Cerebellar Role in Predictive Control of Eye Velocity Initiation and Termination

Shuntaro Miki,¹ Robert Baker,² and Yutaka Hirata^{1,3}

¹Department of Information Science, Chubu University Graduate School of Engineering, Kasugai, Japan, 487-8501, ²Department of Neuroscience, New York University Langone Medical Center, New York, New York 10016, and ³Department of Robotic Science and Technology, Chubu University College of Engineering, Kasugai, Japan, 487-8501

Predictive motor control is essential to achieve rapid and precise motor action in all vertebrates. Visuomotor transformations have been a popular model system to study the underlying neural mechanisms, in particular, the role of the cerebellum in both predictive and gain adaptations. In all species, large-field visual motion produces an involuntary conjugate ocular movement facilitating gaze stabilization called the optokinetic response. Gain adaptation can be induced by prolonged optokinetic visual stimulation; and if the visual stimulation is temporally periodic, predictive behavior emerges. Two predictive timing components were identifiable in this behavior. The first was prediction of stimulus initiation (when to move) and the other was stimulus termination (when to stop). We designed visual training that allowed us to evaluate initiation and termination independently that included the recording of cerebellar activity followed by acute and chronic cerebellar removal in goldfish of both sexes. We found that initiation and termination predictions were present in the cerebellum and more robust than conflicting visual sensory signals. Each prediction could be acquired independently, and both the acquisition and maintenance of each component were cerebellar-dependent. Subsequent analysis of the neuronal connectivity strongly supports the hypothesis that the acquired eye velocity behaviors were dependent on feedforward velocity buildup signals from the brainstem, but the adaptive timing mechanism itself originates within the circuitry of the cerebellum.

Key words: cerebellum; eye movement; goldfish; motor learning; optokinetic response; prediction

Significance Statement

Predictive and rapid motor control is essential in our daily life, such as in the playing of musical instruments or sports. The current work evaluates timing of a visuomotor behavior shown to be similar in humans as well as goldfish. Given the latter species' known brainstem cerebellar neuronal connectivity and experimental advantage, it was possible to demonstrate the cerebellum to be necessary for acquisition and maintenance of both the initiation and termination components of when to move and to stop. All evidence in this study points to the adaptive predictive control site to lie within the cerebellar circuitry.

Introduction

Since the first formal advocacy by Norbert Wiener in *Cybernetics* (Wiener, 1948), it has been recognized that biological motor systems rely on feedback control mechanisms that use vision, proprioception, and other sensory information to precisely achieve desired motor output. It is also known that sensory feedback

loops usually add significant delays that are too long to compensate for rapid and high-frequency components of motor errors and may cause unstable oscillatory motor output (Stark, 1968). Even the vestibulo-ocular reflex, known as one of the fastest sensory-motor transformation systems, takes ~10 ms to initiate eye movement after the onset of head motion (Collewijn and Smeets, 2000). Visuomotor transformations in general require up to an order of magnitude longer (~100 ms) to initiate movements (Woods et al., 2015). To solve this fundamental problem, predictive mechanisms are used in motor systems that use past time sequences of the sensory input and motor output as demonstrated in finger tapping (Repp and Su, 2013), limb movements (Torre and Delignières, 2008), and whole-body oscillations to external rhythms (Miura et al., 2011), as well as visuomotor tracking to periodic visual stimulation (Heinen et al., 2005). Brain imaging studies in humans have implicated the cerebellum, basal ganglia, and numerous primary and secondary

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Correspondence should be addressed to Dr. Yutaka Hirata, Department of Robotic Science and Technology, Chubu University College of Engineering, 1200 Matsumoto, Kasugai, Aichi 487-8501, Japan. E-mail: yutaka@isc.chubu.ac.jp.

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motor cortices as involved in predictive motor controls (for review, see Repp and Su, 2013). However, detailed neural mechanisms as to how the passage of time is represented in these areas independently and interactively for predictive motor output are currently enigmatic.

Predictive visuomotor control has been found in a wide range of species from fish through primates in response to periodic visual stimuli (Sumbre et al., 2008; Broersen et al., 2016). Zebrafish larva, for example, responded to periodic (6 s) visual disturbances with caudal fin motion and the timed escape behavior was continued, even after the periodic stimulation was suddenly terminated (Sumbre et al., 2008). Before this study, predictive optokinetic responses (OKR) were described in goldfish in response to a periodic visual stimulation (Marsh and Baker, 1997). In this case, eye velocity induced by a large-field visual stimulation moving periodically to the left and right at a constant speed started to decrease before the visual stimulation switched direction (see Fig. 1A, Trained). When the visual stimulus was turned off after acquisition of this behavior, the eye velocity profile continued for several periods in the dark (see Fig. 1C).

To achieve timed, predictive, periodical motor output, there may be at least two different prediction mechanisms involved. That is, predictions of stimulus-start timing to know when to move (Initiation) and stimulus-end timing to know when to stop (Termination). One of the animal species most thoroughly studied for visual and vestibular behavior is goldfish whose structural/functional understanding of oculomotor neurons and circuitry has accumulated for the past 2 decades. Collectively, these studies have analyzed the visuo-vestibular signal content and anatomical connectivity of cerebellar Purkinje cells (Pastor et al., 1997; Straka et al., 2006), precerebellar Area II neurons (Beck et al., 2006), and postcerebellar vestibular neurons (Pastor et al., 2015). Hence, the goldfish OKR appeared to be a good model system to begin understanding more precisely the role of the cerebellum in predictive motor control.

Presently, we performed a series of behavioral experiments in goldfish to evaluate separately the Initiation and Termination predictions in predictive OKR. We performed single-unit recording of Purkinje cell to ascertain cerebellar involvement and then evaluated behavior following acute and/or chronic cerebellectomy before or after the same experiments. Our findings show that both the Initiation and Termination timings are under predictive control, and both events can be individually established. Some Purkinje cells clearly showed predictive components in their firing modulations, and fish without a cerebellum lost both the ability to acquire and maintain either predictive component. These results suggest that cerebellar circuitry is necessary, albeit it may not be sufficient, for all aspects of acquiring and sustaining rapid and predictive visuomotor control.

Materials and Methods

General procedures

Goldfish (*Carassius auratus*) of both sexes, 12–15 cm in length, were obtained from an authorized supplier (Meitosuien) and maintained at 26°C on a 12 h light/dark cycle with the water quality monitored bi-weekly. Procedures for animal preparation were adopted from those previously described (Pastor et al., 1994b; Marsh and Baker, 1997; Aksay et al., 2000; Debony and Baker, 2011). Briefly, goldfish were restrained by fitting the mouth onto a tapered tube and holding the body, wrapped in gauze for protection, in a Plexiglas rigging lined by moistened sponges. The circular tank, 30 cm in diameter, had untextured white walls that acted as a blank background for the visual stimulus. Aerated water, at 26°C, was passed over the gills for ventilation. After preparation for re-

cording, fish were acclimated to the apparatus for 60 min before experimental intervention.

