of time since reactivation is not artifactual but reflects a time-locked retrieval process initiated when retrieval cues are noticed during reactivation treatment.

These data raise questions regarding the nature of the retrieval process. Is it a step function or a continuous function? Three aspects of our findings favor the latter: (i) the distribution of individual retention ratios after different delays at each test point (Fig. 1) is rectangular, not bimodal; (ii) progressively higher ratios were attained by infants tested after successively longer delays; and (iii) infants who slept longer during the 8-hour delay had higher retention ratios.

A second question concerns the basis of the reminiscence phenomenon displayed by infants tested after lengthier intervals whose retention ratios exceeded 1.00. We have observed a similar phenomenon after original training: the mean retention ratio peaked at a value greater than 1.00 after a 48-hour delay and significantly exceeded the ratio obtained after only a 24-hour delay (*9*). This phenomenon suggests that the same retrieval process underlies the expression of older (2-week) and younger (2-day) memories.

Third, we must ask what the infants are remembering. Do infants with higher retention ratios remember more about an event (that is, have richer memories)? If attributes are forgotten at different rates, then memories retrieved after different intervals may contain different kinds of attributes (*11*).

Finally, the findings raise questions regarding whether a common, reversible process underlies the actions of reminders and certain amnestic agents that induce progressive forgetting over periods of minutes (for example, electroconvulsive shock) or hours (for example, antimetabolite injection) (*12*).

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Reference and Notes

1. R. Brown and D. McNeill, *J. Verb. Learn.* **5**, 325 (1966).
2. W. K. Estes, *Am. Sci.* **68**, 62 (1980); N. E. Spear, *The Processing of Memories: Forgetting and Retention* (Erlbaum, Hillsdale, N.J., 1978).
3. N. E. Spear, *Psychol. Rev.* **80**, 163 (1973); B. J. Underwood, *ibid.* **76**, 559 (1969).
4. C. K. Rovee-Collier and J. W. Fagen, in *Advances in Infancy Research*, L. P. Lipsitt, Ed. (Ablex, Norwood, N.J., 1981), vol. 1, p. 225.
5. _____, M. J. Gekoski, *Adv. Child Dev. Behav.* **13**, 195 (1979).
6. J. M. Davis and C. K. Rovee-Collier, *Dev. Psychol.* **29**, 353 (1983); C. K. Rovee-Collier *et al.*, *Science* **208**, 1159 (1980).
7. N. E. Spear, in (*3*); _____ and P. J. Parsons, in *Processes of Animal Memory*, D. L. Medin, W. A. Roberts, R. T. Davis, Eds. (Erlbaum, Hillsdale, N.J., 1976), p. 135.
8. J. Gaito, *Psychol. Bull.* **63**, 125 (1965).
9. C. K. Rovee-Collier *et al.*, *Infant Behav. Dev.* **4**, 341 (1981).
10. Alternatively, if sleep also makes stored memory attributes more accessible, it may sum with the reactivation treatment [N. E. Spear and W. C. Gordon, in *Sleep, Dreams, and Memory*, W. Fishbein, Ed. (Spectrum, New York, 1981), p. 183].
11. L. Hasher and M. Griffin, *J. Exp. Psychol.: Hum. Learn. Mem.* **4**, 318 (1978); C. K. Rovee-Collier and M. W. Sullivan, *ibid.* **6**, 789 (1980).
12. B. W. Agranoff, in *Animal Memory*, W. K. Honig and P. H. R. James, Eds. (Academic Press, New York, 1971), p. 243; A. Geller and M. E. Jarvik, *Psychonom. Sci.* **12**, 169 (1968); R. R. Miller and A. D. Springer, *Physiol. Behav.* **8**, 229 (1972).
13. Supported by NIMH grant 32307 to C.R.C. We thank B. A. Campbell, G. Collier, M. R. D'Amato, M. Enright, and A. Glass for critical comments, and M. Enright, M. Hoffman, and S. Thompson for assisting with the data collection.
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5 July 1983; accepted 6 October 1983

