CONDITIONAL DISCRIMINATION AND TEMPORAL BISECTION IN RATS

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Abstract

In conditional discrimination, an animal do or do not responds during a conditioned stimulus if this stimulus was or was not preceded by a conditional stimulus of another sensorial modality. The aim of this work was to investigate if two durations (4 or 16 s) of a same modality stimulus can also acquire the conditional property of modulating rat's responses during a 5 s conditioned stimulus. A 5 s empty interval was included between the conditional and the conditioned stimuli, so as to evaluate the durations processing by short-term memory. All the rats of the experiment learned the task. Then, the rats were tested in a temporal bisection procedure, presenting conditional stimuli in several intermediate durations between 4 and 16 s. We obtained a typical temporal bisection function indicating that duration's representation can modulate rat's responses even if temporally separated from the response opportunity.

Using rats, Ross and Holland (1981) carried out a pavlovian conditional discrimination procedure with: (1) presentation of two stimuli (X and A) simultaneously (XA), followed by an unconditioned stimulus (XA+); and (2) presentation of the stimulus A alone and not followed by the unconditioned stimulus (A-). After training the rats, the authors made a test in which the stimulus A was replaced by a stimulus B of a different sensorial modality. The results showed that the training with the simultaneous arrangement established a direct association between X and the unconditioned stimulus, independently on the stimulus A, because the substitution of the stimulus A did not changed the performance of the animals. In a second experiment of the same work, they used a pavlovian conditional discrimination procedure in which X and A were presented serially and separated by a five seconds empty interval (X→A). In such procedure, the series X→A was followed by the unconditioned stimulus (X→A+), while A presented alone was not (A-). In the test at the end of this training, the substitution of the stimulus A by the stimulus B impaired the performance of the animals, indicating that in serial conditional discrimination procedures, the unconditioned stimulus is directly associated with the stimulus A, while the stimulus X sets the occasion in which A is followed by the unconditioned stimulus. The serial arrangement reported by Ross and Holland (1981; X→A+/A-) is a positive conditional discrimination, because the stimulus X, also called "feature-stimulus", acquires an excitatory conditional property. Nevertheless, animals can also learn a negative serial conditional discrimination, in which the stimulus X acquires an inhibitory feature-stimulus property (X→A -/A+; Holland & Lamarre, 1984). In both types of serial conditional discrimination, the conditioned stimulus A is also referred as "target-stimulus".

Time components in pavlovian conditional discrimination procedures were examined in rats by Holland (1998) through manipulation of the empty interval duration, which separates the feature-stimulus from the target-stimulus in serial arrangements. Bueno, Wagatsuma and Martins (2007) also studied the effects of durations in a pavlovian conditional discrimination procedure with rats, but investigating if different durations of a same-modality feature-estimulus could acquire excitatory or inhibitory properties. Accordingly with the data of Bueno, Wagatsuma and Martins (2007), a single stimulus modality, like light, can acquire excitatory or inhibitory properties depending on the duration with which is presented in positive and negative serial conditional discrimination training.

In further development of the Bueno, Wagatsuma and Martins (2007) procedure, characterized by a serial conditional discrimination of durations with an empty retention interval, the present work submitted rats to a temporal bisection task (Church & Deluty, 1977) introducing an empty interval between the time signal and the reinforcement opportunity signalized by another stimulus. By means of a serial arrangement of conditional discrimination and temporal bisection, the present work investigated how the short-term mnemonic processing can affect the temporal bisection curve obtained through the test with intermediate durations of light.

Method

Conditional discrimination training

Using standard operant experimental chambers, the procedure reported here involved the training of 10 male Wistar rats with the presentation of a same-modality visual stimulus with two different durations (short and long), both followed by an empty retention interval which, in turn, was followed by an auditory target-stimulus (the reinforcement opportunity signal). As in traditional temporal bisection procedures, the reinforcement was contingent to the choice between two levers, in such a manner that the response on the left lever was reinforced only if preceded by the short-duration light and the response on the right lever was reinforced only if preceded by the long-duration light.

