Leverpress Escape/Avoidance Conditioning in Rats: Safety Signal Length and Avoidance Performance

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Leverpress escape/avoidance is an excellent model for assessing coping in rats. Acquisition of the leverpress response is determined by the interstimulus (signal-shock) interval, as well as the type and duration of the aversive event. One factor that has received less research attention is the safety or feedback signal. The safety signal presumably negatively reinforces leverpress responding through fear reduction. Here, we present a parametric manipulation of safety signal length and avoidance performance. All rats were trained with a 60-s tone conditioned stimulus and an intermittent 1-s, 1.0-mA footshock. Training was further accomplished with a 1-, 2-, 4-, or 6-min safety signal. Acquisition of the avoidance response was comparable at all safety signal durations. Rats trained with the shortest safety signal (1 min) exhibited more leverpresses during the safe period, a measure of anxiety. Thus, acquisition of the leverpress avoidance response was efficient regardless of safety signal duration, even though shorter periods were associated with greater anxiety.

ESCAPE/AVOIDANCE (E/A) conditioning involves the acquisition of an operant response that will either terminate (escape) or prevent (avoid) an aversive event. The nature of the arbitrary correct response is critical to acquisition of the avoidance response. Rats will learn a one-way shuttlebox response very rapidly, often within 10 trials (Brush, 1966). Other responses that are less like a species-specific defensive reaction (SSDR; Bolles, 1970) are learned much more slowly. An example of a response learned more slowly than shuttlebox running is pressing a lever to escape or avoid shock. Although the leverpress response is learned more slowly, a significant advantage is that acquisition can be evaluated. It has been noted for decades that the leverpress avoidance response is learned with difficulty (D'Amato & Schiff, 1964; Meyer, Cho, & Weseman, 1960). When a leverpress is the required response, most rats efficiently learn to escape (e.g., Davis, Porter, Burton, & Levine, 1976), but have difficulty making the transition to avoidance performance.

Berger and Brush (1975), however, reported that leverpress avoidance performance is vastly improved with several minor modifications to the procedure. These investigators extended the signal-shock interval to 20 sec or more. They also used intermittent rather than continuous shock, which is also associated with better avoidance performance (e.g., D'Amato, Keller, & DiCara, 1964). Most importantly, Berger and Brush (1975) utilized a

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discrete safety signal. The safety signal was a flashing light mounted on one wall of the operant chamber that remained on for 5 min after a leverpress. A discrete feedback signal, explicitly paired with a period of no shock, is thought to be reinforcing and is associated with improved performance (see Bolles & Grossen, 1970; Dinsmoor, 2001). However, the length of the safety signal appeared to be arbitrary. The purpose of the following study was therefore to parametrically manipulate the length of the safety period following a leverpress response to observe the effect upon escape/avoidance acquisition.

Although the length of the safety period in escape/avoidance conditioning has not been extensively examined, several relevant studies exist. Galvani and Twitty (1978) found no effect of safety signal duration in gerbils using a shuttlebox avoidance procedure. Candido, Maldonado, and Vila (1991), using a jumping avoidance response in female rats, also found no effect of safety signal duration. Although the previous studies found no effect of safety signal duration on avoidance performance, they utilized avoidance paradigms in which acquisition is relatively rapid (shuttle box and jumping). The effect of different safety signal durations using a leverpress paradigm, where avoidance is acquired more slowly, is unknown. Further, fear-induced freezing is purported to interfere with leverpress avoidance response. Since we were modeling our procedure on the Berger & Brush (1975) leverpress paradigm, we chose to study safety intervals that were both shorter and longer than the 5-min interval they employed. We hypothesized that longer safety signal durations would allow the animal more time to "relax" (Denny, 1971), and thus would be associated with superior avoidance acquisition.

Method

Subjects

Subjects were 48 male, Sprague-Dawley rats, obtained from Charles River, Kingston, NY. They were 60–90 days old and weighed 300–350 g at the time of testing. Subjects were maintained on ad lib food and water except during the escape/avoidance sessions. They were individually housed in plastic tubs within boxes that controlled temperature, airflow, and light/dark cycle. Animals were maintained on a 12/12 light dark schedule with lights on at 0700.