How a Nerve Fiber Repairs Its Cut End: Involvement of Phospholipase A_2

Abstract. *Following transection of a giant axon, the nerve membrane at the cut end is resealed within 5 to 30 minutes. This membrane resealing process is highly dependent upon temperature and extracellular calcium ions. The membrane resealing is triggered by excess calcium entering the axoplasm at the site of transection but is prevented by the application of phospholipase A_2 inhibitors. We propose that calcium activated phospholipase A_2 plays a central role in resealing of the ruptured nerve membrane.*

Following transection of a nerve fiber, the proximal cut end regenerates within days to weeks. Before outgrowth and elongation of the proximal cut end the axonal membrane once ruptured must be repaired. It is not known how the cut end of a nerve fiber is resealed or how fast the resealing may occur. As early as 1877, Engelmann showed that the loss of the membrane potential at the injured site of a nerve (demarcation potential) slowly recovers within a few hours (*1*). However, no systematic studies have as yet been made on the membrane resealing process of injured nerve fibers. We have examined the membrane resealing process following transection of the cockroach giant axon, using recovery of changes in the membrane potential and input resistance as criteria. The membrane resealing was found to occur within 5 to 30 minutes after the transection. Our results suggest that the membrane resealing is induced by phospholipase A_2 which is activated by calcium entering at the site of transection.

The cell bodies of the ventral giant axons of the cockroach, *Periplaneta americana*, are located in the sixth abdominal (A_6) ganglion (*2*), and the axons project centripetally through the thoracic ganglia without synaptic interruption. The entire nerve cords and attached ganglia from the first (A_1) through A_6 abdominal segments were excised and placed in a chamber filled with the standard insect saline (*3*). A single giant axon was isolated between the A_4 and A_5 ganglia, leaving its rostral and caudal connections intact. Two microelectrodes were inserted into the isolated axon. While intracellular potentials were recorded from one electrode, the other electrode was used to apply current pulses for measuring the input resistance. The axon was then acutely severed at a point approximately 0.5 to 1.0 mm from the recording site. In order to avoid possible dislodgment of the microelectrodes, the axon was firmly pressed to the bottom of the recording chamber with a broken electrode immediately before cutting. As shown in Fig. 1A, a substantial depolarization was observed at the recording site following the transection. This was accompanied by a drastic decrease in the input resistance, as indicated by potential changes induced by brief hyperpolarizing current pulses applied every 1 minute (Fig. 1A). About 10 minutes later both the resting potential and input resistance recovered to near-normal levels. This behavior is essentially the same as self-resealing of injured cardiac muscle membranes (*4*). The recovery time varied from 5 to 30 minutes in different axons examined at 23° to 27°C. The membrane resealing occurred in the proximal end of the cut distal (terminal side) segment as well as in the distal end of the cut proximal (soma side) segment. Apparently, the resealing that follows immediately after axonal injury is a local event of the nerve membrane and does not depend on the soma (*5*).

The membrane resealing was found to be highly dependent on temperature. Every axon examined above 22°C showed resealing following transection, no resealing occurred below 13°C. This range of temperatures roughly agrees with the phase transition temperatures of membrane lipids (*6*). Because lipids above

their transition temperatures are more susceptible to fusion, these results suggest that resealing of ruptured membrane is achieved by fusion of lipids forming the membrane.

Our standard insect saline contained 9 m*M* Ca^{2+}. When Ca^{2+} was replaced with equimolar Mg^{2+} or Sr^{2+} or Mn^{2+}, the axon failed to reseal the cut end (Fig. 2A). The minimum extracellular Ca^{2+} concentration required for successful membrane resealing was 0.1 to 0.5 m*M*. It is possible that Ca^{2+} might act on the microfilaments or microtubules which, in turn, may be involved in the membrane resealing process. To test this possibility, the axon was exposed to cytochalasin B (5 μg/ml) or cytochalasin D (10 μg/ml) or colchicine (25 μ*M*) for 60 minutes before transection. These treatments, which impair cytoskeletal systems, did not prevent membrane resealing.

