Applied DC Magnetic Fields Cause Alterations in the Time of Cell Divisions and Developmental Abnormalities in Early Sea-urchin Embryos

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Abstract

Most work on magnetic field effects focuses on AC fields. This study demonstrates that exposure to medium-strength (10 mT - 0.1 T) static magnetic fields can alter the early embryonic development of two species of sea urchin embryos. Batches of fertilized eggs from two species of urchin were exposed to fields produced by permanent magnets. Samples of the continuous cultures were scored for the timing of the first two cell divisions, time of hatching, and incidence of exogastrulation. It was found that static fields delay the onset of mitosis in both species, by an amount dependent on the exposure timing relative to fertilization. The exposure time which caused the maximum effect differed between the two species. Thirty mT fields, but not 15 mT fields, caused an eight-fold increase in the incidence of exogastrulation in *Lytechinus pictus*, while neither of these fields produced exogastrulation in *Strongylocentrotus purpuratus.*

Introduction

The resurgence of interest in the interactions between electromagnetic fields and biological systems has mainly focused on AC (time-varying) fields. However, there have been studies showing that DC (static) magnetic fields can also interact with living systems at various levels. Effects on *in vitro* biochemical reactions have been reported (Kim, 1976, Adamkiewicz, 1983, 1987, EPA, 1990, Markov *et al*., 1992, Richardson *et al.*, 1992, Harkins and Grissom, 1994). Perhaps most interestingly, it is seen that a 50 mT DC magnetic field can alter the structure of poly-L-lysine (Verma and Goldner, 1996).

Behavioral effects of DC fields have also been noted. For example, strong static magnetic fields are avoided by mice and worker ants (Kermarrec, 1981), though apparently a DC magnetic field of about 0.1 mT increases bee life-span by more than 60% (Martin *et al*., 1989). Weak static fields affect the choice of motion of flatworms, snails, and paramecia (Dubrov, 1978, Martin *et al*., 1988), while medium-strength static fields have been used as conditioning stimuli for bees and rabbits (Kholodov, 1971, Walker and Bitterman, 1989), and a 7 mT static field disrupted honey-bee dancing (Tomlinson *et al*., 1981). Medium-strength DC fields act as a general stressor in mice (Laforge *et al.*, 1978, Laforge *et al.*, 1986).

Physiological effects, such as changes in leukocyte count in mice (Barnothy, 1957), disruption of the mammalian menstrual cycle (Kholodov, 1973), reduced respiration in cultured embryonic and sarcoma cells (Pereira *et al*., 1967), alterations in growth rate of plants and bacteria (Dycus and Shultz, 1964, Pittman, 1972, Singh *et al.* 1994), and changes in aging rates (Kholodov, 1971, Bellossi, 1986) have also been reported. Likewise, morphological and histochemical alterations in rat spermatogenesis (FBIS, 1983) and CNS microstructure (Abdullakhodzhayeva and Razykov, 1986), cytological changes in paramecia (Kogan *et al*., 1967), reduction of X-irradiation-induced mortality (Barnothy, 1963), retardation of wound

healing (Beischer, 1964), and abnormal mitotic figures and nuclei (Linskens and Smeets, 1978, Mastryukova and Rudneva, 1978, FBIS, 1983) appear to be caused by exposure to medium- to high-strength static magnetic fields.

Some attention has been focused on interactions between static fields and processes involved in carcinogenesis and tumor formation. As with AC fields (reviewed in Bates, 1991), the effects of static fields on tumors can appear contradictory depending upon field parameters. Fields of 730 mT cause cell degeneration in several types of tumor cells (Kim, 1976). Gross (1962) found that a 400 mT field increased the rate of death from transplanted tumors in mice, yet treatment of H2712 mouse tumor cells with a 3.8 T field (with a 1.2 T/mm gradient) caused significant inhibition of the ability to infect a healthy host (Weber and Cerilli, 1971). Recent studies have shown that the oncogene c-fos can be induced by 0.2 T static field in cultured mammalian cells (Hiraoka *et al.*, 1992). DC magnetic fields (0.73 T) applied to tumor cell suspensions can cause a sharp reduction in cell number (König *et al*., 1981).

Especially interesting are the reports that static fields are able to alter embryonic development and morphogenesis, since, in addition to the basic question of mechanisms of field-biosystem interaction, they provide the opportunity to learn more about developmental mechanisms. It has been reported that 1 T fields are lethal to young mice (Kholodov, 1971), and that a 14 T field stopped sea-urchin development, but did not affect *Drosophila* and mouse development (Kholodov, 1971). A weaker, 420 mT field, caused embryo death and dissolution in the wombs of mice (Kholodov, 1971). Klueber (1981) showed that 5 mT static magnetic fields produced dramatic teratogenic effects in the eye and nervous system of developing chick embryos. Static magnetic fields of 0.4 mT retarded development of the pigeon embryo, and exposure of chick embryos to a 500 mT field for just 1 hour produced poor brain development with an open neural tube, shortening of the embryonic long axis, and slight heart displacement (Joshi *et al.*, 1978). Neurath

(1968, 1969) showed that in many organisms, gastrulation is halted in fields with a gradient of 8.35 T/cm, and *Drosophila* cuticular abnormalities resulted from brief exposures to static magnetic fields (Ho *et al*., 1992). 1 T DC fields caused axial anomalies in frog embryos (Ueno, 1984). Regeneration, a non-embryonic example of large-scale morphogenesis is also affected by DC fields, as DC fields accelerate tail regeneration in tadpoles; the effect exhibits exposure time and field strength dependence (Kudokzev and Baranovskiy, 1988).

