

The Effect of Weak Permanent Magnetic Fields on the Electric Properties of Lipid-Bilayers

Alexander Pazur

Botanisches Institut der Universität, Menzingerstr. 67, D-80638 München

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Black lipid membranes were prepared on a Teflon septum separating electrically the two chambers of a Teflon cuvette, using the technique of Mueller *et al.*, (Nature **194**, 979 (1962)). An external, static magnetic field was applied, whose intensity varied from 0 G to 100 G at the membrane location. Field applications higher than 10 G are effecting higher leakage currents, increased capacity and faster breakdown of the bilayer state, as compared to the absence of a magnetic field. If bilayers were doped with chlorophyll *a*, these effects were increased. Quantum mechanical and thermodynamical phenomena on membranes will be discussed as possible origins of these effects.

Introduction:

Static, as well as high and low frequency alternating (electro)magnetic fields are a steady environmental factor for life on earth. Naturally generated components of such fields are the result of a complex interaction between electrical processes in the atmosphere, charged solar and interstellar particles, and the earth's magnetic field (Schumann, 1956; Conley, 1969; Pazur, 1992). The average of these actions generally alters with geological timescales. On the contrary, man-made electromagnetic fields have increased rapidly in various frequency- and intensity-ranges as a consequence of our technical progress in the last decades. The examination of potential biological effects *i.e.* health damages in man and animals caused by magnetic fields should be an effort with public interests (WHO, 1984; WHO, 1987). Examples for their daily presence are wireless telecommunication, electric power distribution, and NMR based medical diagnostics. Compared with investigations on other environmental factors, there are, however, only few results, which can mechanistically link the physics of weak (electro)magnetic fields and the processes of life.

Ferromagnetic particles are well known in some bacteria and algae (Torres *et al.* 1986; Frankel and Blakemore, 1989), where they are the receptors for magnetotaxis. An established perception of magnetic field without the use of ferromagnetic materials is principally also possible by their effect on singlet-triplet mixing (Vrieze and Hoff, 1990). In photosynthetic electron transport, the triplet yield can be modulated by more than 100% with magnetic fields of some hundred Gauss. This effect is also discussed for linking the magnetotaxis and phototactic behavior of birds with processes localized in the retinal membranes of their eyes (Wiltchko *et al.*, 1993; Albe and Able, 1993). Growth and reproduction of microorganisms affected by weak magnetic fields are described by (Pothakamury *et al.*, 1993). Cyclotron resonance of ions affecting bonds between ions and large molecules was given here as one possible explanation.

Some of the aforementioned results link magnetic field perception to membrane bound complexes. There are also currently less established theories, which discuss collective effects of ordered structures like *e.g.* membranes as mechanisms for perception and/or communication (Warneke and Popp, 1979). These results have prompted us to investigate the effect of weak magnetic fields on the bulk properties of membranes. Here results are reported, which show that the electric properties of black lipid membranes (BLM) are changed measurably by fields as low as 10 G.

Abbreviations: PC, phosphatidylcholine; DPPC, dipalmitoyl-phosphatidylcholine; ASOL, soybean-lecithin; BLM, black lipid membrane (lipid bilayer); Chl*a*, Chlorophyll *a*.

Reprint requests to Dr. A. Pazur.

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Materials and Methods

Phospholipids and doping

Asolectin (Soybean-Lecithin, Fluka, Buchs Switzerland 11145) with a primary L- α -Phosphatidylcholine (PC) content of approximately 30% was purified and enriched up to 75% PC by silica-gel-thin layer chromatography after (Klenk, 1961) and detection after (Bartlett, 1959). Egg Lecithin (Merck 1.05331), L- α -dipalmitoyl-PC (DPPC, Sigma P-0763) and purified, lyophilized PC (Sigma P-3556) were used without further purification. Stock solutions of lipids were 10% w/v in a mixture of *n*-hexane (Baker 9303-3) and *n*-octane (Merck 4727)(9:1, v/v), and stored at -18°C until use. For experiments with doped bilayers, chlorophyll (*Chl*) *a* was extracted and purified from dried *Spirulina* after (Svec, 1978) and added at 1/1000 of the molar concentration (respectively approximately an absorbance of OD 0.1 at 660 nm) of the lipids, directly before usage.

