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A New Method For Aversive Pavlovian Conditioning of Heart Rate in Rhesus Monkeys

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KALIN, N. H., S. E. SHELTON, R. J. DAVIDSON AND D. E. LYNN. *A new method for aversive Pavlovian conditioning of heart rate in rhesus monkeys.* PHYSIOL BEHAV **60**(4) 1043–1046, 1996.—Aversive Pavlovian conditioning is an important tool used to investigate neurobiological mechanisms underlying the acquisition and expression of fear. Most studies have used nonprimate species employing electrical shock as the unconditioned stimulus (US). Although important advances have been made in understanding the neural substrates of conditoned fear, the extent to which these findings apply to primates is unclear. Research in primates has not progressed because of the lack of a conditioning paradigm that does not use shock. Therefore, we developed a method that uses a US consisting of a loud noise coupled with a stream of compressed air aimed at the face to aversively condition heart rate response in rhesus monkeys. With this US, rhesus monkeys rapidly acquire a conditioned bradycardia. The availability of an easy, reliable, and efficient method of aversive conditioning that does not require electrical shock, will facilitate studies investigating neurobiological mechanisms underlying the acquisition and expression of fear in primates.

Aversive Conditioning Primates Bradycardia

NUMEROUS studies have used aversive Pavlovian conditioning paradigms in nonprimate species, to study mechanisms underlying the acquisition and expression of fear-related behavioral and physiological responses (1,11,3,4,7,10). In these studies, performed primarily in rodents and rabbits, the unconditioned stimulus (US) is almost always some form of electric shock. Combined with modern site-specific lesioning and drug infusion strategies, this approach has identified neural circuits that mediate the fear response in nonprimates (3,10). Although it is tempting to extrapolate from these data, the requisite studies have not yet been performed to establish the generality of these findings to primates. In addition, performing these studies in nonhuman primates is an important step in understanding the neurobiological underpinnings of human fear and anxiety.

Earlier studies in primates used electric shock to condition a fear response (14–20). However, at that time, reliable techniques to selectively lesion small subcortical brain structures were unavailable. Even though these techniques have recently been validated in primates (12,13,21–23), advances in understanding mechanisms mediating conditioned fear in primates have been impeded by the controversy around the use of electric shock in these species. Consequently, most primate researchers interested in the biology of fear have abandoned aversive conditioning paradigms.

Therefore, we developed an aversive Pavlovian conditioning procedure in rhesus monkeys that does not use shock as the US. An ideal US would be aversive enough to ensure associative learning, but would not elicit significant physical pain. Therefore, our US consisted of a combination of a loud noise paired with compressed air directed towards the monkey's face. Heart rate was measured as the conditioned response.

METHODS

Subjects

Four female rhesus monkeys (M. mulatta) between 1.3 and 1.5 years old were the subjects. The monkeys were housed with their mothers in cages 71.5 cm wide by 84 cm high by 71 cm deep and maintained on a 12-h light/dark cycle (lights on at 0600 h) at the Wisconsin Regional Primate Research Center. Animal housing and experimental procedures were in accordance with institutional guidelines.