We performed a series of experiments to examine the effects of medium-strength static magnetic fields on the development of sea urchin embryos. The sea urchin is an excellent and well-studied developmental system, and the presence of static magnetic field effects on its development would afford a tractable model for studying field-cell interactions, as well as the normal processes of development by providing a new perturbing factor. In one sense, static field effects are more interesting, because unlike AC fields, they cannot cause ionic currents. While our initial studies (Levin and Ernst, 1995) showed that weak AC magnetic fields can affect the mitotic timing of sea urchin embryos, and Kholodov (1971) reports that a high-strength (14 T) static magnetic field arrests sea-urchin development, there have been very few experimental studies of applied medium-strength static field effects on sea-urchin embryogenesis. In this study we report that such fields are able to cause a delay in the mitotic cycle of early embryos, and to greatly increase the incidence of exogastrulation, a well-characterized developmental abnormality in sea urchins. Thus, we show that in the sea urchin model, static magnetic fields are a potent teratogen and that the mitotic cycle is sensitive to these low-energy fields as well as to AC fields.

Materials and Methods

Animals, gametes, and embryos

Strongylocentrotus purpuratus and *Lytechinus pictus* were purchased from Marinus, Inc., Long Beach, CA. Animals were maintained in aquaria at 9 °C and were induced to spawn by intracoelomic injection of 0.5 M KCl. Semen was collected dry from the genital pores with a Pasteur pipette and held undiluted in a tube on ice until fertilization. Eggs were collected by inverting the spawning females onto beakers of Millipore-filtered sea water (MPFSW). The suspension of eggs was filtered through several layers of cheesecloth and settled on ice through fresh MPFSW three times. Eggs were suspended to a final concentration of 1-2% (V:V) in MPFSW.

Fertilization was accomplished by adding a freshly prepared dilute sperm suspension to the eggs to result in a final sperm concentration of about 1:10,000 (V:V). Successful fertilization was determined by elevation of the fertilization membrane, generally within 90 sec. of sperm addition. Fertilization was greater than 95% in all experiments. Experiments were carried out with eggs produced from several females to minimize effects of individual differences. Embryos in 250 ml beakers were cultured with stirring in an incubator at 13-16 °C depending on the species.

Experimental design for static field exposure

The static magnetic fields were produced by a parallel pair of attracting rectangular ceramic magnets positioned opposite each other, with the sample of sperm, eggs, or embryos between them (Figure 1). Field strength was measured with a Gaussmeter (Walker Magnetics, model MG-4D) at the midpoint of the culture. Field strength at the outer edges of the culture was no more that ±15% of this value.

Styrofoam blocks were used to insulate the stirring motors from the cultures to guard against possible differential heating effects due to the motors. Temperature between the test and control cultures in individual experiments did not differ by more than ±0.5 °C, as determined by continuous monitoring. The magnetic environment of the incubator was investigated with the Gaussmeter and was determined not to be different from the ambient geomagnetic field within ±0.01mT. The stirring motors produced no detectable stray fields at the location of the cultures (35 cm away from motors). All supporting material within the incubator was made of non-ferrous material to prevent unwanted leakage of the fields towards the control culture. All aspects of culture except for the presence of magnets were the same between the exposed and control samples (including beakers, stirring motors, etc.).

Sampling and data collection

During the continuous experiments, samples of about 200 embryos were taken without interruption of field exposure approximately every 15 min., fixed in 3% formaldehyde, and scored for the number of blastomeres, the presence of the fertilization membrane, or the position of the gut, with the aid of a Nikon microscope.

Results

Exposure to static fields delays hatching

The first series of experiments was designed to maximize the possibility of detecting effects arising from exposure to static magnetic fields. Immediately following fertilization, *L. pictus* embryos were divided into two equal volumes; one culture was exposed to a 30 mT static field (Fig. 1) for the duration of the experiment. The other culture received no exposure except for the ambient geomagnetic field. All other conditions of culture remained the same. At 26 hours post-fertilization, samples of each culture were taken and scored for percentage of embryos which had hatched. Hatching is an easily recognizable developmental event, resulting from blastula-stage embryos acquiring motility and secreting an enzyme which digests the fertilization membrane (reviewed: Okazaki, 1975). The results are summarized in Table 1, and demonstrate that at 26 hours 82% of the control embryos had hatched, while only 36% of the exposed embryos had done so ($p<0.01$). Additional experiments with *L. pictus* and *S. purpuratus* revealed that in both species, exposure to 30 mT static magnetic fields significantly delays hatching relative to control groups (data not shown).

Exposure to static field delays the 1st and 2nd cell divisions of *S. purpuratus*

Having seen that exposure to the field is able to delay hatching time, we hypothesized that this was due to an increase in the length of the cell cycle, rather than a delay of the hatching mechanism itself. Consequently, we studied the effect of exposure on the time of the first two cell divisions. A batch of eggs was split in two immediately following fertilization, and one culture was exposed to a 30 mT magnetic field (Fig. 1). At 3.75 hours, approximately 100 embryos from each culture were scored for cell division. Four times as many exposed eggs relative to controls remained undivided (Table 2). These results demonstrate that the field in-

creases the length of time between completed cell divisions.

