sites of unusually frequent sister chromatid exchange. These data are unavoidably subject to the influence of selective forces (*10*) operative during the two division cycles required for the analysis described here. The magnitude of this influence is unknown.

While the present method obviates the use of radioactivity for analysis of sister chromatid exchanges, it requires the incorporation of BrdU into chromosomal DNA. BrdU has been observed to cause breaks in some mammalian chromosomes (*11*), but it does not appear to contribute appreciably to them under the conditions employed here. Moreover, the BrdU-induced chromosome breaks reported (*11*) occurred in heterochromatic regions, which are thought to correspond to the quinacrine band regions (*1, 2*), while the observed sister chromatid exchanges occur predominantly in interband regions. This location of sister chromatid exchanges does not correlate with the expected distribution of BrdU along the chromatids, assuming that regions fluorescing brightly when stained with quinacrine are relatively A-T rich (*12*). Nonetheless, at this time, the possibility that BrdU may contribute to the exchange frequency cannot be ruled out.

Chromatid breakage prior to sister chromatid exchange may involve events similar to those of unhealed chromatid breaks, chromosome translocations, and meiotic crossing-over. The preferential location of sister chromatid exchanges in interband regions might then indicate that these other processes follow a similar nonrandom distribution. Since interband regions are enriched for less condensed, early replicating chromatin (*1, 2*), these regions might be the sites of actively expressed genetic loci. Translocations occurring in these interband regions might then subdivide some of these loci, perhaps accounting for some of the pathology observed in patients with apparently reciprocal translocations.

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Chloride Spike: A Third Type of Action Potential in Tissue-Cultured Skeletal Muscle Cells from the Chick

Abstract. *In addition to sodium and calcium spikes, tissue-cultured skeletal muscle cells from the chick can initiate spikes lasting tens of seconds. The peak membrane potential of the spike correlates with the chloride ion concentration, but not with the calcium or sodium ion concentration. The chloride spike is blocked by manganese ion but not by cobalt ion.*

Besides the Na and Ca spikes (*1*), skeletal muscle cells from the chick in tissue culture can generate a spike lasting tens of seconds (*2*). This late spike (LS) has been considered a Ca spike because it is blocked by Mn^{2+} but not by tetrodotoxin (*2*). If the LS were a Ca spike, its size should depend on the Ca^{2+} concentration and Co^{2+} should inhibit it (*3*). These expectations, however, were not verified by the experiments reported here. On the other hand, changes in Cl^- concentration significantly altered the peak potential of the LS. Therefore, the LS may be a Cl spike, similar to that found in certain plant cells (*4*) and in fish electroplaques (*5*).

Monocellular layers of skeletal muscle cells (6 to 30 days old), which were 20 to 50 μm thick and 0.5 to 5 mm long (in the myotubular and striated stage), were obtained by culturing myoblasts dissociated from pectoral muscles of chick embryos (10 to 12 days old) in modified Eagle minimum essential medium (*6, 7*). Fibroblasts were usually eliminated by adding $10^{-5}M$ D-arabinofuranosylcytosine (Ara-C) to the medium (*6*), but there was no essential difference in the results obtained with cells not treated with Ara-C.

Intracellular recordings were obtained in the conventional way by using glass microelectrodes (20 to 60 megohms) filled with either 3*M* KCl or 3*M* potassium acetate solution. Usually a cell was impaled by two microelectrodes with a tip separation of less than 50 μm; one microelectrode was used for passing current and the other for recording membrane potential. The standard extracellular solution contained 128.4 m*M* NaCl, 5.0 m*M* KCl, 5.0 m*M* $CaCl_2$, and 15.0 m*M* tris-Cl or Na-HEPES buffer of *p*H 7.4 (Na-HEPES is the sodium salt of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). The ionic species and concentrations were changed as follows: Na^+ was replaced by tris or tetramethylammonium ion, NaCl by sucrose or urea, Cl^- by acetate, and $CaCl_2$ by NaCl, so as to maintain a constant osmolarity. The extracellular solution was changed within 3 minutes while the microelectrodes remained in the cell (*8*). Solution changes sometimes caused a shift in the reference potential (*9*). After an experiment involving a solution change, therefore, the microelectrodes were always withdrawn from the cell and the new resting, holding, and LS peak potentials were calculated on the basis of the new reference potential.