Eye movement recording

Eye movements were recorded using the scleral search coil technique. Under local xylocaine anesthesia, a 40-turn, 5-mm-diameter insulated coil (IET) was sutured at two points onto the upper scleral margin of both eyes with 8.0 ophthalmic silk. Visual field obstructions were avoided by careful eye coil suturing and testing the normal range of vestibular and visual eye movements (Pastor et al., 1992). The water tank was placed in the center of a magnetic field (DNI) generated by two sets of field coils driven by 2 sinusoids with different frequencies for the measurement of horizontal and vertical eye positions. Each of horizontal and vertical eye position signals from both eyes was digitized in synchrony with visual stimulation motion (see below). Horizontal and vertical eye velocities were calibrated online by assuming near unity gain during vestibulo-ocular reflex in light at 0.125 Hz (McElligott et al., 1995) and by rotation at a constant velocity of 20 deg/s for 1 min (Marsh and Baker, 1997).

Visual stimulation

Visual stimulation was provided by a servo-controlled planetarium that could be rotated 360° around the vertical axis at speeds ranging from 0 to 100°/s constant velocity. The planetarium projected a random light spot pattern on to the walls of the water tank. The rotational axis of the planetarium was carefully aligned to the center of the goldfish head at the level of the horizontal semicircular canals. Horizontal and vertical eyes, including planetarium positions, were measured by a potentiometer (Midori Precisions). The signals were continuously digitized at a sampling frequency of 1000 Hz in 16 bits using a Power 1401 interface (Cambridge Electronic Design) for display and storage using the Spike2 program. The commands to drive the planetarium were also generated in Spike2 and D/A converted by Power1401.

Experimental paradigm

All goldfish were trained by using one of the following optokinetic visual stimulation paradigms. Each stimulus cycle was repeated for 3 h. After training, the visual stimulation was turned off for 2–3 min in the dark followed by post-training testing. The test evaluations were conducted according to the various training paradigm.

Bidirectional velocity step. The planetarium was rotated at a constant speed of 20 deg/s alternately in both clockwise (CW) and counterclockwise (CCW) directions for 8 s each. Before beginning this training, visual stimulation with an extended period (16 s for each of CW and CCW directions) at 20 deg/s was applied for 10 cycles. After training, the same extended period visual stimulation was applied. This paradigm was conducted to confirm and more quantitatively present the results shown in the previous study (Marsh and Baker, 1997).

Unidirectional velocity step. The planetarium was rotated only in the CW direction for 8 s at a constant speed of 20 deg/s, and stopped for 8 s. This ON-OFF cycle was repeated for the duration of the training. This paradigm was implemented to distinguish Initiation and Termination predictions that were indistinguishable by bidirectional velocity step paradigm.

Unidirectional double velocity step. The planetarium was rotated only in the CW direction at a constant velocity of 10 deg/s for 4 s and at 20 deg/s for 4 s, then stopped for 8 s. This paradigm was designed to further characterize Initiation prediction.

Unidirectional velocity step with fixed duration at variable interval. The planetarium was rotated only in the CW direction for 8 s at 20 deg/s, and stopped for uniformly random intervals lasting between 1 and 15 s. This paradigm tested whether Termination prediction alone can be acquired independently from Initiation prediction.

Unidirectional velocity step with variable duration at fixed interval. The planetarium was rotated only in the CW direction for uniformly random duration between 1 and 15 s at 20 deg/s, and stopped for 8 s. This paradigm asked whether Initiation prediction alone can be acquired independently from Termination prediction.

A total of 48 goldfish were used for 6 different behavioral experiments with 8 animals for each experiment.

Surgical procedures

The animal welfare committee of Chubu University approved all these experimental and surgical procedures. Before experimental sessions, animals were anesthetized by immersion in a solution 1:20,000 w/v of MS222 (tricaine methanesulfonate, Sigma-Aldrich). A pedestal of dental acrylic cemented to self-tapping screws was fastened to the frontal bones to provide head stabilization during experiments (Pastor et al., 1992). A 3 mm triangle window was trephined in the occipital bone to allow access to the cerebellum. The bone flap was reattached with cyanoacrylic glue and removed for either recording or cerebellectomy. Circulating aquaria water revived the goldfish. The cerebellum was aspirated in 1–3 min through a 23-gauge needle (Pastor et al., 1994b). Eye movements were recorded throughout the surgery, and visual stimulation was restarted in <1 min after the surgery to evaluate postcerebellectomy OKR. Cerebellectomy for chronic experiments was performed at least a couple of days before the experiments and only studied in fish that swam normally in their home tank.

Purkinje cell recordings

Extracellular recording of the electrical activity of Purkinje cells was performed with beveled glass micro-electrodes filled with 2 M NaCl and impedances in the range of 2–4 Mega-ohms. The extracellular neural activity was amplified by a pre- and main-amplifiers and bandpass-filtered between 300 and 3000 Hz using a neural recording system (Lynx-8 Amplifier, Neuralynx). The filtered signal was monitored and saved in a PC thru the Power 1401 in synchrony with other behavioral data using Spike2 software. Purkinje cells were recorded in the area of the vestibulo-cerebellum identified as responding to visual and/or vestibular stimulation (Pastor et al., 1997). Electrodes were advanced into the vestibulo-cerebellum with a micromanipulator (MO-10, Narishige), and Purkinje cells were usually isolated between 2 and 3 mm from the surface of the corpus cerebelli. Simultaneous recordings of simple and complex spikes responding to visual stimulation around the vertical axis were used as criterion for Purkinje cell recording. However, as reported previously (Pastor et al., 1997), the relative amplitude of the simple and complex spike depended on the position of the microelectrode, and a complex spike was often not distinguishable at the best microelectrode location for stable simple spike recording. A total of 34 animals were used for Purkinje cell recording (for details, see Results).

Data analysis

Data recorded in Spike2 were exported to MATLAB (The MathWorks), and all analyses were done offline in MATLAB. Eye velocity was calculated by applying a 3-point low pass differentiation filter to eye position data sampled at 1000 Hz. High-frequency noise components amplified by the differentiation processing were eliminated by applying twice a 101-point moving average filter with a cutoff frequency of 4.39 Hz in offline analysis. Saccades and postsaccadic drifts, if present, were eliminated by applying a custom-made automatic desaccading algorithm using an acceleration threshold (Hirata and Highstein, 2001). In this study, the eliminated portions of the data were not used for later analyses.

