Special Section

TIMING IN EYEBLINK CLASSICAL CONDITIONING AND TIMED-INTERVAL TAPPING

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Abstract—*The cerebellum is implicated in interval timing for diverse tasks including eyeblink classical conditioning (EBCC) and repetitive tapping. We examined performance on both tasks across identical intervals ranging from 325 to 550 ms. In five weekly sessions, 23 participants used a different interval each week, both as the target for tapping and as the delay interval in EBCC. Changes in variability as a function of the tapping or delay interval were assessed using regression analyses. The slope for repetitive tapping was comparable to two measures of temporal acuity in EBCC, onset and peak latency of the conditioned response. Each of 80 additional participants was assessed in one session at one of four tapping and delay intervals. Results were similar to those observed in the repeated measures group. These findings provide further evidence that EBCC and repetitive tapping utilize common mechanisms for representing temporal information.*

Two tasks that depend on the cerebellum are eyeblink classical conditioning (EBCC) and timed-interval tapping. In EBCC, each trial consists of presentation of a neutral stimulus, the conditioned stimulus (CS), followed by a reflex-eliciting stimulus, the unconditioned stimulus (US). For example, the CS might be a tone and the US a corneal airpuff. Over trials, the organism learns to produce a conditioned response (CR) in anticipation of the corneal airpuff. EBCC has proven to be one of the most fruitful model tasks for studying the neural mechanisms of learning and memory (see Thompson, 1990). Lesions of the cerebellum produce severe impairments in EBCC in both rabbits and humans, although the motor response, the unconditioned response (UR), remains intact (see reviews in Steinmetz, 1996; Woodruff-Pak, 1997).

The cerebellum receives inputs conveying representations of both the CS and the US. However, EBCC does not simply require that these two stimuli be associated. The organism must be able to represent the precise temporal relationship between the CS and US so that the CR occurs just prior to the onset of the US. Lesion studies (Perrett, Ruiz, & Mauk, 1993; Woodruff- Pak, Lavond, Logan, Steinmetz, & Thompson, 1993), as well as computational models (Bartha, Thompson, & Gluck, 1992; Buonomano & Mauk, 1994; Fiala, Grossberg, & Bullock, 1996), indicate that although the cerebellar nuclei are essential for forming the critical associative link, precise timing is dependent on the cerebellar cortex.

Precise timing is also required for the production of coordinated movement. Lesions of the cerebellum produce impairments on a range of experimental tasks that directly assess timing control (see Ivry, 1997). One such task is the repetitive tapping task (Wing & Kristofferson, 1973), in which participants attempt to produce a series of equally spaced intervals, first with a pacing signal (synchronization phase) and then when the pacing signal is terminated (continuation phase). Patients with cerebellar lesions demonstrate increased temporal variability on this task, and this deficit has been attributed to increased noise in the central control of the timing of the responses (Ivry & Keele, 1989; Ivry, Keele, & Diener, 1988; Woodruff-Pak, Papka, & Ivry, 1996).

The cerebellum has been hypothesized to operate as an internal timing system, given its link to EBCC, repetitive tapping, and other tasks requiring the precise representation of temporal information. To examine interactions between these two tasks, we assessed EBCC learning rates under various dual-task conditions, finding that participants who engaged in tapping while they underwent EBCC showed a reduced percentage of CRs compared with participants who engaged in control tasks while they underwent EBCC (Papka, Ivry, & Woodruff-Pak, 1995). Results indicated that simultaneous tapping interfered with EBCC because the two tasks shared a common cerebellar substrate.

Timing variability is a constant proportion of the interval being timed, at least for intervals ranging from 200 ms to 1.5 s (see Getty, 1975; Gibbon, 1991). This relationship, a temporal form of Weber's law, is described by the equation

$$\text{variance} = k^2 * \text{interval}^2 + c \quad (1)$$

The slope, k^2, provides a measure of duration-dependent variability, assumed to reflect noise in an internal timing system. The square root of the slope corresponds to the Weber fraction. The intercept, c, provides a measure of duration-independent variability, such as noise related to sensory processing or motor implementation.

If two tasks share a common timing component, then the slopes should be similar. Ivry and Hazeltine (1995) applied Equation 1 to variability data obtained on tapping and perception tasks with intervals ranging from 325 to 550 ms. Across a series of experiments, the slope terms for the motor and perceptual tasks were correlated and affected by similar manipulations.

In this study, we employed this methodology to explore similarities in EBCC and repetitive tapping. A range of intervals was used, either as the delay between the CS and US or as the target interval on the repetitive tapping task. We predicted that the slopes would be similar for the two tasks. We did not expect the intercepts to be comparable because the two tasks entail different perceptual and motor pathways.