Apparatus

Back lipid membranes (BLMs) were formed by the "Rudin-Mueller"-technique (Mueller *et al.*, 1962; Tien and Diana, 1968; Matews and Holde, 1990). The lipid stock solutions were diluted to 1% w/v in 90% *n*-hexane/10% *n*-octane. This mixture was dispersed with a soft paintbrush over an aperture with a diameter of 1 mm in a Teflon film with a thickness of 0.1 mm. This film was separating the

two compartments (volume approx. 25 ml) of a Teflon chamber, which were filled both with a 100 mM NaCl solution in ultrafiltrated, doubly deionized water. The chamber was mounted on the top of an electromagnet (coil diameter 12 cm, inductivity 5 H, 8 cm diameter iron core). No other components closer than 30 cm to the probe chamber were manufactured from iron or other magnetizable metals or alloys. Magnetic calibration was done with a semiconductor-based Hall magnetometer, next to the aperture of the BLM. The electric connections from the electrolyte to the electronic equipment was provided by two graphite electrodes.

All experiments were performed at $20 \pm 0.5^{\circ}\text{C}$, controlled by a thermostat. A grounded zinc-box was used for electrical shielding. The measuring system consisted of an AT286 personal computer (Vobis, Munich Germany) with a Flytec 12/16 ADA converter (Sander Computer Systeme, Burscheid-Hilgen Germany) providing 12 bit resolution. This device was used to generate a symmetric squarewave-pulse train with 25 Hz frequency and an amplitude of ± 50 mV (Fig. 2B, top). The signal was applied to the sample, after sending it over a high to low impedance converter without any additional amplification. The shortest useful sampling time for the ADA converter is 350 μs . The period time of 40 ms provided a minimum of crossmodulation or interference with the mains frequency (50 Hz) and warranted still a stable timing of the ADA converter. One half period of 20

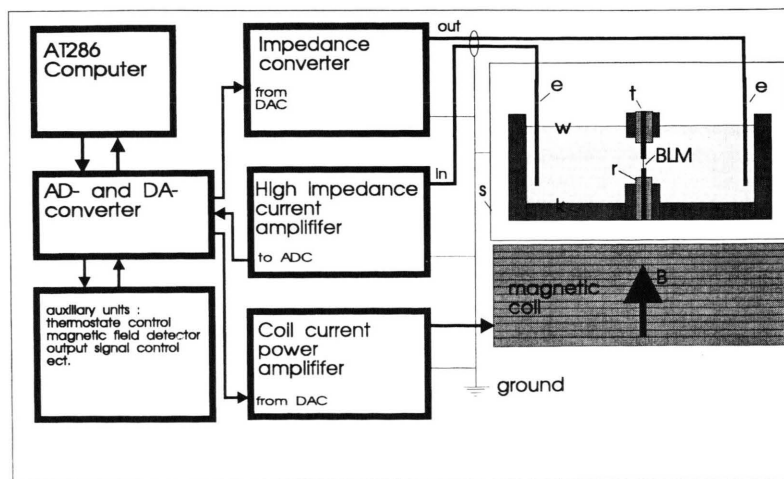


Fig. 1. Schematic diagram of the cuvette with the BLM samples and the measuring system (components are not drawn to scale): e, electrodes; k, cuvette; w, water phase; t, teflon septum; r, rubber seal; s, electrical shielding; in/out, inputs and outputs of the amplifiers; B, magnetic field direction (big arrow); ADC/DAC, analog to digital-digital to analog converter.