To analyze the identified OKR eye velocity components in Figure 1B quantitatively, eye velocity traces during 3 h training were averaged between individual animals ($N = 8$) for all experimental conditions by alignment with the visual stimulus waveforms. Average values were calculated, excluding the portions eliminated due to desaccading. In all visual training paradigms, the 3 h data contained 675 stimulus cycles.

The following OKR parameters were calculated from the averaged eye velocity of 8 fish for each stimulus cycle to quantify Initiation, Termination, Direct and Indirect eye velocity components.

Initiation. The eye velocity waveform for 2 s before the onset timing of the visual stimulus was approximated by a linear regression model ($at + b$, where a and b are variables and t denotes time), and the amount of eye velocity increase during the 2 s ($2a$) period was referred to as the Initiation component.

Termination. The difference between the maximum eye velocity during the stimulation ON period and the eye velocity at the end of the stimulus ON period was measured as the Termination component.

Direct. The Direct component of OKR in each visual stimulus period was quantified by the mean eye velocity between 496 and 505 ms (10 data points) after the onset of the visual stimulation at 0 ms.

Indirect. The eye velocity waveform between 500 ms and 4 s was approximated by the function $c + d \exp(-t/\tau)$. The inverse of τ ($1/\tau$) and c was referred to as time constant and magnitude of the Indirect component, respectively. Variables c , d , and τ were estimated by a nonlinear optimization method using the MATLAB lsqnonlin function.

Results

Similar experiments to those illustrated in a previous study demonstrating that predictive OKR in goldfish were performed to confirm and more quantitatively present the results (see Fig. 1) (Marsh and Baker, 1997). We then present the results of unidirectional velocity step and double velocity step visual stimulations to characterize Initiation and Termination predictions (see Figs. 2, 3). Subsequently, unidirectional velocity step with fixed/variable duration/interval stimulations are presented in which either duration of the stimulus ON period or that of the OFF period (interval) was randomized to evaluate independently the Initiation and Termination predictions (see Fig. 4). Afterward, the results are set out for acute cerebellectomy either before or after the acquisition of the predictive OKR as well as assessing the same stimulus paradigms following chronic cerebellectomy (see Figs. 5, 6). Finally, single-unit recordings of vestibulo-cerebellar Purkinje cells are shown during predictive OKR to assess their participation in the predictive OKR (see Fig. 7).

Predictive OKR trained by bidirectional optokinetic velocity steps

General characteristics during training

Figure 1A illustrates horizontal eye position (top) and velocity (bottom) traces of a typical experiment (Control) and 3 h after (Trained) the beginning of repetitive presentation of bidirectional velocity step visual stimulation. Traces from left (orange) and right eyes (green) are superimposed, showing that both eyes move almost identically during OKR. The eye position traces show slow and fast phases (gray arrows), and the eye velocity traces are shown by thin gray lines. Clearly, the slow phase eye velocity of ± 13 deg/s in the Control did not catch the stimulus velocity of ± 20 deg/s (solid gray lines), whereas it was close to the stimulus velocity after training. Namely, OKR gain adaptation occurred as previously well described (Ito et al., 1979; Nagao, 1983; Marsh and Baker, 1997); however, in addition, there was a noticeable change in the eye velocity profile. In Figure 1A (Control), eye velocity continued to build up until stimulus direction changed, whereas in Figure 1A (Trained) it began to decrease before stimulus direction changes (black arrows), confirming the observations of a previous study (Marsh and Baker, 1997). In respect to stimulus direction, this change in timing produced predictive eye movements.

To evaluate general characteristics of the eye velocity profiles, we calculated average eye velocity from 8 experiments (Fig. 1B) as opposed to single traces evaluated without averaging in the previous study. Figure 1B (left) illustrates averaged Control (blue) and Trained (red) eye velocity superimposed with stimulus velocity trace (gray). After the visual stimulus changed direction, both Control and Trained eye velocity showed a rapid initial jump (①) followed by a gradual buildup (②). Classically, the rapid initial jump has been considered to be generated by the OKR direct pathway, whereas the gradual buildup occurs through the OKR indirect pathway (Waespe and Henn, 1985; Fuchs and Mustari, 1993) as schematically illustrated in Figure 1D (Cohen et al., 1977).

Figure 1. Predictive OKR trained by bidirectional velocity steps. **A**, Performance of eye movement before (left, Control) and after (right, Trained) 180 min bidirectional velocity step training of a representative goldfish in response to the training stimulation whose half-period is 8 s. Top, Orange represents left eye position. Green represents right eye position. Gray arrows indicate slow and fast phases. Bottom, Slow phase eye velocity and visual stimulus velocity (gray). Gray thin lines indicate fast phases in eye velocity. +, Rightward (CW) directed eye positions; −, leftward (CCW) directed eye positions. **B**, Averaged eye velocity over 8 experiments before (blue, Control) and after (red, Trained) bidirectional velocity step training in response to the visual stimulation used for training (left, gray) and during extended period stimulation whose half-period was 16 s (right, gray). Black arrows indicate OKR components consisting of the following: ①, Direct; ②, Indirect; ③, Termination. **C**, Performance of eye velocity in the dark before (blue) and just after (red) bidirectional velocity step training of the same fish as in **A**. Visual stimulation (gray) was turned off at the black down arrowhead. **D**, A system model of the OKR (Cohen et al., 1977; Waespe and Henn, 1987) consisting of direct and indirect pathways whose outputs are Direct and Indirect components in **B**, respectively.

The OKR indirect pathway is considered to include the velocity storage mechanism (VSM) that charges and discharges eye velocity signals like a capacitor in an electrical circuit charging and discharging electrical current (Cohen et al., 1977). The VSM is characterized by its capacity (how much eye velocity can be stored) and the charging and discharging time constants (how fast eye velocity can be charged and discharged). When velocity step visual stimulation is applied, the eye velocity signal gradually builds up; and when the visual stimulation is suddenly turned off, the stored eye velocity signal gradually decreases until the VSM is completely discharged. The alternating slow and fast phase eye velocity profile produced in the dark is called optokinetic after-nystagmus (OKAN). Unlike a passive capacitor in an electrical circuit, the VSM's time constants for charging and discharging are different. Nodular cerebellectomy in monkeys altered the discharging (OKAN) time constant, although it did not affect charging behavior (Cohen et al., 1977; Waespe et al., 1985). Figure 1B shows that, after training, the Direct component increased, whereas the Indirect building-up time constant shortened. Also noticeable is that eye velocity began to slow down at ~ 3 s before the direction of the stimulus changed in Trained compared with Control (Fig. 1B, red trace, ③). This predictive slowing was called the Termination component (③), even though in this paradigm the bidirectional stimulus profile could not distinguish the beginning (①) from the end (③).

