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On the development of inhibition of delay by rats of the Syracuse High- and Low-Avoidance strains

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Abstract. Male and female of the Syracuse strains received 60 trials of avoidance training in a two-way shuttlebox at 90 days of age. Approximately one month later they received additional training at the rate of 30 trials per day for 11 days (330 trials), during which acquisition of inhibition of delay was studied. Animals of the Syracuse High Avoidance (SHA/Bru) strain reached an asymptote of nearly 100% of avoidance responses (AVRs) by trial of 30 of original training, whereas those of a Low Avoidance (SLA/Bru) strain remained at nearly 0 AVRs. During subsequent extended training the SHA/Bru animals remained at 95-100% AVRs. By the end of extended training males of the SLA/Bru strain had approached 40% AVRs, the females 25% AVRs. SHA/Bru animals of both sexes showed a progressive increase in the relative frequency of long latency AVRs, indicating the development of inhibition of delay. In contrast, animals of the SLA/Bru strain, especially the males, showed a progressive increase in the relative frequency of short latency AVRs, presumably reflecting the continued acquisition of the avoidance response. These results are interpreted in terms of two-process theory of avoidance learning and in terms of the development of temporal conditioning or learning as a result of differential extinction of conditioned fear.

Key words: avoidance learning, genetics, Syracuse strains, inhibition of delay, response latency distributions

INTRODUCTION

The Syracuse strains

As their designation suggests, the Syracuse strains were selectively bred for extreme differences in instrumental active avoidance learning (Brush 1977, Brush et al. 1979). The phenotypes resulting from more than 50 generations of selection have been characterized in detail elsewhere (Brush 1991). Briefly, the Syracuse High Avoidance (SHA/Bru) animals, on average, avoid shock on 40 (67%) of their initial 60 trials of training and reach an asymptote of nearly 100% avoidance responses (AVRs) by trial 30. In contrast, the Syracuse Low Avoidance (SLA/Bru) animals avoid shock rarely (0-1%) during their initial training. However SLA/Bru animals are superior to SHA/Bru rats in two forms of Pavlovian aversive conditioning: conditioned suppression (CER) and conditioned flavor/taste aversion (Brush et al. 1988, von Kluge and Brush 1992). In general terms, the SLA/Bru animals appear to be more fearful and/or emotionally reactive and to acquire Pavlovian aversive conditioning more readily than SHA/Bru animals.

Two-process theory divides the dynamics of avoidance learning into two components: (1) Pavlovian conditioning of fear and (2) instrumental learning of the escape and avoidance responses (Mowrer 1939, 1960, Rescorla and Solomon 1967). In this model, Pavlovian conditioning of fear of the warning signal (WS) and of contextual (apparatus) cues is acquired on early escape trials when the electric shock occurs. Instrumental learning of the escape response is indexed by a decrease in its latency, presumably reflecting the primary negative reinforcement of shock termination. Fear conditioned to the WS is the presumptive acquired motivation for the instrumental avoidance response which is negatively reinforced by termination of the WS and the resulting reduction of the fear conditioned to that cue, and by prevention of an impending shock (Kamin 1956, 1957, but see also Bolles et al. 1966). As AVRs increase, frequency of shock decreases and the fear conditioned to the contextual cues undergoes differential extinction because of the long duration of exposure to those cues during the "safe" intertrial interval (ITI) relative to the brief exposures to the WS on avoidance trials, i.e., the animals learn to discriminate between the "dangerous" situation signalled by the WS and the "safe" situation of the ITI. This account may adequately describe the situation for the successful learner, e.g., the SHA/Bru animal. But what of the SLA/Bru animal witch rarely if ever avoids? One possible interpretation is that fear conditioned to the WS and the contextual stimuli is so intense in these emotionally hyperreactive animals that these stimuli elicit only freezing (Bolles 1971).

