Available online at www.sciencedirect.com

ScienceDirect

European Journal of Protistology 51 (2015) 290–298

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Lithium-induced developmental anomalies in the spirotrich ciliate *Stylonychia lemnae* (Ciliophora, Hypotrichida)

Seema Makhija[a,*], Renu Gupta[b], Ravi Toteja[a]

[a]*Acharya Narendra Dev College, University of Delhi, Delhi, India*
[b]*Maitreyi College, University of Delhi, Delhi, India*

Received 14 January 2015; received in revised form 8 June 2015; accepted 10 June 2015
Available online 18 June 2015

Abstract

Lithium is known to have profound biological effects of varying intensity in different life forms. In the present investigation, the effect of lithium was studied on the spirotrich ciliate *Stylonychia lemnae*. Lithium treatment brings about quantitative changes in the patterning of ciliary structures in *S. lemnae*. The dorsal surface of the affected cells develops supernumerary ciliary kineties due to excessive proliferation of the kinetosomes. The ventral surface on the other hand develops fewer than normal cirri formed from reduced numbers of ciliary primordia. The adoral zone of membranelles (AZM) fails to remodel properly as, in certain segments, membranelles become disarranged and misaligned. Lithium-induced changes are transitory as the normal pattern is restored during recovery after the cells are shifted to normal medium, suggesting non-genic regulation of cortical pattern. Lithium also affects the process of cell proliferation as the number of cells undergoing division is negligible as compared to reorganizing cells. The results point to the extremely complex and heterogeneous organization of the cellular cortex (plasma membrane and cytoskeleton) which is capable of exerting autonomous control over the phenotype and cortical pattern.

Keywords: Ciliary pattern; Lithium; Reorganization; *Stylonychia lemnae*

Introduction

Lithium does not occur free in nature and is far from being abundant but it is one of the most effective drugs for the treatment of bipolar disorders. It also exerts profound effect on the development of diverse organisms from cellular slime moulds to human beings (Hill et al. 2013; Kao and Elinson 1998; Malhi et al. 2013; Quiroz et al. 2004). It disrupts cell fate in *Dictyostelium* (Peters et al. 1989; Van Lookeren Campagne et al. 1988), causes vegetalization in sea urchin eggs (Livingston and Wilt 1990; Nocente-McGrath et al. 1991; Ransick et al. 1993; Wikramanayake and Klein 1997), brings about expansion of the dorsal mesoderm leading to duplication of dorsal axis in *Xenopus* and zebrafish (Cooke and Smith 1988; Driever 1995; Kao and Elinson 1989, 1998; Kao et al. 1986; Schneider et al. 1996; Stachel et al. 1993) and deregulates patterning in the regenerates of *Hydra* (Jantzen et al. 1998). Other reported effects of lithium includes

*Corresponding author. Tel.: +91 11 26294542 247; fax: +91 11 26294540.
E-mail addresses: makhija.seema@gmail.com (S. Makhija), guptar17@gmail.com (R. Gupta).

http://dx.doi.org/10.1016/j.ejop.2015.06.006
0932-4739/

teratogenesis in mammals (Nokhbatolfoghahai and Parivar 2008; Tandon et al. 2006; Tsaltas et al. 2007).

In ciliates, lithium ions profoundly affect the ciliary pattern formation by interfering with circular or linear morphogenetic gradients in *Stentor, Paramecium* and *Tetrahymena* (Beisson and Ruiz 1992; Jerka-Dziadosz and Frankel 1995; Tartar 1957). These ciliates have a similar body plan comprising pyriform, elongate, or cone-shaped cell, the surface of which is patterned with linear ciliary stripes (*Stentor*) or uniformly spaced linear ciliary kineties (*Paramecium* and *Tetrahymena)* and a sub-apical buccal apparatus.

The aim of the present investigation was to explore the effects of lithium on the morphology and morphogenesis of the ciliate, *Stylonychia lemnae* which is a dorso-ventrally flattened cell with two surfaces each bearing different types of ciliary structures arranged in a dissimilar but species-specific pattern.

Material and Methods

Ciliate culture

Stylonychia lemnae was originally isolated from a small pond in the Botanical Gardens at Tübingen, Germany. Clonal cultures were maintained monoxenically at 23 °C in Pringsheim's medium (Chapman-Andresen 1958) with the alga *Chlorogonium elongatum* as the food organism (Ammermann et al. 1974).

Lithium treatment

Several preliminary experiments were conducted to select a suitable treatment protocol with lithium. A stock solution (10 mg/ml) of lithium chloride (LiCl; M_W: 42.39 g/mol; Sigma) was prepared in Pringsheim's medium. *Stylonychia lemnae* was exposed to varying concentrations (200–1000 μg/ml) of LiCl to determine its tolerance limits (0% to 100% survivability). Appropriate control experiments (without lithium) with same number of cells were also performed simultaneously. For each experiment, cells were drawn from log phase cultures, washed thoroughly and diluted to a density of 100 cells/ml. Each experiment was carried out in triplicate and was performed without adding fresh food to the medium. The cells were observed under the microscope at intervals of 3 h up to 24 h. After 24 h period, the cells were counted in order to determine percent survival under different concentrations of LiCl (the time period for the toxicity treatment was selected as 24 h so as to allow the cells to undergo 2–3 generations). Cell enumeration was carried out under a Magnüs Stereoscopic microscope at 20–40× magnification. For each dose, twenty-five single cells were isolated in cavity slabs to observe for their capacity to establish clonal progeny and to calculate % clonal viability.

