Short communication

Novel behavioral tasks to explore cerebellar temporal processing in milliseconds in rats

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HIGHLIGHTS

- We devised two novel behavioral tasks for researching milliseconds timing.
- Rats needed only two training sessions to represent 500 ms interval in this task.
- Rats changed strategies to process discrete and continuous timings among two tasks.
- Rats can represent discrete and continuous timings with almost identical movements.

ABSTRACT

Temporal processing in milliseconds has been reported to rely on the cerebellum; however, no detailed neuronal mechanisms have been published yet. This is because there are too few studies on the relationship between temporal processing in milliseconds and cerebellar neuronal spikes that organize behavioral timing. To demonstrate this relationship, it is necessary to record the spike activity while the animal is performing a behavioral task that requires specific temporal processing in milliseconds. In this study, we describe two novel timing tasks. These behavioral tasks comprise the following schedules: fixed ratio (FR) and differential reinforcement of low rate (DRL). This paper describes the behavioral differences between the absolute timing of individual intervals (duration based timing) and the relative timing of rhythmic sequences (beat-based timing) in these novel tasks.

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It is important for organisms to precisely predict when and where an event arises and to adequately act in a timely manner. Previous studies reported that temporal processing has been categorized into 4 timescales: microseconds, milliseconds, seconds, and circadian rhythms. Moreover, these are governed by different neuronal mechanisms [1,2]. For instance, temporal processing in milliseconds has been reported to rely on the cerebellum; however, detailed neuronal mechanisms remain largely unclear [1].

To explore the relationship between cerebellar neural activity and temporal processing, some studies have shown a relationship between the response time of conditioned reflexes and neuronal activity in the cerebellar cortex [3,4]. However, fewer studies have been reported on the relationship between the cerebellar neuronal spikes that organize voluntary-movement timing and temporal processing in milliseconds. Although previous studies have

suggested that the cerebellar cortex is associated with the execution of well-timed voluntary movements [5–7], they observed only the voluntary movements of which timing was animals' autochthonous pace and did not control temporal properties by experimenter. Therefore, it is necessary to analyze spike activity when an animal is working voluntarily with sub-second timing information.

Besides the above problem, the question regarding which temporal coordination during continuous or discrete events precedes cerebellar timing functions remains controversial. Grube et al. [8] used the continuous transcranial magnetic theta-burst stimulation (cTBS) to investigate which cerebellar timing functions occurs between duration-based and beat-based timing. Duration-based timing is a function that counts absolute time between discrete events. Alternatively, beat-based timing measures the continuous inter-event intervals that have a rhythmic sequence. The above authors concluded that the cerebellar obligatory function of time perception lies in the absolute timing of single intervals (duration-based timing) rather than in the relative timing of rhythmic sequences (beat-based timing). In contrast, Ohmae et al. [9] demonstrated that the cerebellar dentate nucleus plays a major role in the

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prediction of repetitive-event timing. Therefore, to detect cerebellar spike activity that contributes to such temporal processing, it is necessary to record the cerebellar spike activity during the timing behavior that requires both duration-based and beat-based timings. Thus far, no study has reported the behavioral characteristics of duration-based and beat-based timings in experimental animals.

To resolve this, we devised 2 behavioral timing tasks. Both behavioral tasks include the fixed ratio (FR) and differential reinforcement of low rate (DRL) reinforcement schedules. These novel tasks enable rats to represent the information regarding duration-based or beat-based timings with almost identical movements. We trained the rats in these tasks and identified their behavioral characteristics that are crucial in comparing the cerebellar activities related to duration-based timing with those related to beat-based timing.

To identify the cerebellar spike patterns for temporal processing, the spikes during temporal processing in milliseconds should be compared with those during supra-second processing. Therefore, we assessed a supra-second temporal processing task. Because sudden shifts in the DRL value can disrupt behavioral performance [10], we provided a DRL criterion for naive rats and evaluated the influence of the shift effect.

10 male Wistar albino rats (Shimizu Laboratory Supplies, Kyoto, Japan) were used in the experiments. All rats were provided lab chow (1–3 h after each daily training session) in amounts sufficient to maintain approximately 80%–85% of their *ad libitum* weight. They were allowed free access to water along with daily light exposure between 08:00 and 21:00 h. All experiments were conducted between 10:00 and 20:00 h as per the Guidelines for Care and Use of Laboratory Animals at Kyoto University (2007), with approval from the Animal Research Committee of Kyoto University.

The rats were trained in the behavioral tasks in an operant chamber 22 cm × 32 cm × 45 cm (Ohara Ika, Tokyo, Japan). One chamber wall had a capacitance touch switch (14 mm × 15 mm) near the center of the wall and 55 mm above the floor to detect behavioral touch responses of the rats. On the opposite chamber wall, a food dispenser behind the wall delivered 25 mg food pellets to a food magazine located at the center of the wall, 10 mm above the floor. A brief tone sounded every time the dispenser delivered a pellet. A personal computer (NEC, Tokyo, Japan) and the Arduino Mega 2560 (Arduino Software, Italy) controlled the apparatuses.