Apparatus

The escape/avoidance sessions were conducted in four boxes obtained from Coulbourn Instruments (model # H10–11R-TC). The chambers are 12" wide X 10" deep X 12" high, and are contained within outer boxes that attenuate sound and contain a fan for airflow. The background noise level inside the box with the fan running is 52 dB. Scrambled shock was delivered to the grid floor by Coulbourn shockers (model # H13–16), and sessions were run on Med PC or Graphic State software.

General Procedures

Animals were placed into an operant chamber with a lever manipulandum mounted into the wall, approximately 10 cm above the grid floor. A houselight was located on the upper portion of the wall opposite the wall containing the lever. After a brief (approximately 1 min) period of acclimation, a 1000 HZ tone, which served as the warning signal (WS) was turned on. The tone increased the sound level inside the chamber to 76 dB. If a leverpress did not occur within 60 sec, a 1.0 mA scrambled shock was presented through the grid floor. One-second shocks continued on a VT–60 sec schedule (range: 30–90 sec) until a leverpress occurred. An animal that did not respond for 20 minutes was given a "free" escape. A leverpress terminated the shock, the WS, and the houselight, and initiated the onset of a discrete safety signal (SS). The SS was a flashing light mounted 12 cm above the lever, on the opposite side of the chamber from the houselight and speaker. A leverpress that occurred during the initial 60-sec WS was defined as an *avoidance* response; any leverpress that occurred after 60-sec was an *escape* response. The major procedural difference between the groups was the *length* of the safety period following an escape or an avoidance response. Groups of 12 animals were trained with a 1-, 2-, 4-, or 6-min safety period. At the conclusion of the safety period, the safety signal terminated and the WS commenced. There was thus no stimulus-free inter-trial interval (ITI). Subjects were run for one 2-hr session per day for four consecutive days. There was a minor computer problem in the 1-min group whereby several of the animals received a small number of trials with less than a 60-sec WS. These trials occurred early in training when animals were primarily escaping and did not appear to affect their acquisition. The data were analyzed via mixed analyses of variance (ANOVA) models, with Newman-Keuls post hoc tests to detect specific differences between groups.

Results

Efficiency Ratios

We expected that animals trained with shorter safety intervals would perform more overall responses (escapes plus avoidances), given the fact that they had more opportunities to respond. This was true as the 1-min group averaged 177 total trials across the 4 sessions, the 2-min group averaged 136 trials, the 4-min group averaged 75 trials, and the 6-min group averaged 62 trials. Therefore, instead of analyzing number of responses, we calculated the efficiency ratio for each subject for each session (as in Steinmetz, Logue, & Miller, 1993). The efficiency ratio was the number of avoidance responses (conditioned responses, CRs) divided by the total number of leverpresses (escapes + avoidances + leverpresses during the safety period). Data from 4 subjects in the 6-min group were lost from the 4th session due to a computer error. The mean (± standard error of the mean) group values are presented in Figure 1. The efficiency ratios increased across sessions, but not differentially between groups. The analysis supported this observation. There was a main effect of session, $F(3, 188) = 24.51$, $p < 0.0001$, but neither an effect of group, $F(3, 188) = 1.58$, $p > 0.05$, nor an interaction, $F(9, 188) = 1.47$, $p > 0.05$. Newman-Keuls follow-up tests revealed that the 3rd and 4th session means did not differ, $p > 0.05$, all other possible differences were significant, p's < 0.05.

Number of Trials from Escape to Avoidance

As a measure of how quickly the animals transitioned to avoidance performance, we calculated the number of trials between the first escape response and the first avoidance response. These data were analyzed via a one-way ANOVA. No group differences were apparent, $F(3, 48) < 1.0$. The grand mean was 12.0 ± 1.5 trials.