Exposure to 400 and 800 μg/ml LiCl for 24 h was chosen for protargol staining as these concentrations permit 70% and 50% survival and clonal viability respectively with significant cellular effects.

Revelation of ciliary structures

Ciliary structures and their developing primordia were revealed by the protargol staining method (Kamra and Sapra 1990) after 24 h of lithium treatment.

Results

The ciliate *S. lemnae* is an oblong flat cell (160 × 60 μm) patterned with species-specific arrangements of ciliary structures on its ventral and dorsal surfaces. The ventral surface bears a prominent feeding organelle, the adoral zone membranelles (AZM), two undulating membranes (UMs), a complex of 18 cirri arranged as eight frontal, five ventral and five transverse (FVT) cirri and two linear marginal cirral rows (RMC and LMC) (Fig. 1A). The dorsal surface (Fig. 2 A) has a longitudinal ciliary pattern arranged as six dorsal rows (DK) and variations in the number are extremely rare among clonal populations.

With the increase in the dose of lithium, there was a decrease in the cell number showing a dose-dependent response following treatment with lithium. A few dividers with a fission furrow were observed during the initial 3 h of lithium treatment but not afterwards (live observation only). However, a steadily increasing number of reorganising cells appeared during the course of the treatment period. Protargol staining at the end of the 24 h of lithium treatment revealed 90% reorganising cells.

Since a normal reorganization cycle in *Stylonychia* is completed in about 3–4 h (Zou and Ng 1991), a high proportion

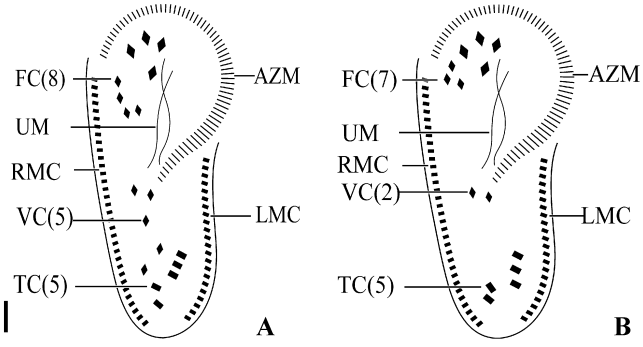

Fig. 1. Schematic diagrams of protargol-stained vegetative cells of *Stylonychia lemnae* showing ventral ciliature. (A) Normal cell showing 8 frontal (FC), 5 ventral (VC) and 5 transverse (TC) cirri forming 18 FVT cirri which are patterned in species-specific manner; adoral zone of membranelles (AZM); undulating membranes (UM); 1 row each of left (LMC) and right (RMC) marginal cirri. (B) Lithium-treated (400 μg/ml) cell showing a reduced number of 14 FVT cirri: 7 frontal, 2 ventral and 5 transverse. Scale bar represents 15 μm.

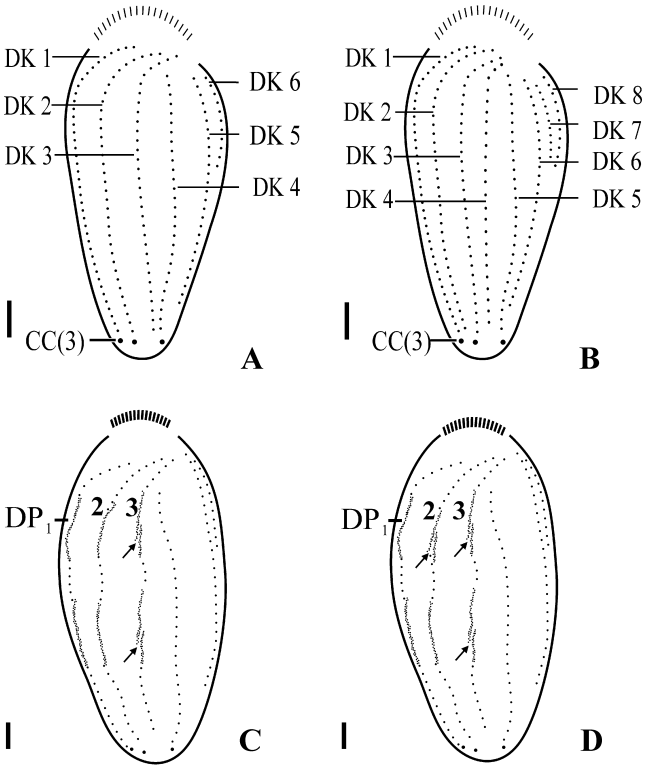

Fig. 2. Schematic diagrams of the protargol-stained cells of *Stylonychia lemnae* showing the dorsal ciliature. (A) Normal vegetative cell: 6 dorsal rows (DK1-6) and 3 caudal cirri (CC). (B) Lithium-treated (400 μg/ml) vegetative cell: 8 dorsal rows (DK1-8) and 3 caudal cirri (CC). (C) Normal dividing cell showing 3 dorsal primordia (DP 1–3) with a split in one primordium in each of the two daughter cells, the proter and opisthe (DP3; arrows). (D) Lithium-treated (400 μg/ml) dividing cell showing splits in two primordia within one daughter cell (DP2 and DP 3; arrows). Scale bar represents 15 μm.

of such cells indicates repetitive cycles of this process during the course of treatment with lithium.

In the reorganizing cells, there was improper alignment of membranelles of the AZM. In extreme cases, membranelles were fragmented and there was a loss of normal order of kinetosomes in the membranelles (Fig. 3A–D).

