offspring of winged parents. The wingless characteristic of these offspring was already determined before birth, but their corpora allata were less active at birth than those of the young of wingless parents were ($P < .001$). This may indicate that they are determined prenatally by a high maternal titer of juvenile hormone (*7*) rather than by maternal activation of the corpus allatum in the developing embryos (*8*).

There is apparently a major difference between the response to crowding and isolation of *B. brassicae* and the responses of *M. viciae* and *A. craccivora*. Adults of the last two species can be crowded briefly and then isolated, and as a result of the crowding they produce winged offspring over a considerable period. If the cabbage aphid is isolated, it immediately switches to the production of wingless young, even though it may have been crowded all its life. This response to isolation is so powerful that it overrides, to a great extent, the effects of other conditions which induce the development of the winged form in other species. In experiments on aphid form determination, it has seemed preferable to cage each treated adult separately and to record the effect of the treatment in terms of the number of parents which respond by producing one form of young or the other form (*3, 5*). Our work indicated that use of this method with the cabbage aphid may obscure the effects of other environmental factors; for example, in earlier experiments (*5*), no response to short-term crowding was detected because of subsequent isolation, and the effects of different temperatures were significant only at a low level.

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Amnesia: A Function of the Temporal Relation of Footshock to Electroconvulsive Shock

Abstract. *When rats received a brief footshock upon stepping off an elevated platform, and an electroconvulsive shock 30 seconds or 6 hours afterward, amnesia was not observed 24 hours later. If a second footshock (noncontingent) was delivered 0.5 second before the electroconvulsive shock, amnesia was observed. The amnesia was temporary if conditioning was strong and permanent if conditioning was weak.*

Compelling arguments have been raised for using a procedure of one-trial learning and a single electroconvulsive shock (ECS) to study the effects of ECS on memory. One-trial learning permits accurate control of the training-ECS interval (*1*), and the single ECS avoids the aversive (*2*) and physiological aftereffects (*3*) that accompany multi-ECS treatments. One of the most common single-trial, single-ECS procedures (*4*) uses as its base-level measure the rat's tendency to step down rapidly from an elevated platform. If the animal receives footshock (FS) immediately upon stepping down or an immediate FS followed 30 seconds later by ECS, it retains the avoidance response and remains on the platform during subsequent trials. However, if it receives an immediate FS followed 0.5 second later by ECS, amnesia is produced and the rat tends to step off the platform on subsequent trials.

The temporal relation between FS and ECS is clear: the shorter the interval between FS and ECS, the less retention observed. There are, however, at least two properties of FS that could interact with ECS: FS-produced learning and FS-produced arousal. Thus the question at issue is whether the differential amnesic effect of varying the interval between FS and ECS is a function of the relation between FS-produced learning and ECS or is due to the relation between FS-produced arousal and ECS. If arousal is critical, and if arousal can be manipulated independently of initial learning, it should be possible to deliver ECS 30 seconds after learning and still produce amnesia as long as FS-produced arousal precedes the ECS by 0.5 second.

To test this prediction, we employed an experimental procedure in which two brief FS's were delivered, one contingent upon the step-down response, the other (noncontingent) delivered 0.5 second before ECS. A 30-second period intervened between the offset of the contingent FS and the onset of ECS. If the critical relation is between FS-produced learning and ECS, the 30-second interval between the contingent FS and ECS should permit retention. If, on the other hand, the critical relation is between FS-produced arousal and ECS, the 0.5-second interval between the noncontingent FS and the ECS should produce amnesia.

Male albino rats (total $n = 220$ in the four studies to be described) of the Sprague-Dawley strain, weighing 225 to 275 grams, were trained in an apparatus patterned after one described by Chorover and Schiller (*4*). The apparatus consisted of a 50-cm square box with Masonite walls 37.5 cm high and a grid floor of 0.6-cm stainless steel rods spaced 1.25 cm apart. Located in the center of the box was a 12.5-cm square platform 5 cm high. The grid floor was connected to a Grason-Stadler shocker that was set to deliver a 0.8-ma FS for 2 seconds. The ECS (35 to 50 ma) was administered through ear clips for 0.3 second.

