

Contributions of restricted length reporting original and significant findings of immediate interest

**ELECTROPHYSIOLOGICAL STUDY OF
SPECIAL CONNECTIONS BETWEEN
CELLS IN THE EARLY CHICK EMBRYO**

JUDSON D. SHERIDAN. From the Department of Neurobiology, Harvard Medical School, Boston, Massachusetts

INTRODUCTION

Electrophysiological methods have provided a quick and useful technique for demonstrating the presence of specialized interconnections between certain cells. In such experiments these interconnections show up as low-resistance pathways for the spread of ionic current between the interiors of the coupled cells. Such "electrical coupling" has been found not only between electrically active cells (e.g. certain nerve cells, cardiac, and visceral muscle cells) (1, 2, 4, 5), but also between certain nonexcitable cells (e.g. glial cells and epithelial cells in insects, amphibians, and mammals) (8-11, 13). In all the cases cited above, electron microscopy has shown that the cells are connected by regions of specialized membrane apposition (e.g. "tight junctions") (3, 7, 12, 15, 20, 21). The widespread presence of such connections, even between cells which do not generate action potentials, has led to the suggestion that these contacts play a role in addition to that of the intercellular transmission of electrical signals (10, 14).

Cell communication in embryonic development has long been of interest, but yet remains poorly understood. The recent demonstration of electrical coupling in the squid embryo between the yolk cell and a variety of other cells, both ectodermal and mesodermal, has opened a new approach to

this question (14), and led to the present investigation of the embryo of the chick, whose development has been widely studied (16). The results of the present study suggest that, in the chick embryo, electrical coupling is extensive, and can occur between cell groups which are developing into different structures.

METHODS AND MATERIALS

Fertilized white Leghorn eggs were incubated at 39°C for 18 to 72 hr. The egg was broken into a dish containing mammalian Locke's solution (17) at room temperature (25°C). The blastodisc was cut away from the yolk and separated from the vitelline membrane, transferred to a small plastic dish containing Locke's solution, and pinned with small tungsten needles to a layer of Sylgard (Dow Corning Corp., Midland, Michigan) covering the bottom of the dish. The electrical measurements and most of the histological procedures were carried out with the blastodisc in the dish.

Standard electrophysiological methods were used to determine electrical coupling between cells. Each of two cells was impaled with a glass micropipette containing a negatively charged, blue dye (Niagara Sky Blue: 6B; 3.5% aqueous solution) as the electrolyte. Both micropipettes could be used for passing current or recording potential with respect to a common bath electrode. A rectangular current pulse was passed through one micropipette, while any re-

