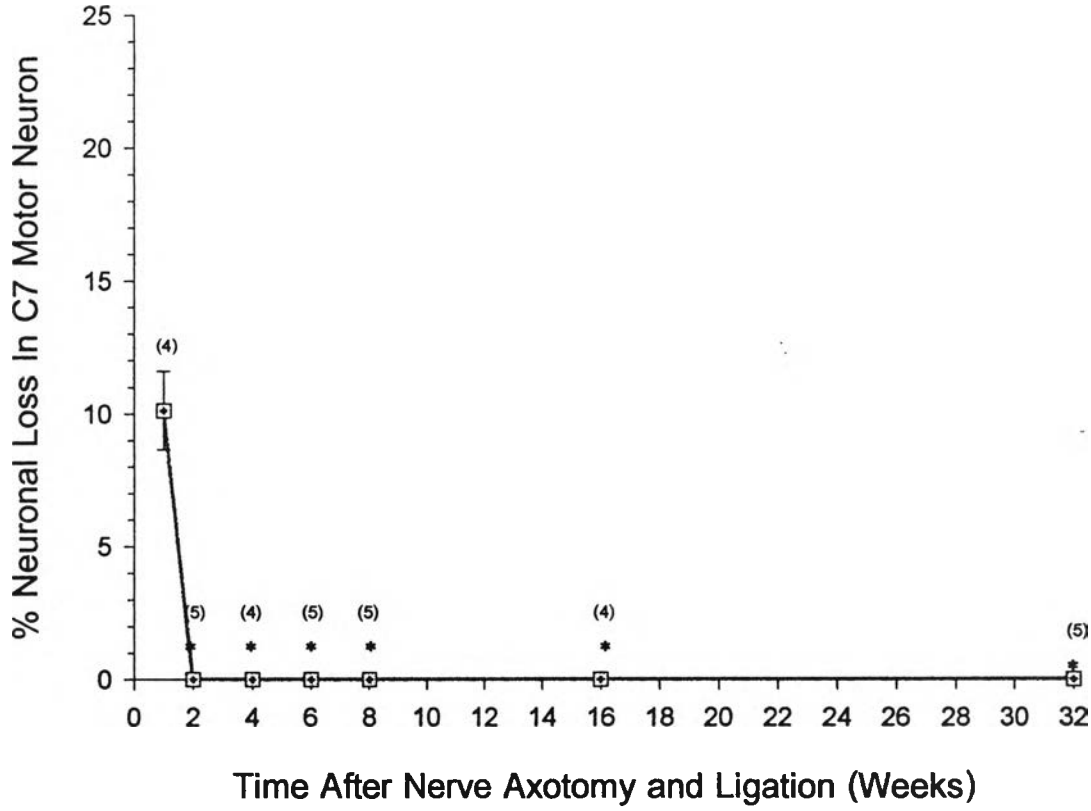


Figure 25 Time course of motor neuron loss in the C7 region for several survival time after median and ulnar nerve axotomy and ligation in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examine in each group. *$p \leq 0.05$ versus 1 week after axotomy with ligation, ANOVA and post-hoc (Duncan) test.