METHOD

Participants

The repeated measures group included 23 undergraduates (10 male, 13 female) ranging in age from 18 to 25 years old (M = 20.4, SEM = 0.39). They received extra credit in a psychology course for participating. Self-reports of handedness indicated that 18 were right-

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handed and 5 were left-handed. The single-session group included 80 undergraduates (53 female, 27 male) ranging in age from 18 to 44 years old (M = 21.7, SEM = 0.60). They received either extra credit or credit in a psychology course for participating. Self-reports of handedness indicated that 76 were right-handed and 4 were left-handed.

Apparatus and Procedures

Repeated measures group

Participants were tested in five separate sessions at weekly intervals. In each session, a single interval was used for both EBCC and repetitive tapping. In Session 1, half of the participants performed the tapping task first and EBCC second, and the rest performed in the opposite order. For both tasks, the critical interval was 625 ms. This session was used as a practice session, and the data were not included in the analyses. In Sessions 2 through 5, the procedure was similar, except the two tasks were administered in random order. The interstimulus interval (ISI) for both tasks was reduced by 75 ms in each successive session. Thus, the ISI was 550 ms in Session 2, 475 ms in Session 3, 400 ms in Session 4, and 325 ms in Session 5. We used a fixed order of descending ISIs to ensure that well-timed CRs in EBCC (i.e., eyeblinks that occurred immediately prior to the US) were specific to the ISI being tested.

Single-session group

Four target intervals were used. Participants were randomly assigned to one of four groups (n = 20/group), with the interval being 325, 400, 475, or 550 ms. Half of the participants in each condition received EBCC first and tapping second, and the order of testing was reversed for the rest.

Eyeblink conditioning

An 80-dB, 1000-Hz tone was the CS, and a 5- to 7-psi corneal airpuff was the US. On paired trials, the tone co-terminated with the airpuff. Each participant received 100 trials per session, grouped into blocks of 10. The intertrial interval was randomly selected from a distribution ranging from 10 to 19 s. Within each block, 8 trials were paired presentations of the CS and US. Trials 1 and 6 of each block were CS-alone trials, but data from CS-alone trials are not presented as there were too few CRs on these trials to achieve reliable temporal measures. For the repeated measures group, 10 CS-alone trials were administered at the beginning of Sessions 2 through 5 to extinguish learning in previous sessions.

Eyeblinks were recorded for 950 ms after the onset of the CS. Only CRs on paired CS-US trials were analyzed. We used two different scoring criteria for CRs: (a) Well-timed CRs were eyeblinks that exceeded 0.5 mm in amplitude no more than 200 ms before US onset; (b) standard CRs were eyeblinks that exceeded 0.5 mm in amplitude at least 150 ms after CS onset but before US onset. Only well-timed CRs were used in regression analyses. There were two reasons for including only well-timed CRs in regression analyses. First, we wanted to maintain a constant scoring interval across changes in the CS-US interval. Second, during acquisition, the timing of CRs is especially variable. For all eyeblinks scored as CRs, two separate measures of timing in EBCC were calculated: CR onset latency, the time when eyelid closure first attained 0.5 mm, and CR peak latency, the time of maximum eyelid closure. For both measures, a mean and variance were obtained at each interval for each subject.

Repetitive tapping task

A trial began with a series of 65-dB, 50-ms tones presented at regular intervals. After the participant's first tap, 12 more tones were presented, and the participant attempted to synchronize taps with the tones. The tones then ended, and the participant continued tapping at the same pace until he or she had generated 30 self-paced intervals. A total of 24 error-free trials was completed in each session. An error-free trial was a trial in which all unpaced taps were within 200 ms of the ISI. The mean and variance of the 30 unpaced intertap intervals were calculated and then averaged across trials for each subject.

RESULTS

In the analyses reported, we included only participants who emitted a large number of well-timed CRs in EBCC in Sessions 2 through 5. The inclusion criterion was four well-timed CRs within five consecutive trials in each of these sessions. A total of 11 participants in the repeated measures group and 50 participants in the single-session group (13 at 325 ms, 17 at 400 ms, 10 at 475 ms, 10 at 550 ms) met this criterion. We adopted this strict criterion because the CR latency data were very noisy for participants who did not demonstrate stable EBCC.

Repeated Measures Group

Regression analyses based on Equation 1 were performed on the variability data. These analyses were performed on both individual data (Table 1) and averaged data (Table 1 and Fig. 1).

