ranks in the hierarchy in each of the three troops were occupied by the very young or else by old females. In general, very young animals were ignored by high-ranking females, while old females avoided interactions with troopmates. In 478 waking minutes that female "M," the oldest female in B-5 troop, was the object of focal-animal sampling during the 1975 study period, she approached another animal only once. She, in turn, was approached on 21 occasions and was displaced on 19 of these. In all three troops, old females tended to move and forage apart from other troop members and occasionally spent periods of several hours, or (as in the case of Sol in 1974) whole days away from the troop. Similar peripheralization of old females was mentioned in the initial langur study by Jay (8). One explanation for the solitary tendencies of old females is the interference they suffer from other females who routinely displace them and who occasionally attempt to take food from their mouths. In contrast to the "timidity" of old females, younger females frequently approached and initiated interactions with other troops members, and almost never left the troop.

Despite their apparent disinclination to compete and their disadvantaged position within the troop, old females participated vigorously in aggressive encounters with other troops, in defending the troop from dogs and harassment by humans, and in protecting troop infants from assaults by infanticidal males. In at least seven assaults on a B-6 infant by an adult male, the two oldest and lowest ranking females in the troop audaciously and repeatedly intervened to defend the infant. The infant's own (young adult) mother played almost no active role in defense of her offspring (6) (cover). Similarly, when a B-3 subadult kidnapped an infant from a neighboring troop, the two oldest among the B-3 females came forward to meet the mother from the other troop and prevented her from retrieving her infant (14). Because of their low reproductive value, such old females have less to lose from risk-taking, in terms of future reproduction, than do younger animals. Of four old females at Abu, one was observed with an infant once during 5 years for which she was identified, two were seen with an infant once each in 4 years of observation, while the fourth (Sol) was never seen with an infant in any of four study periods up until her death. In contrast, young and middle-aged females produced infants roughly once every 2 years. This pattern appears to be due to differences in fecundity, not to differences in mortality; old, middle-aged, and young mothers lost 33 percent of 3 births, 29 percent of 21, and 38 percent of 16, respectively (15).

One explanation for a ranking system that favors youthful females, combined with a defense system in which old females take greater risks, is provided by Hamilton's theory of inclusive fitness (16). A langur near the end of her reproductive career has low reproductive value and may stand to gain more in terms of genetic representation in future generations by "altruistically" investing in close matrilineal relatives than by pursuing an egoistic course. By contrast, young females entering their reproductive prime may stand to gain in fitness by outcompeting female relatives for available resources and by selfishly leaving troop defense to other animals.

SARAH BLAFFER HRDY
DANIEL B. HRDY

Department of Anthropology,
Harvard University,
Cambridge, Massachusetts 02138

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Acetylcholine Hot Spots: Development on Myotubes Cultured from Aneural Limb Buds

Abstract. *The hypothesis that neural induction plays a role in the development of acetylcholine hot spots (high-sensitivity regions) was tested by electrophysiological mapping of the distribution of acetylcholine sensitivity of myotubes derived from aneural hindlimb buds of chick embryos. Hot spots were found. Therefore, hot spot development is not dependent on prior contact with nerve processes.*

A current problem in developmental biology is that of identifying the interactions between developing nerve and muscle which are important in synapse formation. During development, embryonic skeletal muscle fibers are sensitive to acetylcholine (ACh) everywhere on their surfaces (1). After innervation occurs, the extrasynaptic sensitivity disappears, and ACh receptors in the adult are confined largely to the synaptic region (1, 2). Embryonic muscle fibers grown in vitro are also sensitive everywhere to ACh (3, 4). The excellent visual resolution possible with monolayer cultures permits detailed iontophoretic mapping of ACh sensitivity. Although the fibers in such cultures are sensitive to ACh over their entire surface, receptor distribution is not uniform. Rather, small discrete patches of elevated ACh sensitivity, called hot spots, appear. These are about 10 μm in diameter, are scattered at intervals of several hundred micrometers along the length of a fiber, and have a sensitivity about five times higher than background (5–7).

The functional significance of the ACh hot spots is unknown. Since they occur on uninnervated myotubes in primary

Table 1. Data from myotubes with ACh hot spots in 6- to 7-day cultures. Sensitivities are given as mean ± standard error of mean; *n*, number of repeat observations.

