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Experimental Variables in the Effects of Postsession Injections of Strychnine Sulphate on a Classically Conditioned Response

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Summary. Postsession strychnine injections have been shown to retard acquisition rates of the classically conditioned nictitating membrane response (CCNMR) of rabbits trained at a CS-US interval of 1000 msec. The particular interval value employed in CCNMR acquisition was found to be important in the magnitude of the postsession strychnine effect. Strychnine *Ss* trained at a CS-US interval = 1250 msec (nonoptimal) were significantly depressed in acquisition rates relative to saline controls, while strychnine *Ss* trained at a CS-US interval = 250 msec (optimal) were not. Interpretations are based upon the presumed effects of CNS excitants on memory consolidation and possible neurological correlates of the CS-US interval parameter.

Key-Words: Classical Conditioning — Strychnine — Consolidation — Nictitating Membrane.

Cholewiak, Hammond, Seigler, and Papsdorf (1968) have recently reported a depression in the acquisition rates of the classically conditioned nictitating membrane response of the rabbit as a result of immediate postsession i.p. injections (0.3 mg/kg) of strychnine sulphate. Although the Cholewiak *et al.* study is the only one involving postsession injections in a classical conditioning paradigm, numerous other studies have investigated strychnine effects in instrumental approach and avoidance tasks as well as in more cognitive problems. Postsession strychnine *facilitation* has been observed in complex maze learning (McGaugh, Thomson, Westbrook and Hudspeth, 1962), in a visual discrimination problem (Krivanek and Hunt, 1967) in a delayed alternation response task (Petrinovitch, Bradford and McGaugh, 1965), in shuttle box avoidance (Bovet, McGaugh and Oliverio, 1966) and in an oddity discrimination problem (Hudspeth, 1964). Performance facilitation has generally been interpreted in terms of an enhancement of consolidative processes underlying memory formation (McGaugh, 1966).

The evidence accumulated by McGaugh and others of a postsession strychnine facilitation makes the Cholewiak *et al.* observation appear

anomalous. However, the growing evidence concerning the influences of experimental parameters upon postsession effects makes difficult direct comparison of strychnine studies. A relevant factor, for example, appears to be the postsession environment, under which the drug takes effect. In the Cholewiak *et al.* study, the rabbits were returned immediately after injection to the home cage. Unpublished observations from the Michigan laboratory show no depressive effects following postsession injections of strychnine when rabbits were maintained in the experimental enclosure for 30 minutes following drug injection. Calhoun (1966) has varied the postsession environment following postsession injection of strychnine, finding that when there were high levels of stimulation strychnine depressed performance in a spatial discrimination task, but when there were low levels of stimulation strychnine facilitated performance. In addition, Irwin (1967) has observed that postsession injections of metrazol could facilitate retention of a passive avoidance response only if following injections *S*s were maintained up to the time of testing in a plastic cage rather than in the home cage.

In an effort to generalize the postsession strychnine effect of the Cholewiak *et al.* study, experimental parameters were altered in a later study to include a shorter CS-US interval (300 msec instead of 1000 msec), fewer trials per session (5 per session instead of 15 per session), and a higher dosage of strychnine (0.5 mg/kg instead of 0.3 mg/kg), Despite the higher drug dosage and shorter conditioning session, postsession strychnine depression was not observed. As the CS-US interval parameter has been shown to be a powerful determinant of conditioning rates (Schneiderman and Gormezano, 1964) with CS-US intervals of 250 to 400 msec providing most rapid conditioning, the less optimal (1000 msec) interval of the Cholewiak *et al.* investigation might have been critical to their finding. The following experiment was designed to test this possibility.

Method

Subjects. Thirty-one male albino New Zealand rabbits 80—100 days old were obtained through the facilities of the Animal Care Unit of the University of Michigan and maintained on ad-lib food and water.

Procedure. Apparatus and procedure are described in detail by Gormezano (1966). Forty-eight hours prior to the first experimental session, a small nylon loop was sutured into *S*'s right nictitating membrane. On the following day, each *S* was placed in a Plexiglas restraining box for a 10-minute habituation period. During this period, as in the experimental sessions, tailor hooks, which served to retract the eyelids and deliver the shock US, were fastened by an adjustable Velcro strap to the inferior and superior lids of the right eye. Movements of the nictitating membrane were monitored by a small photoelectric transducer

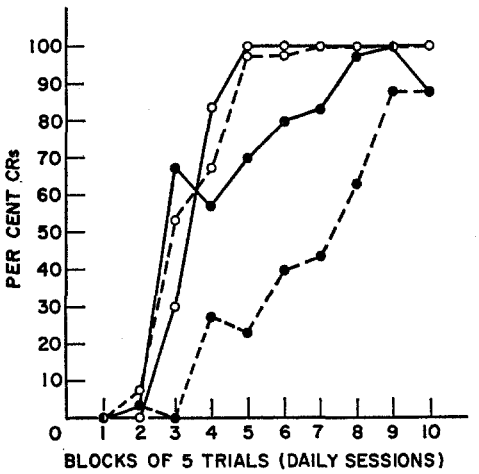

Fig. 1. Percentage of CRs of *Ss* receiving immediate postsession injections of strychnine sulphate or saline at either of two CS-US intervals (in msec). o——o Saline-250; o- - -o Strych-250; •——• Saline-1250; •- - -• Strych-1250

mounted on the *S*'s snout by means of a muzzle-like assembly and mechanically coupled to the loop in the membrane. Signals from the transducer were amplified and graphically recorded by a Beckman Type R Dynograph.