The question then arises whether the SLA/Bru animals are capable of learning to avoid in the shuttle box if they have additional training. Bignami (1965) and Bammer (1978, see also Bond 1984) selectively bred for differences in shuttle-box avoidance learning and used multiple sessions of training as their bases for selection. Their low-avoidance lines (RLA and ALA, respectively) did show modest levels of avoidance responding by the end of training[1]. It is possible, therefore, that SLA/Bru animals simply learn to avoid relatively slowly and that with additional training they could eventually learn to avoid and to discriminate between the aversive WS and "safe" contextual cues of the ITI. If this were to occur, AVRs would be expected to increase slowly, and ITRs would be expected first to increase and then to decrease as differential extinction of contextual fear reduces freezing to those cues.

We have previously reported (Gendron and Brush 1996) the effect of giving additional training, at the rate of 30 trials per day for 11 days, after these animals had received their initial 60 trials of training. SHA/Bru animals had already reached asymptote at nearly 100% AVR by the end of their initial training and remained there during the subsequent 11 days. In contrast, SLA/Bru animals slowly increased their AVRs from around 10% at the end of initial training to around 25% after 11 additional days (330 trials). ITRs made by SHA/Bru animals were elevated when the additional training was started but rapidly declined to the low level shown by SLA/Bru animals throughout training.

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[1] In subsequent research with the Roman lines Bignami et al. (1965) and van der Staay et al (1983), using subsequent generations bred from the Roman lines, found rapid acquisition in SLA animals. In the latter report RLA and RHA animals did not differ from each other. However Durcan et al. (1984) found evidence that the animals tested by van der Staay et al. may have been genetically deviant from the established Roman strains as a result of limited sampling (number unspecified) of animals for breeding stock from the established Roman colonies. Durcan et al. found the expected poor performance of RLA animals in their initial 30 trials of training, although variability was great so that approximately 30% of the RLA animals made more than 50% avoidance responses. However, none of these studies examined the effects of additional training beyond the usual initial session of 30-50 trials.

Inhibition of delay

The concept of inhibition of delay has been around ever since Pavlov (1927) gradually extended the CS-US interval during appetitive conditioning of dogs; he found that the latency of the conditioned salivary response (CR) increased as the CS-US interval increased. He also showed that an external inhibitor could release the CR from its inhibition if it were presented early in the long CS-US interval, thus demonstrating that the phenomenon involved an active inhibitory process. Since then the phenomenon has been reported by Hendry and Van-Toller (1965) and Millenson and Hendry (1967) to characterize the conditioned emotional response of rats using the CER suppression procedure wich was first described by Estes and Skinner (1941) and subsequently elaborated by Kamin (1965). In those studies, conditioned suppression of the operantly rewarded bar-press response gradually disappeared or was markedly reduced if conditioning was continued beyond 50 trials (50 CS-US pairings). This was attributed to a progressive increase in the latency of onset of suppression within the CS-US interval, i.e., to gradual development of inhibition of delay. Brush (1968, unpublished) confirmed this effect in an experiment involving three stimuli (light, clicking sound and white noise) that were shown in pilot experiments to be equally salient by their having equal rates of excitatory aversive conditioning. Two animals received CER conditioning using one of the stimuli (noise) at the rate of four trials per day, five days per week for 18 weeks (360 trials). Suppression ratios showed a progressive decrease during the first few days or weeks of initial conditioning (suppression ratios decreased to approximately 0.1-0.2) followed by a progressive increase in the ratio to approximately 0.5, no measurable change in responding during the 2-min i.e., CS. Both animals then showed a significant decrease in the suppression ratio when conditioning continued for five days using the light stimulus, which suggested there was some degree of stimulus specificity of timing behavior. When conditioning continued with the third stimulus, the clicking sound, both animals showed rapid acquisition to moderate levels of conditioned suppression for about five days followed by rapid increases in the suppression ratio back to around 0.5 during the next 2 weeks. These results may be interpreted in terms of acquisition of timing behavior (inhibition of delay?) which is specific to the stimulus used during the prolonged conditioning, but wich generalizes well to another equally salient stimulus, especially one of the same sensory modality.