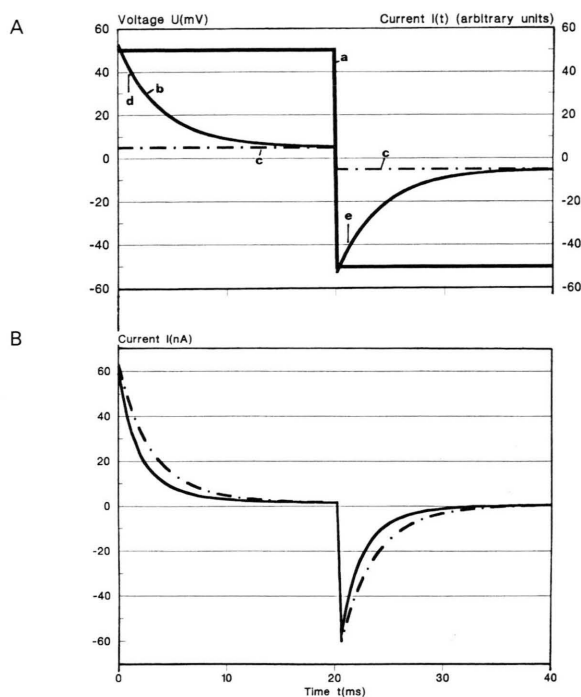


Fig. 2A. Schematic representation of the applied square-wave voltage and an the resulting current signal from a capacitor with a parallel switched Ohm-resistor (passive electric biomembrane model after (Glaser, 1982). a) The square-wave voltage input signal (± 50 mV, duration 40 ms, symmetric half waves); b) Capacitance-current response represented by an exponential decay $I(t) = I_0 \cdot e^{-kt}$, if voltage signal from (a) is applied; c) Remaining current, consisting of the constant leakage current, and a residual of charging current at 20, or 40 ms; d) Marker for $t=1$ ms and e) marker for $t=21$ ms, the current values at these times were used for the calculations. – B. Averages of the normalized BLM currents of all scans in the absence ($B = 0$ G, solid line) and at the maximum of the magnetic field ($B = 100$ G, dotted line). Only experiments with undoped BLMs were taken into account; (for further explanations see text and Table I).

ms was also more than 5 times the observed half-life for the condensator charging current (see Fig. 2B, bottom), the leakage current could therefore be defined as the remaining current on the end of each 20 ms half period. Selecting a sampling time of 400 μ s, resulted in 101 values/period.

The probe current was measured with a home built device, equipped with a high sensitivity operational amplifier in CMOS-technology, in order to obtain a low noise and wide band current input.

Because band-width of data recording was limited by the sampling rate, an impedance of 1 M Ω was selected, in order to get sufficient time resolution of the capacitive currents. Signal recording and all controls were done with the same ADA board in the personal computer, synchronously to the generation of the squarewave signal. The self written program averaged over the desired number of individual cycles within a pulse train, changed the magnetic field strength according to a preset function, and started again the measurement (see circuit block diagram Fig. 1).

After each change of magnetic field strength, a wait cycle of 500 ms was introduced, in order to ensure that possible unpredictable offset effects were damped out and the new field strength had stabilized. Using an internal averaging over ten 40 ms periods for every field strength, the total observation time was approx. 90 s for a scan of the magnetic field strength from 0 to 100 G in 1 G steps.

Results

Several phospholipids of different purity grades were tested (column 1 of Table I). They all derive from phosphatidylcholine, which amounts to 7–39% of the total membrane composition of living cells (Metzler, 1977; Matews and Holde, 1990). The total of 15 experiments included 4 control measurements and four with BLMs doped with Chl *a*.

The main electric response of a BLM is that of a capacitor. It shows however, effects like space charge-limited currents and non-exponential current decay (Braun, 1987) which are not observed by technical capacitors. Fig. 2A (top) shows one periode of the controlling square wave signal of ± 50 mV amplitude used in the experiments (a) and the calculated current response with e^{-t} time course of a capacitor of 0.05 μ F with a circuit resistor of 1 M Ω (b) (approximately the electric data of the samples with the external electronic circuit) in arbitrary units. Curve (c) marks the remaining current, consisting of the constant leakage current and the rest of charging current at 20 and 40 ms becoming zero in the infinite. Marks (d) and (e) show the values corresponding to 1 ms and 21 ms of the timescale, which are used for the calculation of the current amplitude $\Delta I = I(1) - I(21)$.

Table I. Summary of all experiments. Further explanations see in the text. Reference experiments are executed under otherwise identical conditions, as without applying a magnetic field.

