Differential acquisition of specific components of a classically conditioned arterial blood pressure response in rat

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Our group has previously described a discriminative conditioned arterial blood pressure (BP) response in the Sprague-Dawley rat (15, 16). The response to a 15-s pulsed tone that is followed by a 0.5-s tail shock (conditional stimulus (CS+)) in a fully trained animal consists of two components. The first component (C1) is a short-latency pressor response that peaks within ~1.5 s; BP then drops modestly before rising again to manifest the second component (C2) of the response. C2 is more sustained, but lower in amplitude, than C1. The behavioral control (i.e., CS−) is a steady (i.e., nonpulsed) tone of the same frequency as CS+ that is never paired with shock. CS− evokes a C1 BP response, but it elicits no C2. In this traditional paradigm, the amplitude of the C1 conditional BP increase is significantly larger for CS+ compared with CS− in the well-trained subject, and thus C1 demonstrates discrimination between the two behavioral situations. We have found, however, that the C2 increase in BP during CS+, but not CS−, affords a more definitive measure of the difference in response between the two tests (15, 16).

Recordings of renal sympathetic nerve activity (SNA) show why C2 is a more definitive measure of discrimination. That is, the C1 pressor response is preceded by a “sudden burst” in SNA (16), and the amplitude of the C1 increase in BP is proportional to the transient but intense increase in SNA (2). We have suggested that C1 is an open-loop response, probably resulting from a “central command” (16). During C2, there are sustained but smaller magnitude increases in SNA during CS+ that do not occur in response to CS−, making C2 the more sensitive discriminator compared with C1. Another important aspect of C2 is that it appears to be under the control of the baroreflex (16).

We had previously assumed that the conditional changes in BP (C1 and C2) are in response to a perceived stress and not due to the differences in the quality of the tone itself. Experiment 1 tests the hypothesis that the pattern of the BP conditional response does not depend on the nature of the tone. All aspects of the conditioning were kept constant except that the solid tone was now followed by shock and the pulsed tone was used as the behavioral control. We found in fully trained subjects that the amplitude and pattern of the BP conditional response were the same in this “reverse tone” paradigm vs. our traditional procedure.

We also sought to determine the dynamics of the acquisition of C1 compared with C2. We hypothesized that the two appear at different times as the rat learns the conditioning paradigm. To accomplish this, we implanted an arterial catheter in untrained rats to measure BP before proceeding with 3 days of training in the discriminative conditioning paradigm. We found that 1) very early in training both tones evoked a short-latency BP increase that, with continued training, became the C1 component; and 2) the C2 component was acquired later in training; but 3) both components (i.e., C1 and C2) first showed discrimination between CS+ and CS− at the same time (i.e., the end of the third training day). One attractive interpretation of these findings is that C1 is initially part of an orienting response (OR) but, as the animal learns the paradigm, it comes
under the control of the same central processes responsible for expression of the learned C2. A preliminary account of these findings has been published (17).

METHODS

Subjects

Nine Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were used in the reverse tone experiment; eighteen Sprague-Dawley rats were used in the acquisition experiment. Rats weighed between 300 and 450 g at the time of data acquisition. The animals were maintained on standard rat chow and water ad libitum throughout the experiment, except during the time they were actively participating in the conditioning trials. The experimental protocol was approved by the University of Kentucky Animal Care and Use Committee.

Experiment 1: Reverse Tone Paradigm

Behavioral conditioning protocol. The animals were accustomed for 1 h daily over 2 days to handling and to restraint in a comfortable, conical cloth sock. Once adapted to the process, they typically remained quietly snuggled within the sock, although they were free to emerge; on such occasions, they were gently reintroduced to the restraint. Classical, or Pavlovian, conditioning traditionally requires that the CS+ and CS− be initially neutral. Therefore, on the first day of training, each animal was habituated to the audio stimuli eventually to be used as the positive or reinforced tone (i.e., CS+ and the negative or nonreinforced tone (i.e., CS−). Both tones were the same frequency (375 Hz) and length (15 s) and were 8 dB above background noise (66 dB); the only difference was that the CS+ tone was sounded continuously (i.e., steady), whereas the CS− was regularly interrupted (i.e., pulsed: on for 0.064 s, off for 0.16 s). Five pairs of the pulsed and steady tones were presented to each rat in a pseudo-random sequence (e.g., CS− , CS+ , CS− , CS+ , CS− , etc.). A minimum of 4 min elapsed between trials. None of the tones for the first four sets was paired with shock during the habituation trials. The final (i.e., fifth) steady (i.e., CS+) tone was followed with a 0.5-s shock delivered between electrodes on opposite sides of the rat’s tail. The intensity of the shock was adjusted to the lowest value that caused the rat to flinch and vocalize (“squeak”); the intensity usually ranged between 0.2 and 0.3 mA and never exceeded 0.5 mA. Conditioning trials, in which each CS+ was always followed by tail shock, continued for the next 2 days. The pulsed CS− tone was never followed by a shock. Five CS+ and five CS− were given each day.

