The Effect of Neodymium Magnetic Field Intensity on Regeneration in the Planarian *Dugesia tigrina*

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Abstract **The aim of this study was to assess the effects of magnetic field intensity emitted from rare earth neodymium magnets during cephalic and caudal regeneration in planaria *Dugesia tigrina*. For this purpose, cultured planaria were transversely lesioned immediately anterior to the pharynx and immediately exposed to varying magnetic field intensities, namely: 3190, 1920, and 1140 Gauss, under well maintained temperature conditions. Cephalic regeneration and caudal regeneration experiments were conducted separately and were closely monitored over the course of ten days. It was found that all three magnetic fields equally inhibited the rate of caudal regeneration and, to some extent, stimulated the cephalic regeneration. The results of this experiment have shown that the exposure to magnetic fields have various effects, which appear either as stimulation of regeneration or as its inhibition in the planarian *D. tigrina.* It is possible that magnetic fields exert their effect by altering intracellular calcium distribution in neoblast cells.**

Introduction

In planaria, the only known proliferative cell type is the neoblast, which is central to their regenerative capacities (Kreshchenko & Reuter, 2004). Neoblasts are totipotent stem-cells and are capable of generating essentially every cell type in the adult animal, including themselves (Alvarado & Reddien, 2004). When the planarian body is injured, the neoblasts, which are distributed along the anterior-posterior axis, migrate towards the wound surface into the area of the forming blastema and begin to differentiate according to their commitment (Kreshchenko & Reuter, 2004). The blastema is composed of ectodermal cells which cover the wound surface, and mesenchymal cells that proliferate and accumulate beneath the wound epidermis (Alvarado & Newmark, 2001).

During the first 24 hours of injury, biochemical investigations have revealed that elevated serotonin and catecholamine levels initiate a signalling process involving both cyclic adenosine monophosphate and calcium in neoblastic cells (Franquinet & Martelly, 1984). In concert, cyclic AMP and Ca^{2+} initiate the synthesis of DNA and RNA via their respective protein kinases and, ultimately induce proliferation and differentiation (Jenrow *et al.*, 1995). These findings are particularly important because the exposure to low-intensity magnetic fields have been shown to penetrate through the differentiating tissues and impede cytosolic proteins such as calmodulin, by altering membrane permeability to Ca^{2+} (Jenrow *et al.*, 1995). When planarians are grown in medium lacking Ca^{2+}, regeneration does not occur because calmodulin, which is normally found in regenerative cells, requires the binding of four Ca^{2+} ions in order to sequester the kinase helix that normally occludes adenosine triphosphate (ATP) binding (Le Moigne *et al.*, 1983). Once the calmodulin-dependent kinase is phosphorylated by ATP, it subsequently activates DNA and stimulates its synthesis. Overall, magnetic fields can retard planarian regeneration, decrease the rate of cephalic regeneration (Jenrow et al., 1995), and cause anomalies in various tissues (Jenrow *et al.*, 1996).

The underlying purpose of this experiment is to investigate whether magnetic fields emitted from rare-earth neodymium magnets will act to perturb the cell microstructure by disrupting expression of genetic information and resulting in aberrant cell types that are responsible for regeneration, or by disrupting the intercellular signalling events, Ca^{2+}, that direct neoblast differentiation and pattern formation (Jenrow *et al.*, 1996). The rate of regeneration in both the development of the head and the tail will dictate whether any of these two magnet-induced models are taking place during neodymium magnetic field exposure. Therefore, we predict that as magnetic field increases, the rate of cephalic and caudal regeneration will decrease.

Materials & Methods

<u>Lesion and Regeneration Assay:</u> A population of eight adult-stage planaria (*D. tigrina*) were transversely sliced using a scalpel immediately anterior to the pharynx, resulting in eight heads and eight tails. Two of each heads were randomly assigned to four individual water-filled Petri dishes containing all the necessary nutrients for survival and growth; this procedure was also repeated for the tail portions, in which the missing head would regenerate. The controls consisted of two heads and two tails regenerating without the presence of magnets, in addition to two whole planaria, where one was exposed to a magnetic field strength of 3190 Gauss and the other lacking a magnet.