Predictive eye velocity after training manifested by visual stimulus with longer duration

Figure 1B (right) illustrates averaged Control and Trained eye velocity over 8 experiments superimposed on stimulus velocity (gray) whose duration (16 s) is twice as long as the training stimulus duration (8 s). Control (blue) and Trained (red) data were recorded just before the beginning, and then after the end of the 3 h training, respectively. The Termination component (③) appeared in the Trained eye velocity around 8 s after the directional switch of the visual stimulus, even though the visual stimulation continued at the constant velocity. This predictive eye velocity behavior seen after the 3 h training continued, on average, 15–30 min before gradually returning toward control behavior.

Predictive eye velocity after training manifested in the dark

In addition to the predictive decrease in eye velocity before the stimulus directional switch, and as seen previously, an oscillatory eye velocity was observed in the dark when the visual stimulation was suddenly turned off after 3 h bidirectional training (Fig. 1C, red). For comparison, control eye velocity data (blue) in the dark are shown after presenting 10 cycles of the bidirectional visual stimulation before training. Durations of the eye velocity oscillation in the dark after the 3 h training varied from 15 to 60 s in the 8 experiments sampled.

Unidirectional optokinetic step training

After quantifying the behavioral changes associated with bidirectional velocity step visual training demonstrated in the previous study (Marsh and Baker, 1997), we then applied novel unidirectional velocity step visual stimulations to describe details of each predictive component.

Single velocity steps

The bidirectional velocity step visual stimulation shown in Figure 1 did not allow the onset and offset timings of the stimulus to be distinguished because offset in one direction was always onset to the other direction. Although we called ③ Termination in Figure 1, it could have been an anticipatory component generated for

Unidirectional Optokinetic Step

Figure 2. Predictive OKR trained by unidirectional velocity steps. **A**, Performance of eye velocity before (top, Control) and after (bottom, Trained) 180 min unidirectional velocity step training in response to the training stimulation (gray). Left traces, Two stimulus cycles of raw eye velocity whose fast phases were eliminated. Right traces, Eye velocity averaged over 10 first (top) and last (bottom) cycles of the training. Bottom, Black curved arrows indicate decreases in eye velocity before the offset timing of visual stimulation (gray). **B**, Averaged eye velocity over 8 experiments before (blue, Control) and after (red, Trained) unidirectional velocity step training in response to the visual stimulation used for training (gray). Inset, Magnified eye velocity traces just before the onset of the visual stimulation. ①, ②, and ③ indicate Initiation, Direct, and Termination components, respectively, of OKR. **C**, Performance of eye velocity in the dark just after unidirectional velocity step training in the same experiment as in **A**. Visual stimulation (gray) was turned off at the black down arrowhead.

onset of the visual stimulus to the opposite stimulus direction. To discriminate visual stimulus onset from offset, we used unidirectional velocity step visual stimulation in which periodic visual stimulation rotated only in one direction (Fig. 2). Control (Fig. 2A, blue) and Trained (Fig. 2A, red) eye velocity from a typical animal during unidirectional velocity step stimulation is shown in Figure 2. Traces on the left show the first (top) and the last (bottom) 2 cycles of stimulus and eye velocity during the training, whereas the traces on the right show averaged eye velocity over the first (top) and the last (bottom) 10 cycles of the training to better characterize the differences in eye velocity waveforms in this experiment. As observed in Figure 1A for bidirectional stimulation, Trained eye velocity showed a gradual decrease before the offset of each cycle of the visual stimulation (solid curved arrows). Thus, the Termination component (③) is actually a predictive component of the visual stimulus offset. The Termination

component is clearly seen in the averaged Trained eye velocity traces from 8 different experiments (Fig. 2B, red arrow, ③). This behavior was not seen in the superimposed averaged Control eye velocity (blue arrow).

In addition to the Termination component, the averaged traces (Fig. 2B) revealed that Trained eye velocity started to increase before onset of the visual stimulation, as shown in Figure 2B (inset, ⑥). Control and Trained eye velocity at the onset of the stimulus was 0.2 and 1.1 deg/s, respectively. These values were significantly different ($p < 6.47 \times 10^{-8}$, t test). For referral purposes, we have labeled the Initiation component (⑥).

The maximum eye velocity reached during stimulus ON periods was significantly larger in Trained than in Control. Thus, OKR gain increased in response to unidirectional velocity step training as it did to bidirectional velocity step training shown in Figure 1. The mean averaged OKR gains of Control and Trained were 0.54 and 0.89, respectively, and significantly different ($p < 3.04 \times 10^{-13}$, t test). Similarly, the Direct component eye velocity (①) was larger in Trained (13.6 deg/s) than in Control (4.4 deg/s) ($p < 2.05 \times 10^{-6}$, t test). The time constant $1/\tau$ of the Indirect component (②) was shorter in Trained (1.0 s) than in Control (2.3 s) conditions ($p < 0.00228$, t test). These changes in eye velocity parameters during training are further quantitatively evaluated later in Figure 8.

Figure 2C illustrates eye velocity in the dark in the same experiment just after 3 h unidirectional velocity step training. Unlike eye velocity traces after bidirectional velocity step training, more robust eye velocity oscillations were observed in the dark after unidirectional velocity step training. Notably oscillations occurred in only the trained direction, with durations varying between 0.5 and 3 min.

These results from unidirectional single velocity step experiments revealed Initiation and Termination prediction components that, respectively, are eye velocity increases and decreases before stimulus onset and offset. Further, it showed that eye velocity oscillation in the dark after this training occurred only toward the trained direction.

Double velocity steps

To further confirm individuality of the Initiation component shown in Figure 2B (⑥), we used unidirectional double velocity step visual stimulation, as shown in Figure 3 with a format the same as in Figure 2. In this training paradigm, optokinetic visual stimulation (Fig. 3A, gray line) stepped up from 10 to 20 deg/s at 4 s after the onset of each visual stimulation cycle. Figure 3B shows not only a Termination component (③), but also an Initiation component (⑥), to be present in the averaged Trained eye velocity trace before the stimulus velocity stepped up. Namely, Trained eye velocity started to increase gradually and actually exceeded the stimulus velocity (10 deg/s) before the visual stimulus stepped up (inset, ⑥). The average eye velocity over 8 experiments at the onset of the second velocity step was 10.6 deg/s, which was significantly greater than the stimulus speed ($p < 2.32 \times 10^{-4}$, t test). By contrast, the Initiation component for the first velocity step was not as large as that shown in Figure 2B likely due to the 0 to 10 deg/s versus 20 deg/s in the two paradigms. However, the two 10 deg/s steps combined in Figure 3 exceeded the single 20 deg/s step shown in Figure 2.