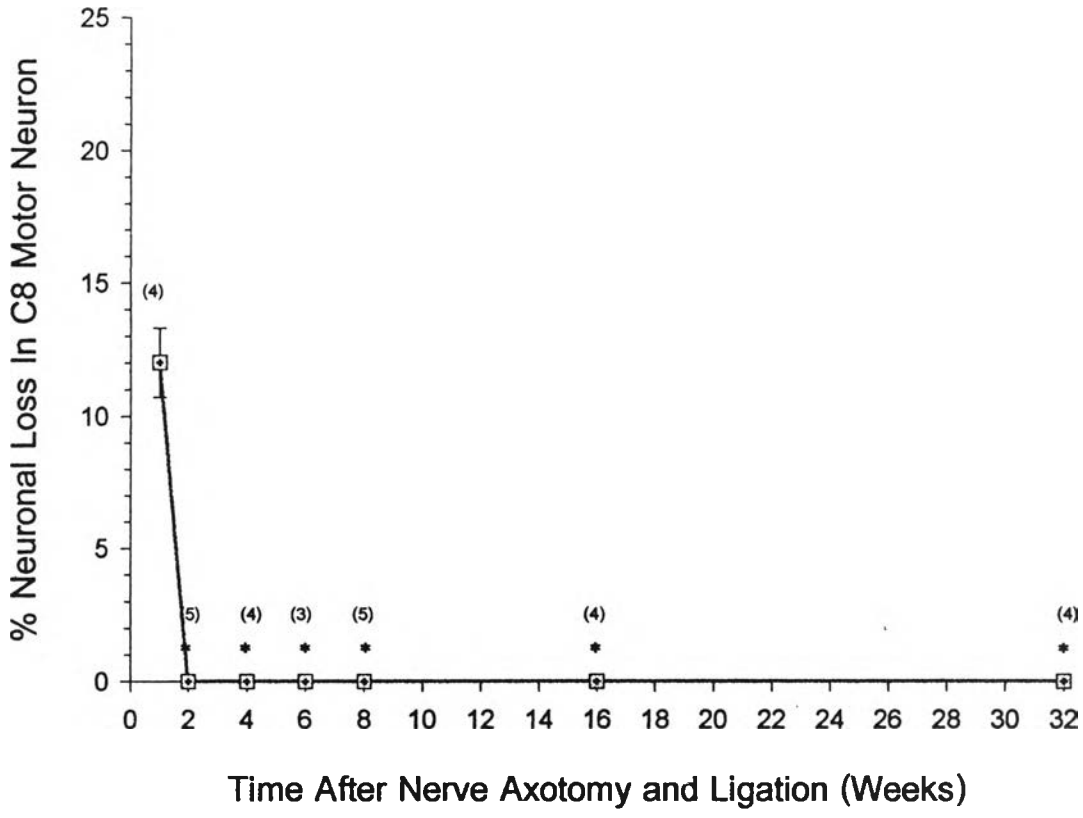


Figure 26 Time course of motor neuron loss in the C8 region for several survival time after median and ulnar nerve axotomy and ligation in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examine in each group. *$p \leq 0.05$ versus 1 week after axotomy with ligation, ANOVA and post-hoc (Duncan) test.

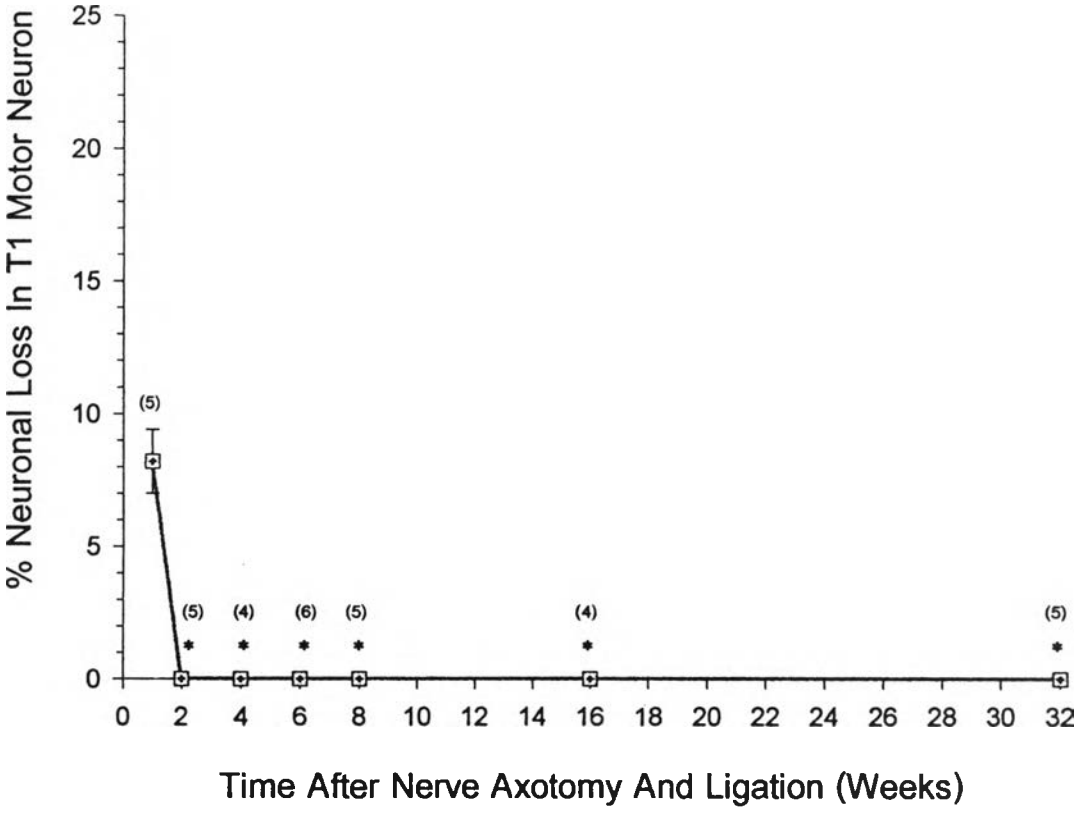


Figure 27 Time course of motor neuron loss in the C8 region for several survival time after median and ulnar nerve axotomy and ligation in adult rats. Data are expressed as percentage (mean±SEM) neuronal loss. Numbers in parentheses above the bar indicate the number of rats examine in each group. *$p \leq 0.05$ versus 1 week after axotomy with ligation, ANOVA and post-hoc (Duncan) test.

5. Effect of LIF treatment on sensory neuron survival

From the time course of neuronal death observed in axotomised animals, we decided to assay the effects of LIF treatment 1, 2, and 4 weeks after axotomy. Median and ulnar nerve transection followed by exposure to gelfoam containing PBS, resulted in the survival of 84% of sensory neuron after 1 week, 85% after 2 weeks, and 86% after 4 weeks, in cervical dorsal root ganglion level C7 (Figure 28, 31,and 34). In cervical dorsal root ganglion level C8 , resulted in the survival of 87% after 1 week, 88% after 2 weeks, and 87% after 4 weeks (Figure 29, 32, and 35). In cervical dorsal root ganglion level T1, resulted in the survival of 88% after 1 week, 86% after 2 weeks, and 86% after 4 weeks (Figure 30, 33, and 36). When the axotomized nerves were treated with LIF the sensory neuron survival was increased in neuronal survival in C7, C8 and T1 sensory neuron compared to the controls; It was found that the group receive LIF treatment at C7 level showed a rescue of 89% after 1 week , 89% after 2 weeks , and 100% after 4 weeks (Figure 28, 31,and 34).