In details, the rats were maintained under water-deprivation during whole experiment, since the water would be used for reinforcement of lever-pressing. Then, the simple discrimination pre-training (three daily sessions with duration of 1 hour) presented pure tones of 5 s, during which the responses at any of the two levers were reinforced by water delivery (Fig. 1). The pure tones were separated from each other by inter-trials intervals of 2 min. Afterwards, the conditional discrimination training (two months of daily sessions of 1 hour) presented random standard trials initiated by the short (4 s) and long (16 s) conditional stimuli (light signals, delivered by a white lamp positioned above the experimental chamber). These temporal signals (short or long) were followed by an empty retention interval of 5 s which, in turn, was followed by a pure tone of 5 s. Although the two levers were always available in the experiment, the pure tone interval (5 s) was the only opportunity of reinforcement. This reinforcement was contingent to the correct responses: pressing the left lever (lever A) during the pure tone preceded by the 4 s light and pressing the right lever (lever B) during the pure tone preceded by the 16 s light (Fig. 1). Independently on correct or incorrect responses, the pure tone was always interrupted by the lever press. The lever A and B positions were counterbalanced for half of the rats. Trials with the pure tone alone (not preceded by light of any duration) were also presented randomly in all the training sessions, but responses at any lever were not reinforced during these specific trials. All the three types of standard training trials (with 4 or 16 s light and without light) were separated from each other by inter-trial intervals of 2 min.

Temporal bisection test

The training was followed by a psychophysical test, in which intermediate durations of white light were presented, allowing the evaluation of the choice pattern between the two levers accordingly with the precedent intermediate duration.

In details, the test sessions introduced probe trials randomly within the sequence of standard trials, while the water-deprivation and the experimental conditions remained unchanged. In these probe trials, the water-reinforcement during the pure tone was always omitted and the light durations were 4.0, 5.0, 6.4, 8.0, 10.0, 12.8 or 16.0 s (Fig. 1). The test was finalized after nine daily sessions.

The data was analyzed with the two-way repeated measures analysis of variance (ANOVA) followed by the Newman-Keuls post-hoc test, and with the Student's t-test.

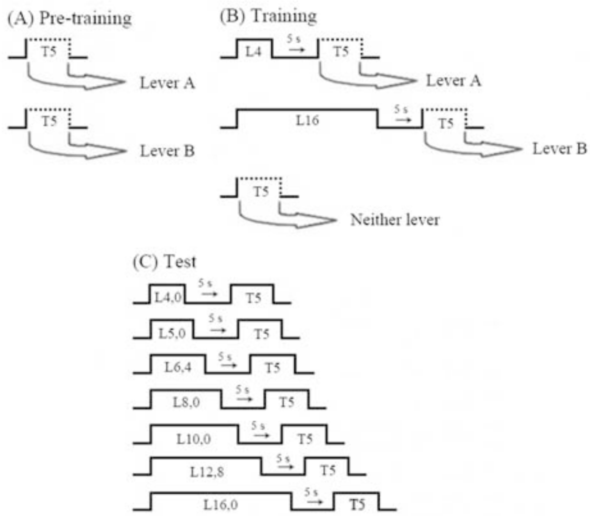

Fig. 1. Experimental paradigms of the simple discrimination pre-training (A), serial conditional discrimination training (B) and temporal bisection test (C). T: pure tone; L: white light. The numbers after T and L indicates the duration of the stimuli. The "5 s" over the arrows represents the empty retention interval. The dashed line above T5 of the pre-training and training indicates that the pure tone could be interrupted by any response.

Results and Discussion

The rats learned to choose one lever or another accordingly with the time signal (short or long duration light) presented before the reinforcement opportunity defined by the pure tone. Fig. 2A shows that until the 3rd block of five sessions there was not a trend in the change of the A and B response proportion, since the B response proportion was maintained around 50% independently on the duration of the precedent light. Then, the curves corresponding to lever B response proportion after 4 s and 16 s light showed opposing tendencies until the end of the experiment: the proportion of B responses after the 4 s light had a gradual decrease (sessions effect: F=21,70; P<0,05) and the proportion of B responses after the 16 s light had an increase (sessions effect: F=12,31; P<0,05).