The absolute requirement of relatively high concentrations of Ca^{2+} also suggests the possible involvement of Ca-activated phospholipase A_2 (*7*) in the membrane resealing process. This possibility was tested by the application of a phospholipase A_2 inhibitor, *p*-bromophenacyl bromide (*8*). When the inhibitor (10 μ*M*) was applied to the recording chamber about 30 minutes before transection, the resting potential and input resistance were unaffected, but the axon failed to reseal the cut end in the presence of 9 m*M* Ca^{2+} at 23° to 27°C. Another phospholipase A_2 inhibitor, mepacrine (*9*), also prevented membrane resealing (Fig. 2B). The two inhibitors exerted their effects in a dose-dependent fashion (*10*).

Since phospholipase A_2 is presumably embedded in the inner layer of the membrane (*7*), it is likely that its activation requires Ca^{2+} entry into the axoplasm. To test this possibility, EGTA was ionophoretically injected into the axon near the cut end while the axon was transected in the presence of 9 m*M* Ca^{2+}. Under such conditions, an increase in the intraaxonal Ca concentration near the cut end would be prevented. As shown in Fig. 1B, the EGTA-treated axons failed to show membrane resealing. When the amount of EGTA injected was reduced by decreasing the frequency of ionophoretic current pulses, the axon showed successful membrane resealing. Thus, the dependence of membrane resealing on extracellular Ca^{2+} and its prevention by the internal EGTA injection suggest that the site of action of Ca^{2+} is the inside of the axon.

Phospholipase A_2 catalyzes the hydrolysis of certain membrane phospholipids to arachidonate and lysolecithin. Since lysolecithin is a fusogen (*11*), the local formation of lysolecithin may facilitate fusion of the ruptured nerve membrane. The phospholipase action increases above the phase transition temperatures of the membrane lipids (*12*). Therefore, the temperature dependence of the membrane resealing process might be due to that of the phospholipase action. Alternatively, the fluid state of the membrane lipids above the transition temperature may be indispensable for initiating membrane resealing (*13*). Whatever the underlying mechanism, our results strongly suggest that activation of phospholipase A_2 plays a central role in resealing of the ruptured nerve membrane.

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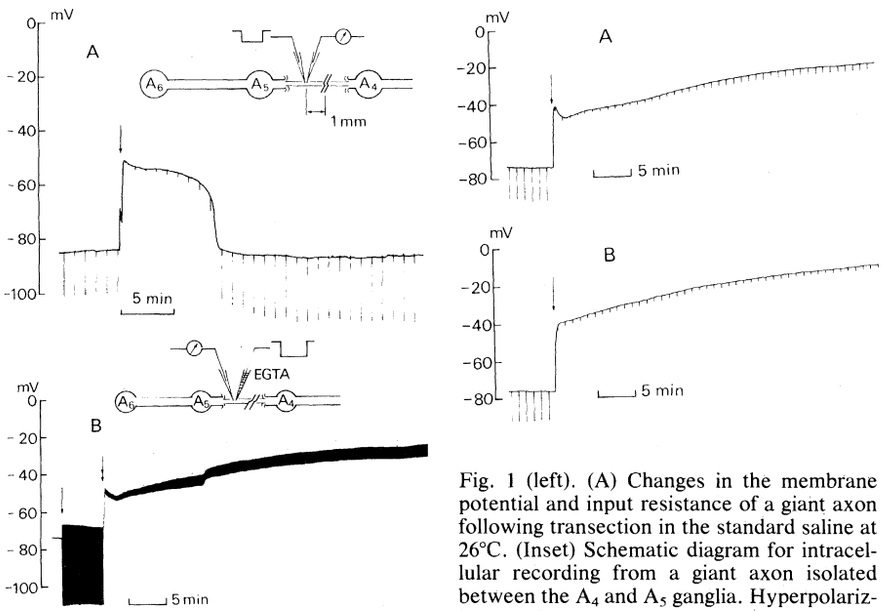