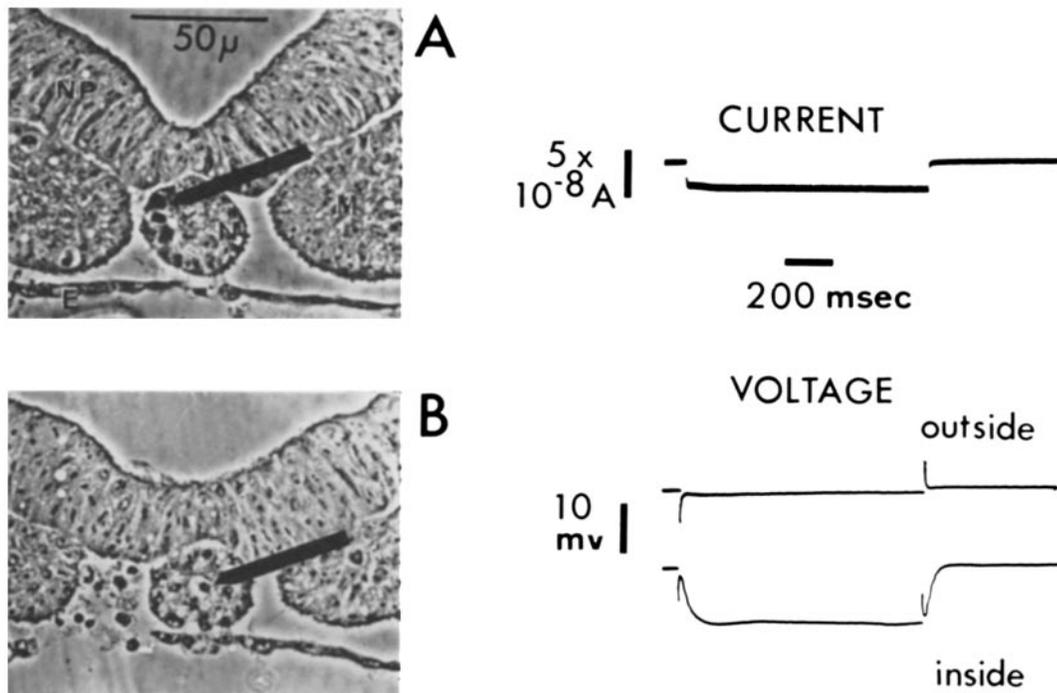


FIGURE 1 Electrical coupling between two notochord cells, 120μ apart, shown by arrows in phase-contrast photomicrographs, *A* and *B*. The 50μ calibration and letters apply to both micrographs. *NP*, neural plate; *N*, notochord; *M*, paraxial dorsal mesoderm; and *E*, endoderm. Electrical records on right show (from top to bottom) current supplied to first cell and the voltage recorded just outside and inside second cell.

sulting changes in membrane potential were monitored by the second micropipette. Then the recording micropipette was withdrawn (or advanced) to a position just extracellular, and the current pulse was repeated. The appearance of an electrotonic potential change restricted to the inside of the cell indicated that the two cells were electrically coupled. The impaled cells were stained by passing hyperpolarizing current through each micropipette, depositing the blue dye. The positions of the marked cells and the distance between them could then be determined in histological sections.

The embryos were fixed overnight at 0°C in a modified Sandborn fixative (18): glutaraldehyde (25% solution), 2.6 ml; acrolein 0.2 ml; 0.1 M acetate buffer, pH 4.0, 5 ml; Locke's solution, 2.2 ml. After dehydration in ethanol, the embryos were cleared in propylene oxide and flat-embedded in Epon, and serial 5μ sections were cut with a steel knife.

RESULTS

Fig. 1 illustrates electrical coupling between two cells, 120μ apart, in the notochord of a nine-so-

mite embryo (approximately 35 hr incubation). The impaled notochord cells are indicated by the arrows in the phase-contrast photomicrographs, *A* and *B*. When viewed with bright field, the blue-stained cells stood out sharply against the nearly colorless background. As shown by the electrical records (on the right), when current was supplied to the inside of the cell in *A* (upper trace), the second electrode recorded an electrotonic potential when it was inside the cell in *B* (lower trace), but only a very small potential when it was just outside (middle trace). The small resting potential (16mv) was registered by the displacement of the lower trace from the middle trace. Similar experiments were made on cells in ectoderm, neural plate, paraxial dorsal mesoderm, somite, Hensen's node, and developing lens. In each case, electrical coupling was observed, provided the cells were less than 200μ apart.

In many cases, particularly in the notochord, several neighboring cells at one electrode site were stained. For example, in Fig. 1*A*, in addition to