Zieliński and coworkers (Zieliński 1966, Zieliński and Walasek 1977, Zieliński et al. 1993) have researched this phenomenon extensively and found evidence that inhibition of delay occurs in a variety of aversive conditioning paradigms, both Pavlovian and instrumental. For example, Zieliński (1966) showed that it was not necessary to extend the CS-US interval gradually to show inhibition of delay in the CER suppression paradigm with rats. Rather, with a long CS-US interval from the onset of conditioning (6-min CS-US interval superimposed on a 2.5 min VI schedule, with or without limited hold [(VI vs. VI-LH), inhibition of delay developed significantly during the course of conditioning. That is, conditioned suppression increased during successive 2-min epochs of the 6-min CS-US interval, and the magnitude of this effect increased as a function of number of conditioning sessions. Furthermore, Zieliński demonstrated that the increase in probability of positive reinforcement that results from any suppression of bar pressing on a VI schedule had little effect on the phenomenon: the degree and temporal pattern of conditioned suppression was the same in groups trained with and without the LH contingency, which controls and roughly equates the probability of reward in the two groups.

This experiment

Zieliński et al. (1993) also showed that inhibition of delay can occur in rats during instrumental aversive conditioning, i.e., avoidance training in a two-way shuttle box. In addition, Zieliński et al. (1995) showed the relevance of salience of the warning signal for the development of inhibition of delay in shuttle-box avoidance learning. The phenomenon was more readily obtained using a strong fear-eliciting auditory stimulus than a less salient visual stimulus. The auditory cue also elevated the overall level of avoidance responding, so it is not clear whether the effect of salience on inhibition of delay is because the auditory stimulus has greater intrinsic stimulus control, including that of inhibition of delay, or it is more effective in eliciting the behavior which mediates the operation of inhibition of delay, i.e., it increases the frequency of avoidance responding and offers, thereby, a more stable base for the measurement of inhibition of delay. Alternatively, the more frequent avoidance responding to the salient stimulus could enable the animal more frequently to contact the temporal characteristics of the WS-shock interval, to become more temporally conditioned and, therefore, to display inhibition of delay.

This report describes the changes in the distribution of latencies of AVRs in SHA/Bru and SLA/Bru animals during the course of extended avoidance training. Our earlier report (Gendron and Brush 1996) described the effects of extended training on the frequency of AVRs and ITRs.

METHOD

Subjects

The subjects were 31 male and 32 female rats from the SHA/Bru strain and 32 males and 37 females from the SLA/Bru strain. At 90 days of age they were tested for the avoidance phenotype (10 pretest trials and 60 trials of avoidance training) and entered this experiment around one month later at approximately 122 days of age. Throughout training the warning signal (WS) was light and white noise presented in the compartment occupied by the rat, the electric shock was scrambled 60-Hz a.c. at 0.25 mA, the WS-shock interval was 5.0 s, the intertrial interval was 1-min., and the maximum shock duration was 35 s. A shuttle response within the WS-shock interval terminated the WS and avoided the shock; a response after shock onset terminated both the WS and the shock and was scored as an escape response (see Gendron and Brush 1996, for details). SHA/Bru animals were from generations CD 28 and 29 and the SLA/Bru animals were from generations CD 26.5 and 27.5[2].

Procedure

After phenotypic confirmation, the animals received training at the rate of 30 trials per day for 11 days. The daily 30 trials of training used the same parameters as the phenotypic determination, i.e., the WS was light and white noise, the WS-shock interval was 5.0 s, the response terminated shock was 0.25 mA, had a 35-s maximum duration, and the ITI was 60 s (Brush et al. 1979).