Experiments with application of magnetic field:

Phospho-lipid	Doping	No. of experiments	Amplitude I [nA]		Current-alteration [nA] δI_{100}	Averaged specific capacitance C_s [$\mu\text{F}/\text{cm}^2$]	
			ΔI_0	ΔI_{100}		0 G	100 G
ASOL	none	3	114.5 ± 8.34	134.7 ± 11.7	20.2 ± 9.45	0.82 ± 0.25	1.28 ± 0.39
PC	none	3	108.2 ± 4.77	122.3 ± 5.95	14.1 ± 5.28	1.17 ± 0.26	1.66 ± 0.05
PC	Chl a	4	122.6 ± 4.10	156.5 ± 5.42	38.9 ± 4.89	0.91 ± 0.06	1.78 ± 0.07
DPPC	none	1	110.5 —	122.2 —	11.75 —	0.65 —	0.829 —

Experiments without application of magnetic field:

			$\Delta I_{\text{scan \#0}}$	$\Delta I_{\text{scan \#100}}$	$\delta I_{\text{scan \#100}}$		
ASOL	none	1	104.0 —	106.71 —	2.71 —	1.23 —	1.24 —
PC	none	2	115.2 ± 2.41	116.95 ± 4.07	1.75 ± 3.30	1.20 ± 0.19	1.185 ± 0.23
DPPC	none	1	117.6 —	119.66 —	2.06 —	0.622 —	0.617 —

Abbreviations: ASOL, soybean-Lecithin (Asolectin); PC, phosphatidylcholine; DPPC, dipalmitoyl-phosphatidylcholine; ΔI , current amplitude $I(1 \text{ ms}) - I(21 \text{ ms})$; δI , magnetic field strength dependent current alteration at a fixed time on the pulse time scale (e.g. 1 or 21 ms); scan #, Scan-numbers instead of magnetic field strength indication for the reference experiments (without magnetic fields).

Electric connection to the electrolyte solution was provided by graphite electrodes, which have an low excess voltage, and are chemically indifferent in comparison with most metals. Distortion of the signal caused by the electrodes should therefore be negligible. The current I was sampled 1 ms after each voltage jump (as described above). This gave nearly maximum current and was sufficiently separated from the pulse slopes to avoid distortion of the signal. The magnetic field strength B was increased by steps of 1 G in the range from 0 to 100 G. The characteristic measurement parameter is the change $\delta I_B = I_B - I_{B=0}$, with I_B determined at increasing field strengths ($0 \leq B \leq 100 \text{ G}$), and $I_{B=0}$ at $B=0 \text{ G}$ in each experiment (see column 6 in Table I, and Figs 3, 4B (bottom)). Temperature dependence was estimated from a control experiment: The current I shifted at $T=18^\circ\text{C} - 22^\circ\text{C}$ about $+0.7 \text{ nA}/^\circ\text{C}$, corresponding to 2.75% of the current change δI .

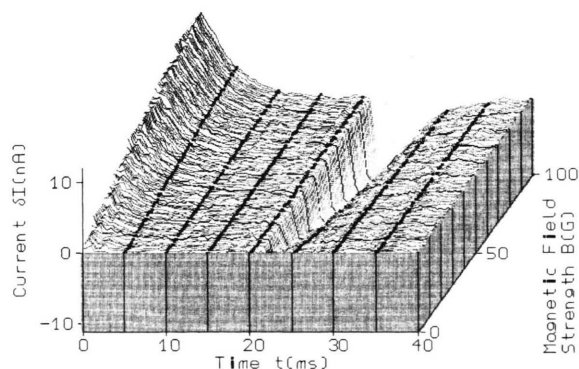


Fig. 3. Complete 3d-representation of the current changes δI over the full length (0–40 ms) and all magnetic field strengths (0–100 G) of a single experiment (one of the 3rd group in Table I, for further explanations see text).

In all experiments currents ΔI were internally averaged over 10 cycles for every new set magnetic field strength during the measurement. In Fig. 2B (bottom) the corresponding 0 G and 100 G scans from all experiments with undoped BLMs are shown, normalized to their peak amplitudes at $t=0$ ms and $t=20$ ms. Their standard deviations were about 1.16 nA ($=2.2\%$ of ΔI) for 0 G and 0.32 nA (0.5% of ΔI) for 100 G. The shape of the response curve is changed in the field, the greatest ratios between magnetic field treated samples and the references occur after 2.2 and 22.2 ms. Fig. 3 shows the complete data set from one experiment (the third one in Table I with PC, undoped) in a differential 3D-representation. There are 101 scans (for $B=0$ to 100 G). The 0 G scan is subtracted from all scans (and becomes so itself zero). The abscissa t represents the elapsed time during the pulse period (0–40 ms), the ordinate marks δI , while the B -Axis (front-to-back) gives the magnetic field strength $B[\text{G}]$. The increase of δI with increasing magnetic field is seen most clearly close to the rising and falling slopes of the exiting square-wave signal, while no significant changes of the leakage current are seen at the ends of the halfpulses, (19 ms and 39 ms respectively).