Surgery. After completion of training, above, the rats were anesthetized with pentobarbital sodium (65 mg/kg ip) in preparation for surgery. A Teflon arterial catheter (ID 0.012 in.) was inserted into the upper abdominal aorta via the left femoral artery in six of the rats. The catheters were tunneled under the skin, exited at the nape of the animal’s neck, and run through a flexible spring tube to the top of the cage. A BP telemetry unit was implanted in three rats. The abdominal aorta was visualized via a laparotomy. The sensory element of a Data Sciences International probe (model TA11PA-C40) was placed into the aorta via a puncture such that the tip pointed toward the heart. The catheter containing the sensitive element of the probe was secured in place with surgical glue. The body of the probe was secured to the interior abdominal wall with sutures. The incision was closed, and the rat was monitored until it regained consciousness. The data were similar, regardless of how the pressure was measured, and were combined for analysis. The animal was returned to the cage and allowed to recuperate for 4 days before any experiment was performed.

Data acquisition and analysis. The rats were reintroduced to the restraint sock, as above, at the end of the recuperation period and tested in the conditioning paradigm for 2 days; five CS+ and five CS− were given each day. BP was digitally sampled at 500 Hz using an analog-to-digital converter (Data Translation 2810) and a micropro-

Statistical analysis. The statistical significances of the changes in BP relative to baseline and of the differences between CS+ and CS− responses were tested by ANOVA followed by post hoc Newman-Keuls’s multiple range tests as allowed by a significant interaction in the ANOVA. Data are given as means ± SE. Statistical significance was accepted for P < 0.05.

RESULTS

Experiment 1

Figure 1 shows a composite analysis of mBP and HR across all nine rats trained in the reverse tone paradigm. Figure 1, top, was constructed by ensemble averaging the “high-resolution” BP files for CS+ trials across all animals. Note, therefore, that the conditional response for any individual animal was represented by a single data file. Likewise, Fig. 1, bottom, was constructed by ensembling the BP and HR data for the CS− trials across rats. The tone began at 15 s and ended at 30 s (dark bar on x-axis). Note particularly in Fig. 1 the “classic” form of the BP conditional response with an obvious sudden but transient C1 for each tone, a delayed, and smaller, C2 pressure response for CS+ but not for CS−. Finally, the shock that followed the CS+ evoked an unconditional increase in arterial pressure.

Figure 2 quantitatively compares the C1 and C2 between CS+ and CS− in the reverse tone conditioning experiment; average changes relative to baseline are given for the peak increase in C1, as well as for the average increase (i.e., average over first 2 s of the tone), and for C2 (i.e., average over the last 10 s of the tone). The steady-tone CS+ evoked significant (F3,24 = 10.1) increases in BP compared with baseline for the peak C1 increase and the average C1 increase, as well as for C2. The pulsed CS− tone also evoked significant changes in pressure (F3,24 = 22.6). Here, the C1 pressor changes (both peak
changes between CS (tone) for CS+ (pulsed tone) and CS- (steady tone) for the first (T1) and fifth (T5) presentation of tones for days 1–3 (D1, D2, D3). mBP was significantly increased over baseline control for all trials except the fifth CS+ and fifth CS− tones (T5) on day 1 and for the first CS+ on day 2 (D2T1). Increase in mBP to CS+ was not significantly greater than that to CS− until the fifth trial on day 3. *P < 0.05, CS+ vs. CS−.

Experiment 2

The standard pulsed tone for CS+ and steady tone for CS− were used for experiment 2. Figure 3 presents means ± SE for the average increases in mBP for C1 (i.e., over first 2 s of each tone) for CS+ and CS− trials. Data are shown for the first and average) were significant, although not that for C2. This is the same pattern of BP change as that evoked in the standard pulsed-tone CS+, steady-tone CS− paradigm (15, 16). Likewise, as is typically seen (15, 16), there was only a modest, but significant, difference from baseline in the average HR during each tone (CS+: −15.5 ± 5.5 beats/min; CS−: −17.8 ± 6.7 beats/min). There was no significant difference in the HR changes between CS+ and CS−.

