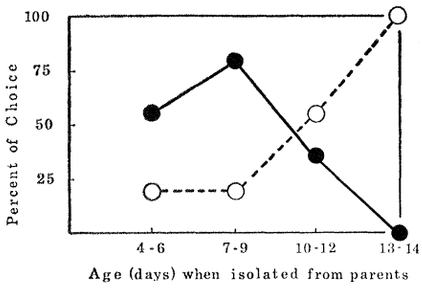

Fig. 2. Percent of choice by 25 ring doves in a free-choice situation between a human being (solid line) and a ring dove (dashed line) of the opposite sex. All birds were kept in visual isolation from other doves from the day they were removed from their parents until they were tested as adults.

tal bird would respond by either assuming the male role in copulation with the choice subjects, showing a decided preference for one, or by exhibiting all courtship behavior except actual copulation. At other times the bird would assume the female role with the appropriate female copulatory behavior patterns. In cases of no preference the choice objects were either ignored or passively avoided. Results for group AA are illustrated in Fig. 2. Only positive choices are included.

The birds in group AB all chose the ring dove. They were so inhibited by the presence of the person in the test cage that they would respond to doves only after the person had left the cage. In group BA two birds chose the human being, two the dove, one bird chose both, and one bird ignored both. Group BB behaved exactly as group AB.

Imprinting occurs in this species of ring dove and the optimum time for its effectiveness seems to be the removal of the young from their parents at 7 to 9 days of age. Squabs removed from their parents after the 12th day chose a dove in a free-choice situation. Only about 50 percent of the birds removed from the nest at days 4 to 6 chose the human being. One might expect that all birds removed before a certain age would choose human beings. The onset of fear lies between the 7th to the 10th day of life (Fig. 1), the period during which the optimum imprinting effectiveness to humans falls. The interaction with a new object, in this case the person who removes the bird from its parents and raises it by hand, may be more effective when this experience is accompanied by fear responses, whereas birds removed earlier have never shown fear to the human. The lack of emotional interaction results in fewer choices for the human being. When all birds are fearful of strange objects, after day 12 the fear is strong enough to prevent establishment of a bond with the human being that might be evident in the adult bird. This hypothesis appears tenable in view of results obtained in chicks by Kovach and Hess (*9*), who showed that more intense arousal, resulting from painful shocks which chicks were given in the presence of the imprinting model, resulted in increased following during and even before the critical period at about 14 to 16 hours.

The birds of group AB behaved similar to birds in group BB. The experience of being raised by hand for several weeks had no detectable lasting effect when these birds again lived with other doves for 7 to 8 months without close contact with human beings. This idea seems to be supported by the behavior of the three birds of group BA who chose the human being. The fact that not all doves in group BA chose the human being may be due to the biologically more adequate object—a dove—competing with such a vastly different one as the human being. This view seems to be borne out by the observation that even human-imprinted birds would nevertheless fight in a species-specific manner with other doves. The competition between biologically appropriate, versus inappropriate, objects may also explain why none of the 11 birds of group AB chose the human being. Another part of the explanation is that in this species, not only the early experience during an optimum period, but also continued experience throughout the bird's life has an effect on adult behavior. That experience around an optimum period between 7 to 9 days of age does have lasting effects is clearly demonstrated by the birds in group AA.

We conclude that imprinting in ring doves is reversible although in some birds its effects are never completely lost. Since both early and subsequent experiences affect the adult sexual behavior, it appears that during evolution two mechanisms evolved which result in pair formation. In nature both must be at work.

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Eel Electroplaques: Spike Electrogenesis without Potassium Activation

Abstract. *Measurements with the voltage clamp technique demonstrate that only an early conductance increase occurs during spike electrogenesis of eel electroplaques. The delayed increase which is characteristic of spike electrogenesis in many other cells is absent. Instead, the membrane resistance increases two- to threefold above its resting value. The brief initial increase in conductance is due to sodium activation followed by rapid sodium inactivation. The influx of sodium causes an inward current of up to 80 ma/cm². The inward current is abolished by eliminating the sodium from the normal medium (substitution of choline chloride for NaCl); by blocking sodium activation with tetrodotoxin; or by causing sodium inactivation through enrichment of the potassium in the medium. The delayed increase in membrane resistance is not affected by eliminating sodium influx, nor by substituting various impermeable anions for the chlorine of the normal medium. Thus, the increase in resistance signifies the occurrence of potassium inactivation which is unmasked by the absence of potassium activation.*

Spikes are produced in most electrically excitable cells by the same mechanism as that which accounts for the response of squid giant axons to electrical stimuli (*1*). An initial depolarizing electrogenic process, sodium activation, is terminated by a subsidiary voltage-dependent process, sodium in-

activation. Meanwhile, due to potassium activation, the growth of repolarizing electrogenesis speeds the return of the membrane potential toward its resting level. Thus, the membrane resistance is low during the falling phase of the spike (*2*).