To gain more information on which phases of the cell cycles were affected, and to have a more quantitative way of determining how the magnitude of the delay varied with field parameters, the continuous cultures were sampled and scored periodically, thereby recording how many cell divisions had taken place in a representative sample of the embryos. This made it possible to plot the number of divisions as a function of time, and then compare exposed and control batches of embryos. Cell divisions in early sea urchin embryos are well synchronized; previously, we have demonstrated that when a batch of fertilized eggs is split and sampled every 15 min., the maximum endogenous variation of the time of cell division is 3 min. (Levin and Ernst, 1995). Thus, differences greater than ±3 min. were taken to be significant in the experiments below.

Since the endogenous variation is known, it was possible to study the effects of exposure to the 30 mT static field on the time of the first two cell divisions. After fertilization, the embryos were split into two cultures, one of which was immediately exposed to the field throughout the experiment, while the other served as a control. The cultures were sampled every 15 min. without interruption of the field, and roughly 200 embryos from each culture were scored for the number of blastomeres. The results are shown in Figure 2. By calculating the time difference between the midpoints of each cell division phase on the plot, it is seen that the field induces an insignificant delay (1 minute) in the first cell division, and a small but significant delay of 6 min. in the second.

Effects of static field applied to sperm on timing of 1st/2nd cell divisions

Having seen that exposure to the field resulted in a delay in the time of cell division, we tested the possibility that some mechanism within the sperm is sensitive to the field. A 30 mT static field was applied to the undiluted sperm sample of *S. purpuratus* for 1 hour. The field was removed immediately prior to dilution of

the sperm sample for fertilization. The resulting culture and a control were incubated without exposure to any field, and samples were taken, scored, and plotted as above. No significant effects on the time of the first two cell divisions were seen (Figure 3). There was also no obvious decrease in the ability of sperm to fertilize eggs since greater than 95% fertilization was achieved within 5 min., as in the control sperm samples. The same result was observed for *L. pictus* (data not shown). However, since sperm were not limiting, we cannot rule out the possibility that a fraction of the sperm were affected by exposure to the field.

Magnitude of cell division delay is a function of timing of field exposure

Since exposure of sperm to a 30 mT static field had no measurable effect, we were interested to see whether pre-fertilization eggs were sensitive to the field, or whether the field only affected activated eggs or developing embryos. It was also possible to study the relationship between the time of onset of field exposure and the magnitude of the delay. A 30 mT static field was applied to the experimental cultures of *S. purpuratus* starting at various times relative to fertilization, and lasting throughout the experiment. The cell division time profiles were calculated as above.

When eggs were exposed to the field beginning 45 min. pre-fertilization, the first cell division was delayed 4 min., and in the exposed cultures the second division was 6 min. behind that measured for the control culture (though these figures are larger than the 3 minute endogenous difference between control cultures, the limited number of experiments precludes determining whether these differences are significant by a comprehensive statistical analysis). Exposure which began 30 min. before fertilization caused a 10 min. delay in the first cell division and a 13 min. delay in the second, while an exposure beginning 15 min. pre-fertilization caused a 17 min. delay in the timing of both cell divisions (Figure 4). When eggs were exposed starting at 6 min. pre-fertilization, there was a 14 min. delay in the

first cell division and a 15 min. delay in the second division. In cultures exposed immediately following fertilization there was an insignificant delay for the first cell division and a 6 min. delay for the second. These results are summarized in Figure 5. When this series of experiments was repeated using *L. pictus*, the same type of relationship was observed between timing of exposure and magnitude of delay, except that the optimal time of exposure was shown to be 30 min. pre-fertilization, and the delay obtained at that exposure was 22 min. (data not shown).

Morphological effects

Given previously-reported teratological effects of magnetic fields (reviewed above), we were interested in determining whether our field exposures had any such effects. In *L. pictus* cultures exposed to a 30 mT field continuously from fertilization and incubated for 48-94 hours, we noticed an apparent increase in the incidence of exogastrulation. Exogastrulation is a well-known developmental abnormality in sea urchins where the archenteron, the primitive gut, evaginates forming outside of the embryo instead of invaginating (Nocente-McGrath *et al.*, 1991). To test directly the effect of a 30 mT static field on *L. pictus* gastrulation, embryos were fertilized and the culture split in half, with one half serving as a control and the other exposed to the field. Gastrulation is normally initiated about 24 hours post-fertilization in *L. pictus*, followed by the morphological differentiation of the gut. Cultures were scored for exogastrulation at 2-3 days.

In exposed cultures, the incidence of exogastrulation rose from control values of 1-2% to as high as 16%. In 7 experiments, the increase in exogastrulation was between 2- and 8-fold, with an average of 6-fold above controls. The results of one such experiment ($\chi^2=8.94$, $p<0.05$) are shown in Table 3, and examples of exogastrulated embryos produced by exposure to a 30 mT static field are shown in Figure 6. A 0.39 mT 60 Hz AC field also resulted in a 3-fold increase in exogastrulation, while 0.195 and 0.016 mT AC fields did not measurably alter the

incidence of exogastrulation (data not shown). In *S. purpuratus* embryos exposed to the same fields, we found no increase in the incidence of exogastrulation.