Examination of protargol-stained reorganizing cells revealed several types of morphogenetic anomalies resulting in their altered ciliary patterns. The ventral surface of the affected cells had 14–18 FVT cirri instead of the usual 18 FVT cirri (Fig. 1B; Tables 1 and 2). In a few extreme cases, the FVT cirri number decreased between 8 and 10. The missing cirri invariably belong to the postoral region but in some cases loss of frontal and transverse cirri was also noticed. By contrast, the dorsal surface of affected cells developed supernumerary cilia (Fig. 2B; Table 3).

Examination of reorganizing cells in lithium-treated cultures showed that the reduction in FVT cirri is due to formation of four or five FVT primordia instead of the normal six (Figs. 4 and 5; Table 2). In such cases, only one primordium (instead of two) originated from the oral primordium (OP). The pattern of cirral differentiation within the primordia was also altered. The usual differentiation pattern of 1,3,3,3,4,4 (18) cirri changed to 1,3,3,4,4 (15) or 1,3,3,3,2, (12) cirri.

The affected cells developed one to three extra dorsal rows involving the formation of up to 45% additional kinetosomes (Fig. 2). Extra kinetosomes were generally accommodated as extra rows. They may also have perturbed the linear kinetosomal order in other dorsal rows (Fig. 6A). Extra dorsal rows generally arose independently in the dorso-marginal region (Fig. 6B) or alternatively may have been formed by extra splitting of the dorsal primordia (Fig. 2D).

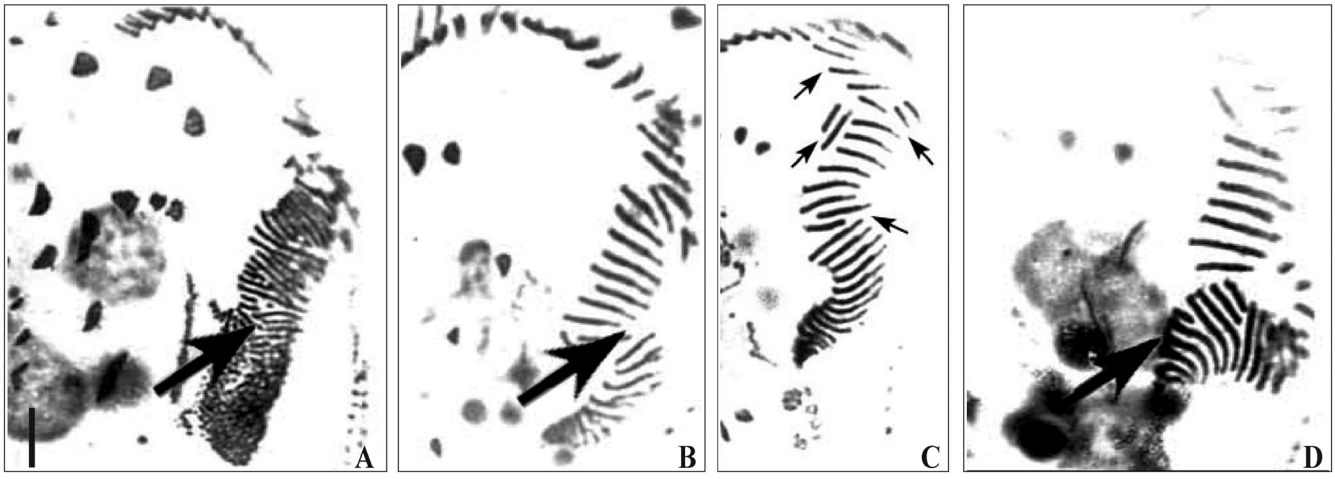

Fig. 3. Photomicrographs of protargol-stained cells of reorganizing *S. lemnae* in the presence of lithium ((A) and (B) 400 μg/ml; (C) and (D) 800 μg/ml) showing a plethora of distortions in the alignment of newly formed adoral membranelles with the parental adoral membranelles (arrows). Scale bar represents 10 μm.

Table 1. Lithium induced effect on morphometry of *Stylonychia lemnae* ($n = 25$).

Character	Control cells				Lithium treated cells							
					400 μg/ml				800 μg/ml			
	Mean	SD	CV	Range	Mean	SD	CV	Range	Mean	SD	CV	Range
No. of FVT cirri	18	–	–	18–18	17.17	1.3	7.7	14–18	15.94	1.79	11.2	14–18
No. of FVT primordia	6	–	–	6–6	5.6	0.56	10	4–6	5.39	0.57	10.5	4–6
No. of dorsal kineties	6	–	–	6–6	6.47	0.93	14.3	6–9	6.7	1.07	15.9	6–9
Total no. of dorsal cilia	227	4.5	1.98	223–280	320.25	33.6	10.4	265–346[a]	325.5	37.8	11.6	260–350[a]
No. of right marginal cirri	33.2	1.83	5.5	30–36	32.6	4.07	12.4	27–38	33	5.2	15.7	25–40
No. of left marginal cirri	21.2	0.97	4.5	20–23	22.2	3.91	17.6	16–28	23	4.13	17.9	16–30

[a] Counted only in cells with increased number of ciliary rows.

Table 2. Lithium induced effect on the number of FVT primordia and their differentiation pattern on ventral surface of *Stylonychia lemnae* ($n = 25$).