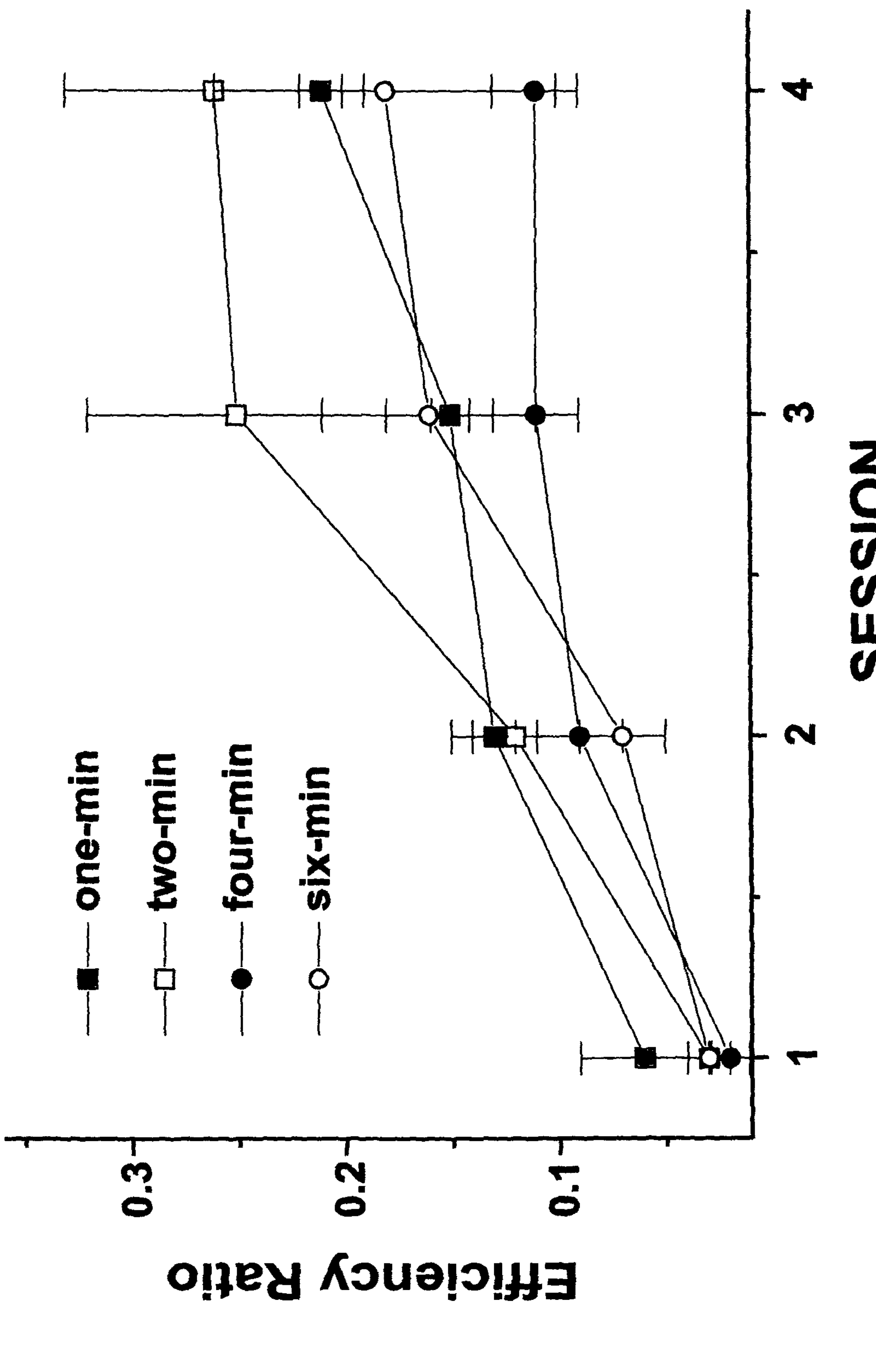

FIG. 1. The effect of length of safety on efficiency ratios across sessions.

Percent Avoidance

Percent avoidance was calculated by dividing the number of avoidances by the total number of responses (escapes + avoidances) per session. The mean (± standard error of the mean) group values are presented in Figure 2. Some of the animals would hit the lever during the very first WS presentation while exploring the chamber. Since these "pseudo-avoidances" occurred prior to any shock presentation, these responses were not counted in any of the dependent measures. Only responses that occurred *after* the first shock presentation are included. All four groups acquired the avoidance response equally well. Manipulation of safety signal length did not affect acquisition of the avoidance response. The ANOVA supported this observation, yielding a significant effect of session, $F(3, 192) = 27.52$, $p < 0.00001$, but neither an effect of group, $F(3, 192) < 1.0$, nor an interaction, $F(9, 192) < 1.0$. Overall, the third and fourth session means did not differ. All other session mean differences were significant, all p's < 0.05.