Interestingly, a morphological abnormality not reported before in either species and never observed in controls was found in the eggs exposed to a 30 mT static field. This abnormality, shown in Figure 7, consisted of embryonal collapse along one axis, resulting in a flat disk rather than the normal sphere. In three experiments where fertilized eggs were exposed to a 30 mT static field at 45, 30, or 0 min. pre-fertilization, and continuing throughout the subsequent 48-94 hours, approximately 1% of the eggs collapsed.

Discussion

In this study we observed that a 30 mT static magnetic field applied to sea urchin eggs produced alterations in the time of cell division and induced two developmental abnormalities, exogastrulation and collapsed embryos. These results are surprising and at present we do not have a unifying model for a mechanism that could account for the diverse effects.

Exposure to a 30 mT static field resulted in delays in the time of the first two cell divisions of sea urchin embryos. Since the slopes of the division profile curves are roughly equal between the exposed and control embryos (e.g. Figure 4), it can be concluded that the field increases the time spent in the G1, G2, or S phases of the cell cycle, rather than slowing down cytokinesis itself. The delay in the time of cell division seems to be an effect of the static field on the egg instead of the sperm.

It is interesting to note that the static field produces relatively equal delays for the 1st and 2nd cell divisions (Figure 5), when compared to our earlier studies where exposure to an AC field produced much larger accelerations for the 2nd cell division than for the first (Levin and Ernst, 1995). The AC field seems to shorten each mitotic cycle, while the static field appears to act once, before the 1st cell division and most likely, before fertilization, since it is seen that earlier pre-fertilization exposures have greater effects.

The static field effect does not show a simple dose-effect relationship with cell division time since longer exposures do not necessarily produce a more pronounced effect than shorter exposures. Rather a bell-curve relationship around a maximal value is observed which may represent some sort of habituation process within egg. This observation is potentially quite important since most EMF-exposure guidelines are designed to limit the field magnitude, and the amount of time a person spends within a field. Thus, our data suggest that the crucial parame-

ter may not be how long a biological system is exposed to the field (since Figure 5 shows, shorter exposures can produce a larger effect), but rather how the timing of exposure relates to key biological events. The exposure timing which produces that maximal delay in *S. purpuratus* is 15 min. pre-fertilization. The 17 minute delay may be a maximum effect that can be achieved by this field, since it is the same for both divisions, whereas the other experiments show slightly greater delays for the 2nd division. The same bell-shaped relationship was seen in *L. pictus* (data not shown), resulting in a slightly different optimal timing (30 min. pre-fertilization), and a somewhat greater delay (22 min.) than for *S. purpuratus.*

L. pictus embryos exposed to static and AC fields exhibited up to an 8-fold increase in the incidence of exogastrulation, while none of the applied fields tested had this effect on *S. purpuratus* embryos. This is consistent with the fact that the natural incidence of exogastrulation in *S. purpuratus* is much lower than in *L. pictus*, and the fact that *L. pictus* exogastrulates at a lower LiCl concentration than does *S. purpuratus* (Nocente-McGrath *et al*., 1991). Thus, this differential sensitivity to magnetic fields likely reflects a genetic difference between the two species.

The collapsed embryos represent an unknown phenomenon. It was observed in single-cell eggs as well as in cultures of hatching embryos. It is unknown, however, whether the collapsed embryos seen in mid-blastula cultures represent embryos which collapsed before the first cell division, or embryos which collapsed at some later time. This defect has been observed in unfixed embryos, ruling out artifacts caused by fixation. No other known teratogenic agent is known to produce similar effects.

The effects described were most likely due to the applied DC field. The small stirring motors produced no detectable AC fields at the level of the magnets. Though we cannot formally completely rule out the possibility of very weak eddy currents being induced in the magnets by stray AC fields, this is extremely un-

likely to cause the effects described above since previous experiments have indicated that somewhat weaker ceramic magnets used in exactly the same experimental setup have no effects (data not shown). The static field cannot induce heating and is several orders of magnitude weaker than fields which have been shown to cause changes in membranes' electrical properties (Kholodov, 1971). Furthermore, ferrous molecules are not known to occur in sea urchins. However, our results demonstrate that static fields containing very little energy (same order of magnitude as average kinetic energy due to thermal motion) can significantly affect important biological processes. DC magnetic fields can, however, alter the velocity of motion of ions.

Under appropriate conditions, small changes in the behavior of ions could cause significant effects. For example, alterations in velocity would affect the interactions between ions and receptor channels. Similarly, small changes in the behavior of ions that are components of signal transduction pathways could produce dramatic changes. Interestingly, lithium, the classic inducer of exogastrulation in sea urchins (reviewed: Nocente-McGrath *et al.*, 1991) is proposed to act by secondary-messenger pathways in several different cell types (reviewed: Berridge *et al.*, 1989). Still, there is no immediately obvious mechanism that can account for the various developmental effects we observe. Some possibilities are induced changes in trajectories of moving ions such as Ca^{++} near membranes, electric currents induced by the motion of the conductive cytoplasm of the urchins through the static field as they are being stirred, and conformational changes in the structure of regulatory proteins (Verma and Goldner, 1996).

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Figure Legends

1. Apparatus for magnetic field exposure. The culture is exposed to a static field produced by two attracting ceramic magnets. Asterisks represent embryos or eggs.

2. Exposure of embryos to a 30 mT field immediately at fertilization. Solid line and asterisks indicate the curve for the control culture and dashed line and empty circles indicate the curve for the exposed culture. The field produces an insignificant delay (1 min.) in the first cell division and a small but significant (6 min.) delay in the second, relative to controls.