RESULTS

Figures 1 and 2 present the cumulative relative frequency distributions of response latencies for SHA/Bru and SLA/Bru males and females, respectively, on days 1, 5 and 11 of training of this experiment, i.e., the data for day 1 are for the first 30 trials of extended training, not original acquisition. The latencies are tabulated in 0.5-s. bins, and latencies greater than 9.5 s are collapsed into the last (9.5 s) bin of these figures. Responses in the latency bins ≤ 5.0 are AVRs, whereas those in bins >5.0 are escape responses. The significance of differences in cumulative frequencies was tested by the Wilcoxon matched-pairs signed-ranks two-tailed tests, and the frequencies in each latency bin were compared between all possible pairs of days within each sex and strain.

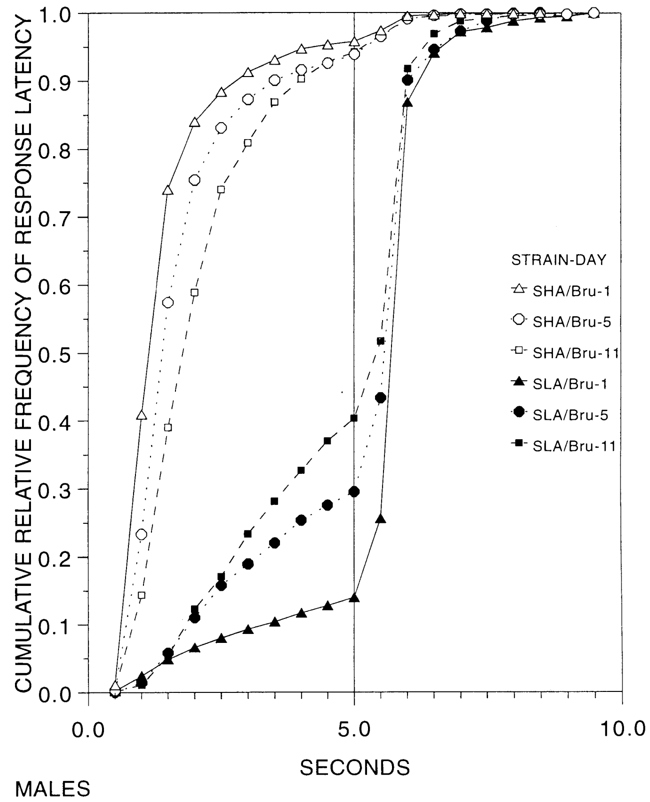

Fig. 1. Cumulative relative frequency distributions of response latency for males of the SHA/Bru and SLA/Bru strains on days 1, 5 and 11 of training.

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[2] The CD designation is because an infection by Mycoplasma pulmonis required that SHA/Bru and SLA/Bru animals, many generations ago, be caesarean derived and fostered to germ-free dams. They were, and are now gnotobiotic, i. e., free of M.pulmonis and other viral infections. The half generation designation for the SLA/Bru animals is because it was necessary once (because of M-pulmonis) to breed them from two adjacent generations. Their offspring were given the half-generation designation, as was done for all following generations, although all subsequent breeding was within a given generation.