Leverpresses during Safety

If a rat pressed the lever during the safety period, the leverpress did not affect either shock presentation or the duration of the safety period. These non-reinforced bar presses are considered to reflect uncertainty and anxiety (see Berger & Starzec, 1988). However, since the subjects spent different amounts of time in safety, we calculated a rate of leverpressing per minute of safety. Subjects that had either 0 trials or 0 leverpresses during safety for a given session received a value of 0 for this measure. An inspection of this data (see Figure 3) revealed that the 1-min group (black squares) appeared to press the lever more during the safety period. The ANOVA confirmed this observation. There was a main effect of group, $F(3, 188) = 8.15$, $p < 0.001$, but no effect of session, $F(3, 188) = 2.04$, $p > 0.05$. These effects were superceded by the significant interaction, $F(9, 188) = 2.35$, $p < 0.05$. The 2-min group (open squares) made more leverpresses during safety than the 6-min (open circles) and 4-min (closed circles) groups during the first session, all p's < 0.05. The 1-min group (black squares) made more leverpresses during safety than the 4-min and 6-min groups during the first and last sessions, and more than the 4-min group during the third session, all p's < 0.05.

Discussion

The present parametric study demonstrated that the length of the safety period following an escape or avoidance response had no effect on acquisition of a leverpress avoidance response. Subjects trained with a 1-min, 2-min, 4-min, or 6-min safety period after an escape or avoidance response all acquired the avoidance response comparably, approaching or exceeding 50% avoidance by the final session. All groups also had equivalent efficiency ratios across the four sessions. Group differences were observed in number of leverpresses per minute of safety. The shortest safety period group (1-min) pressed the lever per minute of safety more than all other groups. Further, the 2-min group pressed the lever more than the 4 and 6-min groups during the first session.

The present results are in agreement with past studies finding no effect of safety signal length in escape/avoidance acquisition. Galvani and Twitty (1978) reported that the length of the feedback safety signal had no effect on acquisition of a shuttlebox avoidance response in gerbils. The longest safety signal used in that study, however, was 10-sec.