If two tasks utilize the same timing mechanism, they should show equivalent Weber fractions, calculated as the square root of the slope term from a linear regression so as to eliminate duration-independent sources of variance. The Weber fraction was calculated for each of the three dependent measures of timing (two EBCC measures and one tapping measure) for each participant. Neither of the mean Weber fractions for the two EBCC measures (CR peak latency: M = 0.068, SEM = 0.016; CR onset latency: M = 0.069, SEM = 0.016) was significantly different from the mean Weber fraction for intertap interval (M = 0.039, SEM = 0.006), $ps > .16$. These analyses support the hypothesis that the same timing mechanism is used in EBCC and tapping.

A second, related comparison can be made by comparing the duration-dependent variances across tasks. The duration-dependent component of each data point was calculated by subtracting the intercept term (Ivry & Hazeltine, 1995). If the intercept was negative, no subtraction was made. Two 2 (task: EBCC measures vs. tapping) × 4 (ISI) mixed-effects analyses of variance were calculated using the duration-dependent variances. A comparison of CR peak latency and intertap interval revealed a significant effect of ISI, $F(3, 30) = 7.23$, $p = .001$, but no effect of task, $F(1, 10) = 0.07$, $p = .33$, and no interaction, $F(3, 30) = 1.48$, $p = .24$. A comparison of CR onset latency and intertap interval revealed a significant effect of ISI, $F(3, 30) = 6.82$, $p = .001$, but no effect of task, $F(1, 10) = 1.46$, $p = .26$, and no interaction, $F(3, 30) = 1.37$, $p = .27$. The results of these analyses provide additional support for the hypothesis that a timing mechanism with similar noise properties is involved in EBCC and repetitive tapping.

A final set of analyses was conducted on the intercept values. The intercept term is assumed to represent duration-independent sources of variance (e.g., sensorimotor transmission and other nontemporal processes) and should be greater than 0. As predicted, the mean

Table 1. *Results of regression analyses of timing data for 11 participants in the repeated measures group*

Timing measure	Slope	Intercept	R^2
CR peak latency			
Regression analyses of individual data			
Mean	0.006	220.66	.50
(*SEM*)	(0.003)	(349.90)	(.07)
Regression analysis of averaged data	0.006	199.90	.99
CR onset latency			
Regression analyses of individual data			
Mean	0.007	1,545.73	.37
(*SEM*)	(0.002)	(301.57)	(.12)
Regression analysis of averaged data	0.006	1,570.87	.80
Intertap interval			
Regression analyses of individual data			
Mean	0.002	195.50	.66
(*SEM*)	(0.001)	(38.31)	(.11)
Regression analysis of averaged data	0.002	199.13	.99

Note. CR = conditioned response.