The survival neuronal cell at C8 level represented a rescue of 89% after 1 week, 89% after 2 weeks, and 100% after 4 weeks (Figure 29, 32, and 35). In cervical dorsal root ganglion level T1, resulted in the LIF-treated group are 93% after 1 week, 90% after 2 weeks, and 100% after 4 weeks (Figure 30, 33, and 36). It is clear that the survival cell in the LIF-treated groups is higher than the PBS group at every level. However, a significant difference is shown only at level of T1 in 1 week , C7 and T1 level in 2 weeks, and all level in 4 weeks.

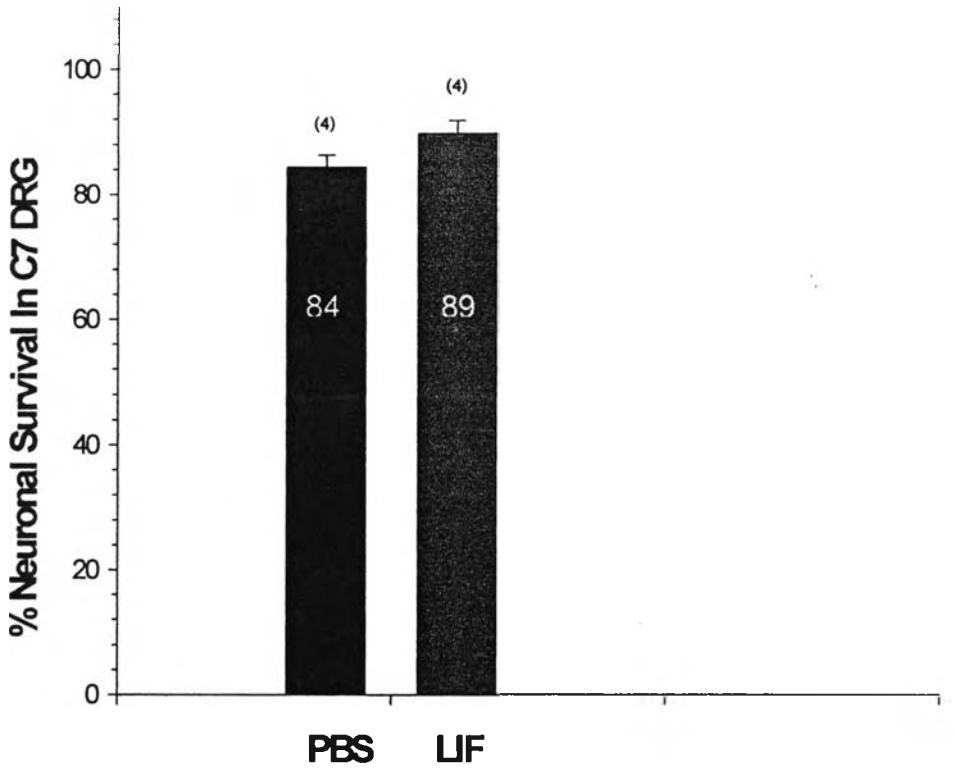


Figure 28 Effects of LIF on adult rat sensory neuron survival 1 weeks after axotomy in C7 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