3. Exposure of sperm alone to a 30 mT static field for 1 hour. Solid line and asterisks indicate the curve for the control culture and dashed line and empty circles indicate the curve for the exposed culture. The field has no significant effect on the duration of the first two cell divisions.

4. Exposure of embryos to a 30 mT field 15 min. pre-fertilization. Solid line and asterisks indicate the curve for the control culture and dashed line and empty circles indicate the curve for the exposed culture. The exposure results in a 17 min. delay in each of the first two cell divisions relative to controls.

5. A non-linear relationship exists between exposure timing and magnitude of cell cycle delay was observed. The optimal exposure time is 15 min. pre-fertilization, which results in a delay of 17 min. in each cell division.

6. Field-induced exogastrulated embryos. *L. pictus* embryos were exposed to a 30 mT static magnetic field at fertilization and the field was maintained throughout the experiment. A and B show examples of field-induced exogastrulated embryos. C is a normal, non-exposed, embryo at the same age.

7. Field-induced collapsed embryos. *L. pictus* embryos were exposed to a 30 mT static magnetic field at fertilization and the field was maintained throughout the

experiment. A shows an example of field-induced collapsed embryos. B is a normal, non-exposed, embryo at the same age.

Figure 1:

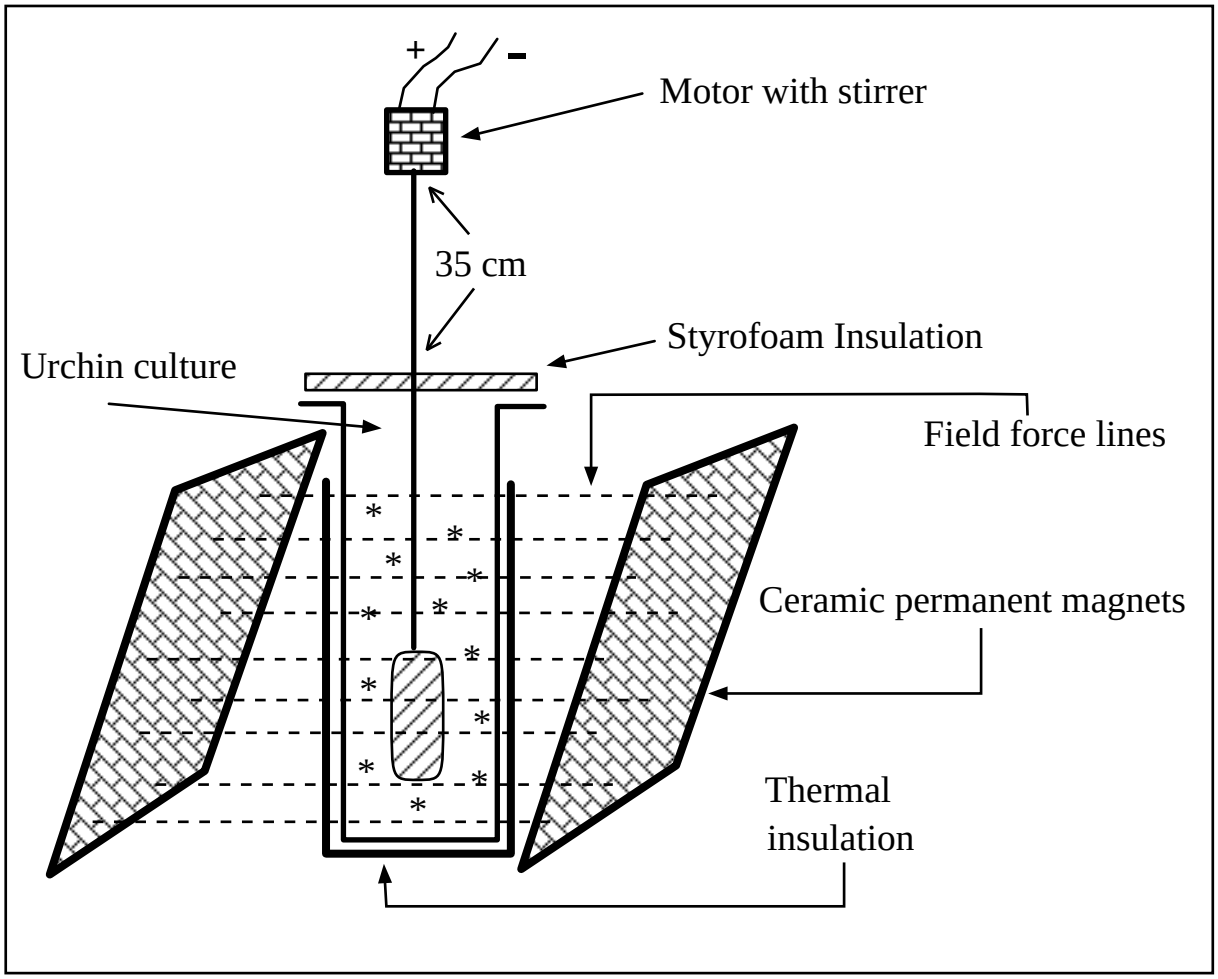
+
-
Motor with stirrer
35 cm
Styrofoam Insulation
Urchin culture
Field force lines
Ceramic permanent magnets
Thermal
insulation