Table I shows the characteristic data from all experiments. The first two columns show the used phospholipid and if BLMs were doped with Chl *a*. The third column indicates the number of averaged independent experiments for this phospholipid or doping. Columns 4 and 5 contain the current differences ΔI , the difference of $I(t)$ between 1 and 21 ms (as described above). These are given for 0 and 100 G field strength condition. Column 6 shows δI_{100} , the field induced difference of $I(1)$ resp. $I(21)$ from 0 to 100 G. These are absolute values, the different polarities of $\delta I(1)$ and $\delta I(21)$ are neglected. Column 7 and 8 show the averaged specific capacitances discussed below.

Fig. 4A (top) shows a field free control scan (asolectin). Timing parameters were the same as in the experiments with magnetic fields. The averaged variation of current δI for these 3 reference experiments were 2.17 ± 1.33 nA, which amounts to 1.5% of the total signal amplitudes ΔI . The averaged standard deviation (single values are drawn as boxes for every presentation in Fig. 4A, 4B) is of the same magnitude as the averaged data, which means, that there is no uniform trend.

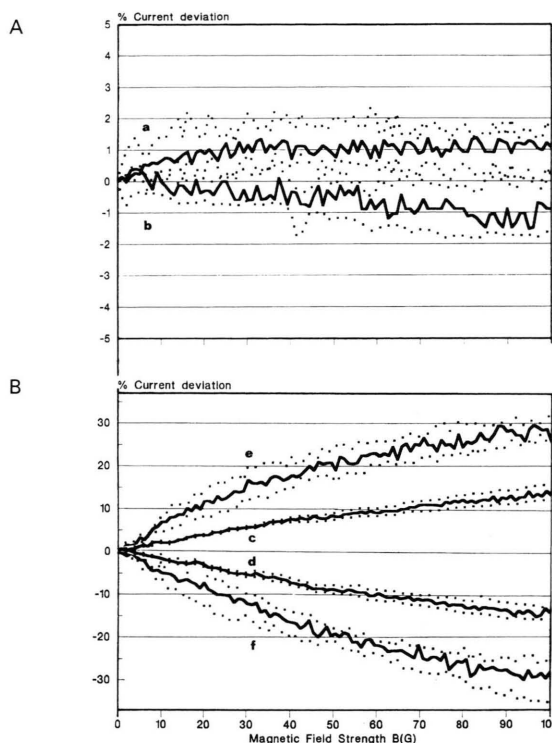


Fig. 4A. All 1 ms (a) and 21 ms (b) single current differences $\delta I(1 \text{ ms})$ and $\delta I(21 \text{ ms})$ for a **reference experiment** (asolectin). Standard deviations (drawn as boxes for every graph in Fig. 4A, 4B) are related to all reference experiments. – B. (inner curve-pair) All 1 ms (c) and 21 ms (d) single current differences $\delta I(1 \text{ ms})$ and $\delta I(21 \text{ ms})$ for an **experiment with undoped BLM** (asolectin). Standard deviations (boxes) are related to all experiments with undoped BLMs). – B. (outer curve-pair) All 1 ms (e) and 21 ms (f) single current differences $\delta I(1 \text{ ms})$ and $\delta I(21 \text{ ms})$ for an **experiment with doped BLM** (phosphatidylcholine). Standard deviations (boxes) are related to all experiments with doped BLMs).

In the presence of a magnetic field, $\delta I(1)$ and $\delta I(21)$ increased monotonously with increasing field strength. Their signs are opposite, but their absolute values were, as expected, the same at each field strength (Fig. 4B, both curvepairs). The current variations here are more than factor 10 larger than in the reference experiments. With a total average of $\delta I=15.35 \pm 3.56$ nA for all experiments with undoped BLMs and magnetic field application, the standard deviations are 1.59% for the positive and 1.72% for the negative branches (related with corresponding ΔI).