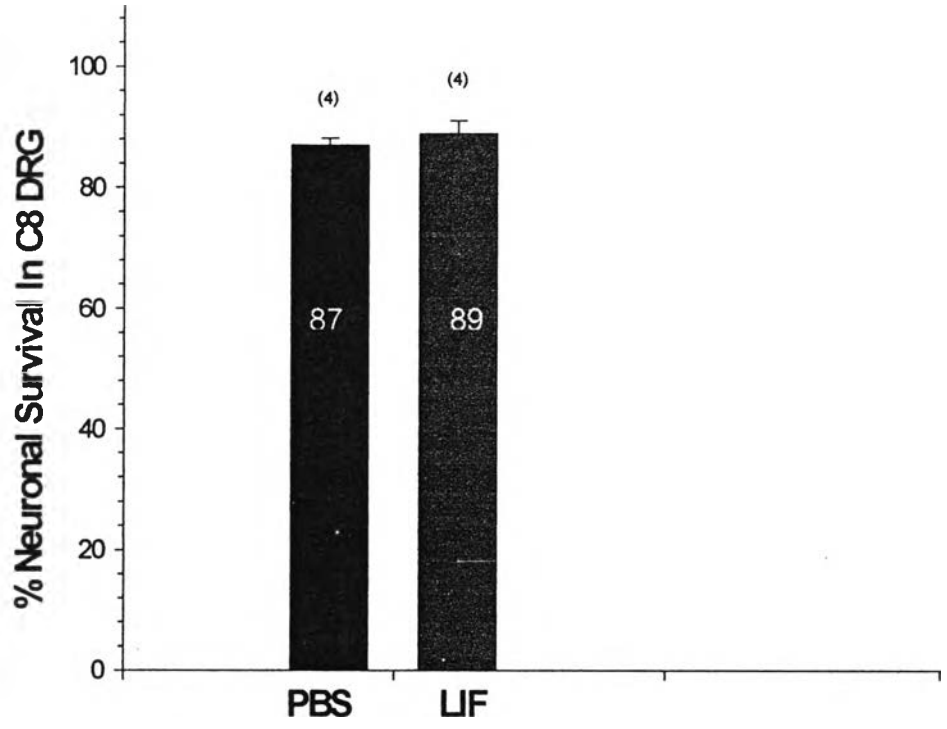


Figure 29 Effects of LIF on adult rat sensory neuron survival 1 weeks after axotomy in C8 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

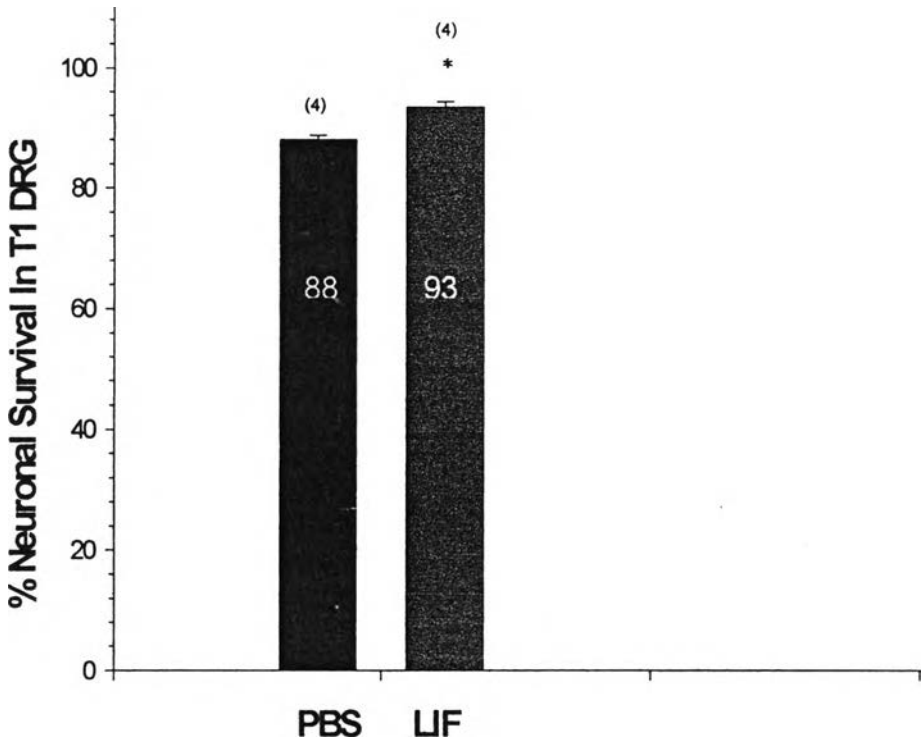


Figure 30 Effects of LIF on adult rat sensory neuron survival 1 weeks after axotomy in T1 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

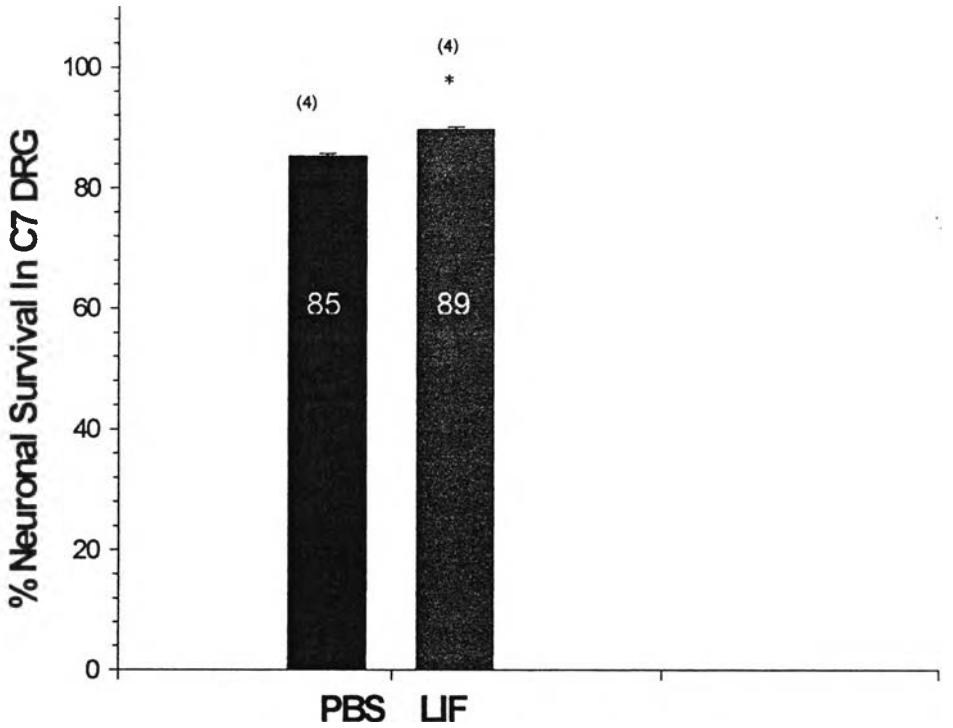


Figure 31 Effects of LIF on adult rat sensory neuron survival 2 weeks after axotomy in C7 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