The behavioral tasks require that a rat reach out 1 of its paws and touch a switch at regular fixed intervals of 500 or 1500 ms. When the rats failed to wait during the fixed intervals, the current trial was canceled by sounding an error buzzer tone and was started again (DRL schedule). Therefore, to succeed in each trial, the rats had to perceive the interval times between the touch responses. When fixed numbers of successive touch responses were successful, the rats were rewarded with a food pellet in that trial. We call that a "tandem FR *x* DRL *y* ms schedule."

If the required number of responses (FR criterion) is 2, then the rats must generate only 1 inter-response time (IRT). When the rats have sufficiently learned the task, they can precisely predict the shortest IRT that passes the criterion (DRL criterion value). Consequently, the behavioral response is based on absolute, duration-based timing. In contrast, when the FR criterion is >2, the rats have to generate multiple IRTs. For example, when the FR criterion is 3, the rats have to generate IRT 2 times. Therefore, the rats touch the switch continuously with regular intervals, and the touches begin to yield some rhythms. We postulate that such behavior corresponds to beat-based timing.

Because it is possible that the rats learned absolute time by repetition even when the FR criterion is >2, it can be said that the duration-based and beat-based timings are interdependent. However, there must be an effect of succession of responses (i.e., relative timing) under the FR ≥3 schedules. While successive responses are

generated, the existence of preceding response(s) affects the timing of next one(s). The effect of succession for temporal processing has been demonstrated in several former studies [8,11]. Therefore, we could infer that the duration-based and beat-based timings are not identical temporal processing events.

At the initial step of training, the rats were required to touch the switch to get a pellet. The rats knew that they actually touched the switch through LED, which lit up when the switch was touched. At this stage, all rats touched the switch using their noses. At the next step, an acrylic plate was inserted between the rats and the switch (Fig. 1A, left). This design prevented the rats from using their noses to touch the switch. The center of the plate had a slit (width 10 mm), and the rats could touch the switch through the slit (Fig. 1A, right). Thereafter, the plate location was set so that the rats could touch the switch using either the right or left paw but not with their noses, and consequently, all rats begin to touch the switch using their paws.

Subsequently, the rats were randomly divided into Group I and Group II and the tandem FR-DRL schedule was introduced into the tasks. In Group I, the rats received a tandem FR 2 DRL 500 ms schedule first. The rats were rewarded when they touched the switch after 500 ms following the preceding response (Fig. 1B). When they responded during the 500 ms, a buzzer tone was presented, and the switch was covered by a guillotine door for 3 s. A session was ended when either the rats had earned 200 food pellets or 1 h had passed.

Every experimental condition was continued for 5 sessions, and we defined these 5 sessions as 1 block. Group I rats received the tandem FR 2 DRL 500 ms schedule first. After a block of the tandem FR 2 DRL 500 ms schedule, a procedure of limited hold (LH) was applied, allowing the rats to further attend the fixed interval. LH comprised a fixed available time period for response following the DRL interval. For example, in the tandem FR 2 DRL 500 ms schedule with LH at 1500 ms, the rats had to respond in 1500 ms after 500 ms following the preceding response. When the rats did not respond in LH, a brief buzzer tone was presented, and the switch was covered by the guillotine door for 3 s. After 1 block of the tandem FR 2 DRL 500 ms schedule with LH at 1500 ms, the tandem FR 3 DRL 500 ms block was performed. Next, LH was added, similar to FR 2 sessions. The same procedure was repeated until a block of a tandem FR 4 DRL 500 ms schedule with LH at 1500 ms was over (Fig. 1C(i)). A tandem FR 2 DRL 1500 ms schedule was conducted following the tandem FR 4 DRL 500 ms schedule with LH at 1500 ms. The subsequent sequence was conducted as described in Fig. 1C(i). The maximum FR value was 3 in the 1500 ms DRL (Fig. 1C(ii)).

The Group II rats received a tandem FR 2 DRL 1500 ms schedule first. Like Group I, LH introduction and increase in the FR criterion were alternately applied to every block (Fig. 1C(ii)). Following a block of the tandem FR 3 DRL 1500 ms with LH at 1500 ms, a block of the tandem FR 2 DRL 500 ms schedule was conducted. Thereafter, LH introduction and increase in the FR criterion were applied similar to that in Group I. The test session was concluded when a block of the tandem FR 4 DRL 500 ms schedule with LH at 1500 ms block was finished (Fig. 1C(ii)).

IRTs of each tandem FR–DRL schedule were recorded and analyzed for each session. IRTs of 0–100 ms were excluded from analysis because these responses may reflect the rats' involuntary quick multiple responses or a response burst. Moreover, to avoid the warm up effect, the initial 3 trials in each session were eliminated from analysis. We analyzed the initial 2 and last 2 sessions of every block and referred to these sessions as "early stage" and "late stage" of learning, respectively. We defined the former as the training session of the block condition and the latter as the test session.

Under the Group I tandem FR 2 DRL 500 ms schedule, incorrect IRTs notably decreased from the early to late stage of learning (blue and red dotted lines in Fig. 2A). In addition, the peak IRTs of