Fig. 1 (left). (A) Changes in the membrane potential and input resistance of a giant axon following transection in the standard saline at 26°C. (Inset) Schematic diagram for intracellular recording from a giant axon isolated between the A_4 and A_5 ganglia. Hyperpolarizing current pulses (10 nA, 120 msec) were applied at 1 per minute through another electrode inserted within 30 μm of the recording electrode for measuring the input resistance. The time of the transection is indicated by the arrow. Vertical lines on the record are the potential changes produced by repeatedly applied 10 nA current pulses. (B) Failure of membrane resealing in an EGTA-treated axon. The transection was made at 26.5°C during perfusion with the standard saline. EGTA was ionophoretically applied through a micropipette filled with 1*M* EGTA. The ionophoretic current pulses (25 nA, 250 msec) were applied at two per second. Single arrow, the onset of the EGTA application. Double arrow, the time of axonal transection. Fig. 2 (right). (A) Failure of membrane resealing of a giant axon which was transected at 25°C in a solution containing 9 m*M* Sr^{2+}, instead of 9 m*M* Ca^{2+}. The axon was perfused with the Sr solution 25 minutes before the transection. The axon showed no recovery in the membrane potential and input resistance for at least 2 hours after the transection. (B) Failure of resealing of a giant axon which was transected at 26°C in the presence of mepacrine (2 m*M*) dissolved in the standard saline. The axon was exposed to mepacrine 25 minutes before transection. No recovery of the membrane potential and input resistance was seen for at least 2 hours after the transection. The transection time is indicated by arrows in (A) and (B).

References and Notes

1. T. W. Engelmann, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **15**, 116 (1877).
2. M. E. Spira, I. Parnas, F. Bergmann, *J. Exp. Biol.* **50**, 629 (1969); C. L. Harris and T. Smyth, *Comp. Biochem. Physiol.* **40**, 295 (1971); D. L. Daley, N. Vardi, B. Appignani, J. M. Camhi, *J. Comp. Neurol.* **196**, 41 (1981).
3. G. A. Kerkut, R. M. Pitman, R. J. Walker, *Comp. Biochem. Physiol.* **31**, 611 (1969). Our solution contained (in micromolars): NaCl, 200; KCl, 3.1; $CaCl_2$, 9; $NaHCO_3$, 1; glucose, 16.7; tris, 5; 1,4-piperazinediethanesulfonic acid, 5; the *p*H was adjusted by NaOH to 7.20 to 7.25 at 5° to 25°C.
4. J. Délèze, *J. Physiol. (London)* **208**, 547 (1970). Decreased input resistance might be due in part to delayed rectification by depolarization. In a few experiments, intracellular recordings were made at two points to measure changes in the length constant for the cable analysis described by Délèze. The observed changes in the input resistance agreed with those predicted from a cable terminated by a short circuit immediately after the transection and from a cable terminated by an infinite resistance after the recovery of the membrane potential.
5. H. Meiri, A. Dormann, M. E. Spira, *Brain Res.* **263**, 1 (1983). In separate experiments, the nerve cords were transected in situ, and 20 to 50 hours later intracellular potentials were recorded from the previously sectioned axon. The resting and action potentials observed 200 μm from the cut end were normal, indicating that membrane resealing is not a transient phenomenon.
6. E. J. Shischick and H. M. McConnell, *Biochemistry* **12**, 2351 (1973); D. Chapman, *Q. Rev. Biophys.* **8**, 185 (1975).
7. H. Van den Bosch, *Biochim. Biophys. Acta* **604**, 191 (1980). Phospholipase A_2 in the nervous system has been described by H. Woelk, G. Goracci, A. Gaiti, and G. Porcellati [*Hoppe-Seyler's Z. Physiol. Chem.* **354**, 729 (1973)] and G. R. Webster [*J. Neurochem.* **21**, 873 (1973)].
8. J. J. Volwerk, W. A. Pieterson, G. H. de Haas, *Biochemistry* **13**, 1446 (1974); M. Gan-Elepano and J. F. Mead, *Biochem. Biophys. Res. Commun.* **83**, 247 (1978).
9. H. B. Markus and E. G. Ball, *Biochim. Biophys. Acta* **187**, 486 (1969); B. B. Vargaftig and N. Dao