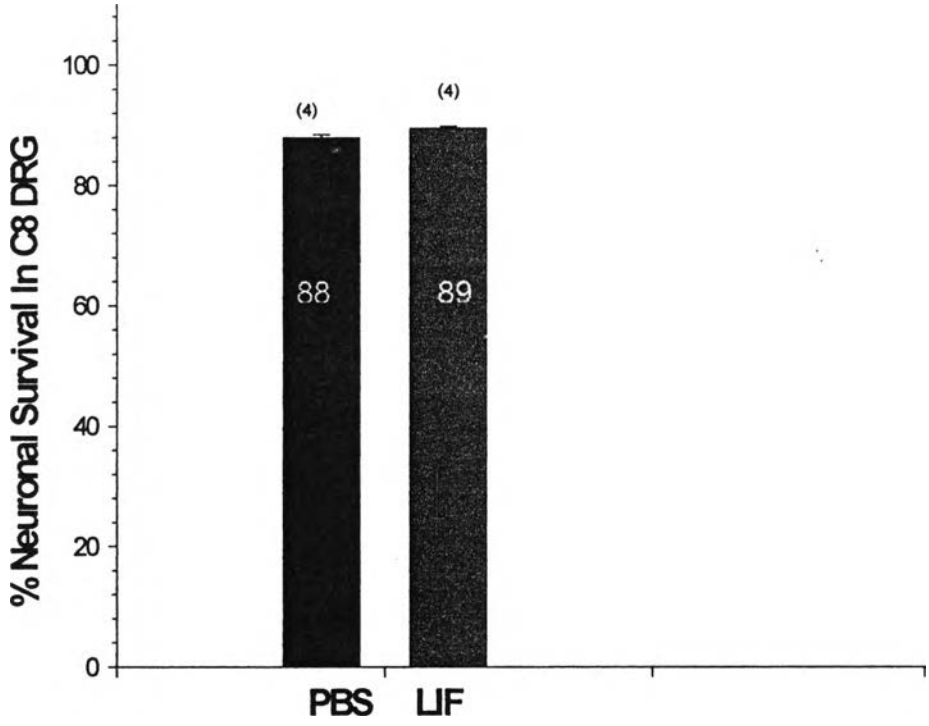


Figure 32 Effects of LIF on adult rat sensory neuron survival 2 weeks after axotomy in C8 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

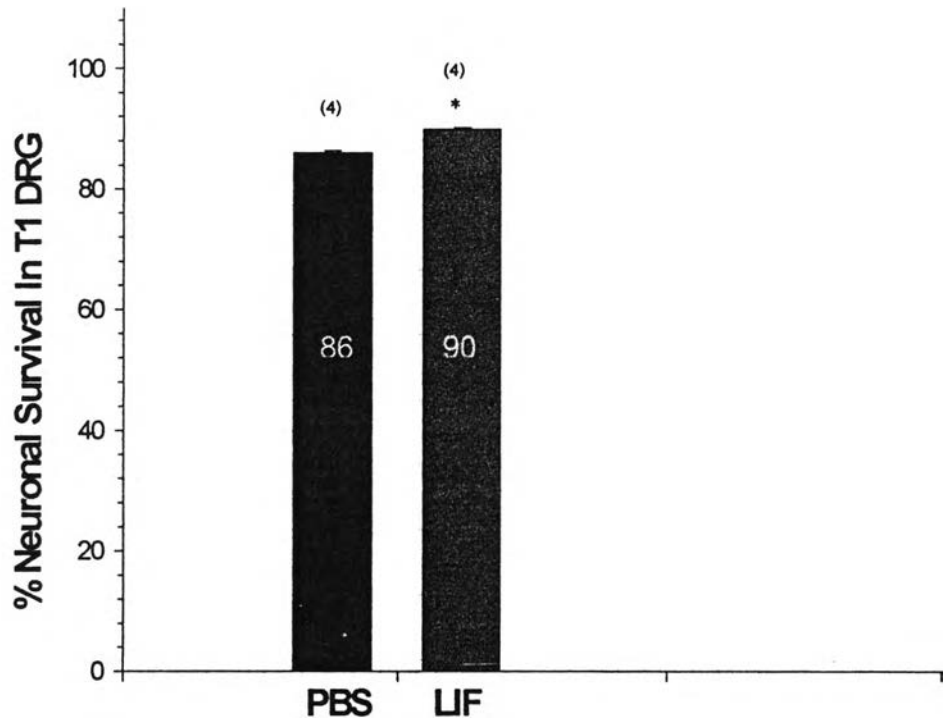


Figure 33 Effects of LIF on adult rat sensory neuron survival 2 weeks after axotomy in T1 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