Fig. 3. Average amplitude of C1 component of conditional blood pressure response to CS+ (pulsed tone) and CS− (steady tone) for first (T1) and fifth (T5) presentation of tones for days 1–3 (D1, D2, D3). mBP was significantly increased over baseline control for all trials except the fifth CS+ and fifth CS− tones (T5) on day 1 and for the first CS+ on day 2 (D2T1). Increase in mBP to CS+ was not significantly greater than that to CS− until the fifth trial on day 3. *P < 0.05, CS+ vs. CS−.

In our previous work, we used a pulsed tone for CS+ and a steady tone for CS− and described the C1 and C2 components of the response pattern (13, 15, 16). In this traditional paradigm, the fully trained animals showed statistically significant differences between CS+ and CS− in both the C1 and C2.

Fig. 2. A comparison between the average CS+ and CS− values for C1 and C2 in the reverse tone experiment. The C1 response is quantified as both its peak increase relative to baseline (left) and the average value over the first 2 s of the tone (middle); value for C2 (right) is the beat-by-beat average over the last 10 s of the tone. Note that the CS+ C2 is significantly larger than for CS−, demonstrating that the rats discriminated between tones in this “reverse” paradigm. *P < 0.05, compared with baseline. *P < 0.05, CS+ vs. CS−.
pressor responses. It is possible that the nature of the tones per se could significantly impact the nature of the BP response. Experiment 1, however, in which we reinforced a nonpulsed tone with tail shock, showed a response pattern that was nearly identical to that evoked using the more traditional technique, except that the amplitude of C1 failed to discriminate between CS+ and CS–.

In our previous work, we did not implant the arterial catheter until the subjects had been fully trained in the behavioral paradigm. Consequently, we had no information on the dynamics of the way in which the animals learned the conditional response. Our present demonstration that C1 and C2 are differentially acquired was possible because we were able to evaluate a given trial as the animals learned the paradigm (e.g., starting with the first tone on the first day of training: D1T1). The major findings of the acquisition study are that 1) there is an increase in BP similar in shape and timing to the C1 component (i.e., as seen in the fully trained animal) in response to the first presentation of both the pulsed and steady tones (i.e., that become the CS+ and CS–, respectively); 2) the first presentation of the tones evokes no significant increase in BP that corresponds in timing and magnitude to the C2 conditional response; 3) as the animal is being trained in the conditioning paradigm, it expresses a short latency but transient pressor response (i.e., that eventually becomes the C1 component of the conditional BP response) before the more latent, smaller pressor response (i.e., that becomes the C2 component); and 4) the rats learn to discriminate between the tones relatively late in the training process. Note that, because both tones are the same frequency, the animals cannot know which tone is pulsed and which is steady for a few tenths of a second (i.e., the duration of a single pulse).

That different aspects of the conditional response can be acquired at different rates is not new. For example, conditional changes in BP, HR, respiration, pupil size, and the galvanic skin response are acquired more rapidly than conditional re- tractions of the nictitating membrane, eyelid, and flexion re- sponses (reviewed in Ref. 11). To the best of our knowledge, however, the finding that different aspects of the conditional response for the same physiological variable, BP in our case, are acquired at a different rate is new. We believe that this study provides new insights into the autonomic control of arterial BP during acute behavioral challenges and that a careful examination of the nature of C1 compared with C2 provides interesting clues as to the explanation for this different rate of acquisition.

The classic view holds that the functional role of the OR is to intensify attentional processes to any new environmental stimulus. C1 showed a number of features characteristic of an OR (reviewed in Ref. 1). First, it was evoked by the very first tone presentation and decreased in magnitude (habituated) with additional presentations of the nonreinforced tones (i.e., for both for CS+ and CS–). Second, a pure OR to two similar tones would have comparable amplitudes, as was indeed the case for D1T1. However, there are also several pieces of evidence that C1 is modified once the CS+ was reinforced. First, although the C1 response decreased during the habituation phase, it started to increase in amplitude during day 2 in response to both tones. This increase in C1 to both CS+ and CS– in all probability reflects a generalization process: the animal had learned that a tone presages a shock but had not yet discriminated between CS+ and CS–. By the end of day 3, however, C1 was significantly larger for CS+ vs. CS– trials, demonstrating that it, too, shows discrimination between tones (see also Refs. 13, 15, 16). We believe, therefore, that the pressor response for the trials during day 1 was a manifestation of an OR. By day 3, it had acquired characteristics of a learned response. If so, our data support the recent conclusion that the OR is “a complex polyfunctional activity, different aspects of which are reflected in different OR components that can be modified rather independently” (8).