Dose (μg/ml)	Percentage of cells showing 6 or less than 6 FVT primordia (No. of Primordia)	Total number of cirri	Pattern of differentiation
Control	100 (6)	18	1,3,3,3,4,4
400	75 (6)	18	1,3,3,3,4,4
	7.5 (6)	16	1,3,3,3,4,2
	12.5 (5)	15	1,3,3,4,4
	5 (4)	14	1,3,3,3,4
800	48 (6)	18	1,3,3,3,4,4
	12 (6)	16	1,3,3,3,4,2
	24 (5)	15	1,3,3,4,4
	16 (5)	14	1,3,3,3,4

Discussion

The alterations induced by lithium treatment in *S. lemnae* are summarized in Table 4. Lithium treatment reduced the cell number at the end of treatment due to inhibition of their proliferation activity and increase in mortality because of faulty patterning and toxicity. Most of the cells did not divide in the presence of lithium. Lithium has been known to interfere with the cell division cycle by blocking G2/M transition (transition from interphase G2 to Mitotic phase) (Mao et al. 2001; Wang et al. 2008). The inhibition of cell proliferation in the presence of lithium has been reported in *Paramecium* (Beisson and Ruiz 1992), sea urchin embryos (Becchetti and Whitaker 1997), L6 myoblasts (Laurenz and Smith 1998), medulloblastoma cell lines (Ronchi et al. 2010), bovine aortic endothelial cells (Mao et al. 2001), vascular smooth muscle cells (Wang et al. 2013) and colorectal cancer cells (Li et al. 2014). Lithium affects Wnt

Table 3. Lithium induced effect on the dorsal surface of *Stylonychia lemnae* ($n = 25$).

Dose (μg/ml)	Percentage of cells showing				Average number of dorsal cilia	Range
	6 DK	7 DK	8 DK	9 DK		
0	100	0	0	0	227	223–280
400	87	9	2	2	320.25	265–346
800	82	10	4	4	325.5	260–350

Table 4. A summary of lithium induced cortical changes in *Stylonychia lemnae*.

Character	Control	Lithium treated cells
AZM	Proper alignment of membranelles	Improper alignment leading to abnormal AZM
No. of cirri	100% of cells show presence of 18 FVT cirri which is a species-specific taxonomic character	25–50% of cells show reduction in the number of FVT cirri (18–14)
Position of cirri	8 Frontal + 3 post oral ventral + 2 Pre-transverse ventral + 5 transverse	8–6 Frontal + 1–0 post oral ventral + 2–0 pre-transverse ventral + 5–2 transverse
No. of FVT primordia	6 Primordia giving rise to 18 FVT cirri	6–4 Primordia giving rise to 18 or less than 18 FVT cirri
No. of dorsal rows	6 Rows	6–9 Rows formed either by abnormal splitting or increased no. of rows from the dorsomarginal region
Alignment of bristles in the dorsal rows	Aligned in a normal pattern	Misaligned and perturb the linear arrangement

(Wingless/Integrase, Wg/Int family) signalling pathways which are a group of signal transduction pathways made of proteins that pass signals from outside of a cell through cell surface receptors to the inside of the cell and are reported to play an important role in cell by effecting GSK-3 (Glycogen Synthase Kinase-3) beta protein. GSK-3 has been known to phosphorylate numerous substrates including cytoskeletal proteins such as the microtubule-associated protein *tau* and the multifunctional protein beta-catenin which controls cell proliferation (Ciobotaru et al. 2005; Hill et al. 2013; Kao and Elinson 1998; Klein and Melton 1996; Ko 2012; Miyoshi et al. 2011; Quiroz et al. 2004).

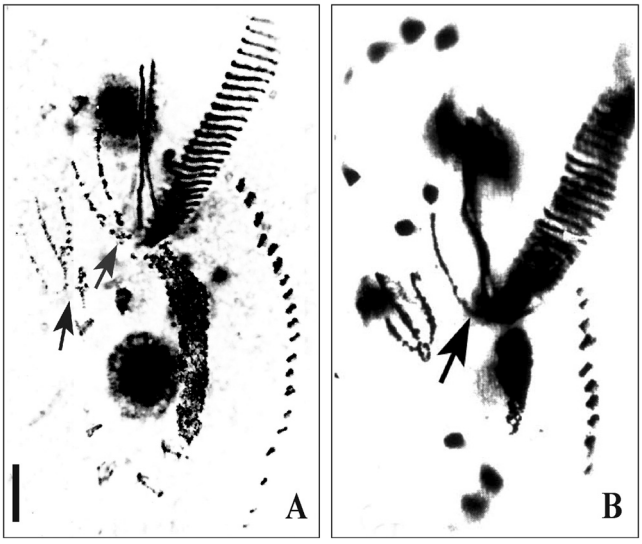

Fig. 4. Photomicrographs of protargol-stained of reorganizing cells of *S. lemnae* showing primordia formation on the ventral surface. (A) Normal cell: first primordium from UM, two primordia (arrow) from the oral primordium (OP) and three from the ventral cirrus (double arrow) resulting in the formation of six primordia (B) lithium-treated cell (400 μg/ml): first primordium from UM, one primordium (arrow) from the OP and three from the ventral cirrus (double arrow) resulting in the formation of five primordia. Scale bar represents 15 μm.

Cells which are initially committed to divide undergo division, but 90% of the cells in presence of lithium undergo reorganization. It is possible that feeding activity may be seriously affected in cells with damaged or altered AZM organization and this may trigger reorganization. Abnormal oral development in ciliates is caused not only by lithium but also by chemicals such as colchicine (Neviackas and Margulis 1969; Rosenbaum and Carlson 1969), wortannin (Kovacs and Pallinger 2003) and roscovitine (Kaczanowska et al. 2012), and by high temperature (Frankel 1964) suggesting the involvement of cytoskeletal elements (Akoev and Sizaya 1970; Ehrlich and Diamond 1980; Lazou and Beis 1993; López Cascales and Torre 1997; Wakefield et al. 2003).