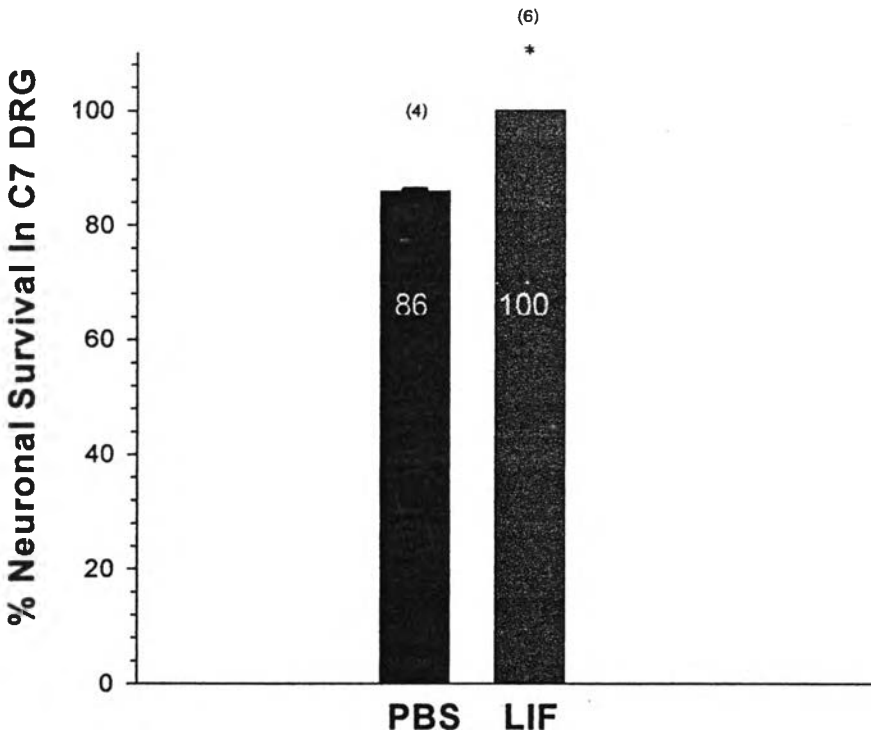


Figure 34 Effects of LIF on adult rat sensory neuron survival 4 weeks after axotomy in C7 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

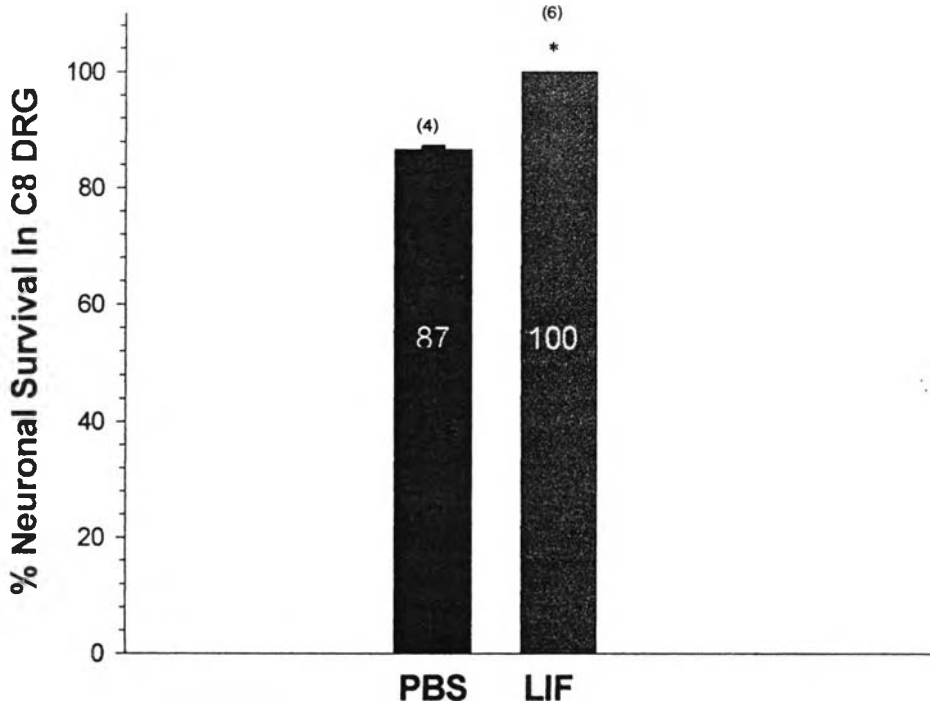


Figure 35 Effects of LIF on adult rat sensory neuron survival 4 weeks after axotomy in C8 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