The rats acquired the conditional discrimination involving the pure tone preceded by light (regardless of its duration) and the pure tone presented alone. Analyzing the training without differentiating the responses at the lever A or B, Fig. 2B shows that in the four initial blocks of five sessions the proportion of tones during which the rats emitted responses at any lever was maintained high for both the tones preceded by light of any duration and the tones presented alone. This indicates that the contingency established in the simple discrimination pre-training was still affecting the behavior of the rats in the beginning of the conditional discrimination training. After that, the curve corresponding to the post-light tones were maintained around 95%, while the curve of tones presented alone decreased gradually (sessions effect: F=17,31; P<0,05). Considering that the light of any duration acted as feature-stimuli (conditional stimuli), and the pure tones acted as target-stimuli (conditioned stimuli), this result shows the consistent acquisition of a positive conditional discrimination.

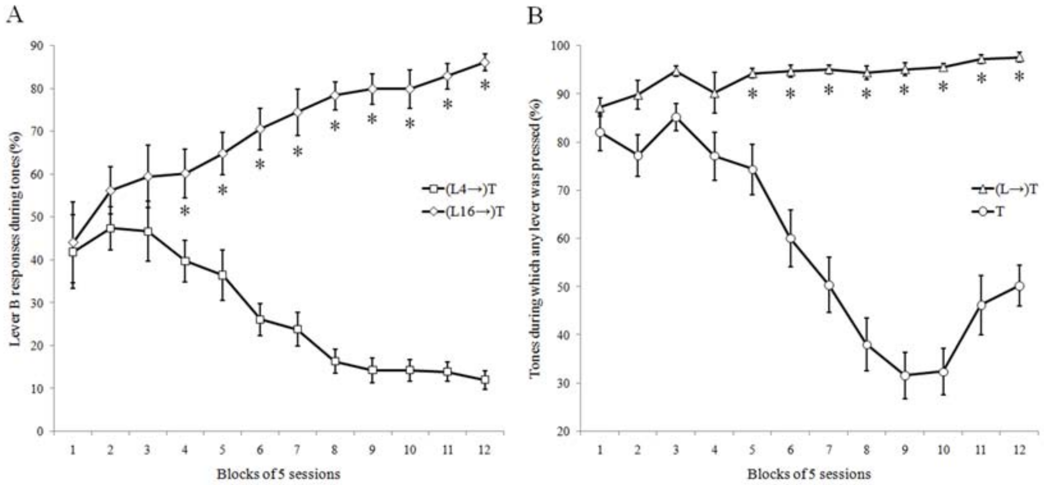

Fig. 2. Acquisition of the serial conditional discrimination of durations. A: graph differentiating the lever B response proportion during the pure tone (T) accordingly with the precedent light duration (L4 or L16). B: graph showing pure tones during which any lever was pressed, differentiating tones preceded by light of any duration [(L→)T] from tones presented alone (T). *: significant differences (P<0,05).

The conditional discrimination acquisition was also suggested by an increase in the response rate during the empty interval across the two months of training. Fig. 3A shows that, without differentiating the responses at lever A or B, the number of responses emitted during the 4 s light did not changed from the first to the last training session. In the other hand, the number of responses during the empty interval posterior to the 4 s light was significantly higher in the last training session (t=-4,66; P<0,05). A similar result was found in the comparison between the last 4 s of the 16 s light and the empty interval posterior to the 16 s light (Fig. 3B): the rats emitted significantly more responses in the post-16 s light empty interval during the last training session than in the first session (t=-3,47; P<0,05). These results indicates that, once the conditional discrimination was learned, the response rate was modulated by the termination of the feature-stimulus, indicating an association between the past stimulus (light) and the incoming reinforcement opportunity signalized by the pure tone.

The acquisition of discrimination between two durations of a same-modality stimulus based on the choice of left of right lever, associated with the observations that the rats acquired the positive serial conditional discrimination, suggests as unlikely the hypothesis that a simple interval timing (independent on the pure tone and the empty interval between light and tone) could control the choice behavior. Therefore, instead of obeying a simple interval timing triggered by the light (indifferent to the empty retention interval and the pure tone), the behavior of the rats was apparently modulated by an "occasion setting" relation between two durations of a same-modality feature-stimulus (light) and a target-stimulus of a different sensorial modality (sound). Traditionally, the training procedures for discrimination between two durations based on the choice between two possible operant responses by rats uses retractable levers, that are inserted in the operant chambers only at the end of the time signal, and then retracted again immediately after the correct or incorrect response (for example, Church & Deluty, 1977; Meck, Church & Olton, 1984; Siegel, 1986; Al-Zahrani et al., 1997). Differently, in the present work the two levers were permanently available inside de operant chambers, allowing the evaluation of how the operant behavior evolves from the beginning of the trial (light) until de conditioned stimulus (pure tone), including the retention interval. This particular analysis showed the ability of rats in acquiring the discrimination between two durations, choosing one lever or another, even when submitted to a complex learning procedure, like serial conditional discrimination. Thus, the results suggests that the rats retained the light duration information during the 5 s empty interval, aiming to respond correctly within the interval signalized by the auditory stimulus.