The resting potential of the muscle cells varied from − 35 to − 80 mv and appeared to correlate with cell length (*7, 10*). When the membrane potential was held around − 80 mv with steady hyperpolarizing current, a depolarizing current pulse could elicit a Na spike and a LS (Fig. 1A). This Na spike was eliminated in Na-free solution or in solution containing $10^{-5}M$ tetrodotoxin (*1*), but the LS remained in these solutions. In Na-free sucrose solution a depolarizing pulse elicited a small

spike 20 to 100 msec in duration (Fig. 1B). This spike was obliterated when 10 m*M* $CoCl_2$ was added to the solution (Fig. 1C), which suggests that it was a Ca spike (*11*). The LS, however, still persisted in the presence of Co^{2+} (Fig. 1C).

Like the Na and Ca spikes, the LS had the all-or-none characteristic (Fig. 1D). When membrane depolarization by a current pulse exceeded the threshold, the LS grew slowly, reached its peak in 1 to 5 seconds, and then decayed to the holding membrane potential (Fig. 1D). The duration of the LS was almost constant in a particular cell but varied between 10 seconds and 2 minutes from cell to cell. After a LS, the cell appeared to fall into a "refractory period" several times longer than the duration of the LS. The input resistance of the cell during the LS, measured by hyperpolarizing current pulses (Fig. 1E), decreased to less than one-tenth of that in the resting state. In addition, some LS's were followed by an "afterhyperpolarization" of 2 to 5 mv (arrow in Fig. 1D), which was associated with an increase of input resistance of about 10 percent. Occasionally LS's occurred spontaneously.

When Cl^- was continuously injected into the cell with a steady hyperpolarizing current through a KCl-filled microelectrode, the peak height of the LS slowly increased and reached an almost steady level in 10 to 20 minutes (Fig. 1F). In this steady state, the peak potential of the LS was not affected by the holding potential (Fig. 1G). This slow increase of the LS peak was not observed when acetate was injected into the cell (Fig. 1F). Since acetate ions do not pass through Cl-permeable membranes (*12*), this experiment suggests that the LS is mediated by an increase of membrane permeability to Cl^-.

When Cl^- in the extracellular solution was partially replaced by acetate ion, the peak potential of the LS shifted to a more depolarized level. These changes in the LS peak potential are plotted in Fig. 2A against the logarithm of the extracellular Cl^- concentration ($[Cl^-]_o$). The average slope (for six cells) of a tenfold decrease in $[Cl^-]_o$ is 43 mv, which is smaller than the 58 mv expected according to the Nernst equation for a Cl electrode. No significant change in the LS peak potential occurred with alteration of $[Ca^{2+}]_o$ (Fig. 2B) or of $[Na^+]_o$ (not illustrated). However, when $[Ca^{2+}]_o$ was increased to more than 40 m*M* (up to 90.6 m*M*), the LS often lost its regenerative characteristic. Addition of 2 m*M* $MnCl_2$ to the standard extracellular solution or the Na-free sucrose solution inhibited the LS (*2*). Addition of 10 m*M* $CoCl_2$ to these solutions did not affect the LS peak potential.