Spike electrogenesis in electroplaques of *Electrophorus electricus* (electric eel) appears to differ from this more common mechanism. During the falling phase the resistance of the reactive membrane increases two- to threefold over the resting value (*3*). Thus, the potential of the spike would appear to decay passively. It was suggested (*4*) that the difference might be due to participation of a fourth process, potassium inactivation, which is formally analogous to the process of sodium inactivation. Depolarizing potassium inactivation is a fairly common response of electrically excitable membranes (*5*), but it is normally masked by the activation processes of spike electrogenesis. Since the inactivation seems to be particularly prominent in eel electroplaques, we have analyzed the components of the spike electrogenesis of these cells by means of the voltage clamp technique (*1, 6*). The analysis has demonstrated both the absence of potassium activation and the occurrence of potassium inactivation. It has also demonstrated that the inward current for the depolarizing electrogenesis is carried by sodium. Furthermore, the data show that the membrane components which are implicated in the processes of sodium and potassium conductance have distinctively different pharmacological properties.

The electrically excitable component of the reactive membrane of the electroplaques responds with spikes only to currents which flow rostro-caudally (*7*). The isolated electroplaques which were used in this study all could respond in this manner, and spikes up to 152 mv in amplitude were obtained (Fig. 1). Conventional voltage-clamp presentations were recorded oscillographically. The peak initial currents and the subsequent currents for different clamping voltages were plotted (Fig. 1). An inward flow of current was initiated when the reactive membrane was depolarized by about 30 mv, with peak currents occurring at a membrane potential of about −20 mv. The membrane conductance increased two- to fivefold above the resting value. The equilibrium potential, inside-positive by as much as 75 mv, was somewhat higher than the potential at the peak of the spike.

The phase of a subsequent ("delayed") increase in outward current which is prominent in squid giant axons (*1*) was absent in the electroplaques. Instead, the linearly increasing "leakage" current which alone remained after sodium inactivation had occurred, deviated from the ohmic relation for higher depolarizations. The deviation, which indicates an increase in the membrane resistance, signifies the occurrence of depolarizing potassium inactivation (*5*). The chord resistance increased two to four times above the resting value.

The initial inward flux could be abolished by replacing the NaCl of the standard medium with choline chloride, but the onset of potassium inactivation was not influenced by the absence of the sodium influx. The influx was also abolished by applying tetrodotoxin without affecting the process of potassium inactivation. Tetrodotoxin blocks specifically the sodium activation process of electrically excitable membranes (*8*) and eliminates spike electrogenesis in various cells (*8, 9*). Thus, tetrodotoxin does not block the processes of potassium inactivation or potassium activation (*8*). These findings support the view that the membrane sites at which the different ionic events occur are spatially separate (*5*), since they have distinctive pharmacological properties. The effects of choline chloride and tetrodotoxin could be produced by changing the solution outside only the innervated surface of the electroplaque.

The membrane of the electroplaques becomes temporarily inside-positive by as much as 70 mv during the peak of the spike, approximating very closely the equilibrium potential for sodium as determined from the voltage clamp data. In the absence of potassium activation repolarizing electrogenesis is absent. Thus the lack of potassium activation may represent an adaptation by means of which eel electroplaques can deliver a higher voltage to make the discharge of the electric organ more