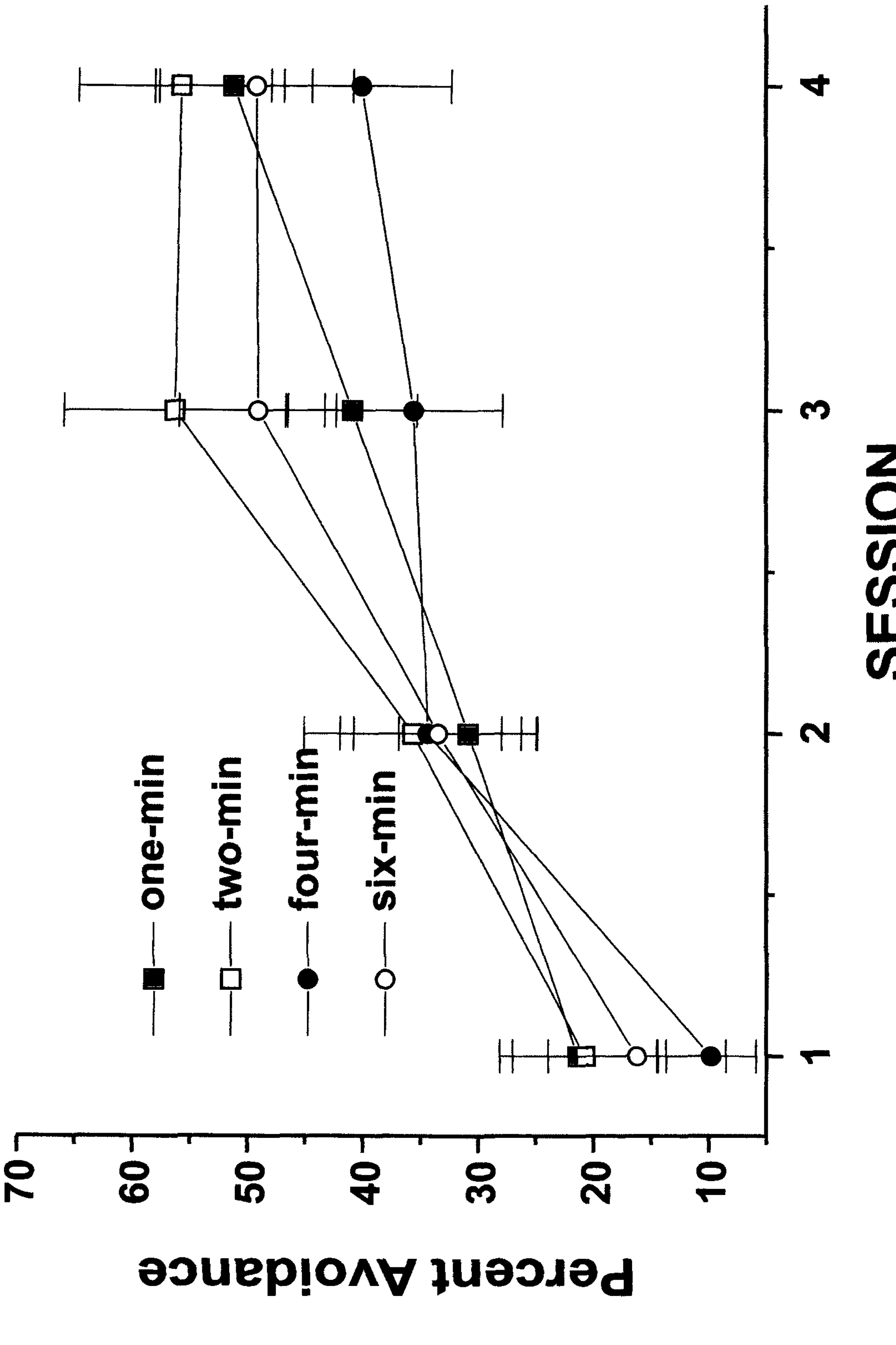

FIG. 2. The effect of length of safety on percent avoidance across sessions.