The present investigation has also shown that lithium affects the ciliary pattern of the cell surface of *S. lemnae* which is an otherwise stable feature and a major taxonomic character for species identification and classification. Detailed description of the cortical patterning process has been well documented in *S. lemnae* (Wirnsberger et al. 1986) but the regulatory mechanisms are not known. Results of the present study demonstrate that lithium interferes with the quantitative regulation of development of surface organelles. Such alterations are transitory as cells form a normal pattern after they are shifted to a lithium-free medium. Lithium-treated cells consistently show a reduction in the number of FVT cirri on the ventral cell surface and an increase in the number of DKs on the dorsal surface. Ciliate morphogenesis is guided at some level by the cell membrane (Hufnagel, 1981, 1982, 1983) and any configurational and structural change in the membrane could lead to improper interpretation by the

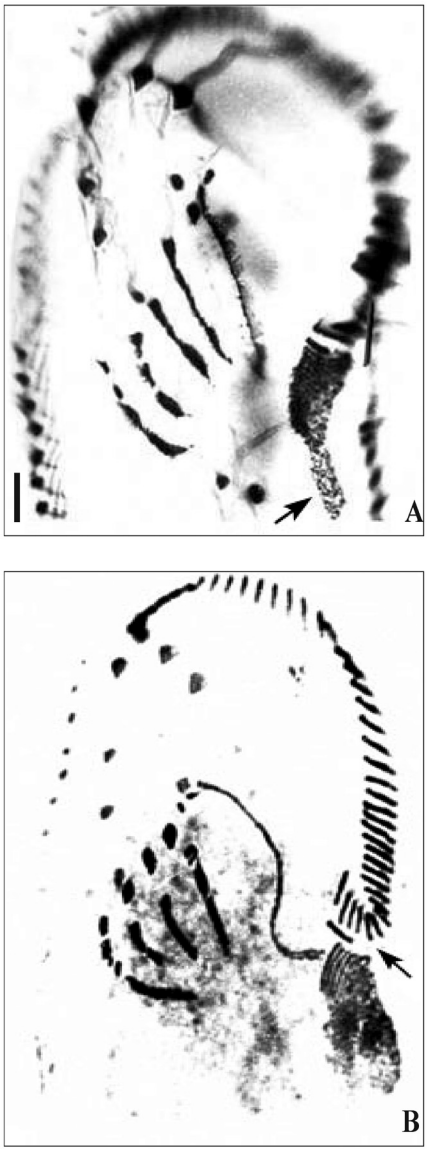

Fig. 5. Photomicrographs of protargol-stained lithium treated (400 μg/ml) cells of *S. lemnae* showing five primordia and an abnormal alignment of adoral membranelles (arrows) in the reorganizers. Scale bar represents 10 μm.

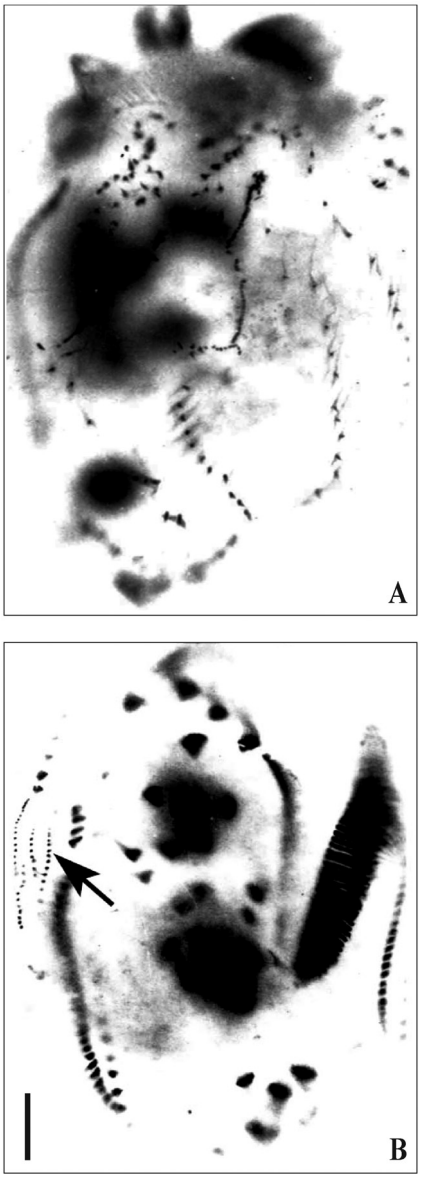

Fig. 6. Photomicrographs of protargol-stained lithium-treated (800 μg/ml) reorganizing cells of *S. lemnae.* (A) Irregular arrangement of bristles on the dorsal surface due to excessive proliferation of kinetosomes. (B) Formation of four dorsomarginal rows near the RMC (arrow) instead of two. Scale bar represents 10 μm.

cell leading to anomalous development. By virtue of its interaction with membrane and cytoskeletal components (Akoev and Sizaya 1970; Beisson 1993; Ehrlich and Diamond 1980; Iftode et al. 2000; Lazou and Beis 1993; López Cascales and Torre 1997; Marziale et al. 2008; Yanagita et al. 2007) lithium somehow creates abnormal conditions leading to cortical changes. A direct demonstration of lithium affecting the membrane organization in ciliates, however, has not been reported.