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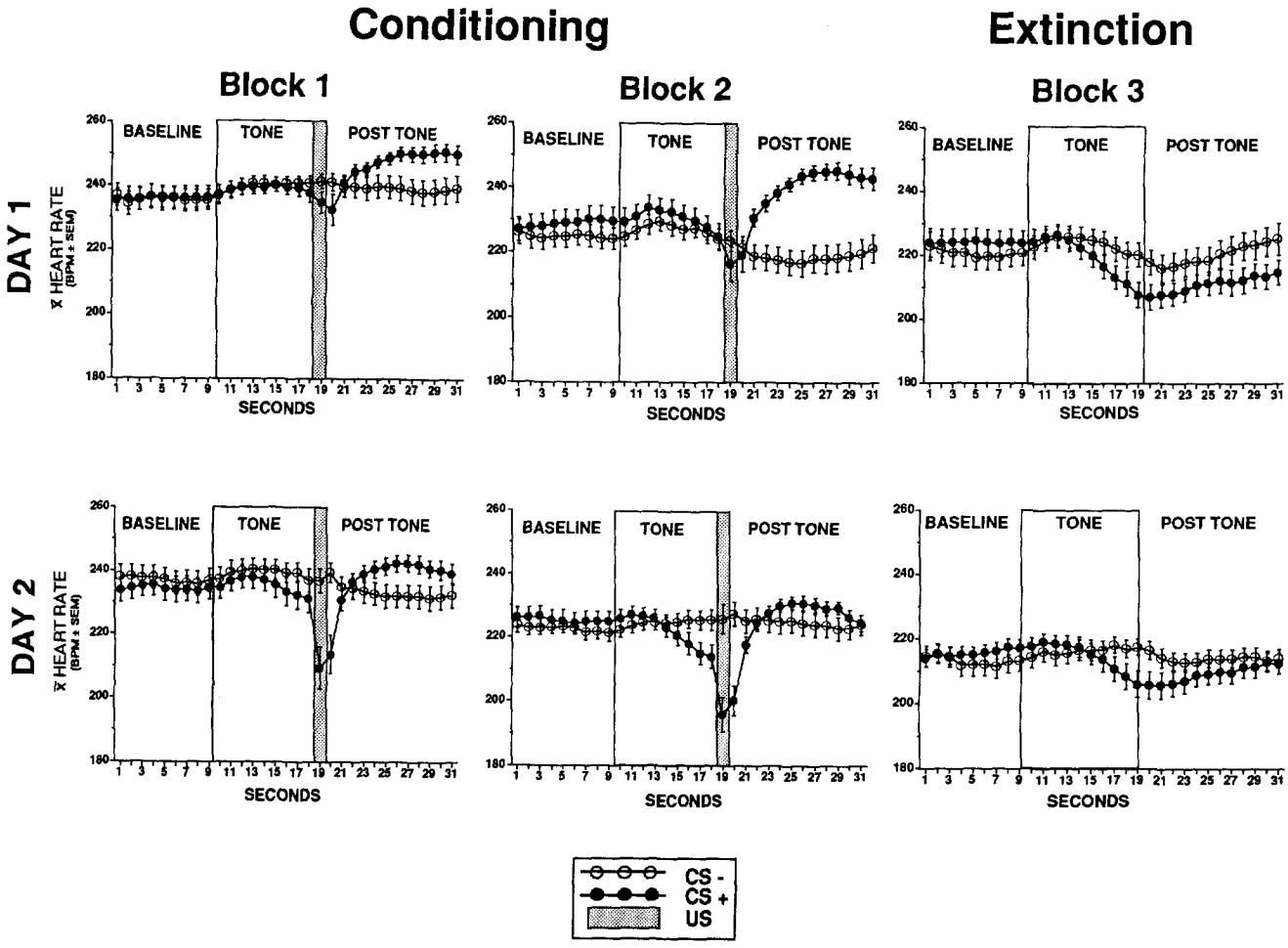

FIG. 1. Mean HR response of 4 Rhesus monkeys exposed to 2 blocks of conditioning and 1 block of extinction daily for 2 days.

Conditioning Procedure

Monkeys were adapted to a primate restraining chair 1 h per day on 4 consecutive days. Chair restraint was used because, in pilot experiments with freely moving animals, we found that activity levels greatly complicated the interpretation of the heart rate (HR) data. Two days following adaptation, and immediately preceding chair restraint, silver/silver chloride ECG electrodes (Medi-Trace, Buffalo, NY) were taped to the previously shaven chest and left flank. Animals were tested twice, with 1 day between tests. On each day, the animal was presented with 2 blocks of conditioning, followed immediately by 1 of extinction. Each conditioning block consisted of 6 positive conditioned stimulus (CS+), and 6 negative conditioned stimulus (CS−) trials. The CS+ and CS− were either a 500 or 1000 Hz 75 dB tone. To control for the possible effects of the tones, the 500 Hz tone was the CS+ for 2 of the subjects; alternatively, the 1000 Hz tone was the CS+ for the other two. The CS+ and CS− were presented randomly with no more than 4 consecutive presentations of each. Each tone was presented for 10 s with a random interval of 45 to 90 (mean 60) s. During the last s of the CS+ trial, the US was presented. It consisted of a 110-dB broad band white noise (1 ms rise and fall time) combined with a stream of compressed air (65 PSI) randomly delivered to the left or right side of the monkey's face. The extinction block consisted of 6 CS+ and 6 CS− presentations. In contrast to conditioning trials, CS+ presentations during extinction were not followed by the US.

Stimulus Presentation and Data Acquisition

For each trial, HR data was collected for 9 s of baseline, 10 s of CS presentation, and 12 s during the post-CS period. The ECG signal was collected with a DOS-based system, using an Analog Devices RTI-800 A/D board, running HEM Data Corporation's (Southfield, Michigan) and Snapshot/Snapstream® software, which streams the data to the PC hard drive. Two channels of data were collected, the ECG signal and an event-marking channel containing TTL (Transistor Transistor Logic) level pulse synchronized to stimulus events.

Data transformation utilized custom written programs and analysis software from the James Long Company (Bedford Hills, NY). This software detects the R-spikes in the ECG waveform. The data are visually inspected and edited and, then, interbeat intervals are transformed to beats/minute.