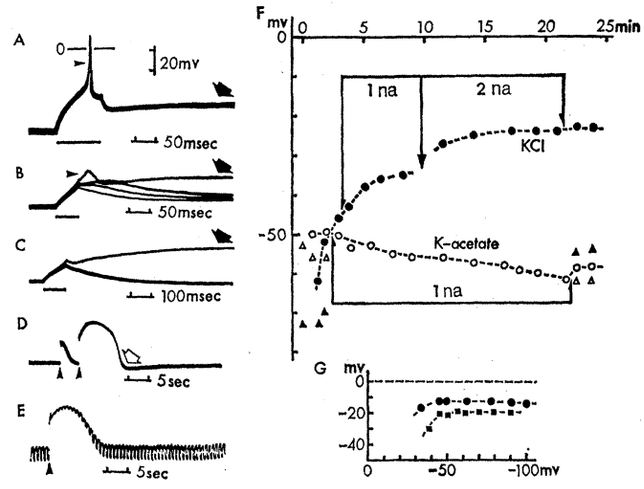

Fig. 1. A Na spike (wedge in A), a Ca spike (wedge in B), and LS's (arrows in A to C). The bars in (A) to (C) show the duration of the depolarizing pulses, and the wedges in (D) and (E) show depolarizing pulses 0.1 second in duration. (D) The cell was stimulated twice; the first stimulus was below the LS's threshold, but the second exceeded it. (E) Hyperpolarizing current pulses of 0.8 cycle per second and 0.2 second in duration were applied to the cell. (D and E) The extracellular solution was Na-free tris. (F) LS peak potential (●, ○) and the resting potential (▲, △). Steady hyperpolarizing currents were injected through microelectrodes filled with KCl (●, ▲) or potassium acetate (○, △). (G) Peak potentials of two LS's (ordinate) plotted against holding membrane potential (abscissa). (A to E and G) Microelectrodes filled with KCl were used. For further detail see text.

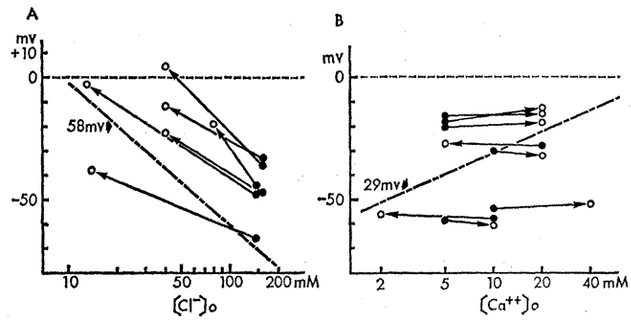

Fig. 2. Effect of (A) a decrease in $[Cl^-]_o$ and (B) a decrease in $[Ca^{2+}]_o$ on the peak membrane potential of LS's [(●) before and (○) after the concentration change]. Each change in the LS peak potential is indicated by an arrow. The slopes expected from the Nernst equation for such ionic species are (A) 58 mv and (B) 29 mv. Microelectrodes filled with KCl were used.

The difference between the observed slopes in Fig. 2A and the slope from the Nernst equation could be explained if Cl^- in the cell partially leaked out during the 2 to 3 minutes required for solution change (*13*). In fact, these muscle cells have a relatively high permeability to Cl^- in the resting state (*8*). Another possibility is that the permeability of the cell membrane to K^+ also increases during the LS.

The Cl spike found in plant cell membranes [*Nitella* and *Chara* (*4*)] has characteristics similar to the LS described in this report. Among animal cells, only electric organ cells (a muscle cell derivative) of some fish exhibit Cl spikes (*5*). Recently, however, potential-dependent movement of Cl^- has been reported in frog skeletal muscle cells (*14*) and in Purkinje cells of sheep hearts (*15*), which suggests even these matured muscle cells may retain an ability to generate the Cl spikes found in the primitive muscle cells.

Since the Na, Ca, and Cl spikes can be differentiated from one another by the procedures described above, the membrane of the muscle cells in tissue culture can be considered as a mosaic containing the three types of the excitable membranes.