The ventral and dorsal cell surfaces of *S. lemnae* respond differently to the presence of lithium clearly indicating independent regulation of the regions of the cell. The strategy of development involves interplay of genetic information and heterogenic properties of the cell surface. Different regions of the cell surface are non-equivalent, an idea originally proposed by Wolpert for metazoan development (Kerszberg and Wolpert 2007; Wolpert 1989, 2011). Frankel (1989, 2008) applied this principle in ciliate development, a property which may govern the formation and number of ciliary primordia, their differentiation into cirri and the subsequent positioning of these cirri. One can thus propose that in *Stylonychia*, the cell surface has different domains on the ventral and dorsal sides with different positional values that determine the quantitative characters of the ciliary primordia and extent of kinetosome proliferation in the primordial derivatives. Thus, alteration in the ciliary pattern could be due to interaction of

lithium with the cell surface (cortex) components resulting in quantitatively altered character states. The cell cortex, which includes both the plasma membrane and cytoskeleton, plays an important role in ciliate cell development. However, there appears to be no molecular basis of regulation as occurs in the development process of multicellular organisms (Beisson 1993; Frankel 2008; Perović 2013). The anomalous development also affirms the importance of positional information as demonstrated in a variety of developing systems from ciliates to chordates. A further feature of universality is that many of the features of regulation and regeneration described by the polar coordinate model apply to organisms as diverse as insects and amphibia (Bryant et al. 1981) and even to protozoa (Frankel et al. 1984). Also, there are an increasing number of reports in which proteins (morphogens), such as EGF-like (epidermal growth factor) molecules (Wolpert 1989), receptors, Wnt proteins (Bajard et al. 2014) and Mob1 (Mps one binder) (Slabodnick et al. 2014) seem to be involved in patterning. Wnt signalling pathway has also been reported to play an important role in positional information in zebrafish somitogenesis (Bajard et al. 2014).

The present study can be extended to understand the molecular mechanisms regulating ciliary pattern (both ventral and dorsal surfaces) in hypotrich ciliates. The involvement of genes cannot be completely ruled out as lithium has been known to effect the expression of Wnt signalling pathway which needs to be explored.

Acknowledgements

The authors appreciate the facilities provided by the Principal, Acharya Narendra Dev College, University of Delhi for carrying out the present study. The support extended by the Principal, Maitreyi College, University of Delhi is thankfully acknowledged. We are indebted to Professor G.R. Sapra for his continuous help, support and guidance in preparing the manuscript. Authors wish to express their sincere gratitude to Dr Alan Warren for his forthright constructive suggestions, help and support for overall improvement of the manuscript. This work was supported by University Grants Commission (UGC), India [F. No. 41-15/2012 (SR)] and Department of Science and Technology (DST), India [SERB/F/1891/2012-13].

References

Akoev, G.N., Sizaya, N.A., 1970. Influence of lithium ions on the electrical activity of nerve cells of the Leech. Neirofiziologiya 2, 484–489.

Ammermann, D., Steinbruck, G., Von Berger, L., Hennig, W., 1974. The development of the macronucleus in the ciliated protozoan *Stylonychia mytilus*. Chromosoma (Berlin) 45, 401–429.

Bajard, L., Morelli, L.G., Ares, S., Pécréaux, J., Jülicher, F., Oates, A.C., 2014. Wnt-regulated dynamics of positional information in zebrafish somitogenesis. Development 141, 1381–1391.

Becchetti, A., Whitaker, M., 1997. Lithium blocks cell cycle transitions in the first cell cycles of Sea Urchin embryos, an effect rescued by myo-inositol. Development 124, 1099–1107.

Beisson, J., 1993. Cytoskeleton and molecular basis of patterning in ciliates. In: Proceedings of IX International Congress of Protozool, Berlin, pp. 15–24.

Beisson, J., Ruiz, F., 1992. Lithium-induced re-specification of pattern in *Paramecium*. Dev. Genet. 13, 194–202.

Bryant, S.V., French, V., Bryant, P.J., 1981. Distal regeneration and symmetry. Science 212, 993–1002.

Chapman-Andresen, C., 1958. Pinocytosis of inorganic salts by *Amoeba proteus* (*Chaos diffluens*). C. R. Trav. Lab. Carlsberg Chim. 31, 77–92.

Ciobotaru, D., Munteanu, C., Zamfirescu, G., Zarnescu, O., 2005. Lithium mineral waters effect on Wnt pathway signalling of glial cells. In: Proc. Balkan Sci. Conf. Biol., Bulgaria, pp. 625–636.

Cooke, J., Smith, E.J., 1988. The restrictive effect of early exposure to lithium upon body pattern in *Xenopus* development, studied by quantitative anatomy and immunofluorescence. Development 102, 85–99.

Driever, W., 1995. Axis formation in zebrafish. Curr. Opin. Genet. Dev. 5, 610–618.

Ehrlich, B.E., Diamond, J.M., 1980. Lithium, membranes, and manic-depressive illness. J. Membr. Biol. 52, 187–200.

Frankel, J., 1989. Pattern Formation: Ciliate Studies and Models, vol. XVIII. Oxford University Press, New York, NY.

Frankel, J., Jenkins, L.M., Nelsen, E.M., Bakowska, J., 1984. Mutational analysis of patterning of oral structures of *Tetrahymena I*. Effects of increased size on organization. J. Embryol. Exp. Morphol. 82, 41–66.

Frankel, J., 1964. The effects of high temperatures on the pattern of oral development in *Tetrahymena pyriformis*. GL. J. Exp. Zool. 155, 403–430.