Figure 2:

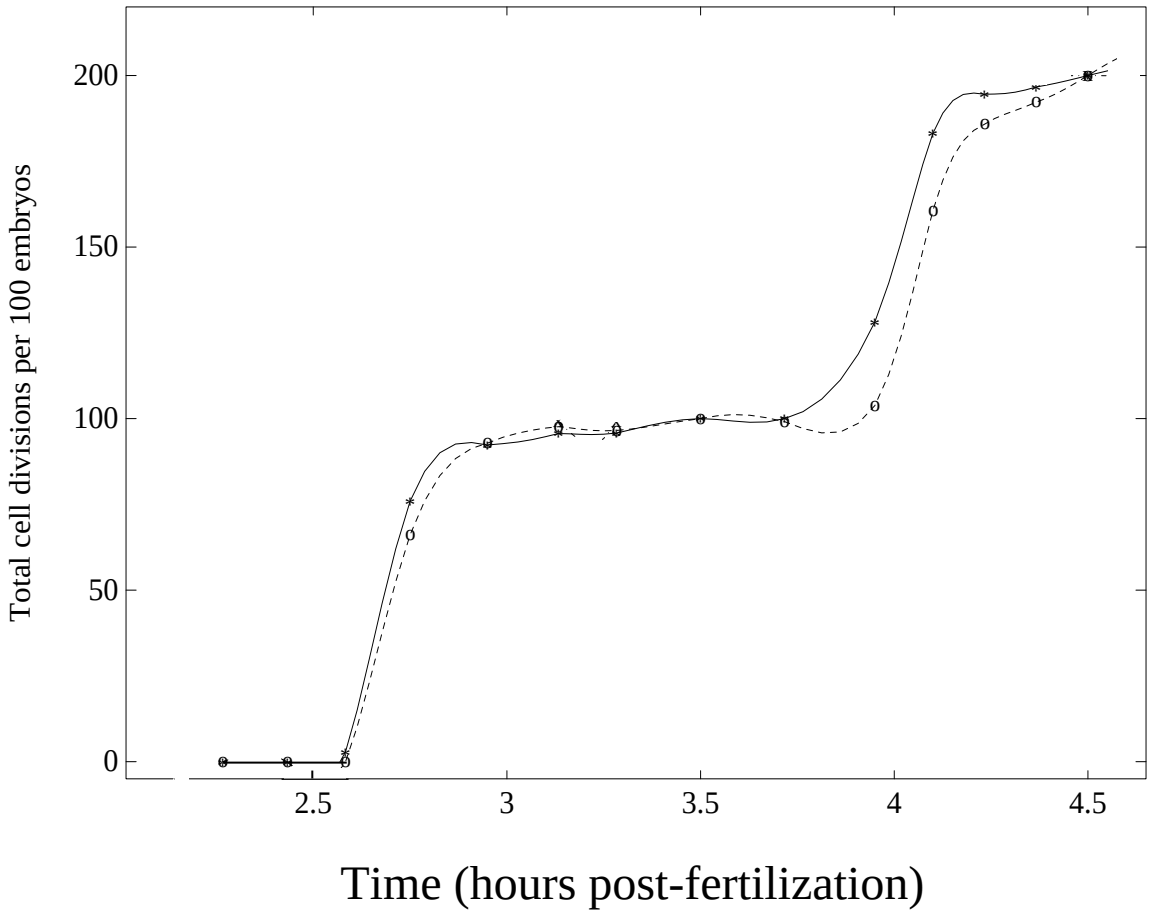
Total cell divisions per 100 embryos
200
150
100
50
0
2.5
3
3.5
4
4.5
Time (hours post-fertilization)

Figure 3:

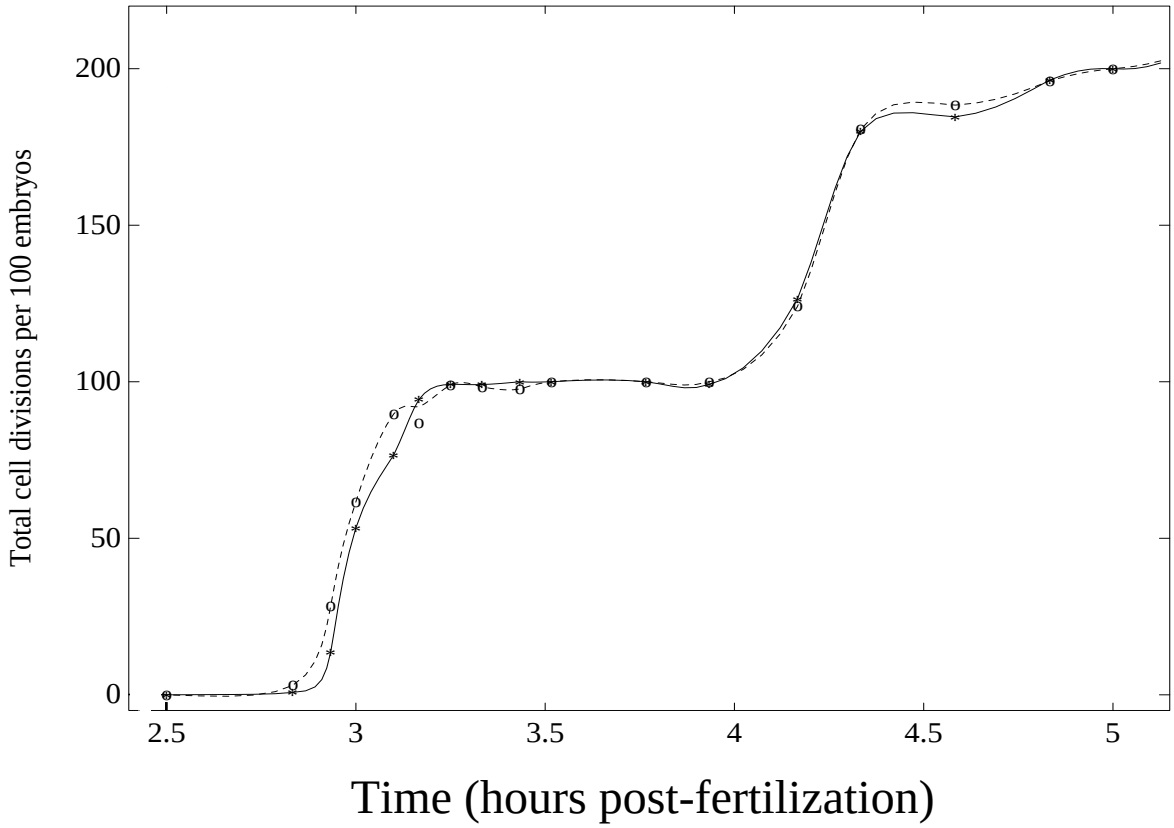
Total cell divisions per 100 embryos
200
150
100
50
0
2.5
3
3.5
4
4.5
5
Time (hours post-fertilization)