Magnetic Field Exposure: Exposure to magnetic fields was initiated immediately following the lesioning process. Magnetic field exposure was continuous throughout the experiment, except for approximately one hour periods during which the extent of regeneration for each population was measured and the water was replaced with a fresh new supply. Measurements were collected on day zero, four, seven, and on day ten. To ensure that the planaria worms were constantly within the magnetic field, they were gated using water bottle caps placed within the Petri dish.

The rare-earth neodymium magnets used in this experiment were obtained from *K&J Magnetics, Inc*. and were specifically calibrated to three varying strengths of 3190, 1920, and 1140 Gauss. The magnetic strengths listed are considered to be within the *safe* range when exposed topically (Carriço *et al.*, 2006). For each treatment, magnets were placed directly equidistant from the center of Petri dish lid to the center of base and oriented so that the north end was pointing upwards while the field emitted by the south end pointed directly on the planaria (Figure 1). An endpoint marker was selected to determine precisely the extent of regeneration: the appearance of bilateral eye pigmentation would indicate complete cephalic regeneration and the appearance of planaria expressing stage 3 development would indicate complete caudal regeneration (Figure 1).

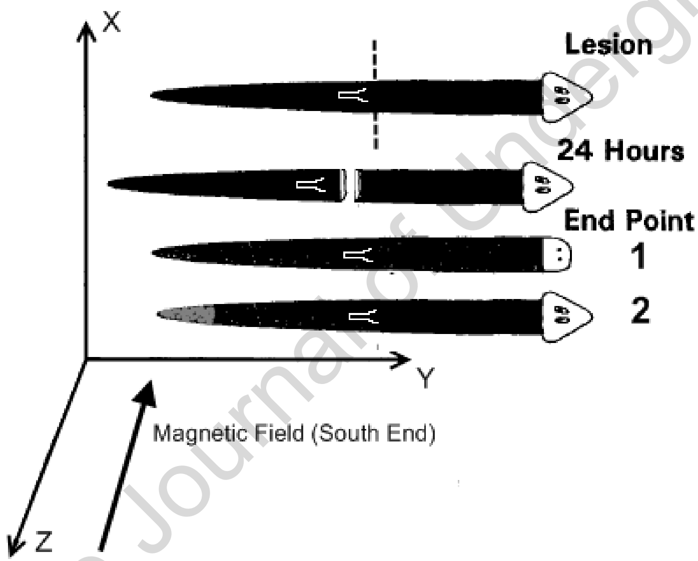

Figure 1. Representation of the cephalic and caudal regeneration process examined in this experiment. Successive stages of planarian regeneration are shown top to bottom. Unshaded regions represent tissue either removed by lesioning or newly regenerated. The endpoints are defined as (1) the appearance of bilateral eye pigmentation and (2) the appearance of stage 3 tail development (pointed). The Z direction corresponds to the geomagnetic North-South direction, the X direction corresponds to the North-South plane, and the Y direction corresponds to the East-West plane.

Results

Caudal regeneration in the presence of neodymium magnets: The average length of planaria used to measure caudal regeneration growth is summarized in Table 1. Under all three experimental treatments, tail growth expressing stage 3 development was fully achieved as early as day seven. When no magnet was present, the planaria grew an average of 3 mm; that is, when taking the difference in length from day ten to day zero. On the contrary, the difference in length was very minimal for the experimental planaria, ranging from 0.5 mm to 1.0 mm.

Table 1. The average length of lesioned planaria heads undergoing caudal regeneration during continuous exposure to varying magnetic field strengths over the course of ten days.

	Day 0	Day 4	Day 7	Day 10
	Length (mm) ± Standard Error			
No Magnet	4±0	4.5±0.5	4.5±0.5	7±1
Magnetic Strength 1†	4.5±1	4.25±1.25	4±0.5	5±1
Magnetic Strength 2†	2.5±0.5	3±0	3.5±0.5	3.5±0.5
Magnetic Strength 3†	5±0	6.75±0.25	6.5±0	6±0.5

* Population size per experimental group, (n) = 2.
† Magnetic strength 1,2,3 = 1140 Gauss, 1920 Gauss, and 3190 Gauss, respectively.