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Choice in Free-Ranging Wild Pigeons

Abstract. *A flock of free-ranging wild pigeons were trained to peck at buttons which, when operated, allowed brief access to grain. Although only one bird at a time could have access to the buttons, the pecks of the group were treated as an aggregate. When they chose between two buttons, each of which could occasionally produce grain, the ratios of pecks at the buttons approximately equaled the ratios of the grain presentations obtained from them. This accords with a relation well substantiated in the laboratory, the matching law. It suggests that the matching law may apply to the behavior of higher organisms in natural environments.*

In a typical laboratory or wildlife situation, an animal generally can engage in any of several alternative activities. Since at any moment one alternative occurs to the exclusion of others, behavior generally implies choice. This does not mean that behaving organisms have free will, but that the laws of behavior must be laws of choice among alternatives.

A law of behavioral distribution that has been well established in the laboratory is the matching law

$$\frac{B_1}{B_2} = \frac{r_1}{r_2}$$

where B_1 and B_2 are the rates of engaging in two alternative activities, and r_1 and r_2 are the rates of reinforcement (for example, food presentation) obtained from the two alternatives. The ratio of choices matches the ratio of reinforcement (*1*). Originally found with pigeons obtaining food by pecking at two buttons, the law has been extended to more alternatives, other types of activities, rats and human beings, and other reinforcers (*2*, *3*).

The laboratory conditions that have produced these findings differ substantially from the natural situations of behaving organisms. Pigeons and rats in the laboratory are typically maintained at a fixed body weight, a certain percentage of that maintained by free feeding. The animals are exposed to the experimental situation for a small fraction of the day, and are never allowed to eat their fill. One may question, therefore, whether the matching law could apply to behavior outside these peculiar conditions.

An earlier study (*4*) provided a partial answer. Pigeons living continuously in the experiment, allowed to satiate and grow hungry again according to the needs of the body, distributed their choices between two sources of food according to the matching law.

A pigeon isolated from the world at large and others of its kind, however, remains in a highly artificial situation. In the experiment described here the inquiry was extended to wild pigeons in a natural habitat. It differed additionally from laboratory research in treating an aggregate of organisms, instead of isolated individuals.

The subjects belong to a flock of about 20 pigeons that live in a wooden-frame house in Cambridge, Massachusetts. An opening about 10 cm high and 1.5 m long allows them to freely enter and leave the attic of the house. The space in which they live, between the finished rooms, the roof, and the eaves, is roughly prismatic in shape, about 1.5 m at the high side, 1.5 m across, and 9 m long.

A version of the standard laboratory apparatus was placed in the living space, opposite the opening to the outside. The front panel contained three translucent buttons (response keys) and an opening through which a hopper full of mixed grain could be made available. Initially, a platform allowed the birds to stand in front of the panel at a height where they could reach the keys and grain hopper.

Preliminary training consisted of a modified form of autoshaping (*5*). The two side keys were covered, leaving only the center key available. At irregular intervals, averaging 1 minute, the key was transilluminated for 8 seconds, during which a peck at the key produced the reinforcer, access to grain for 4.5 seconds. If no peck occurred, the grain was presented at the end of the interval. The response key was dark during grain presentation. If a peck produced the grain, a new 8-second illumination of the key began as soon as the grain was withdrawn. This procedure continued for 6 days, until it appeared that the number of pecks and the amount of food eaten each day had reached a maximum. A perch wide enough (7 cm) to allow only one pigeon at a time access to keys and food replaced the platform. The schedule of grain presentation changed to one in which the key was lit continuously, except during grain presentation, and each peck produced grain. This continued for 3 days, and then the schedule became one which allowed grain presentation once every 30 seconds on the average, at variable intervals. This continued for 16 days, and then the center key was covered, the two side keys uncovered, and the first choice introduced.

Henceforth, pecks at each of the two keys produced grain on an independent schedule at variable time intervals. At first, each schedule allowed pecks to produce food at an average interval of 1 minute. In the succeeding choices, the average intervals for the left versus the right key, respectively, were 2 minutes versus 40 seconds, 2 minutes versus 30 seconds, and