Unidirectional eye velocity modulation in the dark was observed after the 3 h double step visual stimulation training (Fig. 3C). Noticeably, in this paradigm, the eye velocity profile did not show a clear double step-up in the dark (Fig. 3C), resembling more the unidirectional single step-up (Fig. 2C).

Unidirectional Optokinetic Double Step

Figure 3. A–C, Predictive OKR trained by unidirectional double velocity steps. Formats are the same as in Figure 2.

These results from unidirectional double velocity step experiment revealed that the Initiation component can also be acquired when the eyes are following the visual stimulus and become even more accentuated than when the visual stimulus is stationary. Notably, eye velocity at the Initiation of stimulus step-up timing exceeds stimulus velocity, which hardly occurs in ordinary OKR.

Single velocity steps with fixed/variable duration at variable/fixed interval

To test whether Initiation and Termination components could be acquired independently, we used unidirectional velocity step visual stimulation in which either duration (ON period) or interval (OFF period) was randomized so that the offset or the onset of the stimulation was not predictable. Fixed duration at variable interval experiments is shown in Figure 4A from one representative experiment and in Figure 4B for 8 experiments averaged. The stimulus ON duration was constant at 8 s while stimulus OFF duration was randomized between 1 and 15 s such that onset timing could not be predicted. After presentation of this stimulus for 3 h, a Termination component was acquired (Fig. 4B, ③) comparable with that after the fixed duration/interval training (Fig. 2B). However, clearly no Initiation component was observed (Fig. 4B, ⑥).

By contrast, after presentation of variable duration at fixed interval stimulation for 3 h in which duration of the stimulus ON

Unidirectional Optokinetic Step with Randomized Interval or Duration

Figure 4. Predictive OKR trained by unidirectional velocity steps with randomized interval or duration. **A, B**, Results of fixed duration at randomized interval experiments. **C, D**, Those of randomized duration at fixed interval experiments. **A**, Performance of eye velocity before (top, Control) and after (bottom, Trained) 180 min training in response to the training stimulation (gray). **B**, Averaged eye velocity over 8 experiments before (blue, Control) and after (red, Trained) the training in response to the visual stimulation used for training (gray). Averaged eye velocity traces during the stimulus interval are shown only for 1 s after the offset and before the onset of the stimulus. ①, ②, and ③ indicate Initiation, Direct, and Termination components, respectively, of OKR. **C, D**, Formats are the same as **A** and **B**, respectively.

period was randomized, an Initiation component was clearly acquired (Fig. 4C from one representative experiment; and Fig. 4D for 8 experiments averaged). However, because the stimulus offset could not be predicted, a Termination component was not seen (Fig. 4C,D). Therefore, the Initiation and Termination predictions can be independently acquired. Notably, Direct (①) and Indirect (②) components changed after both the fixed duration at variable interval (Fig. 4B) and the variable duration at fixed interval training (Fig. 4D) as after unidirectional fixed duration/interval training (Fig. 2B). Gain and prediction behaviors are further evaluated quantitatively in Figure 8.

These results confirmed that the Initiation and Termination components can be acquired independently.

Effects of cerebellectomy

The cerebellum has been implicated in OKR adaptation and predictive motor control in general (Sokolov et al., 2017). Thus, we performed cerebellectomy after training (acute) or days before (chronic) training with unidirectional velocity step fixed duration/intervals.

Cerebellectomy after unidirectional velocity step training

Figure 5 illustrates results of acute cerebellectomy experiments in the same format as in Figures 2 and 3. Acute cerebellectomy after 3 h of unidirectional fixed duration/interval training resulted in significant changes in eye velocity profile. Both Control and Trained eye velocity in Figure 5A is similar to those shown in Figure 2A for the same training paradigm in which both Termination and Initiation components were present in the Trained condition. After acute cerebellectomy (Fig. 5A, Cerebellectomized), overall eye velocity became slower than that in the Trained condition, closely resembling the Control eye velocity

profile. Comparison of averaged eye velocity profiles of Control (Fig. 5B, blue), Trained (red), and Cerebellectomized (green) revealed that acquired Initiation, Direct, and Termination components (①, ②, ③) were removed by cerebellar removal. Notably, maximum eye velocity (OKR gain) that is closely related to the Indirect component (②) decreased to a lower value than Control eye velocity.

Eye velocity oscillation in the dark acquired during the 3 h unidirectional velocity step with fixed duration/interval training (Fig. 5C, red) also was immediately removed after acute cerebellectomy (green). The record shown was acquired in the dark 80 min after additional unidirectional velocity step fixed duration/interval training.

These results demonstrated that maintenance of acquired Initiation and Termination predictions as well as that of Direct component are fully cerebellar-dependent. However, the changes in the Indirect component after training may involve a neuronal substrate other than the cerebellum.

Cerebellectomy before unidirectional velocity step training

Figure 6 shows results of chronic cerebellectomy experiments illustrated in the same format as in Figures 2, 3, and 5. Figure 6A shows an eye velocity trace sampled before (Control, blue) and after 3 h unidirectional fixed duration/interval training (Trained, red) in an experiment in which the cerebellectomy was performed 1 week earlier. Without the cerebellum, no clear changes occurred in eye velocity after 3 h training, even in the cycle averaged traces (Fig. 6A, right). However, averaged over 8 experiments, Control (Fig. 6B, blue) and Trained (red) eye velocity traces showed quite similar differences as seen in those following acute cerebellectomy (Fig. 5B, blue vs green). Namely, while the Initiation (①), Direct (②), and Termination (③) components

Figure 5. Effects of acute cerebellectomy on predictive OKR trained by unidirectional velocity steps. Formats are the same as in Figure 2, except that eye velocity traces after acute cerebellectomy that were conducted just after 180 min training are shown in green in **A–C** as “Cerebellectomized.”

were hardly altered, maximum buildup eye velocity reached during the stimulus ON period (OKR gain) appeared significantly smaller in cerebellectomized experiments. To the best of our knowledge, this is the first demonstration showing OKR gain modification in cerebellectomized animals.

None of the cerebellectomized experiments ($N = 8$) showed eye velocity oscillation in the dark after 3 h training as exemplified in Figure 6C from a representative experiment, as observed in cerebellar intact experiments (Fig. 2C).

These results demonstrated that the acquisition of Initiation and Termination prediction as well as the increase in the Direct component are fully cerebellar-dependent, whereas changes in the Indirect component may involve a neuronal substrate other than the cerebellum.

Figure 6. **A–C**, Effects of chronic cerebellectomy on predictive OKR trained by unidirectional velocity steps. Formats are the same as in Figure 2.