Statistical Analysis

For each experimental day, HR data from conditioning blocks 1 and 2 and the extinction block were individually analyzed. Repeated measure ANOVAs, with 2 within-subject factors: stim-

ulus (CS+ vs. CS−) and time, were performed on data from the baseline, CS, and postCS periods of each block. The Hunyh-Feldt correction was used to adjust for violations of sphericity.

RESULTS

Figure 1 displays the conditioning and extinction data from both days. On day 1, during the baseline and tone presentation periods of block 1, HR did not significantly differ between CS+ and CS− trials. Analysis of the postCS period revealed a significant main effect of the US ($F = 10.135$; df = 1,3; $p < 0.05$), as well as a stimulus by time interaction ($F = 60.032$; df 12,36; $p < 0.05$). As can be seen, HR immediately decreased and then increased in response to the US. Heart rate changes during the second conditioning block were similar. Again, HR during CS+ and CS− trials did not differ during baseline and tone periods, but did differ during the posttone period. Both a main effect of the US ($F = 38.125$; df = 1,3; $p < 0.009$) and a stimulus × time interaction ($F = 9.664$; df = 12,36; $p < 0.009$) occurred. During the Day 1 extinction block, baseline HR did not significantly differ between CS+ and CS− trials. However, during tone presentation, a main effect ($F = 17.979$; df = 1,3; $p < 0.03$) and a stimulus × time interaction ($F = 39.877$; df = 9,27; $p < 0.0006$) were found. In contrast to the CS−, the CS+ induced a decrease in HR over time. This HR reduction continued into the posttone period; main effect of stimulus ($F = 70.987$; df = 1,3; $p < 0.004$).

On day 2, during the first conditioning block, no significant differences in HR between CS+ and CS− trials occurred during baseline and tone periods (see Fig. 1). As on day 1, HR decreased in response to the US. Analysis revealed a posttone stimulus × time interaction ($F = 8.178$; df = 12,36; $p < 0.02$). During the second conditioning block, differences in HR emerged during the tone period; stimulus × time interaction ($F = 10.127$; df = 8,24; $p < 0.03$). As can be seen, the CS+ resulted in a HR decreased from s 14–18, and no change occurred during CS− presentation. Again, exposure resulted in a reduction in HR over time ($F = 12.428$; df = 12,36; $p < 0.006$). During extinction, no differences in baseline HR were seen in CS+ compared to CS− trials. As on day 1, CS+ presentation resulted in a significant decrease in HR over time ($F = 4.969$; df = 9,27; $p < 0.04$).

DISCUSSION

To ultimately understand neurobiological mechanisms underlying the fear response in nonhuman primates, we focused our initial efforts on establishing a simple and reliable conditioning paradigm that uses a minimally aversive US. The data presented demonstrate the feasibility of using loud noise combined with compressed air as an effective US. This is important because most paradigms using nonhuman primates have relied on electric shock which, in primates, has become a controversial modality. It is not clear from this work how quickly subjects would habituate to the US. It may still be necessary to use shock as a US when studying conditioning for longer time periods.

As can be seen in Fig. 1, the response to the US was a decrease followed by an increase in HR. As reflected in their HR response to the CS+ during the day 1 extinction period, the monkeys appeared to learn the CS+-US association rapidly. The extinction phase was not prolonged enough to uncouple this association. Day 2 data replicated effects seen on day 1. On both days, the conditioned response was characterized by a reduction in HR. This is consistent with findings from other species using different conditioning paradigms (5,6,11). It is important to emphasize that mechanisms underlying the conditioned bradycardia observed in our paradigm, are unclear. Future studies will employ pharmacological and other noninvasive strategies to parse the contributions of the sympathetic and parasympathetic systems (2,20).

Our laboratory has already established the utility of employing an ethologically relevant fear stimulus in studies related to fear in rhesus monkeys. This apporoach, combined with simple aversive conditioning, will allow us to assess, in primates, the extent to which individual differences in innate and learned fear are related (8,9). For example, we will examine whether or not an association exists between the propensity of an animal to freeze when confronted by a human intruder with an averted gaze (8), and the magnitude and duration of aversively learned bradycardia. Future studies will also examine whether the rate of acquisition, the magnitude of an aversively conditioned HR response, and the rate of extinction are stable within individual monkeys. Finally, considerable work in nonprimate species has used aversive conditioning as a model to examine neurobiological mechanisms underlying the acquisition and expression of fear. To begin to understand these findings in relation to humans, similar studies must be performed in primates. This can now be accomplished by testing the effects of small subcortical lesions, in regions such as the central nucleus of the amygdala, on the acquisition and expression of the conditioned HR response in the aversive conditioning paradigm described in this paper.

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