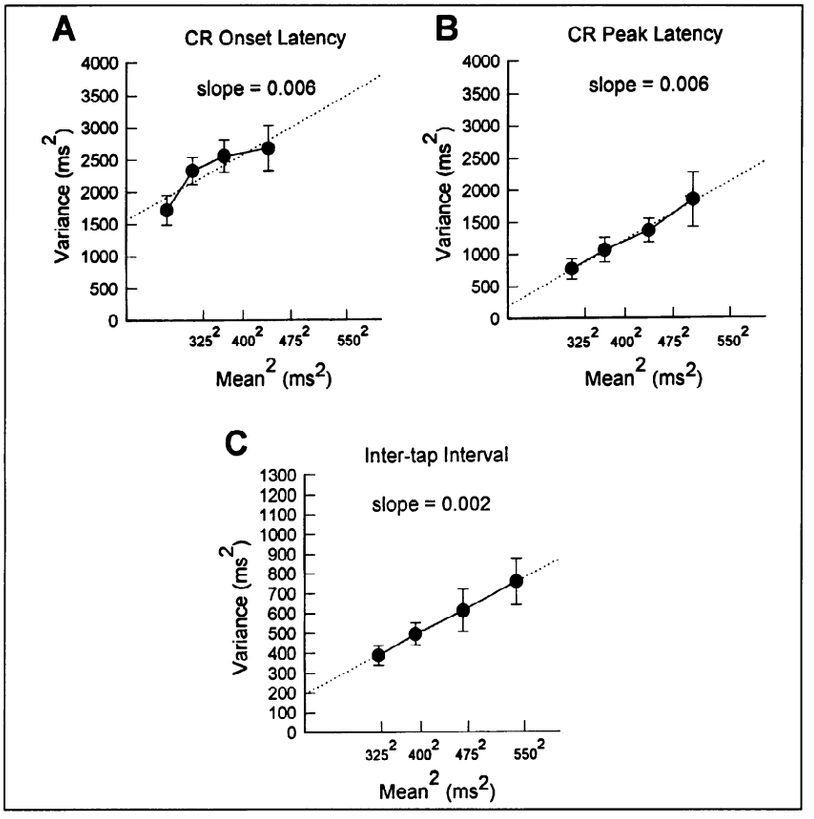

Fig. 1. Variance of (a) conditioned response (CR) onset latency, (b) CR peak latency, and (c) intertap interval as a function of mean interval squared for 11 participants in the repeated measures group who met a criterion of four well-timed CRs within five consecutive trials. Each data point represents the averaged data of these 11 participants. Dashed lines represent linear regressions. CR onset latency and CR peak latency were calculated from CRs emitted within 200 ms of the corneal airpuff unconditioned stimulus. Error bars show the standard error of the mean.

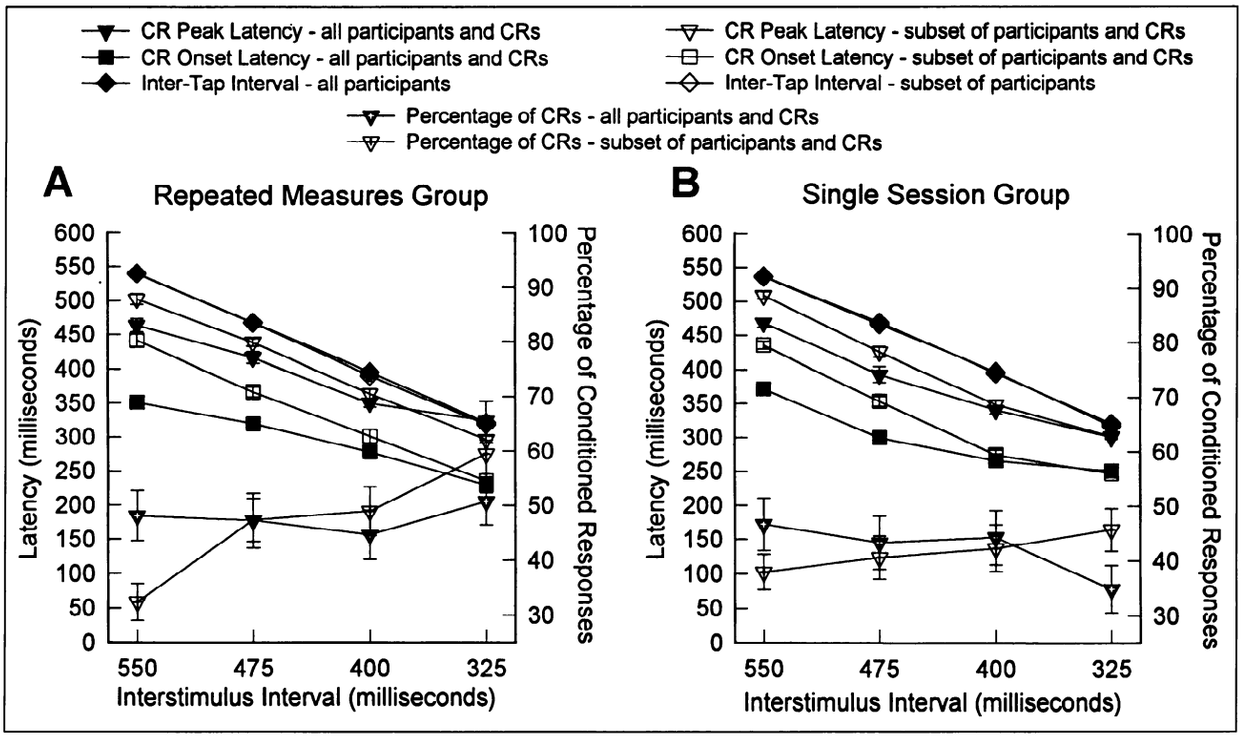

Fig. 2. Mean conditioned response (CR) onset latency, CR peak latency, and intertap interval and mean percentage of CRs as a function of interstimulus interval for (a) participants tested at all interstimulus intervals and (b) participants tested at one interstimulus interval. Black symbols depict data from all participants (n = 23 for the repeated measures group; n = 20 per interstimulus interval for the single-session group). For these data, CR latencies were calculated using our standard CR definition: an eyeblink at least 150 ms after the onset of the tone conditioned stimulus but before the onset of the corneal airpuff unconditioned stimulus. White symbols depict data from participants who met a criterion of four well-timed CRs within five consecutive trials in eyeblink classical conditioning (n = 11 for the repeated measures group; n = 10, 10, 17, and 13 for interstimulus intervals of 550 ms, 475 ms, 400 ms, and 325 ms, respectively, for the single-session group). For these data, CR latencies were calculated using our definition of a well-timed CR: an eyeblink that began no more than 200 ms before the corneal airpuff unconditioned stimulus. Error bars show the standard error of the mean.