Firing modulation of Purkinje cells

Forty-eight Purkinje cells showing horizontal eye velocity sensitivity were recorded from the left vestibulo-cerebellum of 34 animals during visual training. The Purkinje cell population exhibited firing rates that increased during contralateral eye velocity (positive values in ordinate). According to the Pastor et al. (1997) terminology, these Purkinje cells would be classified as Eye velocity Type II (eII), Head velocity Type I, or Eye velocity Type II (hII). During predictive OKR visual training, among the 48 Purkinje cells recorded, 24 cells showed firing modulation clearly correlated with eye velocity as previously described (Pastor et al., 1997; Hirata and Highstein, 2001), including the predictive components. Figure 7A shows modulation of a representative Purkinje cell's simple spikes (top) and eye velocity (bottom) simultaneously recorded during bidirectional step velocity training (left, Training Stimulus Period). Records on the right show a bidirectional extended period stimulation after the training as shown in Figure 1 (Extended Stimulus Period). The continuous gray trace on the left shows first simple spikes and then instantaneous firing rates indicated by the gray dots. The black traces superimposed on Figure 7A (left, right) show mean firing rates of the Purkinje cell activity. Cycle Average shows simple spikes' firing rate and eye velocity averaged over 10 last cycles of the visual training and extended period stimulation. Notably, during the Extended Stimulus Period, these neurons increased their firing rate, not only with contralateral eye velocity, but also

Purkinje Cell Firing Modulation

Figure 7. Purkinje cell firing modulation after acquisition of predictive OKR. **A**, Firing modulation of a Purkinje cell in the left vestibulo-cerebellum (top) and eye velocity (bottom) simultaneously recorded during bidirectional velocity step training (left) and those during bidirectional extended period stimulation after the training (right). In Firing Rate, gray traces and dots represent simple spikes and instantaneous firing rate calculated from two consecutive simple spikes, respectively. Black trace represents mean firing rate averaged over ± 250 ms. The visual stimulations are the same as in Figure 1. Cycle averages on the right show firing rate and eye velocity averaged over 10 stimulus cycles. **B**, Simple spike firing rate of another Purkinje cell and simultaneously recorded eye velocity during the same training paradigm (left) and their cycle average during the training (middle) and extended stimulus period after the training (right). **C**, Firing modulation of the same neuron as in **A** and eye velocity in the dark after the visual training.

with suppressed ipsilateral eye velocity when predictive eye velocity suppression was manifested. Moreover, firing rates in the dark after bidirectional velocity step training in this Purkinje cell showed a clear modulation in parallel with eye velocity (Fig. 7C). Figure 7B shows the firing modulation of another typical Purkinje cell (top) and eye velocity (bottom) corroborating the overall predictive behavior during both the Initiation and Termination components as well as during the Extended Stimulus Period (instantaneous firing rates gray dots and mean firing rates black same as in Fig. 7A).

Collectively, these results demonstrate that at least a subset of Purkinje cells in the vestibulo-cerebellum encodes all of the predictive components illustrated in Figure 1B after the acquisition of predictive OKR.

Time courses of changes in each eye velocity component during each training paradigm

Eye velocity components during different training paradigms

To quantitatively further evaluate how each OKR component changed during the various training paradigms, we calculated a representative value, as described in Materials and Methods, from each component during the 3 h training paradigm consisting of 675 stimulus periods. The evaluated parameters are illustrated in Figure 8A: eye velocity at stimulus onset, Initiation in deg/s (①), maximum value of the Direct component in deg/s (②),

building-up time constant for the Indirect component (2τ) in seconds and magnitude (③ gain) in deg/s, and eye velocity reduction extent at stimulus end timing from the maximum eye velocity for Termination in deg/s (④).

Figure 8D shows the time course changes in each of the 5 parameters throughout the 3 h for unidirectional velocity step with fixed duration/interval training. In each panel, gray dots represent an estimated value from a single stimulus period, and the colored traces represent the moving averages spanning 15 periods. During this training, all of the values changed significantly. Namely, Initiation (①) changed from 0.2 to 1.1 deg/s, Direct (②) from 4.4 to 13.6 deg/s, Indirect (2τ) from 2.3 to 1.0 s, gain from 14.0 to 19.3 deg/s, and Termination (④) from 0.1 to 4.1 deg/s. These changes were all statistically significant (p values, Fig. 8).

During the variable interval training in which the onset of the visual stimulus was unpredictable (Fig. 8B, same format as Fig. 8D), Initiation (①) remained unchanged from the initial value. By contrast, Indirect (2τ) and gain, and Termination (④) showed almost identical changes to those in unidirectional velocity step with fixed duration/interval training (corresponding values and statistical significance, Fig. 8D). In contrast, the change in Direct (②) was significantly less than that during unidirectional velocity step with fixed duration/interval training (D), suggesting that the

Changes in Eye Velocity Components

Figure 8. Changes in eye velocity components during each kind of predictive OKR training. **A**, Definition of OKR eye velocity components. ①, Initiation eye velocity at the stimulus onset timing; ②, peak velocity of Direct component; ③, time constant of building-up Indirect component; ④, gain, peak velocity of Indirect component; and ⑤, amount of decrease in Termination eye velocity. **B**, Unidirectional velocity step with fixed duration at randomized interval training. Values that changed from pretraining to post-training: Initiation (①) from -0.5 to -2.1 deg/s ($p < 0.167$, t test); Direct (②) from 3.7 to 10.5 deg/s ($p < 1.08 \times 10^{-6}$, t test); Indirect (③) from 1.7 to 0.9 s ($p < 0.000560$, t test), gain from 11.9 to 18.9 deg/s ($p < 6.46 \times 10^{-8}$, t test); and Termination (⑤) from 0.0 to 3.2 deg/s ($p < 5.01 \times 10^{-9}$, t test). **C**, Unidirectional velocity step with randomized duration at fixed interval. Values changed from pretraining to post-training: (Figure legend continues.)

Direct component contains a predictive factor that could not be acquired when onset timing of visual stimulation was unpredictable.

During unidirectional velocity steps with variable duration at fixed interval training (Fig. 8C, same format as B,D), the stimulus for OFF timing could not be predicted. As a result, Termination (③) changed very little, whereas Direct (①), Indirect (② τ), and gain showed comparable changes with those seen during unidirectional step with fixed duration/interval training (for values and statistics, see Fig. 8D). A possible reason for small change in Termination during this training paradigm was likely the limited random range of the variable stimulus from 1 to 15 s. As a result, the predictive mechanisms adapted with uncertainty. Initiation (②) also changed less than that during unidirectional velocity step with fixed duration/interval training (Fig. 8D), suggesting that this predictive component depended not only on the interval time, but also on the preceding duration of the stimulus ON period.