Figure 4:

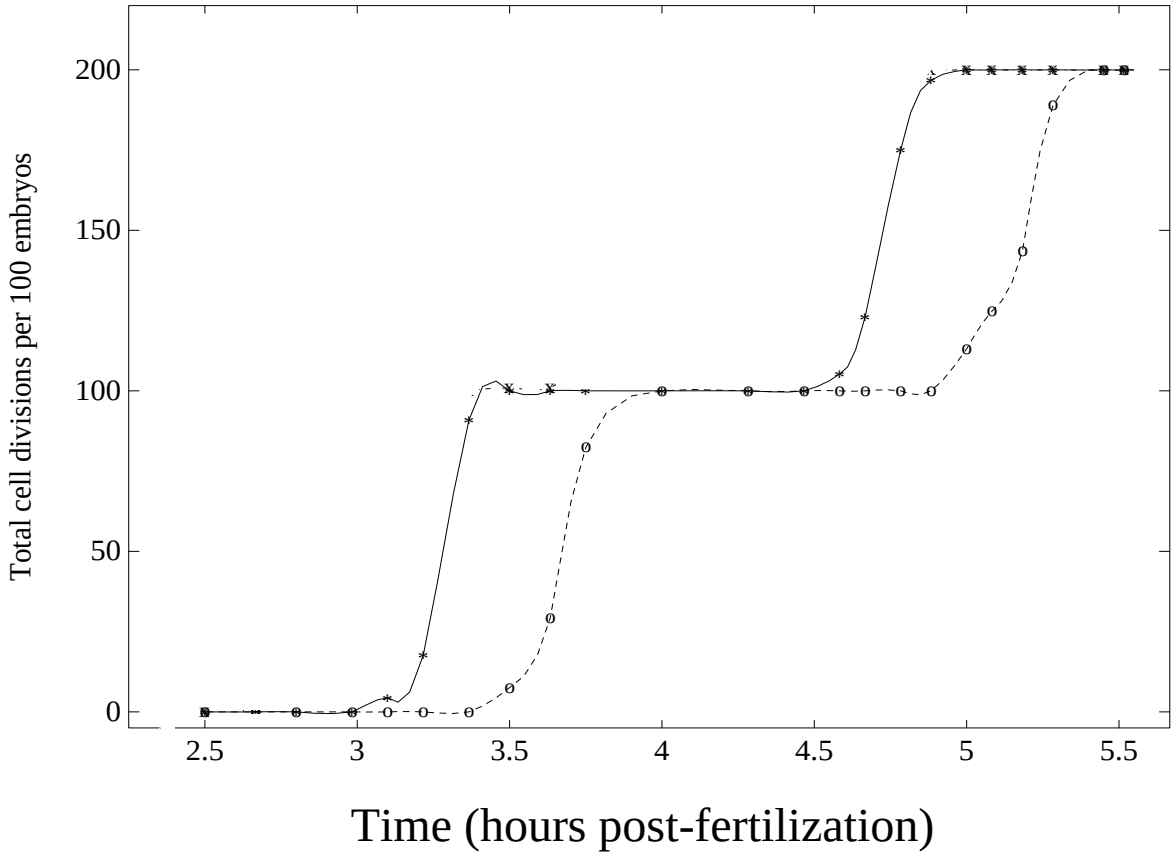
Total cell divisions per 100 embryos
200
150
100
50
0
2.5
3
3.5
4
4.5
5
5.5
Time (hours post-fertilization)

Figure 5:

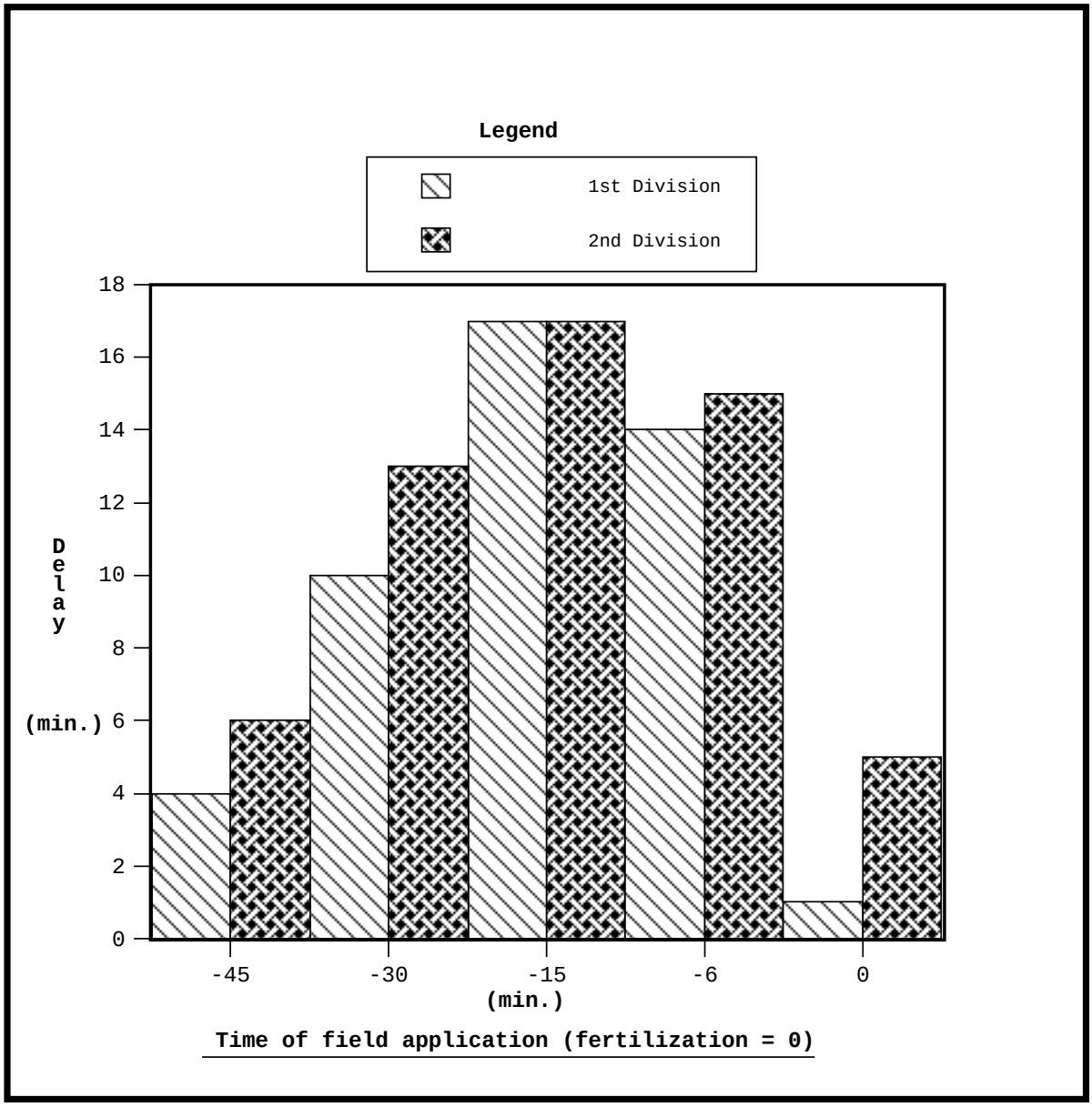
Legend
1st Division
2nd Division
18
16
14
12
10
8
6
4
2
0
Delay
(min.)
-45
-30
-15
-6
0
(min.)
Time of field application (fertilization = 0)

Figure 6:

A

B

C

Don’t worry about the picture quality - this was just to settle order, placement, etc. I have the original pics and can scan them (mount, etc.) on our system so that they will look like my figures for the LR asymmetry paper. Will that be ok?

Figure 7:

A

photo of collapsed embryo

B

Photo of control embryo will go here.

Don’t worry about the picture quality - this was just to settle order, placement, etc. I have the original pics and can scan them (mount, etc.) on our system so that they will look like my figures for the LR asymmetry paper. Will that be ok?

Table 1: *L. pictus* embryos were exposed to a 30 mT static field immediately after fertilization. Twenty-six hours later, samples from the control and exposed batches were scored for hatching (absence of fertilization membrane).

	Control	Exposed
hatched:	82%	36%
not hatched	15%	60%
arrested before hatching	3%	4%
total embryos:	109	111

Table 2: *L. pictus* embryos were exposed to a 30 mT static field immediately after fertilization. At 3.5 hours post-fertilization, samples from the control and exposed cultures were scored for the number of blastomeres.

	Control	Exposed
1 cell	9%	36%
2 cell	91%	64%
4 cell	0%	0%
other	0%	0%
Total embryos:	104	111

Table 3: *L. pictus* embryos were exposed to a 30 mT static field immediately after fertilization; the exposure lasted throughout development. Two to three days later, samples of the control and exposed cultures were scored for the incidence of exogastrulation.

	Control	Exposed
normal gastrula	98%	88%
exogastrula	2%	12%
Total embryos:	109	111