Stage at explanting	Resting potential (mv)	Background		Hot spots		Ratio of hot spots to background
		ACh sensitivity (mv/ncoul)	Points (No.)	ACh sensitivity (mv/ncoul)	*n*	
18	80	184 ± 38	18	927 ± 74	13	5.0
22	58	208 ± 25	31	868 ± 20	16	4.2
24	70	178 ± 15	45	743 ± 73	5	4.2
24	60	168 ± 14	41	1072 ± 92	5	6.4
24	70	328 ± 19	84	1132 ± 129	5	3.5
18*	30	94 ± 10	20	385 ± 41	8	4.1
24*	40	114 ± 27	17	792 ± 95	8	6.9
24*	45	209 ± 17	47	1157 ± 107	9	5.5
24*	50	240 ± 27	34	1401 ± 142	9	5.8
24*	50	163 ± 17	11	642 ± 55	5	3.9

*Lumbosacral spinal cord extirpated at stages 15 to 16.

cultures, they might serve as preformed target sites for ingrowing motoneurons (*5*, *8*). On the other hand, hot spots do not appear in clonal lines of muscle cells unless the muscle is cocultured with neurons (*7*). Therefore, Harris and co-workers (*7*) suggested that ACh hot spots are induced on cloned muscle fibers by nerve terminals. Moreover, Harris (*9*) suggested that the presence of hot spots on uninnervated muscle fibers in primary cultures does not rule out the neural induction hypothesis. This is because most muscle for primary cultures has been obtained from 10- to 11-day chick embryos, in which motoneuron axons have already been in contact with the developing muscle for several days. It is possible that these nerves provide a message required for hot spot formation and that hot spot appearance later in culture reflects this early in vivo nerve-muscle interaction.

To determine whether or not early in vivo contact with nerve processes is necessary for the formation of hot spots, we measured the ACh sensitivity of myotubes derived from aneural hindlimb buds of chick embryos (*10*). It was crucial in these experiments that the muscle be removed from the embryo before it was contacted by motoneuron axons. Chick embryo hindlimb buds begin to form at stage 16 [51 to 56 hours (*11*)], and a few of the most anterior lumbosacral motoneurons complete their final mitoses at stage 17 [52 to 64 hours (*12*)]. However, light microscopic studies of silver-stained sections (*13*) indicate that nerve processes do not reach the hindlimb until stages 24 to 25 (4.5 to 5 days). Thus, in the current experiments, the mesodermal cores of hindlimb buds were removed from embryos between stages 18 and 24. Explant cultures were prepared and maintained essentially according to the method of Dienstman *et al.* (*14*). Cells grew radially from the explants, and small clusters of bare myotubes formed near the edges of the explants.

On the basis of the morphological data cited above (*13*), we considered it unlikely that any nerve processes had reached the developing muscle at the time of explanting. Nevertheless, to ensure that the limb buds were completely aneural, we conducted a second set of experiments in which lumbosacral spinal cord segments 21 to 30 were surgically extirpated at stages 15 to 16. This operation completely removed the motoneurons destined to innervate the hindlimb buds (*15*). The embryos were incubated for 24 to 48 hours after the operation to allow limb buds to develop, and then explant cultures were prepared as described above. Individual myotubes in the two groups of cultures were morphologically indistinguishable. However, fewer myotubes were present in the second group of cultures, and they were more easily damaged by microelectrode impalement than were those in the first group.

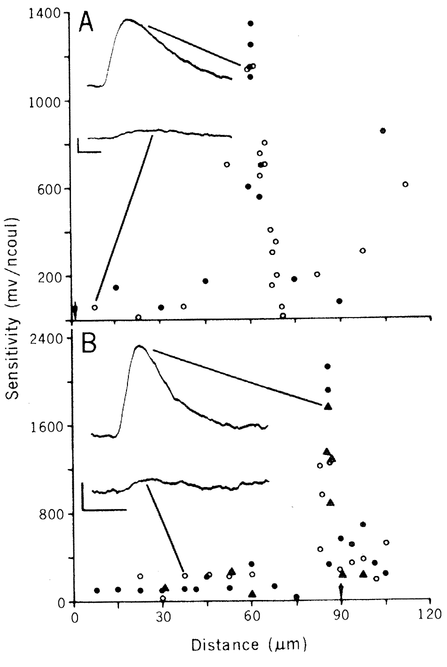

Fig. 1. Acetylcholine sensitivity plotted against distance along the fiber for two myotubes cultured from aneural hindlimb buds of chick embryos. Each point represents one electrode placement. Arrows on abscissas indicate recording electrode positions. Representative ACh potentials are shown as insets. (A) The myotube was cultured from a stage 18 embryo (ACh pulses: 5 na, 4 msec). (B) The myotube was cultured from a stage 24 embryo from which the lumbosacral spinal cord had been extirpated at stage 16 (ACh pulses: 3 na, 3 msec). Closed circles, first map; open circles, second map; triangles, third map; calibration for ACh potential traces, 5 mv and 10 msec.

Electrophysiological experiments were performed on 4- to 7-day cultures. Myotubes were observed at × 780 magnification with Nomarski optics. Standard intracellular recording and ACh iontophoretic techniques were used (*16*). A 100- to 150-μm segment of each myotube was mapped at least twice with identical ACh pulses, at intervals of 10 μm or less. Sensitivities were calculated as millivolts of depolarization per nanocoulomb of charge passed through the ACh electrode. The criteria for identification of a hot spot were (i) that the spot be at least three times more sensitive to ACh than the surrounding muscle surface (background), (ii) that no detectable response be observed after reversal of the current polarity, and (iii) that the measured sensitivity be reproducible after mapping other regions of the fiber.