Effects of cerebellectomy on eye velocity components

The acute cerebellectomy experiments, first, confirmed that changes in the four OKR features during the 3 h unidirectional velocity step with fixed duration/interval training (Fig. 8E) were statistically identical to those during the same training paradigm in cerebellar intact experiments (Fig. 8D). After acute cerebellectomy, Initiation (②), Direct (①), and Termination (③) acquired by 3 h training returned to pretraining values (corresponding values and statistics in Fig. 8 legend). By contrast, the acquired change in Indirect (② τ) (0.8 s) was only slightly affected by acute cerebellectomy and did not return to pretraining values (1.6 s). Indirect (②) gain decreased after acute cerebellectomy to a significantly lower value than its original value ($p < 0.000541$, t test), suggesting that presentation for part of the modified memory regarding the Indirect component does not require the cerebellum. The absence of training during the acute cerebellectomy, averaging at most 4 min (Method), should have minimal effects on these parameters as a similar paradigm in a previous study showed the learned predictive eye velocity persisted for up to 50 min (Marsh and Baker, 1997).

The results from the chronic cerebellectomy experiments showed that all the pretraining values were comparable with

those of the cerebellar intact. Initiation (②), Direct (①), and Termination (③) did not change during 3 h training in the chronic cerebellectomy experiments. These were the same three parameters that returned to pretraining values after acute cerebellectomy. In contrast, Indirect (② τ), which did not return to pretraining values after acute cerebellectomy, decreased significantly in the chronic cerebellectomy experiments. The final value (0.8 s) was equivalent to that of cerebellar intact after 3 h training (1.0 s), but the rate of change during the 3 h training was slower in the chronic cerebellectomy experiment (compare Fig. 8D, E; ② τ). The Indirect (②) gain also gradually decreased to values comparable with that after acute cerebellectomy. The amount of decrease during 3 h training from 12.5 to 8.3 deg/s was significant ($p < 0.000179$, t test).

Together, the cerebellum was demonstrated to be necessary for acquisition and maintenance of Initiation and Termination components as well as changes in Direct component; although it was not necessary for maintaining the acquired Indirect component, the acquisition process was slower without the cerebellum.

Discussion

One of the most distinctive features of any nervous system is the ability to predict incoming sensory inputs based upon past experiences (Clark, 2015). The ability to foresee future sensory inputs and produce predictive motor output is well represented throughout the animal kingdom from fish to humans. Predictive visuomotor control has been shown in a wide range of species in response to periodic sensory stimuli (smooth pursuit: Collins and Barnes, 2009; Deno et al., 1995; ocular following response: Miles and Kawano, 1986; saccades: Zorn et al., 2007; Takeya et al., 2017). In these studies, the stimulus periods were < 1 s for which the cerebellum plays a crucial role (Golombek et al., 2014). Our study focused on the predictive OKR behavior in the goldfish that extends the stimulus periods up to 2 orders of magnitude longer, and demonstrated that the cerebellum is also required for this predictive behavior.

What is predicted in predictive OKR and roles in eye movement control

By using novel visual stimulation, we demonstrated that both of the onset and offset timings of the stimulation are predicted as shown by the Initiation and Termination component, respectively (Fig. 2). The onset timing prediction was pronounced in response to the double velocity step stimulation, such that Initiation component of eye velocity gradually increased and even exceeded the speed of the visual stimulation before onset of the second velocity step (Fig. 3). When we randomized either interval or duration of the unidirectional velocity step stimulation to make onset or offset timing unpredictable, only one of the predictive components was clearly acquired (Fig. 4), suggesting that the onset and the offset timing predictions are independent processes.

Both Initiation and Termination predictions result in increasing image slip on the retina. This strategy would work against the basic function of the OKR that is thought to minimize retinal image slip by matching eye velocity to visual stimulus velocity via a visual feedback loop (Fig. 1D). One possible benefit of increasing retinal slip during the predictive behavior would be to reduce slip following the onset and offset of the stimulation. In the case of the Initiation component, much less retinal slip increase is seen, and this would amplify the gain of the Direct component with the same mechanism as seen with smooth pursuit in which the gain is higher when the eyes are moving than when stationary

←

(Figure legend continued.) Initiation (②) from -0.1 to -0.4 deg/s ($p < 0.00516$, t test); Direct (①) from 3.5 to 10.9 deg/s ($p < 2.69 \times 10^{-7}$, t test); Indirect (② τ) from 1.9 to 0.6 s ($p < 9.81 \times 10^{-5}$, t test), gain from 11.8 to 17.4 deg/s ($p < 0.000291$, t test); and Termination (③) from 0.1 to 1.2 deg/s ($p < 0.00159$, t test). D, Unidirectional velocity step. Values changed from pretraining to post-training: Initiation (②) from 0.2 to 1.1 deg/s ($p < 6.47 \times 10^{-8}$, t test); Direct (①) from 4.4 to 13.6 deg/s ($p < 2.05 \times 10^{-6}$, t test); Indirect (② τ) from 2.3 to 1.0 s ($p < 0.00228$, t test), gain from 14.0 to 19.3 deg/s ($p < 6.83 \times 10^{-5}$, t test); and Termination (③) from 0.1 to 4.1 deg/s ($p < 1.76 \times 10^{-10}$, t test). E, Acute cerebellectomy after unidirectional velocity step. Values changed from pretraining to post-training: Initiation (②) from 0.1 to 0.7 deg/s ($p < 0.000384$, t test); Direct (①) from 4.8 to 12.4 deg/s ($p < 2.70 \times 10^{-7}$, t test); Indirect (② τ) from 1.6 to 0.8 s ($p < 0.0121$, t test), gain from 12.7 to 19.3 deg/s ($p < 7.86 \times 10^{-6}$, t test); and Termination (③) from 0.2 to 4.1 deg/s ($p < 9.23 \times 10^{-7}$, t test). The values changed from pretraining to postcerebellectomy: Initiation (②) from 0.1 to -0.1 deg/s ($p < 0.249$, t test); Direct (①) from 4.8 to 3.9 deg/s ($p < 0.0914$, t test); Indirect (② τ) from 1.6 to 1.0 s ($p < 0.0869$, t test), gain from 12.7 to 8.67 deg/s ($p < 0.000541$, t test); and Termination (③) from 0.2 to 0.1 deg/s ($p < 0.147$, t test). F, Chronic cerebellectomy before unidirectional velocity step. Values changed from pretraining to post-training: Initiation (②) from 0.2 to 0.1 deg/s ($p < 0.463$, t test); Direct (①) from 4.8 to 3.3 deg/s ($p < 0.00263$, t test); Indirect (② τ) from 1.8 to 0.8 s ($p < 0.00527$, t test), gain from 12.5 to 8.25 deg/s ($p < 0.000176$, t test); and Termination (③) from 0.1 to 0.0 deg/s ($p < 0.141$, t test). B–F, Top, Averaged eye velocity over 10 last cycles of each training of 8 experiments (black) and visual stimulation used for each training (gray).