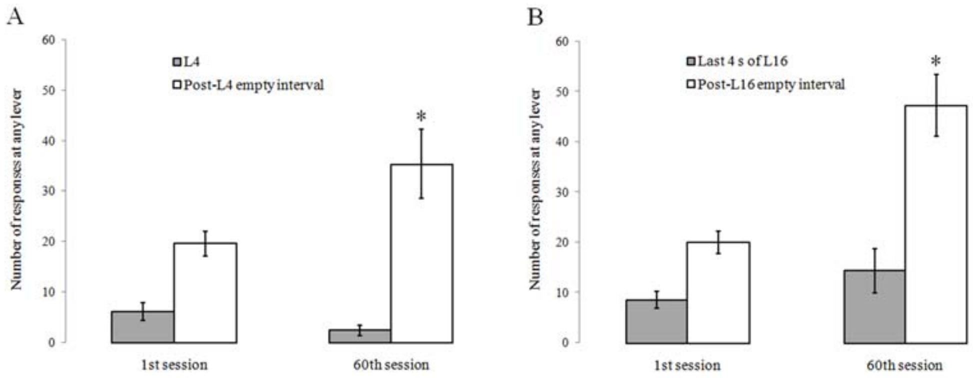

Fig. 3. Increase in the number of responses during the empty retention interval from the first to the last training session. A: comparison between the 4 s light (L4) and the 5 s empty interval posterior to L4. B: comparison between the last 4 s of the 16 s light (L16) and the 5 s empty interval posterior to L16. *: significant difference (P<0,05) between the number of responses during the empty intervals presented in the last and the first training session.

In the temporal bisection test (Fig. 4), the response curves showed an increase of the lever B response proportion across the crescent scale of intermediate probe durations (F=88,98; P<0,05). This result indicates a robust cognitive time processing established by the two-months training, consistently with the temporal bisection curves obtained by Church and Deluty (1977) and many subsequent reports. However, the present result indicates a bisection point (50% probability of lever B response, or point of subjective equality) at the arithmetic mean (10 s) between the two extreme durations presented in the training (4 and 16 s), while the traditional bisection experiments also using 4 and 16 s as extreme durations indicates bisection points at the geometric mean (8 s), showing that the time perception increases in logarithmic function of the physical time. One could argue that the subjects of the present study had learned to add the 5 s of the empty retention interval with the 4 and 16 s of light in the training, biasing the time processing of all the probe intermediate durations in the test. But the training result showing that the rats were not processing time indifferently to the empty interval raises the possibility that the atypical temporal bisection point found in the present work appears to be, somehow, due to a complex cognitive processing involving serial conditional discrimination and temporal bisection.

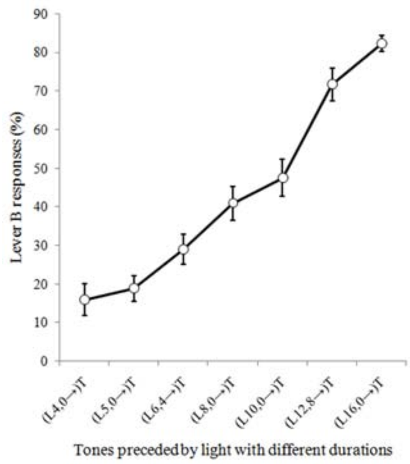

Fig. 4. Temporal bisection curve showing a bisection point at 10 s. Only the responses emitted during the pure tones were quantified.

Further works involving new behavioral paradigms of complex learning and interval timing, allied to manipulation of neurobiological substrates may shed some light on the particular cognitive and mnemonic processes assessed by the present report.

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