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Retrograde Amnesia Gradients by Subconvulsive and High Convulsive Transcranial Currents in Chicks*

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Abstract. Either a subconvulsive or a high convulsive current was delivered transcranially at various intervals after a learning trial to independent groups of newly hatched chicks. The one-trial learning involves suppression of the chicks' predilection to peck at a small shiny bead. The effect of current on retention was measured one day later. Results indicate a current-sensitive memory consolidation period of about 30 seconds that cannot be appreciably prolonged by a tenfold increase in current intensity and a simultaneous twofold increase in current duration. On the other hand, at comparable trial-to-current intervals within this brief consolidation period, more retention deficit was caused by the higher current. Beyond this critical period, both currents still effected some retention deficit, but it did not seem to vary with the trial-to-current interval from one minute to 24 hours. Overt muscular convulsion was unnecessary for the retrograde amnesia effect. The subconvulsive current may be a more pure agent for consolidation studies because the high, convulsive current may affect test performance in ways other than disrupting the consolidation processes.

Retention of one-trial learning in chicks has been found to suffer from post-trial administration of a low-intensity electroconvulsive shock (ECS).[1,2] Within the 30-second period immediately following the learning trial, the sooner the current was delivered, the greater the retrograde amnesia. The current also impaired retention when given after longer delay intervals, but the deficit was smaller and did not vary with the trial-current interval from one minute to 24 hours. The results have been interpreted to indicate an ECS-sensitive memory consolidation process of about 30 seconds in duration, plus some general "brain damage" effect of the current that impairs retention but is independent of the trial-current interval.

In a subsequent study where the trial-current interval was held constant at ten seconds and the current intensity was varied, a definite amnesic effect, comparable to that of low-intensity ESC, was obtained with some subconvulsive currents (SCC). Nevertheless, retention deficit did increase gradually with current intensity. Comparing SCC with high-intensity ECS, it was clear that the latter caused more retrograde amnesia when both were given ten seconds after the learning trial.[3]

The present study consists of three related experiments. Experiment I compares two retrograde amnesia gradients obtained with a SCC and a high ECS, respectively. Experiment II checks the effect of the above two respective currents on the pecking response among naive chicks. Experiment III tests whether the currents will affect retention performance when given before instead of after the learning trial.

Experiment I. Three questions were asked, as follows: First, does a subconvulsive current have amnesic effects similar to that of a low-intensity electroconvulsive shock at not only ten seconds, but also at other trial-current intervals? Second, does the duration of the apparent "consolidation period" increase with current level, or does a high current merely cause more retention deficit within a fixed period? Third, is the amount of retention deficit found beyond the critical consolidation period also a function of current level? More specifically, will the deficit be reduced or even disappear with SCC, and become larger with a high-intensity ECS? Two extreme currents, one subconvulsive and the other about ten times above the convulsion threshold, were delivered after various trial-current intervals to independent groups of chicks. The two resulting retrograde amnesia gradients were compared with each other, as well as with the one obtained earlier with a low ECS.

Method: The general method has been reported in detail.[3] In brief, subdermal electrodes with fine wire leads were implanted bitemporally before 11 AM in day-old White Leghorn cockerels (*S*s) obtained the same mornings from a nearby hatchery. Afterwards, the *S*s were housed individually in white, open-top cylindrical cartons in the experimental room where the temperature was 85–90°F and the humidity 30–50%. All *S*s had about 5 hr to adapt to their cartons and electrodes before presentation of the learning trial or other experimental manipulations.

The one-trial learning involves suppression of the *S*s' predilection to peck at a 3-mm metal bead (the lure) neatly soldered to the tip of a thin probe. The bead was lowered into the carton and presented to the *S* about 1 cm in front of its beak, after having first been dipped in methyl anthranilate. This chemical is a transparent liquid apparently unpalatable to the chicks. Typically, a *S* pecks at the lure in 1 or 2 sec, then shakes its head vigorously, and will not peck again. The lure was withdrawn at the start of the head shake. About 15% of the chicks failed to peck at the lure in 5 sec and shake their head in 10 sec after pecking, and they were discarded.