Frankel, J., 2008. What do genic mutations tell us about the structural patterning of a complex single-celled organism? Eukaryot. Cell 7 (10), 1617–1639.

Hill, E.J., Nagel, D.A., O'Neil, J.D., Torr, E., Woehrling, E.K., Devitt, A., Coleman, M.D., 2013. Effects of lithium and valproic acid on gene expression and phenotypic markers in an NT2 neurosphere model of neural development. PLoS ONE 8 (3), e58822.

Hufnagel, L.A., 1981. Particle assemblies in the plasma membrane of *Tetrahymena*: relationship to cell surface topography and cellular morphogenesis. J. Protozool. 28 (2), 192–203.

Hufnagel, L.A., 1982. Mechanisms for assembly of membrane structure during morphogenesis in *Tetrahymena*: freeze fracture evidence. J. Cell Biol. 95, 269a.

Hufnagel, L.A., 1983. Freeze-fracture analysis of membrane events during early neogenesis of cilia in *Tetrahymena*: changes in fairy ring morphology and membrane topography. J. Cell Sci. 60, 137–156.

Iftode, F., Clerot, J.C., Levilliers, N., Bre, M., 2000. Tubulin polyglycylation: a morphogenetic marker in ciliates. Biol. Cell 92, 615–628.

Jantzen, H., Hassel, M., Schulze, I., 1998. Hydroperoxides mediate lithium effects on regeneration in *Hydra*. Comp. Biochem. Physiol., C: Pharmacol. Toxicol. Endocrinol. 119, 165–175.

Jerka-Dziadosz, M., Frankel, J., 1995. The effects of lithium chloride on pattern formation in *Tetrahymena thermophila*. Dev. Biol. 171, 497–506.

Kaczanowska, J., Kiersnowska, M., Fabczak, H., Kaczanowski, S., Kacznowski, A., 2012. Effect of roscovitine on schedule of divisional morphogenesis, basal bodies proliferation and cell division in *Tetrahymena thermophila*. Acta Protozool. 51 (2), 87–107.

Kamra, K., Sapra, G.R., 1990. Partial retention of parental ciliature during morphogenesis of ciliate *Coniculostomum monilata* (Dragesco & Njine, 1971) Njine, 1978 (Oxytrichidae, Hypotrichida). Eur. J. Protistol. 25, 264–278.

Kao, K.R., Elinson, R.P., 1998. The legacy of lithium effects on development. Biol. Cell 90, 585–590.

Kao, K.R., Elinson, R.P., 1989. Dorsalization of mesoderm induction by lithium. Dev. Biol. 132, 81–90.

Kao, K.R., Masui, Y., Elinson, R.P., 1986. Lithium-induced respecification of pattern in *Xenopus laevis* embryos. Nature 322, 371–373.

Kerszberg, M., Wolpert, L., 2007. Specifying positional information in the embryo: looking beyond morphogens. Cell 130, 205–209.

Klein, P.S., Melton, D.A., 1996. A molecular mechanism for the effect of lithium on development. Proc. Natl. Acad. Sci. U.S.A. 93, 8455–8459.

Ko, H.W., 2012. The primary cilium as a multiple cellular signalling scaffold in development and disease. BMB Rep. 45, 427–432.

Kovacs, P., Pallinger, E., 2003. Phosphatidylinositol 3-kinase-like activity in *Tetrahymena*, effects of wortmannin and LY 294002. Acta Protozool. 42, 277–285.

Laurenz, J.C., Smith, S.B., 1998. Lithium chloride does not inhibit the proliferation of L6 myoblasts by decreasing intracellular free inositol. J. Anim. Sci. 76, 66–73.

Lazou, A., Beis, A., 1993. Lithium induces changes in the plasma membrane protein pattern of early amphibian embryos. Biol. Cell 77, 265–268.

Li, H., Huang, K., Liu, X., Liu, J., Lu, X., Tao, K., Wang, G., Wang, J., 2014. Lithium chloride suppresses colorectal cancer cell survival and proliferation through ROS/GSK-3β/NF-κ B signalling pathway. Oxid. Med. Cell. Longevity 2014, 1–8.

Livingston, B.T., Wilt, F.H., 1990. Range and stability of cell fate determination in isolated sea urchin blastomeres. Development 108, 403–410.

López Cascales, J.J., Torre, J.G., 1997. Effect of lithium and sodium ions on a charged membrane of dipalmitoylphosphatidylserine: a study by molecular dynamics simulation. Biochim. Biophys. Acta 1330, 145–156.

Malhi, G.S., Tanious, M., Bargh, D., Das, P., Berk, M., 2013. Safe and effective use of lithium. Aust. Prescriber 36, 18–21.

Mao, C.D., Hoang, P., Dicorleto, P.E., 2001. Lithium inhibits cell cycle progression and induces stabilization of p53 in bovine aortic endothelial cells. J. Biol. Chem. 276, 26180–26188.

Marziale, F., Pucciarelli, S., Ballarini, P., Melki, R., Uzun, A., Iiyin, V.A., Detrich, H.W., Miceli, C., 2008. Different roles of two γ-tubulin isotypes in the cytoskeleton of the Antarctic ciliate *Euplotes forcardii*: remodelling of interaction surfaces may enhance microtubule nucleation at low temperature. FEBS J. 275, 5367–5382.

Miyoshi, K., Kasahara, K., Miyazaki, I., Asanuma, M., 2011. Factors that influence primary cilium length. Acta Med. Okayama 65, 279–285.