Cephalic regeneration in the presence of neodymium magnets: The average length of planaria used to measure complete cephalic regeneration is summarized in Table 2. Under all three experimental treatments, the appearance of bilateral eye pigmentation was verified as early as day seven of analysis. When no magnet was present, the planaria grew an average of 0.5 mm in overall length. In addition, magnetic fields had very little influence on their regeneration as it yielded a very minimal difference in overall average length, ranging from 0.875 mm to 1.25 mm.

Table 2. The average length of lesioned planaria tails undergoing cephalic regeneration during continuous exposure to varying magnetic field strengths over the course of ten days.

	Day 0	Day 4	Day 7	Day 10
	Length (mm) ± Standard Error			
No Magnet	6.5±1.5	6.75±0.75	5.5±0.5	7±1
Magnetic Strength 1	4±1	4.5±0.5	4.25± 0.25	5±1
Magnetic Strength 2	5.5±1	5.25±0.75	5.25± 0.75	6.75±0.25
Magnetic Strength 3	6±1	5±0	6±0	6.875±0.125

* Population size per experimental group, (n) = 2.
† Magnetic strength 1,2,3 = 1140 Gauss, 1920 Gauss, and 3190 Gauss, respectively.

Whole planarian in the presence of neodymium magnets: No change was observed in length after exposing the planarian to the magnet field.

Discussion

The main purpose of this experiment is to investigate whether magnetic fields produced by neodymium magnets can affect the biological mechanism involved in the regeneration process in adult planaria, in terms of rate and degree of regeneration. Analysis of caudal regeneration indicates that the magnet-lacking control group grew longer than all three experimental treatments. This suggests that the magnetic field may have induced an inhibitory effect on cells responsible for regeneration. By taking into consideration what is presently known about calcium signaling events associated with planarian regeneration, this effect could reflect magnetic field interaction at several levels. If the observed effect is specific to calcium physiology within the cell, then the neoblasts may have experienced an altered membrane permeability to Ca^{2+} (Jenrow *et al.,* 1996). Alternatively, the magnetic field may have impeded the binding of calcium to cystolic proteins such as calmodulin, which is required to bind to DNA and initiate its synthesis (Jenrow *et al.,* 1996, Le Moigne *et al.,* 1983).

Not all neoblastic processes are necessarily calcium dependent. Ornithine decarboxylase enzyme has been shown to play an important role in planaria regeneration, especially since it is responsible for the synthesis of certain growth factors (Baguñà & Saló, 1989). A connection has been reported between the exposure to extremely low-frequency magnetic waves and ornithine decarboxylase activity in vitro (Adey *et al.,* 1987). Although further biochemical investigations are required to accurately monitor this process, it can assumed that from the results obtained, the magnets used in this experiment may have inhibited the action of this enzyme, impeded the regenerative cells from proliferating, and in turn, caused a decrease in the rate of caudal regeneration.

The effects of neodymium magnetic field exposure on cephalic regeneration after its amputation are generally variable and inconsistent. Analysis of the results obtained in this experiment and of those obtained from literature suggests that high doses of magnetic field exposure specifically favour cephalic regeneration, as demonstrated here, while low doses lead to a distinct inhibitory effect. For instance, rather than using magnets with extremely low magnetic fields (<700 Gauss) as employed by Jenrow *et al.,* (1995), the magnets used in this experiment were slightly higher in strength. In fact, depending on the *intensity*, *orientation*, and *duration* of magnetic field exposure, the pattern of fluctuations of the effects observed may vary (Fesenko *et al.,* 2000). A combination of these factors may have influenced the neoblasts by stimulating their migration towards the site of injury and promoting regeneration of the head.

Conclusion

This study concludes that neodymium magnetic fields, at intensities higher than previously tested, can decrease the rate of caudal regeneration or retard regeneration in *D. tigrina*, and increase the rate of cephalic regeneration. In order to better understand the biochemical processes involved in regeneration during magnetic field exposure, further histological and biochemical investigations are required to resolve these details. In addition, these results also suggest that cephalic and caudal regeneration in the planarian represents an excellent model system for investigating teratogenic effects of continuous magnetic field exposure during pregnancy.

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