Hai, *J. Pharm. Pharmacol.* **24**, 159 (1972).
10. The doses of *p*-bromophenacyl bromide and mepacrine examined were 1 to 20 μ*M* and 0.1 to 5 m*M*, respectively; the doses at which 50 percent of the axons tested failed to reseal were 2 to 5 μ*M* in the former inhibitor and 1 m*M* in the latter.
11. A. R. Poole, J. I. Howell, J. A. Lucy, *Nature (London)* **227**, 810 (1970).
12. J. C. Wilschut, J. Regts, H. Westenberg, G. Scherphof, *Biochim. Biophys. Acta* **508**, 185 (1978).
13. D. Papahadjopoulos, G. Poste, B. E. Schaeffer, *ibid.* **323**, 23 (1973).
14. We thank Dr. Yasunobu Okada for helpful discussion. This study was supported by research grant (564-40-026) from the Ministry of Education, Science and Culture of Japan.

26 September 1983; accepted 8 November 1983

The Thymus-Adrenal Connection: Thymosin Has Corticotropin-Releasing Activity in Primates

Abstract. *Endotoxin-free thymosin fraction 5 elevated corticotropin, β-endorphin, and cortisol in a dose- and time-dependent fashion when administered intravenously to prepubertal cynomolgus monkeys. Two synthetic component peptides of thymosin fraction 5 had no acute effects on pituitary function, suggesting that some other peptides in thymosin fraction 5 were responsible for its corticotropin-releasing activity. In agreement with these observations, total thymectomy of juvenile macaques was associated with decreases in plasma cortisol, corticotropin, and β-endorphin. These findings indicate that the prepubertal primate thymus contains corticotropin-releasing activity that may contribute to a physiological immunoregulatory circuit between the developing immunological and pituitary-adrenal systems.*

Glucocorticoids have immunomodulatory effects. High concentrations of corticosteroids induce thymic involution, reduce mitotic activity in thymus-dependent (T) lymphocytes, and inhibit phagocytic activity of human leukocytes (*1*). In contrast, lower concentrations of glucocorticoids enhance thymocyte differentiation, stimulate antibody formation in vitro, and seem necessary for modulating the immune response (*2*). The regulatory mechanisms controlling this interaction between the hypophyseal-adrenal axis and the immune system are unknown. Recent studies indicate that at least two families of biologically active products of immunogenic tissues influence adrenal steroidogenesis. First, thymosin fraction 5 (TSN-F5), a family of peptides known to induce maturation of T lymphocytes and other thymic extracts, stimulates adrenal cortical secretion in rats and rabbits (*3*). Second, lymphokines appear also to elevate serum corticosteroid concentrations, while a macrophage product has been reported to be inhibitory (*4*).