Each rat received one trial per day with the ear clips attached. On each trial the rats were placed on the platform and their step-off latencies were recorded. The first 3 days consisted of habituation trials in which the rats were permitted to step off and to explore the test chamber for 10 seconds. On day 4, 70 rats were divided into five groups. Three of the groups received two FS's (FS--FS conditions), one FS immediately upon stepping off and another FS approximately 27.5 seconds later: the FS--FS group received no ECS; the FS--FS,ECS group received ECS 0.5 second after the second FS; and the FS--FS--ECS group received ECS 30 seconds after the second FS. The rats remained on the grid floor during the 27.5-second FS-FS interval after which the second FS was delivered automatically. Two control groups were employed: the ECS-alone group received ECS 30 seconds after step-off (both FS's were omitted); the NT group received no treatment upon

Table 1. Median step-off latencies before (trial 4) and after (trials 5 and 6) the 27.5-second FS-FS treatment. See text for details.

Group	Latencies (seconds)			
	Rats (No.)	Trial 4	Trial 5	Trial 6
FS--FS	20	2.3	21.3	12.5
FS--FS,ECS	20	2.1	3.7	15.4
FS--FS--ECS	10	1.6	15.8	9.9
ECS alone	10	1.7	1.5	2.2
No treatment	10	1.2	2.1	1.8

step-off. All animals receiving ECS were removed from the test chamber while still unconscious. The animals in the two groups that received no ECS underwent the following treatments: those in the FS--FS group were removed from the chamber immediately after the second FS; those in the NT group were allowed to explore the chamber for 30 seconds after step-off. On each of the two subsequent days the animals were tested for retention. Each retention trial consisted of the same procedure as that employed for the habituation trial unless the rat remained on the platform for 30 seconds, in which case the trial was terminated.

The latencies on the first retention day (day 5) are a function of the interval between the second FS and the ECS; retention is poorest when the interval is reduced and improves when the interval is increased (see Table 1). The latency for the FS--FS,ECS group was significantly (*5*) shorter than that for either the FS--FS or the FS--FS--ECS group. The permanence of ECS-produced amnesia was assessed on the second retention day (day 6). The significant (*6*) increase in latencies in the FS--FS,ECS group from 3.7 seconds on day 5 to 15.4 seconds indicates that retention is only temporarily suppressed and recovers 48 hours after training.

Table 2. Median step-off latencies before (trial 4) and after (trials 5 and 6) the single-FS treatment. See text for details.

Group	Latencies (seconds)			
	Rats (No.)	Trial 4	Trial 5	Trial 6
FS	10	1.1	12.5	6.3
FS,ECS	20	1.7	2.2	2.4
FS--ECS	10	1.2	16.2	5.9

Table 3. Median step-off latencies before (trial 4) and after (trials 5 and 6) the 6-hour FS-FS treatment. See text for details.

Group	Latencies (seconds)			
	Rats (No.)	Trial 4	Trial 5	Trial 6
FS--FS	20	1.4	10.9	6.8
FS--FS,ECS	20	1.3	2.5	4.5
FS--FS--ECS	20	1.5	11.1	12.21
FS---ECS	10	1.8	11.0	7.5

Since the double-FS procedure was employed to distinguish between two variables, FS-produced learning and FS-produced arousal, presumably confounded in the single-FS procedure, questions regarding the comparability of the single and double-FS procedures may be raised. For example, does a double FS produce greater conditioning than a single FS and, if so, are the amnesic and recovery effects of ECS different when ECS is delivered after a single FS? To answer these questions, on day 4 we gave a second sample of 40 additional rats a single FS upon step-off. One group (FS) received no ECS; two groups received ECS either 0.5 second (FS,ECS) or 30 seconds after FS (FS--ECS). On each of the two subsequent days the animals were tested for retention.