Each day the newly arrived *S*s were divided into several different treatment groups, and for each group data were accumulated over a number of days. Subjects in the 22 experimental groups were given the learning trial followed after various intervals by a transcranial 60 Hz current. A subconvulsive current of 12 ma, 0.25 sec was used with half of the groups (the SCC groups) and a high convulsive current of 120 ma, 0.50 sec was used with the other half (the high ECS groups). The eleven trial-current intervals, from start of the *S*'s head shaking to start of current, were 0, 5, 10, 30, 45, 60, and 240 sec, and 1, 2, 3, and 24 hr. Retention of the one-trial learning was measured in a test trial given in PM of Day-2, about 22 hr after the learning trial, in all except the two 24-hr groups, for which the test trial was given at 10 AM on Day-3. The number of *S*s in each group (N) ranged from 100 to 108, except for the two 24-hr groups, where N was 240 in the SCC group and 244 in the high ECS group.

To assess normal retention without post-trial current, two learning-only control groups were used. Both groups had the learning trial around 3 PM in Day-1, and were tested for retention, in PM of Day-2 (N = 382) and AM of Day-3 (N = 255), respectively.

To obtain baseline incidences of pecking without a prior learning trial, six additional control groups, with N's ranging from 100 to 108, were used. Three Day-2 baseline groups were presented with the lure for the first time in PM of Day-2, during a test trial. One group had had SCC, another high-ECS, and the third no current, in PM of Day-1.

Three Day-3 baseline groups were given a test trial in AM of Day-3. Two of the groups had had a current, one SCC and the other high ECS, in PM of Day-2.

During the test trial, the lure was again first dipped in methyl anthranilate and then presented to each *S* for 5 sec, and whether the *S* pecked at it or not was recorded. The *E* did not know of the *S*'s group designation while testing.

Results and comment: Incidence of pecking was high during the test trial in all six baseline control groups that had had no learning trial, no matter whether they had previously received a SCC, or a high ECS, or no current. Percentage of *S*s in each group that did not peck (the no-peck percentage) ranged from 5 to 7 per cent in the three groups tested on Day-2, and was 6 per cent when combined. The no-peck percentage for the three groups tested on Day-3 ranged from 1 to 5 per cent, and was 3 per cent when combined.

In contrast, pecking was infrequent in the two learning-only control groups that had had a learning trial, but no subsequent current, indicating good retention. The no-peck percentage was 71 per cent in the group tested on Day-2, and 64 per cent in the group tested on Day-3.

The no-peck percentage of the experimental groups are plotted against trial-current interval in Figure 1. For comparison, some results from the earlier

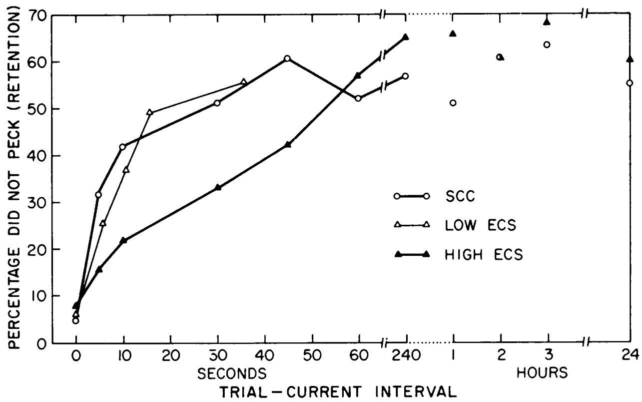

FIG. 1.—No-peck percentage as a function of trial-current interval. Each data point based on about 100 *S*s. Low-ECS data are from a previous experiment.[1, 2]

study[1, 2] with a low ECS (60 Hz, 28 ma, 0.45 sec) were also included. Together with the control values presented earlier, the following findings are indicated:

First, the subconvulsive current indeed has amnesic effects similar to that of a low electroconvulsive shock at not only ten seconds, as has been found before,[3] but also at other trial-current intervals. In the earlier study with low ECS,[1, 2] the procedure was slightly different in that the lure was presented for a fixed 10-second period during the learning and the test trials; retention in the control groups was about 80 per cent, and the asymptotic level of the experimental groups was about 60 per cent. Despite these differences, it is clear that the two retrograde amnesia gradients, one produced by a SCC and the other, a low ECS, are remarkably similar. Both currents apparently caused complete amnesia when given immediately after the learning trial. Both gradients were quite steep, reaching their respective asymptotic levels in about 30 seconds. These findings further confirm the effectiveness of SCC as a consolidation-disrupting agent, and the irrelevance of motor convulsions.