In 4- to 5-day cultures no hot spots were found, although "warm" areas (1.8 to 2.6 times higher than background) were detected in three of the eight fibers studied. However, by 6 to 7 days in culture, a hot spot was clearly detected in the mapped segment of ten of the 22 fibers studied, independent of the stage of explanting and independent of whether or not the spinal cord had been extirpated. Figure 1 shows data from two fibers in which hot spots were detected, a myotube in an explant from a stage 18 embryo (Fig. 1A) and a myotube in an explant from a stage 24 embryo from which the lumbosacral cord had been extirpated at stage 16 (Fig. 1B). Data from all ten fibers on which hot spots were found are summarized in Table 1.

The results of the present experiments are similar to those obtained with dissociated 10- to 11-day embryonic chick muscle (*5*, *6*). These data differ, however, from those obtained with a clonal muscle cell line derived from primary muscle cell cultures exposed to a carcinogen, in which ACh hot spots do not appear unless the muscle is cocultured with neurons (*7*). Other differences in membrane properties, such as resting potential and specific membrane resistance, have also been noted when primary cultures of muscle cells are compared to the clonal cell line (*17*). These differences may reflect a departure from normal development in the clonal cell line. In conclusion, our data demonstrate that in primary cultures of chick myotubes, ACh hot spots can form on muscle fibers that have never been contacted by nerve processes. This suggests that neural induction of ACh hot spots may not be an essential step in neuromuscular synapse formation.

ANNE BEKOFF,* WILLIAM J. BETZ
Physiology Department, University of Colorado Medical School, Denver 80220

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* Present address: Department of Environmental Population, and Organismic Biology, University of Colorado, Boulder 80309.

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Evolving Strategic Arms and the Technologist

Gustavson (*1*) does not go far enough in his descriptive example of strategies in a nuclear battle. Carrying the example to its conclusion suggests a simple model that leads to some interesting observations regarding disarmament.

The results discussed below indicate that participants in disarmament talks, such as the SALT talks, should concentrate not only on reduction or equalization in total reentry bodies, but that they also direct their attention to the multiplicative MIRV factors that tend to destabilize the system, to the effectiveness of their missiles, and to the perceived quality of information.

The model shows that escalation is more likely (or occurs at a faster rate) if the perceived quality of information is lower, if P, the single kill probability of a reentry body, is higher, or if the number of reentry bodies per missile is higher. Since the interchange associated with SALT would tend to improve the perceived quality of information, it is encouraging to find that this will act as a damper on escalation. It is not clear whether SALT will have the desired effect on P or the MIRV factors.

Although P can be reduced in several ways, its reduction is unlikely. Reducing P of one's own missiles is antithesis to the technologist, and it is dangerous as a unilateral policy. Reducing P of the opponent's missiles can be accomplished with ABM's or through the hardening of silos. Curiously, both of these alternatives have been precluded to a large extent by SALT.

Gustavson's example purports to show that the dynamic analysis he proposes will yield results different from and more realistic than the more traditional analysis which compares static capabilities. Whereas such a traditional analysis may show that the side (A) with the first strike in a nuclear battle has the advantage, Gustavson's dynamic analysis shows that the other side (B) can gain the advantage if it is able to determine which of its silos are threatened and launch those ICBM's before they are destroyed.

However, Gustavson permits each side only one strike. Since A does not use all of its missiles in the first strike, some of its remaining missiles will be threatened by B's retaliation, and the same logic that caused B to retaliate with threatened missiles will now cause A to launch those missiles threatened by B's retaliation.

The example is carried to its completion in Table 1. As in Gustavson's example, the table is based on a single-kill probability of .8, a perceived quality of information of 8/9, and an assumption that missiles cannot be retargeted.

In Gustavson's scenario, the second strike is unanswered by A, and B ends up with the advantage because its missiles destroy most of A's remaining force. In my extension of the example, the exchange is terminated when one side runs out of missiles, and here A has the final advantage from at least two points of view.

By "street fighting" standards, A is the winner, having delivered both the first and the last punches, although the last punch is somewhat meaningless since it was initiated after all target silos were empty.

The final tally also favors A, with 17 remaining missiles, although neither side destroyed any primary targets (missiles). In terms of secondary targets (silos), A comes out ahead, retaining an expected 40 of its silos unscathed. B retains only an expected 1.15 of a silo. Note that A's advantage in remaining missiles and silos (given that both sides must initially have the same number of reentry bodies) is due to its lower MIRV factor, giving it more silos. A's advantage does *not* result from the fact that it executed the first strike.

Below is presented a simple model that incorporates the dynamics of

Table 1. Missile exchange carried to completion.

Strike	Initial status			
	Missiles		Reentry bodies	
	A: 1000	B: 500	A: 2000	B: 2000
1 Used by A	250		500	
2 Launched by B in return to strike 1		450		1800
3 Launched by A in return to strike 2	729		1458	
4 Launched by B in return to strike 3		50		200
5 Launched by A in return to strike 4	4		8	
Remaining	17	0	34	0