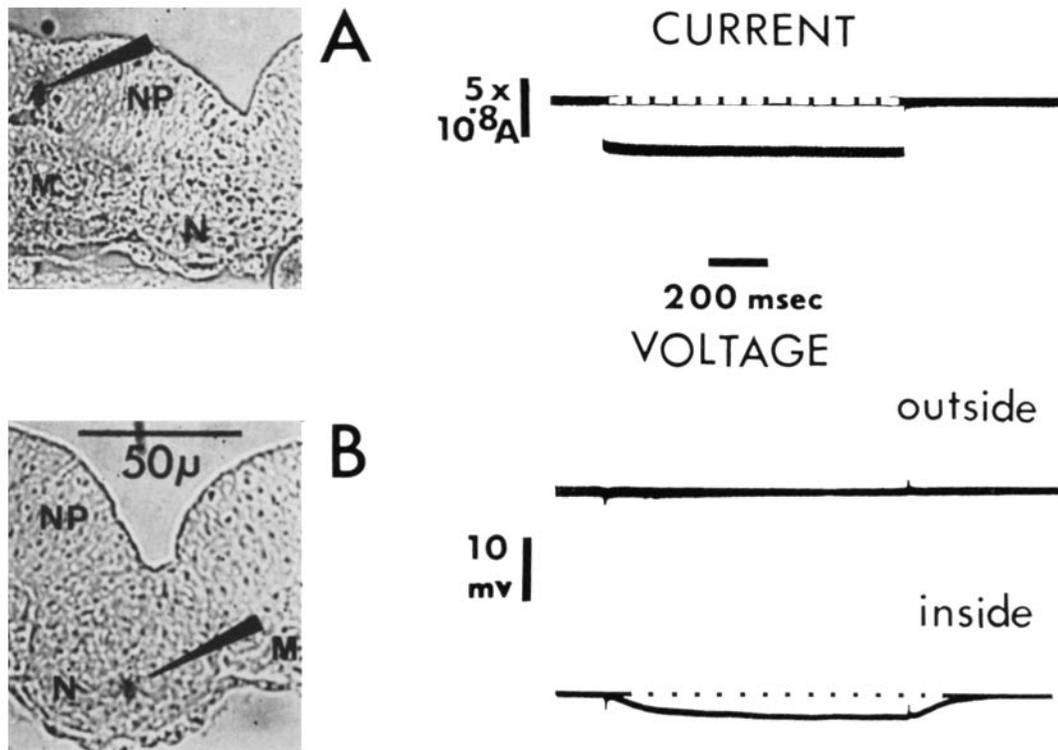


FIGURE 2 Electrical coupling between presumptive notochord cell and neural plate cell, 91μ apart, indicated by arrows in brightfield photomicrographs, *A* and *B*. The 50μ calibration applies to both micrographs. Letters refer to same structures as in Fig. 1, except for *N*, presumptive notochord. Electrical records as in Fig. 1. Small resting potential in presumptive notochord cell (91 mv) shown by deflection of lower from middle trace.

the darkly stained cell indicated by the arrow, three cells ventral to the first also contained blue dye. As in similar cases of dye spread in the squid experiments, these findings may indicate that molecules larger than inorganic ions flow between coupled cells. However, because of its proteophilic nature, the dye may not be a suitable tracer.

In addition, electrical coupling was found between cells of unlike development potential. For example, it was consistently found that, in the region just anterior to Hensen's node, cells of the developing notochord were coupled both to cells of the overlying neural plate and to cells of the paraxial dorsal mesoderm. Fig. 2 shows an example of coupling between a cell in the developing notochord and a neural plate cell in a six-somite embryo (approximately 30 hr incubation). When current (upper trace) was supplied to the inside of the neural plate cell, an electrotonic potential change appeared in the presumptive notochord

cell (lower trace), but not just outside (middle trace). The arrows in *A* and *B* show, respectively, the neural plate and presumptive notochord cells which were impaled.

In almost all experiments, many cells intervened between the two cells under study (see legends for Figs. 1 and 2). Studies of the fine structure of embryonic chick tissues (see reference 19) show that cells in notochord, ectoderm, and mesoderm do not have long processes extending over 100μ distances. Therefore, in the electrical experiments, the current passed successively through the intervening cells via low resistance cell junctions. In the case of coupling between two cells of the same tissue, the intervening cells were all of the same type, e.g., all notochord cells in the case of Fig. 1. However, several possible current pathways exist in the case of coupling between tissues of different developmental potential. The coupling illustrated in Fig. 2 might have been mediated directly, by

connections between presumptive notochord and neural plate cells, or indirectly, by way of Hensen's node, or by both pathways. There is a similar problem in determining the current pathways between notochord and mesoderm cells, and in neither case do the electrical recordings serve to distinguish between the possibilities.

DISCUSSION

It is clear from the results that electrical coupling between embryonic cells is not peculiar to the squid. It is interesting in this regard that the ectoderm and endoderm cells in the *Xenopus laevis* embryo (mid-neurula stage) are also coupled (unpublished), as are earlier stages of the *Triturus* embryo (6).

As in many previous instances of electrical coupling, electron microscope studies of cell contacts in the early chick embryo have revealed the widespread occurrence of specialized cell interconnections (19).

Although the electrical coupling in the chick embryos is likely to depend upon low resistance interconnections between cells, as found in other cases of coupling (cf. references 10, 11, 14), certain alternatives should be mentioned. The existence of a high resistance barrier surrounding the whole

blastodisc is excluded since virtually no potential changes were recorded unless both current and recording micropipettes were intracellular. In addition, light and electron microscopy indicate no anatomical barrier (19). A second alternative, a very high interstitial resistance (cf. reference 14), would not produce coupling between surface cells; however, such coupling was easy to demonstrate.

The intercellular current is doubtless carried mainly by inorganic ions spreading directly from one cytoplasm to the next, and electrical coupling may help to regulate the distribution of ions between cells. However, it is possible that the connections which provide a low resistance to ion flow also pass larger substances important in embryogenesis (cf. references 6, 14). Further studies on the chick embryo are now in progress to determine the possible presence of electrical coupling in other instances of interacting cells.

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