Second, the high ECS did not appreciably prolong the observed consolidation period, but it did cause more retention deficit than SCC when given within 45 seconds after the learning trial. This finding strongly suggests a critical current-sensitive consolidation period of about 30 seconds in duration that is largely invariate with current level. Within this short period, retention deficit is a function of both the level of the current and the time of its delivery.

Third, as had been found in the earlier study with low ECS, both SCC and high ECS still caused some retention deficit beyond the consolidation period, even when current was delayed for as long as 24 hours. (This finding will be repeatedly referred to later on as "the small gap between the control and experimental groups.") Quite unexpected, however, was the additional finding that here the SCC seemed to have caused more retention deficit than the high ECS! The no-peck percentage was higher in the high ECS groups for five of the six trial-current intervals ranging from 60 seconds to 24 hours. At the other interval, both currents gave the same results. If the small gap between the control and experimental groups were caused by some relatively permanent brain damage inflicted by the currents, as was hypothesized before,[1, 2] it would be reasonable to expect more damage from the high ECS, which has ten times the intensity and two times the duration of the SCC.

Experiment II. It was quite puzzling that subconvulsive current seemed to have caused more retention deficit than high electroconvulsive shock beyond the consolidation period. This would be understandable if, in addition to disrupting memory consolidation, the electric currents also have a disinhibiting effect[4] on pecking, and that somehow SCC disinhibits more than high ECS. The purpose of Experiment II, then, was to examine whether the currents did cause some disinhibition of pecking independently of disrupting memory consolidation. More specifically, Experiment II was designed to check whether the currents given on Day-1, unprecedented by any learning trial, would enhance pecking of a naturally unattractive or even aversive lure on Day-2, among naive chicks that had not been exposed to the lure before.

Finding a naturally unattractive lure for two-day-old chicks turned out to be quite difficult. The chicks' predilection to peck is much stronger on Day-2 than on Day-1. Many lures that elicited low incidences of pecking on Day-1 were pecked by nearly all chicks on Day-2. After trying out a large variety of lures, we selected two. These were presented in very dim illumination which itself tended to reduce pecking. In addition, they were dipped in 100 per cent alcohol, the vapor of which seemed to irritate the chicks and further inhibited pecking.

Method: All *S*s were implanted with bitemporal electrodes in AM of Day-1, and then housed in individual cartons, as in Experiment I. They were divided into two sets of three groups each. For each set, one group was given the high ECS, one the SCC, and the other no current, at about 3 PM on Day-1. Pecking was measured during a 5-sec test trial at about 2 PM on Day-2, by an *E* who did not know of the *S*s' previous treatment. A different lure was used with each set of chicks. One lure was a 1-cm section of black rubber tube, with outer diameter of 3 mm, fitted over one end of a 2-mm diameter dull-gray metal stick (the black tube lure). The other lure was the handle end of a metal blade holder (Bard-Parker, No. 7), which was about 5 mm wide, with a slightly rounded end. During the test period, the room light was dimmed so that illumination at area of lure presentation was reduced from a regular 17 to 3 ft-c. The lure was first dipped in 100 per cent alcohol, then immediately presented to the *S* for a 5-sec period, and whether the *S* pecked or not was recorded.

Results and comment: From earlier screening tests for unattractive lures using independent groups of naive day-old chicks, the no-peck percentage was 72 per cent ($N = 115$) for the black tube lure, and 74 per cent ($N = 114$) for the blade holder lure. The no-peck percentage became much lower when tested on naive two-day-old chicks, indicating a higher pecking tendency on Day-2. Results presented in Table 1 show clearly, however, that delivery of either a high

TABLE 1. *Percentage of Ss that did not peck during a Day-2 test trial. All Ss had not been given a previous learning trial.*

Day-1 treatment	Black-Tube Lure (%)	Black-Tube Lure (*N*)	Blade-Holder Lure (%)	Blade-Holder Lure (*N*)
No current	25	166	27	162
SCC	24	180	28	188
High ECS	37	185	45	179

