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Food deprivation and startle magnitude: inhibition, potentiation, or neither?

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The role of tissue deprivation as a determinant of acoustic-induced reflexive startle responses is unclear, variously having been shown to potentiate, retard, or have no effect at all. The present is a preliminary evaluation of certain procedural features upon which various apparently contradictory outcomes have rested. Two large groups, one satiated and the other made 46-h hungry, were divided so that half of each received nine consecutive startle test sessions beginning with the initial 46- to 48-h hunger cycle and spaced every 2 days thereafter. Testing was delayed until the last two sessions for the remaining group halves. Although standard startle habituation functions across sessions and trials within sessions were obtained, reliable differences traceable to hunger did not occur for any test circumstance. External validity remains a difficult commodity to come by for any of the hunger-startle effects thus far reported.

Of considerable interest to motivation theorists is whether the strength of an innate response to its adequate stimulus can be modified by variations in seemingly irrelevant emotional and/or appetitive states. Beginning with the innovative demonstration of Brown, Kalish, and Farber (1951), there have been numerous studies showing that a stimulus previously paired with electric shock, a CS for conditioned fear, can enhance the magnitude of an unlearned startle response (Anderson, Johnson, & Kempton, 1969a, 1969b; Chi, 1965; Davis & Astrachan, 1978; Galvani, 1970; Kurtz & Siegel, 1966; Wagner, Siegel, & Fein, 1967; Anderson, Crowell, & Brown, Note 1; Meryman, Note 2). However, much more controversial has been whether unlearned conditions ("states?") such as food deprivation also can potentiate startle responses. Meryman (Note 2) reported, among other things, that startle responses were significantly augmented by 46 h of food deprivation, and Mellgren (1969) confirmed this outcome. However, Anderson et al. (Note 1), in duplicating Meryman's study and several of his major findings, did not discern a reliable difference in startle magnitude traceable to hunger per se. To complicate matters, Fechter and Ison (1972), Ison and Krauter (1975), and Szabo (1967) reported that their deprived rats exhibited smaller startle reactions than satiated animals did.

Clearly, these discrepancies underscore the need for further replicatory attempts. Unfortunately, procedural differences exist between the studies of Anderson et al. (Note 1) and Meryman (Note 2), and between the former and those of Ison and Krauter (1975) and of Szabo (1967). In the former case, many of these differences appeared to bias against Anderson et al.'s (Note 1) obtaining any effects that were as marked as those reported by Meryman. Indeed, Anderson et al. (Note 1) appealed to several of these procedural variances as possible reasons why their hungry animals evinced only nominally, but not reliably, larger mean startle amplitudes than their nonhungry rats. However, the procedural differences between the Anderson et al. and Meryman studies and those of Ison and Krauter and Szabo appear to be of even greater magnitude, if not qualitative in nature. Animals of the Ison and Krauter study, for example, received a great many more startle trials per day and many more trials overall than did the subjects of either Anderson et al. or Meryman. In addition, these two sets of studies differed markedly with respect to deprivation regimens, test schedules, body weights at time of tests, and so forth.

Because of these many potentially important variances, it is difficult to immediately identify any single item as possibly more important than any other in accounting for the disparate results reported. Accordingly, the present study represents only a beginning attempt to discern possible critical procedural variations in this connection. For example, since Ison and Krauter (1975) report similar results when deprivation regimens differed markedly across control and experimental groups, the present study entailed a deprivation routine only for the latter condition. Further, since Anderson et al. (Note 1) compared subjects deprived of food for only 40 h, as opposed to 46 h in the Meryman study, deprivation level in the present study was extended to the latter. Third, at least one of Ison and Krauter's studies entailed startle testing following the first complete deprivation cycle, rather than after

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full acclimation to the deprivation routine. Neither Meryman nor Anderson et al. tested until subjects had been deprived for at least 1 week. Thus, in the present study, half of the subjects were tested beginning with the first 48-h deprivation cycle and the other half were not tested until 15 days had elapsed, in an attempt to assess whether time of test, in relation to the deprivation regimen, might be an important transexperimental difference.

METHOD

Subjects

Twenty-four male naive rats, supplied by Kind Laboratories, Oregon, Wisconsin, were used. They were 90-100 days of age at the beginning of the study, acclimated to the laboratory for 7 days prior to beginning the project, caged singly, and weighed and handled daily during this period.