intercepts for CR onset latency and for intertap interval were both significantly greater than 0, $t(10) = 5.13$, $p < .001$, and $t(10) = 5.10$, $p < .001$, respectively. These two values were significantly different from each other, $t(10) = 4.20$, $p < .01$, consistent with the prediction that the two tasks involve different nontemporal sources of variability. The nontemporal variability associated with CR onset appears to be substantial. The comparison between the intercepts for CR peak latency and intertap interval was not significant, $t(10) = 0.07$, $p = .95$. In fact, the mean intercept for CR peak latency was not significantly greater than 0, $t(10) = 0.63$, $p = .54$. As can be seen in Table 1, the individual differences were quite substantial for the intercept measures of CR peak latency.

Single-Session Group

The single-session group allows an assessment of the effects of repeated measurements at different intervals on EBCC and repetitive tapping. There were no systematic differences between the repeated measures and single-session groups, in terms of either the means or the variances of the timing measures. We compared the repeated measures and single-session groups using data only for those participants and CRs included in the regression analyses. Sixteen analyses were carried out to compare means and variances for CR peak and onset latencies at each ISI. Of these numerous analyses, only one achieved statistical significance. Mean CR onset latency at a 400-ms ISI was significantly longer in the repeated measures group than in the single-session group, $F(1, 26) = 4.76$, $p = .04$. Among the eight comparisons made for the tapping measures (means and variances for four ISIs), there were no significant differences between the two groups.

Figure 2 depicts mean latencies for EBCC and timed-interval tapping and the percentage of CRs in EBCC as a function of ISI. Both the entire data set (any eyeblink exceeding 0.5 mm in amplitude at least 150 ms after CS onset but before US onset) and the restricted data set (any eyeblink exceeding 0.5 mm in amplitude within 200 ms before US onset) are graphed.

DISCUSSION

The slope values obtained for temporal variability in EBCC and the repetitive tapping task were similar. This finding is in accord with the hypothesis that the two tasks invoke the operation of a common timing system. Although caution is always warranted when considering null results, this conclusion is bolstered by two aspects of the data. First, given the very different nature of the two tasks, it is impressive

that the variability functions are so similar. We expect that the difference in mean slope for the EBCC and tapping measures would be even further reduced if the two tasks were made more similar. Ivry and Hazeltine (1995) observed that the Weber fraction for tapping became larger as the number of consecutive intervals was decreased. EBCC requires that the timing be based on a single stimulus.

Second, the finding of a significant difference between the intercept values for tapping and CR onset latency suggests that the regression analyses were sufficiently sensitive to detect differences between these two tasks. Although we had no a priori prediction concerning the magnitude of duration-independent variability for EBCC and tapping, the results suggest that a large nontemporal component of variability is associated with the onset of the CR. This measure is likely dependent on many factors that are likely to fluctuate, such as the extent of learning, the subjective intensity of the CS and US, and the arousal state of the participant.

Patients with cerebellar lesions have impaired EBCC (Daum et al., 1993; Lye, O'Boyle, Ramsden, & Schady, 1988; Solomon, Stowe, & Pendlebury, 1989; Topka, Valls-Sole, Massaquoi, & Hallett, 1993; Woodruff-Pak et al., 1996) and show increased variability during repetitive tapping (Ivry & Keele, 1989; Ivry et al., 1988; Woodruff-Pak et al., 1996). The current results provide novel evidence indicating that a common neural system is associated with EBCC and tapping. We hypothesize that the association with the cerebellum reflects the fact that this neural structure is capable of providing the requisite precise temporal representation. This does not mean that temporal processing in the two tasks invokes the same neural elements. One possibility is that the cerebellum can be conceptualized as an array of task- and domain-specific timing elements (Ivry, 1997). Similarities across tasks likely reflect similar noise characteristics across this system.

The kind of regression analysis we employed should prove beneficial to other studies of the psychological and neurological mechanisms of timing. By allowing a separation of duration-dependent and duration-independent sources of variability, this methodology provides a valuable tool for determining commonalities and differences between tasks, as well as for comparing the effects of lesions in different neural systems. For example, lesions of the basal ganglia have also been associated with increased variability in temporal processing tasks, including tapping (Harrington, Haaland, & Hermanowicz, 1998; O'Boyle, Freeman, & Cody, 1996) and EBCC (Woodruff-Pak & Papka, 1996). Examining performance over a range of intervals should allow greater specification of the source of the impairment.

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