On each of 10 subsequent days, *Ss* received 5 CS-US pairings at a variable intertrial interval averaging 1 min. The CS, a 1000 Hz tone at 93 db. SPL, was presented against a continuous white noise background of 70 db. SPL for either 350 msec or 1350 msec. The US was a 3 ma. 60 Hz shock delivered across the eyelids and overlapping the 100 msec of CS presentation.

Ss were randomly assigned to four groups in a 2×2 design with CS-US intervals of 250 msec (optimal) and 1250 msec (nonoptimal) orthogonal to 0.5 mg/kg i.p. injections of a 1.0 mg/cc solution of strychnine sulphate or a comparable injection of physiological saline. Injections were administered one to two minutes following each conditioning session. *Ss* were returned to home cages immediately after the injections.

Results

Percentage of CRs across blocks of 5 trials is plotted in Fig. 1.

Over sessions 3—9, at CS-US interval = 250 msec, performance of strychnine *Ss* and saline *Ss* were essentially equivalent. However, at CS-US interval = 1250 msec the CR level of strychnine *Ss* was from 12% to 67% below that of saline *Ss*. Analysis of variance yielded a significant CS-US interval effect ($F = 9.76$, $df = 1/20$, $p < 0.01$) CS-US interval $\times$ Trials interaction ($F = 4.33$, $df = 9/180$, $p < 0.01$), and CS-US interval $\times$ Drug $\times$ Trials interaction ($F = 2.49$, $df = 9/180$, $p < 0.05$), the latter indicating that the magnitude of postsession strychnine depression was determined by the CS-US interval employed.

The mean percentages of CRs summed over sessions 2—10 for Saline-250 and Strychnine-250 *Ss* were 79.3% and 80.0% respectively. Equivalent measures for Saline-1250 and Strychnine-1250 *Ss* were 71.5% and 41.5% respectively.

A conservative *a posteriori* analysis of variance (Winer, 1962) regarding the conditional effect of the drug at CS-US interval = 1250 msec was statistically significant ($F = 4.10$, $df = 2/20$, $p < 0.05$).

Discussion

In agreement with the finding of Cholewiak *et al.* (1968), postsession injections of strychinne sulphate produced a significant depression in the rate of conditioning of *S*s trained at a nonoptimal CS-US interval. Three possible hypotheses accounting for this depression may be considered: 1. A drug-induced enhancement of central sensitivity to peripheral stimulation (Smolin and Samko, 1968) might make enviromental conditions of the home cage disruptive for memory consolidation, 2. the environmental conditions of the home cage might evoke an emotional response incompatible with that elicited by the experimental enclosure which, when in the presence of the CNS excitant, might compete with the task-related consolidative processes, or 3. there might exist a generalization of inhibition from excessive non-reinforced blinking in the strychnine *S*s.

The generalization of inhibition hypothesis is initially appealing due to its success in accounting for the ISI-strychnine interaction. Hartman and Grant (1962) have shown data suggesting longer recruitment times for inhibitory processes than for excitatory ones. Longer CS-US intervals would provide the needed time for inhibition recruitment to occur. However, the prediction would have to be made that *S*s returned to the experimental enclosure for a period of time following injection would show a greater strychnine depression than those returned to home cages after injection. Unpublished data from the Michigan laboratory as indicated earlier show the opposite result. A somewhat similar difficulty resides in the hypothesis of consolidation of competing response tendencies. It would be difficult to identify the cues in the experimental enclosure that would be able to elicit the competing response tendencis acquired in the home cage.

Thus, the most promising hypothesis for the depression effect appears to involve increased sensitization to peripheral stimulation. In order to account for the parametric interaction, this hypothesis must assume that the optimal and nonoptimal interval groups differ in the neural configuration coding the CS-US contingency, so as to involve a differential vulnerability to drug-produced interfering neural events. As a proposed mechanism for simple classical conditioning, John (1967) has suggested that the reverberatory overlap of coherent neural activity in a US dominant focus as a consequence of afferent input from a CS dominant focus underlies the neural basis of associative development. If a long CS-US interval activated a more complex common mode of activity

relative to a short interval, with a greater number of synapses where neuronal interaction could occur, the long CS-US interval system could be considered more vulnerable to interfering activity.

To the extent that CS-US interval influences the characteristics of the neuronal coding of the CS-US event, the classical conditioning paradigm, where the parameter can be brought under experimental control, should be helpful in more detailed analyses of the processes underlying memory consolidation.

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