(Ono, 2015). Even a small increment of eye velocity, as seen with the Initiation component, may increase initial rapid jump of velocity step OKR, namely, the Direct component. Indeed, increases in Initiation and Direct components during 3 h visual training appear highly correlated (Fig. 8D), but the amount of increase in the Direct component is ~10-fold. Therefore, Initiation may be beneficial to reduce total amount of retinal slip around the onset of visual stimulation.

By contrast, the Termination component sacrifices a significant amount of retinal slip, and it does not appear to reduce a comparable amount of retinal slip after the offset of visual stimulation. This result suggests that the predictive mechanism is so powerful that it can override the general function of the OKR gain adaptation to minimize image slip on the retina.

OKR gain and direct and indirect components during acquisition of predictive OKR

Gain adaptation is one of the most studied visuomotor behaviors. In naive animals, OKR eye velocity is not compensatory but approaches unity gain by prolonged exposure to optokinetic visual stimulation. It has been widely confirmed that the modification is always toward increased gain asymptotically approaching 1, and the cerebellum is necessary for this visual gain modification and maintenance (van Alphen et al., 2001, 2002; Kheradmand and Zee, 2011). In all the species tested to date, OKR eye velocity can be decomposed into two characteristically different components of Direct and Indirect (Fig. 1D). In goldfish, both the Direct and Indirect components are present on all hindbrain neurons involved in the visuo-vestibuloocular pathways, in particular, those in vestibular nucleus (Pastor et al., 2015), Area II (Beck et al., 2006), and vestibulo-cerebellum (Pastor et al., 1992).

In the present study, we showed that both Direct and Indirect component gain changed during the 3 h exposure to periodic visual stimulation. That is, the Direct component increased its magnitude, whereas the Indirect component shortened its building-up time constant and increased its magnitude. In most previous studies on OKR gain adaptation, sinusoidal visual stimulation was used (Nagao, 1989; van Alphen et al., 2001; Yoshida et al., 2007). Because the nature of sinusoids did not easily allow the Direct and Indirect components to be separated, the current results are the first to demonstrate that both components adapt during OKR gain adaptation.

Roles of the cerebellum in predictive OKR

A subset of Purkinje cells sampled in the vestibulo-cerebellum of goldfish presented both Direct and Indirect components of step velocity OKR in their simple spike firing modulation (Pastor et al., 1992) (Fig. 7). Neurons in a precerebellar nucleus, called Area II, which provide major mossy fiber input bilaterally to vestibulo-cerebellum (Straka et al., 2006), carry both Direct and Indirect components of OKR in their firing modulation (Beck et al., 2006). Further, neurons in the vestibular nucleus that are the target of the ipsilateral vestibulo-cerebellar Purkinje cells also present both components in their firing modulation (Pastor et al., 2015). Therefore, it was not surprising to find Purkinje cells that code Direct and Indirect OKR eye velocity information. The current results now demonstrate, for the first time, that Purkinje cells in the vestibulo-cerebellum exhibit clear firing modulation correlated with acquired predictive components of eye velocity (Fig. 7). These findings suggest that the predictive mechanism was generated within the cerebellar neuronal circuitry and presents itself throughout the recurrent neural loop consisting of vestibular nucleus, Area II, and the cerebellum (Beck et al., 2006).

Acute cerebellectomy after 3 h visual training reset both of the acquired Initiation and Termination components to their pretraining values (Fig. 8E). Similarly, the animals cerebellectomized before visual training did not acquire either of these predictive components following the same 3 h visual training (Fig. 8F). Although Initiation and Termination predictive components could be acquired independently in normal animals, these results demonstrate that both predictions are cerebellum-dependent.

Chronic cerebellectomy could have initiated neuronal degeneration in precerebellar Area II, which we view as unlikely for the following reasoning. Pastor et al. (1994a) showed that bilateral pharmacological inactivation of Area II greatly affected the OKR and completely compromising OKAN; however, all our chronically cerebellectomized animals had normal OKR and OKAN (Miki et al., 2014). Therefore, Area II neurons appear to survive chronic cerebellectomy.

OKR gain acquired after 3 h visual training decreased significantly below the pretraining gain after acute cerebellectomy (Fig. 5B). The increased Direct component after the visual training decreased to a value comparable with pretraining Direct. By contrast, the shortened time constant of the Indirect component did not change, whereas the Indirect magnitude decreased further below the pretraining value after acute cerebellectomy (Fig. 8E). Along the same line, the animals cerebellectomized before visual training did not change the Direct component during the same 3 h visual training, yet the time constant of Indirect component decreased to a comparable value for normal animals, but at a much slower rate. In these experiments, the Indirect component magnitude slightly decreased asymptotically to a value comparable with that after acute cerebellectomy. These results demonstrate that the cerebellum is not necessary to generate either the Direct or Indirect components of the OKR per se, but acquisition and maintenance of changes in the Direct component are totally cerebellum-dependent, whereas those of the Indirect component (both time constant and magnitude) only partially depend on the cerebellum. The paradoxical OKR gain decrease to a value lower than the pretraining value in the cerebellectomized animals seems to be caused by the shortened time constant and decreased magnitude of the Indirect component (Fig. 8E,F). These results show that characteristics of the hindbrain VSM are modifiable without the cerebellum and suggest that the modified state can be maintained, unlike conventional OKR gain modification, which has been demonstrated to be totally cerebellar-dependent.

In conclusion, an intact cerebellum-brainstem loop is necessary to acquire and maintain predictive eye velocity Initiation (when to move) and Termination (when to stop). The predictive behaviors originate in the cerebellum and even take precedence to the primary proposed purpose of eye movements (i.e., to stabilize what we see). A subset of vestibulo-cerebellar Purkinje cells conventionally identified to code eye velocity information and adjust eye velocity gain to compensate for blurred vision, in addition to code for predictive components. The VSM generated within the noncerebellar hindbrain (Beck et al., 2006) was modified in parallel with acquisition of the predictive eye movements and without cerebellar influence. Together in the context of the well-defined neuronal connectivity of the goldfish OKR, our findings argue that the acquired predictive eye velocity behaviors appear to be dependent on feedforward VSM signals from the brainstem to the cerebellum, but the adaptive timing mechanism, itself, originates within the cerebellar circuitry.

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