ECS or a SCC in PM of Day-1 did not further enhance pecking. To the contrary, while SCC did not influence pecking, the high ECS actually reduced the pecking tendency. For either lure, the no-peck percentages were almost identical between the no-current and SCC groups, but higher in the high ECS group. The differences between high ECS versus either SCC or no-current group are significant at the 0.05 level for the black-tube lure, and at the 0.01 level for the blade holder lure, by the chi-square test. Analysis of the distributions of pecking latency, i.e., the interval from start of lure presentation to pecking, indicates that high ECS caused a higher no-peck percentage not by prolonging the pecking latency in all *S*s, but rather by changing some *S*s from pecking to not pecking. Similar depression effect of ECS on activity has also been demonstrated in rats, where a single ECS reduced bar pressing and open field activity, but did not prolong descend latency from a small platform.[5]

The finding that high ECS tended to reduce pecking of the less attractive lures could explain why SCC seemed to have caused more retention deficit than high ECS beyond the consolidation period: The higher no-peck percentage in the high ECS groups did not reflect better retention, but rather the additional effect of high ECS on pecking. In fact, if the additional effect were eliminated, the asymptotic level of the high ECS groups might have been even lower than that of the SCC groups. It should be emphasized, however, that the present results do not tell us exactly how many percentage points should be subtracted from each of the high ECS groups in Experiment I to get rid of the confounding effect. The observed amount of peck suppression by high ECS apparently depends on the appeal of the lure to the particular group of chicks being tested. For instance, when pecking was tested with the very attractive metal bead lure on Day-2 or Day-3 among naive chicks, as was the case in the no-learning baseline control groups in Experiment I, no suppression effect was detected. The effect did show clearly, however, in Experiment II, in which less attractive lures were used. Presumably the effect was also present in the experimental groups of Experiment I, especially when the current was delivered after the consolidation period, because the learning experience on Day-1 grossly reduced the attractiveness of the metal bead lure. The present results demonstrate the subtlety sometimes involved in finding appropriate control groups.

Since the present experiment did not show any facilitation or disinhibition of pecking by either SCC or high ECS, the small gap between the control and the experimental groups found in Experiment I remained unexplained. The gap was also clearly present in the earlier study with low ECS.[1, 2] Obviously it is not a result of statistical sampling error. Whether the gap is also present in similar experiments using rats or mice is unclear. Since most such studies use fewer trial-current intervals and fewer *S*s in each group, a small but true difference between experimental and control groups may not become obvious.

Experiment III. Results of Experiment II showed that neither SCC nor high ECS increased pecking in addition to and independently of disrupting memory consolidation, therefore the small gap between control and experimental groups found in Experiment I cannot be explained in terms of disinhibition. For more precise quantitative analysis and model fitting of the consolidation data, it is important to determine whether the gap is related to processes of consolidation or not. Experiment III was conducted to find out if the gap would still be present when the currents were delivered long before the learning trial, instead of after it. If so, we could be reasonably sure that the gap was not due to disruption by the currents of ongoing consolidation processes.

Method: All *S*s were implanted with electrodes and housed individually as in Experiments I and II. They were divided into three groups. A high ECS group received the high ECS, a SCC group the SCC, at 11 AM of Day-1. A control group received no current. All three groups were given the learning trial at about 4 PM on Day-1. Retention was again measured in a test trial given at about 2 PM on Day-2.

Results and comment: Currents delivered in the morning of Day-1 did not affect pecking of the metal bead lure during the learning trial given five hours later, indicating no different selection of *S*s by this procedure. The percentage of chicks that failed to peck in five seconds and shake their head within ten seconds after pecking was 11 per cent each for the high ECS and the SCC groups, and 12 per cent for the control group, and these chicks were discarded.

The currents also did not appear to affect retention of the one-trial learning. The no-peck percentage during the retention test was 69 per cent ($N = 204$) in the high ECS group, 70 per cent ($N = 221$) in the SCC group, and 72 per cent ($N = 195$) in the control group. In other words, the gap between experimental and control groups disappeared when the currents were delivered before instead of after the learning trial. These results left open the possibility that the gap observed in Experiment I was indeed related to the consolidation processes.