Apparatus

A startle test chamber, a sound-deadened, ventilated, and light-controlled housing enclosure, a proximal 10.3-cm (diameter) Quam speaker, and a Grason-Stadler (Model 901B) white-noise generator were the major pieces of equipment. Ancillary devices included an inexpensive oscilloscope to monitor the voltage output of the startle-transduction system, a General-Radio sound-intensity meter to aperiodically calibrate level of the startle stimulus, an amplifier to increase the intensity of the startle stimulus, and a modified Digital Equipment Corporation (PDP-8I) laboratory computer located at some distance from the laboratory. The computer digitized the analog voltage output of the startle transducer, sampled the peak reading, and punched the value in 1-mV units on paper tape. The stabilimeter was modeled exactly after Hoffman and Fleshler (1964). The transduction system was akin to an accelerometer in which a permanently magnetized rod extended through the center of a solenoid coil. The chamber was tightly sprung so that even vigorous movements by subjects did not visibly cause its displacement. This markedly restricted movement of the magnetic core within the coil, the result being an approximate linear output over the range of forces exerted by rats in response to the acoustic startle stimulus.

Calibration of this device entailed dropping a bolt (via solenoid operation) from 5.1 cm above the chamber prior to testing. No measurement deviations of consequence occurred with repetitions of this procedure. The visual oscillographic output of this transduction device to a startle response is an initial upward (positive) spike followed by a markedly attenuated opposite voltage swing and little electrical activity thereafter, suggesting highly effective damping by the system. Since all of the several hundred rats that have been startle tested in this chamber have invariantly evinced the full startle within 30 msec of the acoustic input, only this period was sampled for peak reading during the experiment. Sampling rate was 1 kHz/sec.

The dimensions of the chamber were 8.0 x 7.5 x 22.0 cm (height x width x length), and subjects could be inserted or removed through a hinged door that was not physically connected to the chamber per se. The speaker was located 20.0 cm directly to the side and on the same horizontal plane, slightly to the left of the center of the chamber, so that no obstruction existed between subject and acoustic stimulus. Stimulus duration was 20 msec, and peak intensity was approximately 110 dB (A-weighted measurement). Considerable experimentation in our laboratories has shown this to be a highly effective startle stimulus.

Design

Half of randomly formed hungry and satiated groups were startle tested on each of nine consecutive sessions, beginning with the first 48-h deprivation cycle. The design for this aspect of the study conformed to a 2 by 9 by 5 ANOVA in which these consecutively tested hungry and nondeprived groups were given five startle trials on each of the nine consecutive sessions. Testing was delayed for the remaining halves until Test Days 8 and 9. This permitted two 2 by 2 by 2 by 5 factorial configurations in which deprivation condition and delay of test from onset of food removal constituted the first two between-groups variables and test days and startle test trials constituted the remaining within-subjects dimensions. One of these analyses compared startle performances with the consecutively tested groups for the first two and the other for the last two test sessions.

Procedure

All subjects were handled and weighed every other day during the experiment. Body weight on the day before experimentation served as a criterion variable to rank all rats. For each consecutive set of four, subjects randomly were assigned to one of the four groups using a randomized-blocks practice. (A further division included random assignment of half of each group to one of two replications, run on alternate days. This conformed to a random-replications procedure following Lindquist, 1953.) Food removal for deprived rats was timed so that testing for respective replications could be consummated at the same time each day. Food intake for these subjects was regulated so that body weights approached 80% of respective ad-lib levels at about the 5th deprivation day. (About 2 h of ad-lib access to food every 2 days was needed to maintain this criterion. Free access to water was maintained throughout the study for all rats. Control groups were on an ad-lib feeding schedule throughout the study.) Order of testing was randomized by group, condition, and replication, and testing was conducted at the same time of day by replication.

A given test session consisted of placement of the subject into the test chamber followed by a 5-min quite period. The initial acoustic stimulus was then delivered, and four additional presentations followed at approximately 2-min intervals (±30/sec). Subjects were returned to their cages and weighed and fed, if appropriate. For one 46-h hungry and one control group, this procedure was repeated nine consecutive times; sessions took place 46-48 h apart. Testing was delayed for the remaining two groups until Day 8, and two consecutive sessions spaced about 46-48 h apart followed thereafter for these subjects.

RESULTS

The startle scores for each subject for each trial, indexed in terms of 1-mV units, were subjected to a square-root transformation following the suggestion of Winer (1971). This transformation also made the data somewhat more comparable with those of Anderson et al. (Note 1), as well as reducing heterogeneity of varaince. These data, averaged by trials for each day and group, are shown in Figure 1.