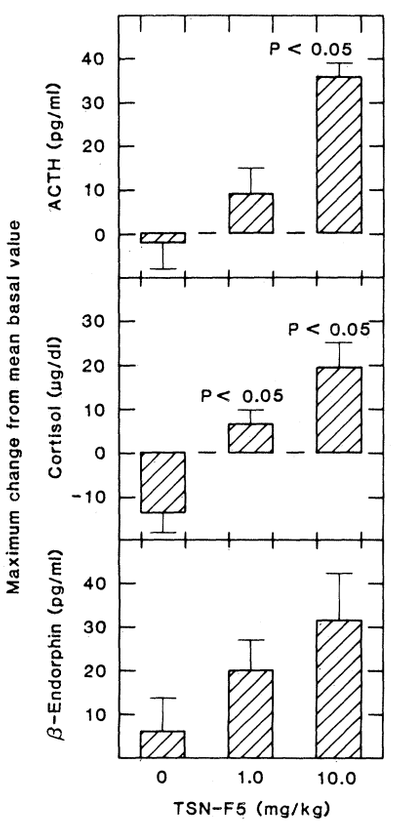

Fig. 1. Maximum change from the mean basal concentrations of plasma ACTH, cortisol, and β-endorphin after intravenous administration of TSN-F5 (1.0 or 10.0 mg/kg) or bovine serum albumin (10.0 mg/kg) in normal saline to three groups of five monkeys. Error bars indicate ± 1 standard error.

We report experiments in juvenile cynomolgus monkeys demonstrating that the thymus contains corticotropin-releasing activity. This activity may function as a subsidiary prepubertal and preadrenarcheal stimulus of the release of pituitary adrenocorticotropic hormone (ACTH). Our findings indicate a thymo-pituitary-adrenal axis in juvenile primates.

Eighteen premenarcheal cynomolgus monkeys (*Macaca fascicularis*) with a median age of 22 months (range, 11 to 27 months) were fitted with a vest and mobile tether assembly that permitted long-term cannulation of the femoral vein for serial blood collection. This allowed plasma to be harvested from monkeys that were unanesthetized, freely moving, and undisturbed by the experimenters, who were located in an adjacent room (*5*). Experiments began 24 hours after cannulation.

In the first experiment pyrogen-free TSN-F5 (10.0 or 1.0 mg/kg), crystalline bovine serum albumin (10 mg/kg), or normal saline were injected through the cannula. Plasma was collected for measurement of all anterior pituitary hormones and cortisol by radioimmunoassay (*5–7*). Red blood cells were returned to the monkeys during each experiment. Statistical significance of the changes in hormone concentrations was determined by Student's *t*-test for unpaired data. Results are expressed as means ± standard errors.

Figure 1 shows that TSN-F5 produced dose-dependent increases in plasma ACTH, cortisol, and β-endorphin; basal values were 24.06 ± 3.91 pg/ml, 35.3 ± 3.16 μg/dl, and 37.3 ± 7.65 pg/ml (N = 16), respectively. By contrast, no change in the concentration of plasma prolactin, growth hormone, thyrotropin, follicle-stimulating hormone, or luteiniz-

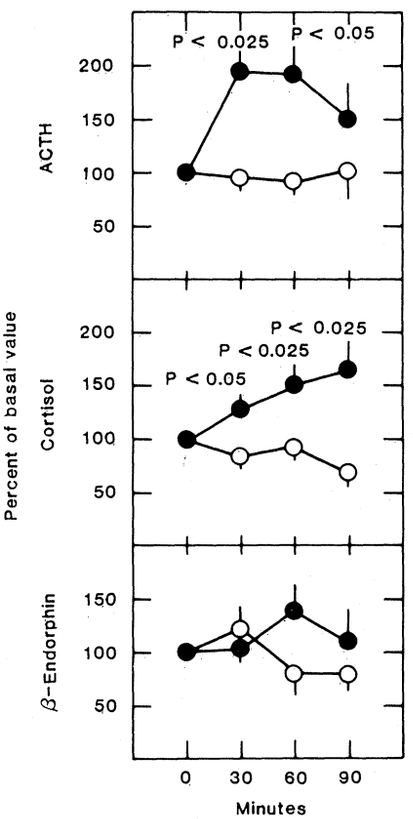

Fig. 2. Circulating concentrations of ACTH, cortisol, and β-endorphin after the administration of TSN-F5 (10 mg/kg) (●) or placebo (○) to eight premenarcheal cynomolgus monkeys at 0700 hours. Hormone concentrations are expressed as the percent of the transverse mean of 24 basal values: ACTH (22.3 ± 3.6 pg/ml), cortisol (31.9 ± 2.9 μg/dl), and β-endorphin (36.8 ± 7.6 pg/ml). Error bars indicate ± 1 standard error.