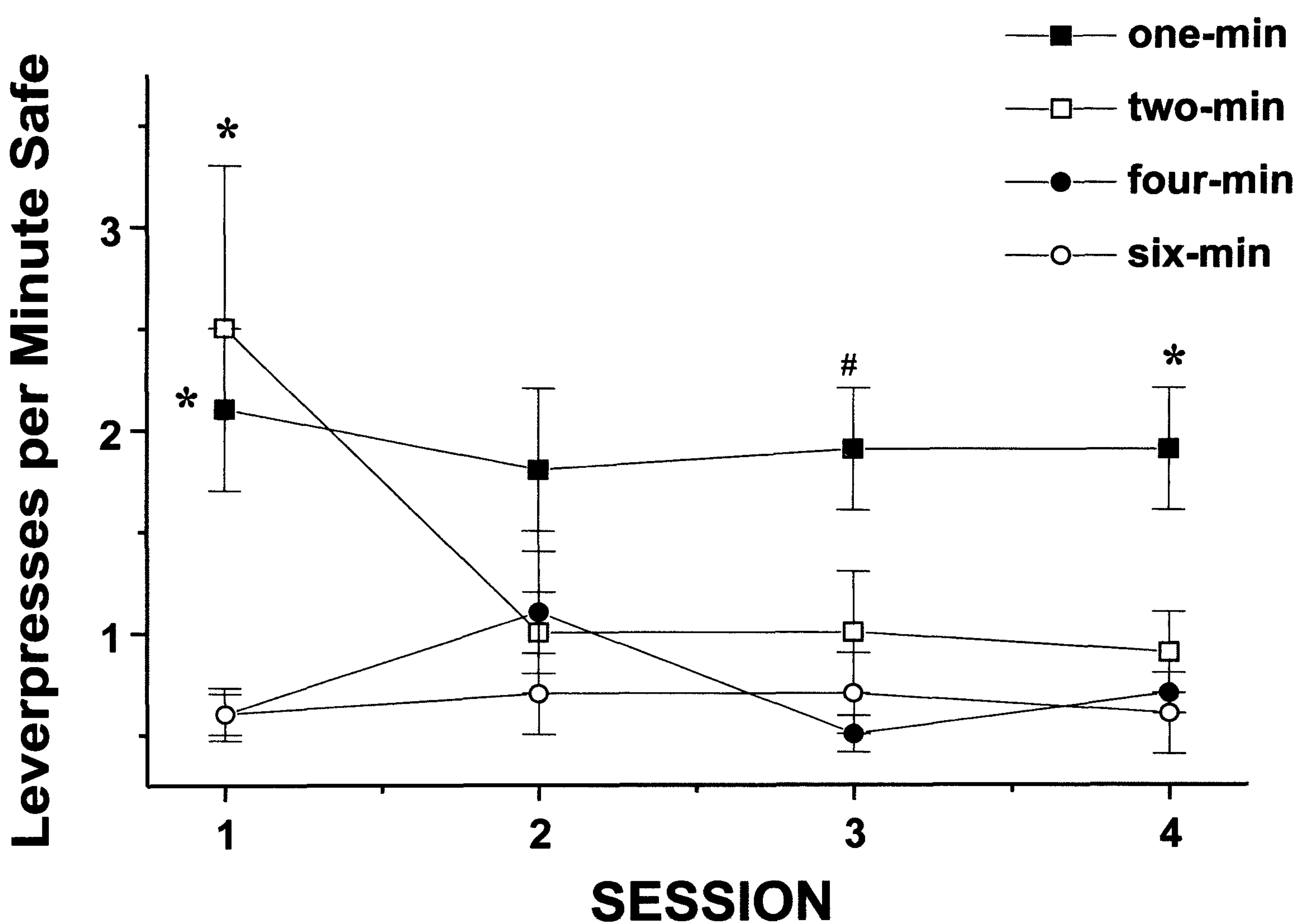

FIG. 3. The effect of length of safety on leverpresses per minute of safety across sessions. *Different from both the 4 and 6-min groups. #Different from the 4-min group.

Candido, Maldonado, and Vila (1991) extended the length of the safety periods studied from 3 to 60 sec. They found that all led to successful acquisition of a jumping avoidance response in female rats. A feedback or safety signal can be construed as having a cognitive or informational function, by virtue of predicting a period of no shock (Galvani, 1979). Further, a safety signal could also have a fear-reducing component. The classic "two-factor theory" of Mowrer (1947) postulated that a warning signal presented prior to the aversive event becomes a conditioned stimulus (CS) for the aversive unconditioned stimulus (US). Performance of the response also terminates the warning signal; fear reduction is presumed to negatively reinforce the avoidance response. Further, a discrete safety signal paired with a period of no shock will acquire reinforcing fear-reducing properties (see Dinsmoor, 2001). It is apparently not even critical what form the safety signal takes, as long as it predicts a period of no shock; rats trained with a period of darkness for the safety period acquired the avoidance response similarly to rats trained with the flashing light safety signal (unpublished observations; also see Bower, Starr, & Lazarovitz, 1965).

In the current study, all safety intervals should have had equal informational value in reliably predicting a shock-free period. What may have differed were the fear-reducing properties of different lengths of safety. Different feedback signal lengths are associated with different amounts of both contextual fear conditioning and inhibition conditioned to the safety signal (Rosellini & DeCola, 1988). Since there were no group differences in avoidance performance, the current data supports the idea that the cognitive or informational role of the safety signal is more important than the fear reduction component (see Candido, Maldonado, & Vila, 1991, for a discussion).

The 1-min group did press the lever more during safety than the other groups. The greater number of leverpresses might simply reflect the tendency for rats to press the lever immediately after an escape/avoidance response (i.e., response perseveration). Longer safety periods would then dilute the concentration of leverpresses during the safety period. However, a stepwise decrease in leverpresses per minute of safety in the 2-min, 4-min, and 6-min groups was not apparent. Further, rats trained with the 1-min safety period animals appeared to vocalize more to shock and were more difficult to handle at the conclusion of each session, contributing to the impression that these rats were in fact more anxious. It is interesting to note that this apparent heightened state of anxiety did not interfere with acquisition of the avoidance response.

The neural basis of escape/avoidance conditioning is beginning to be understood. Regions of the amygdala have a role in both contextual fear conditioning (Blair, Schafe, Bauer, Rodrigues, & LeDoux, 2001) and avoidance conditioning (Holahan & White, 2002), and thus must play some role in the acquisition of the leverpress avoidance response. Lesions of cerebellar nuclei also blocked acquisition of a leverpress avoidance response; and retarded escape learning as well (Steinmetz et al., 1993). Future work from our laboratory will attempt to further elucidate the factors affecting acquisition of leverpress avoidance, as well as the underlying neurobiology of the response.

Note

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