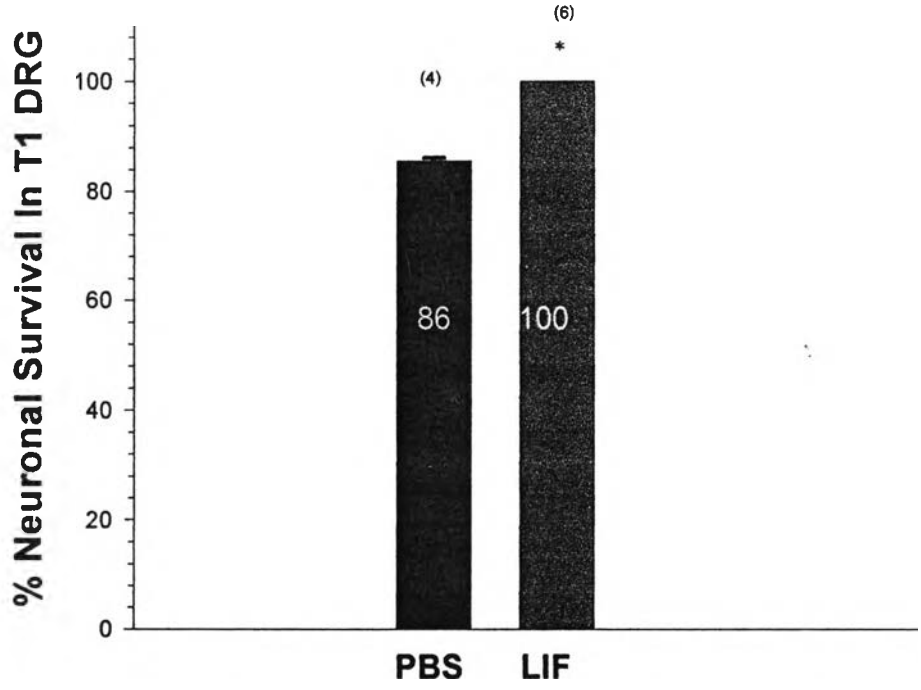


Figure 36 Effects of LIF on adult rat sensory neuron survival 4 weeks after axotomy in T1 DRG. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in sensory neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

6. Effect of LIF treatment on survival of axotomized motoneurons

The ability of LIF rescues motoneurons from axotomy-induced cell death. Median and ulnar nerve transection followed by exposure to gelfoam containing with either the PBS or LIF at peripheral end. After 1 week period the motor neuron of the PBS group at C7 , C8 , and T1 levels resulted 90 , 90, and 92 percent (Figure 37, 38, and 39). When the axotomized nerves were treated with LIF the motor neuron survival was increased in neuronal survival in C7, C8, and T1 motor neuron compared to the controls; It was found that the group which receive LIF treatment at C7, C8, and T1 level showed a rescue of 95%, 92%, and 94 % of motoneurons respectively (Figure 37, 38, and 39). However, a significant difference is shown only at level of C7.

In contrast to the developing motoneurons, axotomy of the median and ulnar nerve at a point 3-mm elbow to the spinal cord did not cause any significant motor neuronal loss 2 and 4 weeks after axotomy. Similar results have been obtained following axotomy in adult rats.

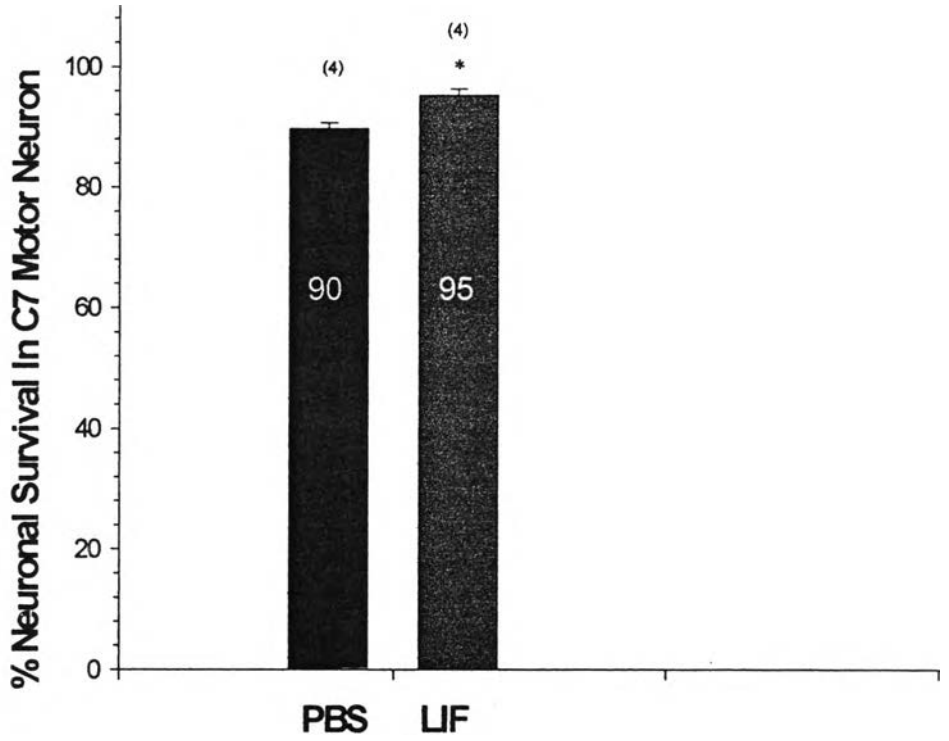


Figure Effects of LIF on adult rat motor neuron survival weeks after axotomy in C level Data are expressed as percentage mean ±SEM neuronal survival Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in motor neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