Table 2 shows that conditioning occurred on day 5 in the FS group but to a significantly lesser degree than that observed in the FS--FS group (Table 1). Thus, although the second FS in the FS--FS group was delivered 29.5 seconds after step-off, it contributed to conditioning. Comparison of the results for the two ECS groups receiving a single FS (FS,ECS; FS--ECS) with their corresponding double-FS groups (FS--FS,ECS; FS--FS--ECS) in Table 1 indicates that the amnesic effects of ECS on day 5 are the same for the corresponding groups, but that the recovery effects on day 6 are quite different: recovery is evident in the FS--FS,ECS group but not in the FS,ECS group.

That recovery occurs with the double but not the single FS may be due to the fact that conditioning is stronger in the double- than in the single-FS procedure. To examine this possibility we employed a double-FS procedure designed to produce conditioning comparable to that of the single FS. This was accomplished by giving rats on day 4 two FS's separated by approximately 6 hours rather than 27.5 seconds, as in the first experiment; the rats were given the first FS upon step-off, were returned to their home cages during the 6-hour interval, and were placed directly on the grid floor for the second FS. A third sample of 70 additional rats was divided into four groups. One group (FS--FS) received no ECS; two groups received ECS either 0.5 second (FS--FS,ECS) or 30 seconds (FS--FS--ECS) after the second FS. A fourth group (FS---ECS) received a single FS upon step-off and ECS 6 hours later. On each of the two subsequent days the animals were tested for retention.

Table 4. Median step-off latencies before (trial 3) and after (trials 5 and 6) the 27.5-second FS-FS treatment given outside the apparatus. The step-off trial on day 4 was omitted. See text for details.

Group	Latencies (seconds)			
	Rats (No.)	Trial 3	Trial 5	Trial 6
FS--FS	20	2.0	2.4	5.8
FS--FS,ECS	20	1.2	1.5	3.7

The two FS's separated by 6 hours produced (see Table 3) conditioning comparable to that of the single FS (Table 2) and significantly less than that of the two FS's separated by 27.5 seconds (Table 1). Comparison of the FS--FS,ECS groups in Tables 1 and 3 shows that the amnesic effects of ECS on day 5 were not affected by the 6-hour interval in spite of the significant decrease in conditioning; in the 6-hour procedure the latency for the FS--FS,ECS group was significantly shorter than that for the FS--FS, the FS--FS--ECS, or the FS---ECS group. That the second FS in this 6-hour procedure has minimal effects on conditioning but is still integrally related to the amnesic effects of ECS, further indicates that the arousal property of FS is a critical variable in ECS-produced amnesia. The recovery effects on day 6, on the other hand, were affected by the 6-hour interval: in contrast to the 27.5-second FS--FS,ECS group, no evidence of recovery was observed in the 6-hour FS--FS,ECS group, which indicates that with a decrease in strength of conditioning there is a corresponding decrease in recovery.

One final question can be raised regarding the presence of recovery in the 27.5-second, but not in the 6-hour, FS--FS,ECS group. Recovery of retention is characterized by short step-off latencies on day 5 and long step-off latencies on day 6. Perhaps this sequence of responses does not reflect recovery of the conditioned response but rather reflects a gradual increase in motor impairment produced by the interaction of the two FS's and ECS. Of course this motor effect would have to be specific to a 27.5-second FS-FS interval and to a 0.5-second FS-ECS interval since no increase in latencies is observed with either the 6-hour FS-FS interval or the 30-second FS-ECS interval.