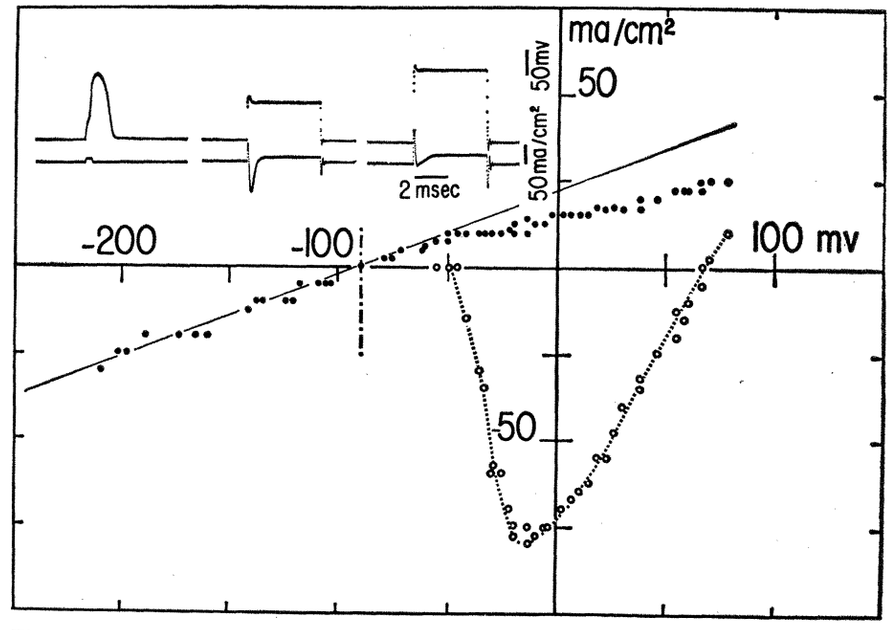

Fig. 1. Voltage-current relations of eel electroplaque under voltage-clamp conditions. D-Tubocurarine (50 μg/ml) was applied to the innervated surface. The resting potential (−90 mv) is shown by the broken line vertical to the abscissa. The cell responded with spikes like that shown in the left record in the upper part of the figure. The two records beside it are samples of the voltage clamp data. The center pair show the maximum inward current of this experiment (about 80 ma/cm²) during the early part of a depolarization by 75 mv, and lasting 4.5 msec, which raised the membrane potential to −15 mv. The other pair of records were for a voltage step which carried the membrane potential to +70 mv and abolished the initial inward current. Thus, E_{Na} was about +70 mv. The graph shows the data of the complete experiment. Open circles represent the peak initial current for the different values of the membrane potential. Filled circles show the steady-state current just before the end of the pulse. The current-voltage relation at this time was linear (ohmic) except for depolarizations greater than about 40 mv. When the voltage became more positive the current was smaller than predicted from the linear relation, signifying the occurrence of K-inactivation. A delayed increased conductance was not observed.

effective. The intracellular concentration of sodium may be estimated from the equilibrium potential as about 9 mmole/liter. From the measurements of the peak currents (80 ma/cm^2) the influx of sodium during a single spike is of the order of 0.01 mmole/liter. These calculations indicate that eel electroplaques may possess a very active "pump" mechanism for extruding sodium.

Despite the absence of an active repolarizing process the duration of the spike of eel electroplaques is only 2 to 3 msec (*7*). However, the time constant of the cell membrane is only about 75 μsec, as was also confirmed in the present work. Thus, passive decay of the membrane potential from the peak voltage of the spike should be rapid. Even if, due to potassium inactivation, the time constant increases three- or fourfold, the potential should decay to negligible values in about 1 msec after depolarizing electrogenesis is terminated by sodium inactivation. This is confirmed by the voltage-clamp data, since the phase of inward current lasted only 1 msec or less (Fig. 1).

An earlier finding on eel electroplaques (*7*), which seemed to be anomalous in the light of data on other cells, is accounted for by the coupled occurrence of potassium inactivation and the absence of potassium activation. When neural stimuli generate depolarizing postsynaptic potentials during the falling phase of a directly elicited spike the two depolarizations sum. The summation results because the falling phase of the spike represents a passive decay of the membrane potential.

Depolarizing inactivation has been observed in crayfish (*10*) as well as frog (*11*) skeletal muscle fibers and in cardiac muscle (*12*). It has been analyzed with voltage clamp techniques in electroplaques of several species of the weakly electric Gymnotid relatives of the electric eel (*13*) and in supramedullary neurons of the puffer (*14*). Eel electroplaques, to our knowledge, present the first example in which the depolarization of the electrically excitable spike-generating membrane does not cause the repolarizing electrogenesis and delayed rectification which are attributable to potassium activation.

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Activating and Synchronizing Centers in Cat Brain: Electroencephalograms after Lesions

Abstract. *Electroencephalographic changes occur after small unilateral lesions have been made in the pontine tegmentum of cats with permanently implanted electrodes. Lesions in the area of the nucleus reticularis pontis oralis produce electroencephalographic synchronization (sleep pattern). Lesions in the pontine and caudal midbrain tegmentum, dorsal, lateral, and caudal to the lesions producing synchronization, produced electroencephalographic activation (waking pattern).*

The states of sleep and wakefulness in adult animals are generally expressed in the electroencephalogram (EEG) by synchronization (high-voltage, slow waves) and activation (low-voltage, fast waves), respectively (*1*). An exception is the activated EEG of paradoxical sleep. Within the limits of the pons there are structures that produce synchronization or activation when destroyed (*2*). We have sought to determine more precisely the location and limits of these structures.

We used 30 cats under general anesthesia. Silver-ball electrodes were implanted on the dura of both sides of the brain over the motor, auditory, and visual cortex to record electrical activity and stereotactically oriented steel electrodes were implanted to produce diathermocoagulation. Ten days later EEG control records were obtained from the free unanesthetized animals in an electrically shielded box. Since synchronization and activation periods vary considerably in adult cats, the control records were of long duration. The records, taken periodically for a month after the localized lesions had been made, were of the same duration as the control records and were made under the same conditions.

Quantitative analysis of synchronization and activation periods in the records of animals with lesions, relative to the amount of synchronization and activation found in control records, allowed us to differentiate between the lesions that produce synchronization and those that produce activation. The unilateral lesions in the pontine tegmentum were 2 to 3 mm in diameter. Figure 1 (*A*, *C*, *D*, and *E*) shows the combined areas where lesions gave rise to the two types of EEG activity.

The degree of activation in the control records (before the lesions were made) in all the animals of each group was considered to be zero (Fig. 1*B*). The absolute values of zero were different for the two groups of animals. The curves represent the percentage of activation (positive values) or synchronization (negative values) as deviations from the control level of activation.

Lesions in the reticular formation of the pons (nucleus reticularis pontis ora-