The two groups tested over nine consecutive sessions (Groups EC and CC) evinced nearly identical startle magnitudes throughout, although the nondeprived rats (Group CC) showed nominally larger responses on 6 of the 9 days. These larger responses primarily were due to the somewhat larger difference favoring nondeprived rats on the initial trial of most sessions. This same relationship was duplicated in the delayed-test groups (Groups ED and CD). That is, the nondeprived rats (Group CD) of this comparison also showed a tendency to evince marginally greater overall startle responses

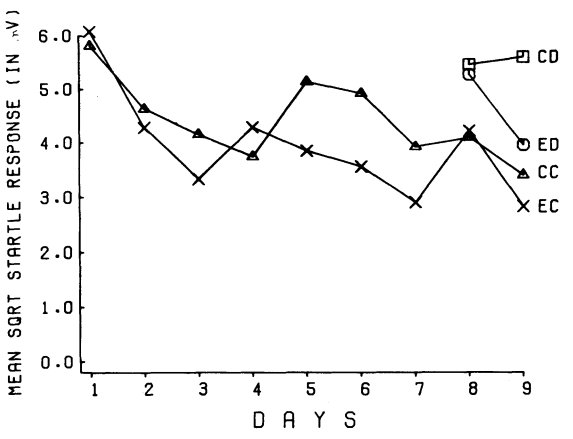

Figure 1. Mean square-root startle amplitudes (in millivolts) for satiated (C) and 46-h hunger-deprived (E) groups, tested either nine consecutive (C) sessions following onset of deprivation or only on Sessions 8 and 9 (D). Respectively, the four groups were consecutively tested hungry (EC) and satiated (C) and delay-tested hungry (ED) and satiated (CD) groups.

than the 46-h deprived rats. Also, although performances of the delay groups were more like those of the consecutively tested groups on initial than on terminal test sessions, no reliable group differences emerged for comparisons based on performances for either of these session sets. Finally, progressive declines in startle magnitude occurred both over test sessions and over trials within sessions for all but the CD group. The latter showed a slight increase in magnitude from Test Day 8 to Day 9.

A 2 (Groups EC vs. CC) by 9 (test sessions) by 5 (trials) ANOVA was applied to the transformed scores of the consecutively tested rats. The effects for trials [$F(4,40) = 6.8$, $p < .01$], days [$F(8,80) = 3.45$, $p < .01$], and for the Trials by Days interaction [$F(32,320) = 1.6$, $p < .03$] were reliable. No other effects emerged as significant, although the Groups by Trials interaction approached reliability [$F(4,40) = 1.98$, $p < .12$]. This was due to the tendency, noted above, for nondeprived rats to show somewhat larger startle magnitudes than hungry subjects on the first trial of each day (all $ps > .06$).

Two additional major analyses were performed, each being a 2 (deprived vs. nondeprived) by 2 (consecutive vs. delayed testing) by 2 (test sessions) by 5 (trials within sessions) ANOVA involving all groups. The first was applied to the startle data of Sessions 1 and 2, and the second to Sessions 8 and 9. The days and the Trials by Test Sessions effects were reliable for both ($p < .035$ and $p < .001$, respectively). Moreover, the Trials by Groups by Days triple interaction approached reliability for the initial-sessions analysis [$F(4,80) = 1.95$, $p = .11$] However, follow-up paired comparisons for hungry vs. nondeprived groups for each trial of each of Sessions 1 and 2 yielded no reliable group effects for any trial (all $ps > .08$). For those trials on which group differences approached significance, that is, a p value of .10 or less, absolute startle magnitudes for the nondeprived rats exceeded those of the 46-h hungry.

DISCUSSION

The major point of this report was to show that the effect upon startle magnitude of an unlearned appetitive "state," hunger, is not clear, consistent, or easy to duplicate. The present study contributed yet another indecisive outcome in this connection, since, strictly speaking, no effects other than the standard startle-habituation functions were obtained. And, while generally consistent with the null hunger-startle outcome of Anderson et al. (Note 1), a further seeming puzzle is that the direction of the present findings were more in accord with those of Ison and Krauter (1975) than with those of Meryman (Note 2) on this occasion. Whenever group differences herein approached significance, nondeprived rats evinced the larger startle magnitudes. However, these tendencies toward reliability were not related to aspects of the testing regimen, nor was it possible to discern from the design of the present study whether the use of nondeprived controls or of a deprivation cycle somewhat longer than that of Anderson et al. (Note 1) contributed to these somewhat nebulous group differences.

The major conclusion regarding whether startle is potentiated, retarded, or unaffected by an irrelevant, unlearned appetitive "state" seems to be that, based upon the present results and the conflicting findings previously reported, there is too little consistency across outcomes by which to reach resolution on this issue. Until those variables can be isolated that may have contributed to the apparent contradictions in deprivation-startle effects that thus far have been reported, confidence regarding the general meaning of any of these outcomes seems unwarranted.

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