To test for this motor effect, we eliminated conditioning but still maintained the 27.5-second FS-FS pattern. The step-off trial on day 4 was omitted and a fourth sample of 40 additional rats was given two FS's outside the platform apparatus; one group (FS--FS) received no ECS, and a second group (FS--FS,ECS) received ECS 0.5 second after the second FS. Table 4 shows no evidence of conditioning in either group on day 5 and a slight but nonsignificant increase in latencies on day 6. Thus, for step-off latencies to increase significantly from day 5 to day 6, conditioning must occur, and therefore the increase in step-off latencies observed in the 27.5-second FS--FS,ECS group in the first experiment does indeed reflect recovery of a conditioned response.

The experimental results indicate (i) a noncontingent FS given after initial learning suppresses retention when delivered 0.5 second before ECS but does not block retention when delivered 30 seconds before ECS; and (ii) recovery of retention following ECS-produced amnesia varies directly with strength of conditioning.

Previous studies using a single-FS, single-ECS procedure have taken the time-dependent data (that is, amnesia following a 0.5-second FS-ECS interval, retention following a 30-second FS-ECS interval) as evidence for the process of memory consolidation (*4*). Our present findings, however, bring the consolidation notion into question, since they demonstrate that amnesia can be produced 30 seconds or 6 hours after initial learning as long as a noncontingent FS precedes the ECS by 0.5 second. The time-dependent data obtained in the present experiment and those obtained in earlier studies can be accounted for by a single assumption: the 0.5-second FS-ECS interval produces aftereffects that interfere with subsequent retention but the 30-second FS-ECS interval does not produce such aftereffects. On the basis of recent findings in other laboratories (*7*), it may be further speculated that the aftereffects interfere with retention of the avoidance response by reducing "freezing" behavior. Although the aftereffects notion is in need of further tests, the ease with which it accounts for both the time-dependent and recovery data suggests that it is the most parsimonious explanation available.

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Lipids and the Assembly of Chloroplast Membrane

Rosenberg (*1*) postulates that galactosyl diglyceride serves to stabilize the orientation of chlorophyll in the chloroplast membrane. He suggests that this is made possible by a lock-and-key fit between the methyl groups in the phytol portion of the chlorophyll molecule and the methylene-interrupted, *cis* double bonds of the fatty acids in the galactosyl diglycerides. Induced polar interaction of double bonds with methyl groups is proposed as a force that favors binding of the two components. London—Van der Waals forces are also considered by Rosenberg to contribute to this binding. These latter forces alone appear to be powerfully attractive between closely packed molecules in biological systems (*2*). However, the widespread occurrence of methyl groups in proteins and *cis* double bonds in lipids suggests that any unique forces of attraction between such groups and bonds would not only be important in the chlorophyll-galactolipid interaction but of general significance to the formation and function of all lipid-protein complexes.

Another attractive feature of Rosenberg's theory is that it suggests a possible relation between an ordered program of syntheses and an ultimate state of structure and function resulting from the syntheses. Given the high degree of organization existing in the chloroplast membrane (*3*), it seems improbable that such a structure could result by random self-assembly from a mixture of its components (*4*). Rather, when one finds molecules that make an extremely good fit in an organized structure, it is reasonable to assume that one molecule may have served as the template on which an adjoining (bound) molecule was synthesized. In this way each structural-synthetic event determines the next such event and the entire sequence of events determines the functional capability as well as the structure of the whole.

Thus, in the case of Rosenberg's postulation the unsaturated fatty acids of the galactosyl diglycerides may serve as the template for synthesis of the phytol (*5*) which in turn serves as the acceptor for the chlorophyllide. The alternative, that phytol or related isopentyl-containing structures serve as templates for unsaturated fatty acid synthesis, may also deserve consideration.

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4. The presence of genetic material in the chloroplast [see E. Stutz and H. Noll, *Proc. Nat. Acad. Sci. U.S.* **57**, 774 (1967)] also suggests organized developments of its membranes.
5. According to Rosenberg, other chloroplast compounds composed of isopentyl units, such as the carotenoids and plastoquinones may also be bound by the galactosyl diglycerides in the manner he proposes for phytol.

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