The rate of extinction of the OR, and acquisition of the conditional response for different response systems, can differ significantly. With respect to the former, the autonomic components of the OR (HR, respiratory changes, and galvanic skin responses) habituate first followed by the EEG components (alpha desynchronization and vertex potential; Ref. 8). Latash (8) believed that this difference can be explained by different “habituation thresholds” in different brain systems. Powell (14) proposed that the differential acquisition represents different stages of the learning process, perhaps mediated within different regions of the brain. The evolution of the characteristics of C1 from an OR to a learned response might be explained by a progressive shifting of the mediation of this initial response from one to another of the hypothesized brain regions.

In contrast to C1, C2 specifically appears to be acquired only as the animal learns the conditioning paradigm. Recall, for example, that a statistically significant C2 had not appeared even by D2T5, probably because at least some of the rats had not yet learned the association between tone and shock. (This latter statement is also supported by the observation that there was no difference in the amplitudes of C1 for the CS+ and CS– tones throughout day 2.) By this logic, it was only during day 3 that the animals had learned the association between the CS+ tone and shock and could therefore discriminate between the reinforced CS+ and nonreinforced CS–. This acquisition of a discriminative autonomic conditional response on day 3 was evidenced by 1) a significant difference in the C1 response to CS+ vs. CS– and 2) the emergence of a significant C2 to the CS+ but not to the CS–. We believe that these findings indicate that C2 is attributable to central nervous processes directly indicative of associative learning.
A great deal is known about the role played by various parts of the central nervous system in the classical aversive conditioning of autonomic responses (reviewed in Ref. 9). It is widely accepted that the key neuronal systems receive information from both the conditional stimulus (i.e., the tone) and the unconditional stimulus (i.e., the shock). A great deal of experimental work has identified the amygdala as one of the critical centers for fear conditioning (e.g., Refs. 3, 9). Kapp et al. (6) were among the first to show, for example, that placing lesions within the central nucleus of the amygdala in the rabbit attenuates the bradycardia. LeDoux et al. (10) placed lesions in regions of the rat brain to which the amygdala projected (lateral hypothalamic area, midbrain central gray, and bed nucleus of the stria terminalis); the animals were classically conditioned to shock 2 wk later. The lateral hypothalamic area lesions interfered with the conditional BR response but not the freezing response; conversely, the central gray lesions disrupted the conditional freezing but not the learned BP increase. In another important study, Gallagher et al. (4) showed that lesions placed in the central nucleus of the amygdala impaired acquisition of behaviors attributable to orienting-type responses; conversely, these animals did acquire conditional behavioral responses directly related to food delivery.

**Perspectives**

C1 is undoubtedly a “complex polyfunctional activity” (8) that cannot be attributed to any single process. We have suggested that at least the initial portion of the C1 response results from what we termed an open-loop relationship. For example, the sudden burst in SNA precedes the C1 increase in BP (16), and preliminary findings indicate its amplitude is not significantly changed before vs. after sinoaortic denervation (18). We now propose that the initial, or rising phase, of C1 is an OR, much like the sudden conditional tachycardia in dog (12). It is clear, however, that the peak amplitude of C1 differs in CS+ vs. CS− trials, so it can be modified by learning. C1 is probably terminated by the baroreflex responding to the sharply elevated pressure (16). C2 seems to be under the control of closed-loop biofeedback (i.e., baroreflex) mechanisms; in fact, our group (16) speculated that it may be due to an upward resetting of the baroreflex. These differences in the fundamental nature of the two components of the BP conditional response would be much easier to explain if C1 and C2 were mediated by different pathways within the nervous system. Moreover, this would make it easier to understand why C1 appears to be less immediately reflective of actual associative learning processes than does C2. Our recent finding (7) that chronic nicotine exposure depresses C2 but not C1 also appears to be in concert with the notion that the central mediation of these two components is fundamentally different. In any case, our finding that different components of the BP conditional response are acquired at different rates further extends the notion that classical conditioning is a complex, “polymodal” process (e.g., Ref. 5).

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