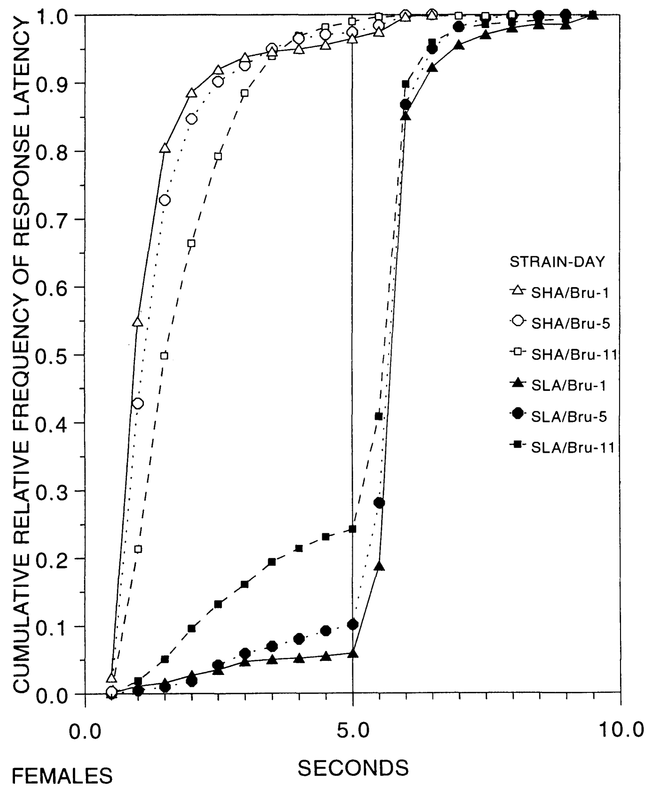

Fig. 2. Cumulative relative frequency distributions of response latency for females of the SHA/Bru and SLA/Bru strains on days 1, 5 and 11 of training.

It is apparent in Fig. 1 that the proportion of short-latency AVRs by SHA/Bru males decreases progressively from day 1 to day 5 and from day 5 to day 11. The frequency of AVRs, however, remained constant at approximately 95% throughout training (Gendron and Brush 1996). The differences between days 1 and 5 are significant ($P \leq 0.05$) for all latency bins except the first (0.0-0.5 s). The differences between days 5 and 11 are significant only between 1.0 and 3.0 s. Those between days 1 and 11 are all significant except the first. None of the day-wise comparisons among escape latencies are significant.

For females of the SHA/Bru strain a similar pattern emerges in Fig. 2, namely a progressive, albeit not as dramatic, decrease in the relative frequency of short latency AVRs from day 1 to day 5 and from day 5 to day 11. Wilcoxon tests indicated that the differences between days 1 and 5 are significant only up to 1.5 s. Those between days 5 and 11 are significant from 1.0 through 3.0 s and again at 5.0 s. The differences between days 1 and 11 are all significant except those at 3.5 and 4.0 s. Escape latencies at 5.5 s are significantly different between days 5 and 11 and between days 1 and 11.

In contrast to those findings for the SHA/Bru animals, the distributions of the latencies of AVRs made by SLA/Bru males (Fig. 1) show the reverse effect of days, i.e., there is a progressive increase in short latency responses from day 1 to day 5 and again from day 5 to day 11. The differences between days 1 and 5 are significant for latencies 3.0-5.5 s. Those between days 5 and 11 are also significant from 3.0-5.5 s, whereas the differences between days 1 and 11 are significant from 2.5-6.0 s.

A similar pattern, although again not as dramatic, occurs for SLA/Bru females as seen in Fig. 2. As in the males, the distributions of the response latencies of the SLA/Bru females show a progressive increase in the proportion of short-latency responses from day 1 to day 5 and more markedly from day 5 to day 11. Wilcoxon tests indicated that the differences between days 1 and 5 are significant only in the escape range from 5.5-9.5 s. The differences between days 5 and 11, on the other hand, are significant from 1.0-5.5 s except for the 6.0-s bin.[3]

DISCUSSION

What do these results tell us about the acquisition of inhibition of delay? First, it should be clear that the absence of inhibition of delay in SLA/Bru animals is not because of a genetic defect in those animals nor is its presence in SHA/Bru animals because at the action of a "super" gene in them. Granted, there is a genetic difference between the animals of the two strains that leads to the differential performance of the avoidance response seen in this experiment. However, the genetic variation in performance of animals of these strains enables exploration of process involved in learning and

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[3] Given the clearly correlated nature of the data within each strain and sex and the need to compare days 1 vs. 5, 5 vs. 11 within each strain and sex and for each time bin, it would be desirable to make some correction for the unavoidable correlations wich influence these multiple comparisons. We know of no such corrective procedure. However, with respect only to avoidance latencies, there are 10 0.5-s time bins for each of the three between-day comparisons. Of these 30 statistical comparisons for each strain and sex, 73% and 57% are significant ($P \leq 0.05$) for the SHA/Bru males and females. These percentages so exceed the 0.05 probability of the alpha rejection region that erroneous rejection of H_0 because of these correlations seems exceedingly unlikely.

performance that aid or impede development of inhibition of delay.