Neviackas, J.A., Margulis, L., 1969. The effect of colchicine on regenerating membranellar cilia in *Stentor coeruleus*. J. Eukaryot. Microbiol. 16, 165–171.

Nocente-McGrath, C., McIsaac, R., Ernst, S.G., 1991. Altered cell fate in LiCl-treated Sea Urchin embryos. Dev. Biol. 147, 445–450.

Nokhbatolfoghahai, M., Parivar, K., 2008. Teratogenic effect of lithium carbonate in early development of Balb/C mouse. Anat. Rec. 291, 1088–1096.

Perović, S., 2013. The rebirth of the morphogenetic field as an explanatory tool in biology. Filozofija I Društvo. XXIV (4), 181–198.

Peters, D.J.M., Van Lookeren Campagne, M.M., Van Haastert, P.J.M., Spek, W., Schaap, P., 1989. Lithium ions induce prestalk-associated gene expression and inhibit prespore gene expression in *Dictyostelium discoideum*. J. Cell Sci. 93, 205–210.

Quiroz, J.A., Gould, T.D., Manji, H.K., 2004. Molecular effects of lithium. Mol. Interventions 4, 259–272.

Ransick, A., Ernst, S., Britten, R.J., Davidson, E.H., 1993. Whole mount *in situ* hybridization shows Endo 16 to be a marker for the vegetal plate territory in sea urchin embryos. Mech. Dev. 42, 117–124.

Ronchi, A., Salaroli, R., Rivetti, S., Bella, E.D., Tomaso, T., Voltattorni, M., Cammelli, S., Ceccarelli, C., Giangaspero, F., Barbieri, E., Cenacchi, G., 2010. Lithium induces mortality in medulloblastoma cell lines. Int. J. Oncol. 37, 745–752.

Rosenbaum, J.L., Carlson, K., 1969. Cilia regeneration in *Tetrahymena* and its inhibition by colchicine. J. Cell Biol. 40, 415–425.

Schneider, S., Steinbesser, H., Warga, R.M., Hausen, P., 1996. Beta b-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. Mech. Dev. 56, 191–198.

Slabodnick, M.M., Ruby, J.G., Dunn, J.G., Feldman, J.L., DeRisi, J.L., Marshall, W.F., 2014. The kinase regulator Mob1 acts as a patterning protein for *Stentor* morphogenesis. PLoS ONE 12 (5), e1001861.

Stachel, S.E., Grunwald, D.J., Myers, P.Z., 1993. Lithium perturbation and goosecoid expression identify a dorsal specification pathway in the pregastrula zebrafish. Development 117, 1261–1274.

Tandon, A., Bhalla, P., Nagpaul, J.P., Dhawan, D.K., 2006. Effect of lithium on rat cerebrum under different dietary protein regiments. Drug Chem. Toxicol. 29, 333–344.

Tartar, V., 1957. Reactions of *Stentor coeruleus* to certain substances added to the medium. Exp. Cell Res. 13, 317–332.

Tsaltas, E., Kontis, D., Boulougouris, V., Papakosta, V.M., Giannou, H., Poulopoulou, C., Soldatos, C., 2007. Enhancing effects of chronic lithium on memory in the rat. Behav. Brain Res. 177, 51–60.

Van Lookeren Campagne, M.M., Wang, M., Speck, W., Peters, D., Schaap, P., 1988. Lithium respecifies cyclic AMP-induced cell type specific gene expression in *Dictyostelium*. Dev. Genet. 9, 589–596.

Wakefield, J.G., Stephens, D.J., Tavare, J.M., 2003. A role for glycogen synthase kinase-3 in mitotic spindle dynamics and chromosome alignment. J. Cell Sci. 116, 637–646.

Wang, X., Li, J., Feng, X., Wang, Q., Guan, D., Shen, Z., 2008. Involvement of the role of Chk1 in lithium-induced G2/M phase cell cycle arrest in hepatocellular carcinoma cells. J. Cell. Biochem. 104, 1181–1191.

Wang, Z., Zhang, X., Chen, S., Wang, D., Wu, J., Liang, T., Liu, C., 2013. Lithium chloride inhibits vascular smooth muscle cell

proliferation and migration and alleviates injury-induced neointimal hyperplasia via induction of PGC-1α. PLoS ONE 8 (1), e55471.

Wikramanayake, A.H., Klein, W.H., 1997. Multiple signalling events specify ectoderm and pattern the oral–aboral axis in the sea urchin embryo. Development 124, 13–20.

Wirnsberger, E., Foissner, W., Adam, H., 1986. Biometric and morphogenetic comparison of the sibling species *Stylonychia mytilus* and *S. lemnae* including a phylogenetic system for the oxytrichids (Ciliophora, Hypotrichida). Arch. Protistenkd. 132, 167–185.

Wolpert, L., 1989. Positional information revisited. Development 107 (Supplement), 3–12.

Wolpert, L., 2011. Positional information and patterning revisited. J. Theor. Biol. 269, 359–365.

Yanagita, T., Maruta, T., Uezono, Y., Satoh, S., Yoshikawa, N., Nemoto, T., Kobayashi, H., Wada, A., 2007. Lithium inhibits function of voltage dependent sodium channels and catecholamine secretion independent of glycogen synthase kinase-3 in adrenal chromaffin cells. Neuropharmacology 53, 881–889.

Zou, S.F., Ng, S.F., 1991. Physiological reorganization and post-traumatic regeneration in *Stylonychia mytilus*: reflections on developmental constraints and the evolutionary origin of alternative modes of asexual morphogenesis. J. Eukaryot. Microbiol. 38, 182–192.