Data summarized in Table I for the third group of experiments indicate, that doping the phospholipid with Chl *a* causes stronger increase of the current, when the magnetic field is applied, (Fig. 4B, outer curves with greater magnitude). The observed $\delta I = 38.9 \pm 4.89$ nA is roughly twice as large as in an undoped membrane, standard deviations of δI are 3.9% of ΔI for the positive (1 ms) and 3.03% of ΔI for the negative (21 ms) branch. Conversely the synthetic, very pure DPPC with an uniform chain length of 16 carbon atoms shows a lower current increase as the mixtures of heterogeneous chain lengths like PC. This may indicate, that the magnetic field effect decreases with increasing order of the membrane.

Specific membrane capacitances reach typical values in the order of $1 \mu\text{F} \cdot \text{cm}^{-2}$, assuming uniform capacitance over the entire hole spanned by the BLM. In the reference experiments, the variations were in the order of 1–2%. Upon applying magnetic fields, these values rose at $B = 100$ G to 143% (undoped BLMs) and 195% (doped BLMs) of the values at $B = 0$ G. Capacitances were calculated by integrating the current (total charge, SI-unit: $Q[\text{A} \cdot \text{s}]$) for nearly completely (dis)charging the BLMs at a given voltage and the assumption of uniform current density. Specific capacitance C_s is defined as

with the area $A [\text{m}^2]$ and capacitance

$$C_s = C/A \quad [\text{F}/\text{m}^2] \quad (1)$$

$$C = Q/U \quad [\text{F}] \quad (2)$$

with the voltage $U [\text{V}]$ and the charge

$$Q = \int_{t_0}^t I dt \quad [\text{A} \cdot \text{s}] \quad (3)$$

Discussion

There are several reports in the literature, which point to the involvement of membrane structures in biological effects of weak magnetic fields. Able and Able (1993) and Wiltshko *et al.* (1993) found a correlation in birds between visual orientation and static magnetic fields with the strength of the earth magnetic field (0.5 G). They concluded, that the membranes involved in light perception are probably also involved in the perception of the

magnetic field. Marron *et al.* (1988) investigated the mitotic cycle, moving activity and respiration rate of *Physarum polycephalum*, where they found changes under the influence of a magnetic field of only 1 G. They discuss a decrease of the hydrophobic character of the cell surface, caused by exposure to the magnetic field. Traikov *et al.* (1994) discuss a change of the binding properties of lecithin to proteins at a static field strength of 5 mT (50 G), based on investigations of antigen-antibody reactions of erythrocytes under magnetic field influence.

We have extended in this study the investigation to isolated lipid membranes. To our knowledge, this is the first report of an effect of weak magnetic fields on the bulk electric properties of pure lipid bilayers. Obviously, neither of the two established mechanisms potentially useful for magnetic field perception, *viz.* ferromagnetic particles (Torres *et al.*, 1986; Frankel and Blakemore, 1989) and singlet triplet mixing (Vrieze and Hoff, 1990) in radical pairs, can account for the observed behavior.

Some studies are aimed at different mechanistic explanations for magnetic field effects on membranes. The orientation of phospholipids in liquid crystals has been reported by Sakurai *et al.* (1980), which is influenced by a magnetic field. However the used field strength of 3000 G was larger by at least one order of magnitude compared with the one used for the biological experiments. Xiang and Anderson (1994) suggested, that structural changes occur at the hydrophobic-hydrophilic interphase of membranes, on the basis of a molecular dynamics simulation. Magnetic fields <100 G might generate an additional pressure tensor, which could affect the conformational energy of the lipid molecules. Thermo- and electrodynamic descriptions of membranes (which might be the base of magnetic property calculations), are reported by Requena and Haydon (1975), Zilker (1991), Scholz *et al.* (1984), Seelig *et al.* (1985).

In recent time, quantum mechanical coherence enhancing effects have been discussed of electromagnetic waves on biological matter (Warnke and Popp, 1979; Pazur, 1991). Molecules, which are coherently excited by electromagnetic waves from the environment or as a consequence of chemical and physical conversion processes inside the living system are suggested to attain energy levels with comparably long life times, if their magnetic mo-

mentums are non-zero. The potential consequence is a measurable influence of weak magnetic fields. Biological membranes and the DNA are such structures, envisioned for coherent properties, because they have a high spatial order. However, the BLM studies presented here show, that the magnetic field effect decreases with increasing order of the membrane, and may argue against this in-

terpretation. Future investigations are needed to elucidate details of these connections.

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