Results from Experiment II have shown that, while SCC did not by itself affect pecking, high ECS did reduce pecking of the less attractive lures independently of learning and retention. Taking this additional inhibition effect into account, the "true" no-peck percentage in the high ECS group of the present experiment might be lower than the observed value, suggesting that high ECS also has some general long-term effect that impairs learning or retention even when given before the learning trial. Inferior performance in a learning situation as well as physiological changes in the brain have been demonstrated in the rats after repeated ECS.[6] Although this possible general effect is only indirectly inferred in the present study, it could be tested in an active-avoidance learning situation where presumably the inhibition and the general impairing effects would add instead of cancel.

While results from Experiments II and III did not explain the small gap between experimental and control groups found in Experiment I, they did implicate effects of high ECS in addition to the disruption of consolidation. These results suggest the use of SCC as a more pure agent for consolidation studies. They also give warning about the likely contamination of retrograde amnesia gradients obtained with high doses of disruptive agents.

Discussion. Results from the present study have indicated a current-sensitive consolidation period of about 30 seconds which cannot be appreciably prolonged by simultaneous tenfold increase in current intensity and twofold increase in current duration. Within the critical period, however, retrograde amnesia is a function of the electrical energy dissipated as well as the learning-current interval. Beyond this period, the current still caused some retention deficit, but it did not seem to vary with either its delay or its magnitude.

The similarity between retrograde amnesia gradients by SCC and by low ECS demonstrated in Experiment I shows that motor convulsions are unnecessary for the amnesic effect. A recent study has shown, however, that behaviorally subconvulsive currents may nevertheless elicit spike activities as measured on the electrocorticogram.[7] Although the chicks appear normal right after SCC stimulation, pecking responses are suppressed for about five minutes.[8]

The finding that increasing current magnitude did not much prolong the apparent consolidation period, but did cause more retention deficit within the critical period, is consistent with findings from a similar study with rats.[9] Other studies indicate that a higher current may impair retention at longer learning-current intervals when lower currents are no longer effective.[10, 11] One possible explanation for such instances is that high currents may affect test performance in ways other than disrupting the consolidation processes, as was found in Experiments II and III of the present study.

Both intensity and duration of the current were varied in the present study because a pilot study with chicks indicated that both variables are important, with the magnitude of retention deficit depending on their interaction. The same study also showed, however, that for either intensity or duration, little gain in retrograde amnesia effect can be obtained by further increases above a sensitive region. Similar findings have been obtained in a parametric study with mice.[12] Discrepancies in the literature with regard to the importance or unimportance of variations in current intensity or duration may well depend on whether the range covered in individual studies included the critical regions or not.

The "consolidation" interpretation has been challenged repeatedly, mostly on the grounds that ECS has effects other than disrupting consolidation. Indeed, alternative interpretations have been presented to account for long temporal gradients of performance deficit caused by post-trial administration of ECS.[13] In order to avoid possible contaminating effects of ECS, two recent studies used response choice measure in a T maze instead of the usual response latency measure in avoidance situations to indicate retention. Both studies obtained graded retrograde amnesia effect only when the currents were delivered within 60 seconds, or less, after the learning trial.[14, 15] These studies offer strong support for the consolidation hypothesis. Together with our own findings, they also indicate that the current-sensitive consolidation period is quite brief, in the order of seconds.

Taking all evidence into consideration, the consolidation interpretation seems to remain the most reasonable for our own data. Alternative interpretations either do not apply in our experimental situation, or have been checked and found to be inadequate. For instance, a recent challenge claimed that retrograde amnesia could be obtained by delivering ECS long after the initial learning trial, as long as the memory trace is activated immediately before current delivery by reexposing the *S*s to some aspects of the training situation.[16, 17] However, no retention deficit has been observed following such procedure in the chicks. Another challenge proposed that ECS sometimes affects memory in its retrieval rather than its consolidation.[18, 19] In a joint study with G. Magnus, no retrieval amnesia gradient was obtained when SCC was delivered five minutes to two hours before retention test. These experiments will be reported in detail separately.

Although the memory consolidation interpretation cannot be easily dismissed, cumulating evidence does indicate the complexity involved in interpretation of data. Observed effect of post-learning delivery of current on later performance depends on not only the trial-current interval and current level, but also many other factors, including species or even strain differences,[20] procedure of training,[21–23] type of response measured,[24] etc. All these findings suggest the desirability of intensive and systematic studies in each animal form, and caution against sweeping generalizations.

Abbreviations used: ECS, electroconvulsive shock; SCC, subconvulsive current.

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