One enormous difference between the animals of Syracuse strains in this experiment is that SHA/Bru rats develop inhibition of delay during the course of their nearly 100% avoidance responding, whereas SLA/Bru animals do not. Indeed, the change of the distribution of AVR latency shown by the SLA/Bru animals was quite reverse of that shown by SHA/Bru rats, i.e., rather than a progressive increase in AVR latency, SLA/Bru animals show a progressive decrease in response latency over a course of training.

Inhibition of delay is presumed to be a property of Pavlovian conditioning. In that context we noted previously that SLA/Bru animals are superior to SHA/Bru rats in two forms of Pavlovian aversive conditioning: CER suppression and conditioned taste aversion (Brush et al. 1988, von Kluge and Brush 1992). It cannot be concluded, therefore, that the absence of inhibition of delay in SLA/Bru rats is because they have not been adequately conditioned to fear the WS and/or the contextual cues that are associated with shock. Indeed, that association was made on nearly 300 occasions during the course of this experiment, i.e., on their many escape trials. During conditioning in the CER-suppression paradigm, only 3 or 4 days (12-16 trials) were required to establish significant conditioned suppression in SLA/Bru animals, whereas SHA/Bru rats required nearly 50 CS-US pairings and an increase in shock intensity to establish conditioned suppression (Brush et al. 1988). Similarly, in establishing a conditioned flavor aversion, only two trials were required to establish a significant aversion in animals of both strains (von Kluge and Brush 1992). Thus, the rate of aversive Pavlovian conditioning may, to some extent, be situation specific, but clearly the SLA/Bru animals are not deficient in this capacity.

The other enormous difference in the behavior of the SHA/Bru and SLA/Bru animals in this experiment is the level of performance of the avoidance response. This difference is, of course, genetically determined, at least in part. As can be seen in the figures, where the latency distribution functions intersect the vertical line designating the end of 5 s CS-US interval, SHA/Bru animals of both sexes were close to 100% AVRs throughout the experiment, whereas SLA/Bru males were at about 45% AVRs and females at about 25% AVRs by the 11th day of training. This suggests that development of inhibition of delay may be dependent on a high level of performance of the index response.

This relationship suggests that inhibition of delay is not just an artifact of measurement in the sense that more frequent responding makes it easier to detect the occurrence of the phenomenon. In fact, the orderly progression of increasing response latencies seen in all demonstrations of inhibition of delay, whether the behavior is appetitively or aversively based, suggests that learning is involved. What form of learning? Temporal conditioning is the Pavlovian candidate of choice. Temporal discrimination, also referred as timing behavior, is the instrumental candidate of choice. Whatever terminology or paradigm one prefers, it seems obvious that differential discrimination is involved. In Pavlovian paradigms temporal conditioning requires the operation of differential excitation and extinction: initial excitatory conditioning of the onset and of the entire duration of the CS, with subsequent differential extinction of excitation to the onset and early portions of the CS that are temporally remote from the reinforcing US. In instrumental paradigms similar mechanisms apply: operant timing paradigms, e.g., fixed interval schedules of food reward, Sidman avoidance schedules, differential reinforcements of low rate (DRL) schedules, all involve excitatory control of the operant response at the beginning of the timed interval with subsequent differential excitation of that behavior in favor of behavior later in the timed interval.

It appears that the development of inhibition of delay in discrete-trial active avoidance learning may involve these same principles. The genetic models used in this experiment provide an individual-difference manipulation of independent groups, rather than within-group correlation, that permits clarification of this process.

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