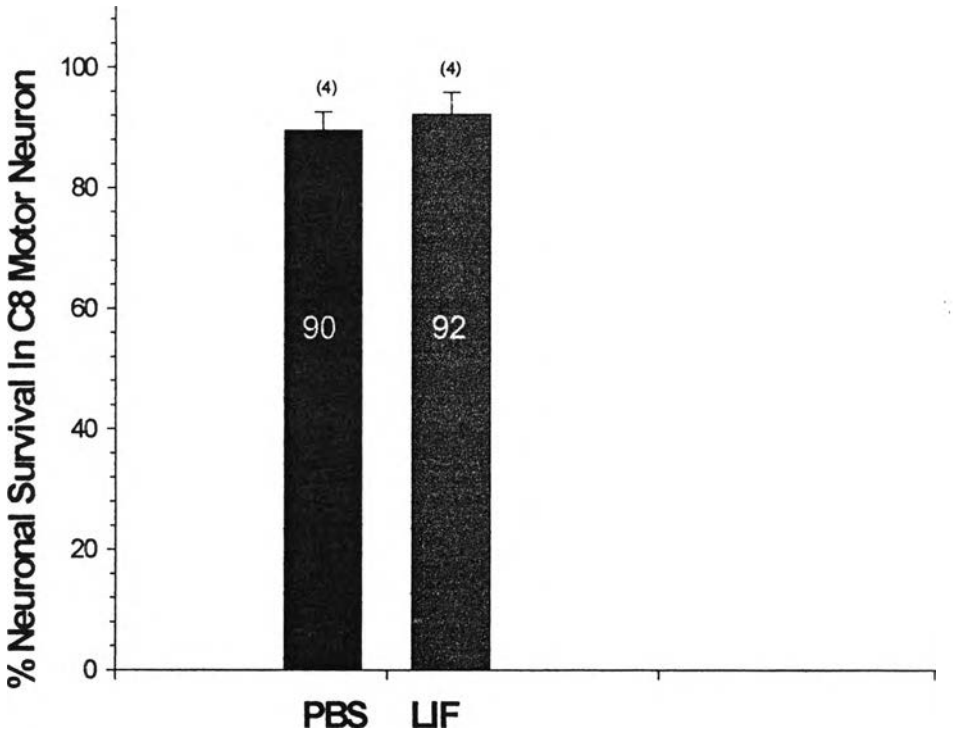


Figure 38 Effects of LIF on adult rat motor neuron survival 1 weeks after axotomy in C8 level. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in motor neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).

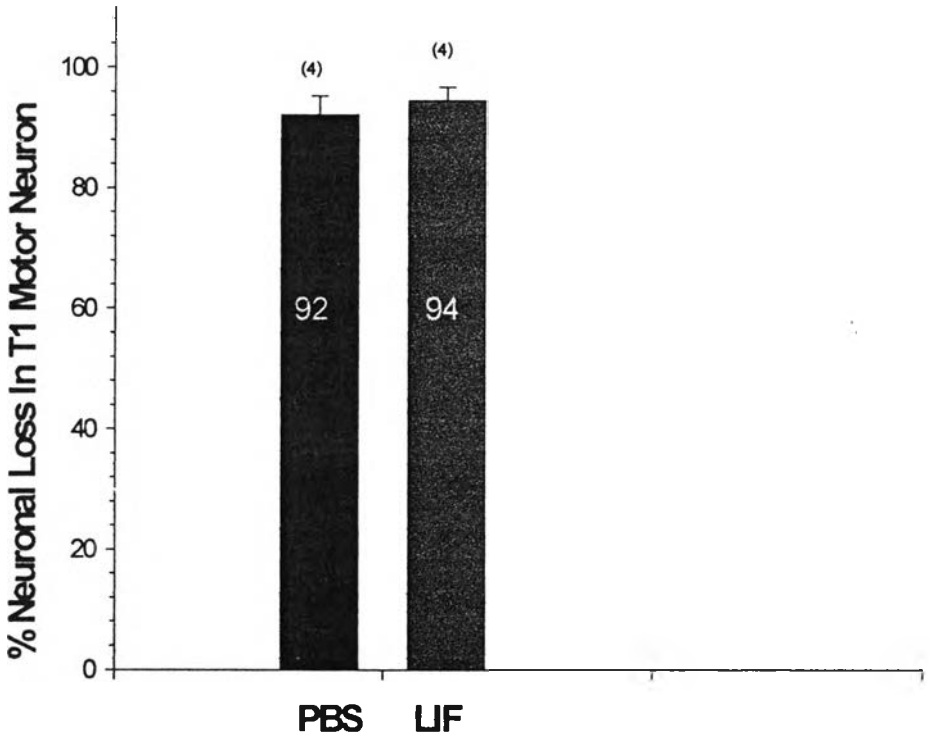


Figure 39 Effects of LIF on adult rat motor neuron survival 1 weeks after axotomy in T1 level. Data are expressed as percentage (mean ±SEM) neuronal survival. Numbers in bar represent numbers of percent neuronal survival and numbers in parentheses above the bars indicate the number of animals examined in each group. All the LIF-treated groups showed significant increase in motor neuron survivals compared with PBS-treated controls as determined using student's t-test ($p \leq 0.05$).