Purkinje Cell Spike Firing in the Posterolateral Cerebellum: Correlation With Visual Stimulus, Oculomotor Response, and Error Feedback

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INTRODUCTION

The trigger features and effects of the Purkinje cell (Pc) complex spike (CS), caused by discharge of its climbing fiber input, are controversial. Some postulate that Pc CSs of the lateral cerebellum are specifically movement related (Fu et al. 1997; Graf et al. 1998; Kobayashi et al. 1998; Ojakangas and Ebner 1992; Stone and Lisberger 1990), whereas others argue that the CS may encode sensory parameters alone (Apps 2000; Bower 1997; Gao et al. 1996; Paulin 1993; Rushmer et al. 1976). Still others hold that the CS signals performance error (Albus 1971; Kitazawa et al. 1998; Ojakangas and Ebner 1994; Wang et al. 1987) or that it provides a teaching signal to the Pc to recognize novel sensory inputs, adapting the motor output (Marr 1969).

Stein (Stein 1978; Stein and Glickstein 1992) suggested that the lateral cerebellum is important in processing and transforming visual information into a motor command. Martin et al. (1996a) established that patients with specific cerebellar lesions of the lateral hemisphere showed no signs of arm ataxia but demonstrated impaired adaptation while throwing with laterally displacing prism glasses. To further test the hypothesis that the cerebellum implements visuomotor adaptation, we designed a task in which a monkey was required to detect a visual target presented at random locations on a video screen, shift gaze to, reach to, and touch the target. The task required a correction for the visual-gaze error when gaze was off-target. We hoped to better distinguish the role of the complex spike in visuomotor error detection and the correction of ongoing movement.

Our results show that CSs were evoked when a randomly placed visual target appeared (target appearance, TA) on the screen in a preferred direction and location displaced from the center of gaze. The occurrence of CS firing was independent of which arm was used to reach and touch the target, and whether movement of the arm occurred at all. When target shift location was in the preferred location for a particular Pc CS, the CS often occurred on the first trial of a block of novel shift trials. The CS tended to fire in the same way throughout the block, irrespective of any short-term reach-touch adaptation within the block or long-term learning across days and weeks. When a target appeared off-gaze, in the preferred direction, there was a saccade to the target (91% of such trials). When the gaze happened to be foveated on or near the screen location of TA, the T1 CS peak tended not to occur, and there was no saccade (84% of such trials). We conclude that CS firing in the posterolateral cerebellar cortex occurs during the training and performance of a visually guided movement of eye and arm to the off-gaze appearance of the visual target. We discuss the role that the CS (and also the SS) may play in visuomotor error detection and correction.

METHODS

Setup

Two male rhesus monkeys (Macaca mulatta) each performed a visually guided reach task. All surgical and experimental procedures were in accordance with National Institutes of Health and U. S. Department of Agriculture guidelines and were approved by the Animal Studies Committee at Washington University School of Medicine under Protocols 98081 and 20010036. The monkeys sat in a custom-built primate chair that restrained the head and allowed free movement of both arms. Two capacitance switches were fixed on the chair so that the monkey’s arms were positioned by its side when its hands were on the switches. A 15-in touch-sensitive video monitor was positioned vertically ~20 cm from the monkey’s eyes to present a visual target dot and register the monkey’s touch response. A 10 × 10 cm target was presented at a distance of 20 cm from the monkey’s eyes. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
10-mm white box continuously appeared in one of the upper corners of the screen to indicate the respective reach hand.

**Behavioral task**

Figure 1 shows the reach task. At the onset of a trial, the monkey held both its hands on capacitance switches for a random initial hold time (500–1,000 ms). A red target dot, 6 mm in diameter, appeared on the video screen. The monkey was required to gaze to, reach, and touch the target. Once the monkey began its reach, it had 300 ms to touch the screen with the instructed hand. When the monkey touched the screen, a white dot 6 mm in diameter appeared on the screen at the location of touch, and the red target dot remained. The monkey then had 400 ms to return the reach hand back to the correct capacitance switch. A final random hold time (500–1,500 ms) was required before the monkey received a liquid reward for a correct trial. The interval between reward and the beginning of the next trial’s initial hold period was randomized (1–2 s).

A trial was considered correct if the target and touch dots overlapped, a maximum center-to-center distance of 6 mm. If the target and touch dot did not overlap, the monkey did not receive a reward. During a trial, the hand contralateral to the reach was required to remain on the switch at all times. If this hand was removed from the switch, the trial was immediately aborted and recorded as incomplete. An incomplete trial was also recorded if any of the time constraints were exceeded throughout the reach. After an incomplete trial, the screen was immediately cleared until a new trial began after a 3-s delay.

Trials were performed in blocks of each of three conditions: baseline, shift, and de-adaptation. In each trial of each condition, the target initially appeared (TA) at a random location on the screen. In the baseline condition (10 complete trials), the target disappeared when the monkey removed its reach hand from the switch. Then, after a fixed interval (50–150 ms), the target reappeared (target reappearance, TR) in the same location. By contrast, in the shift condition (30 complete trials), the target disappeared when the monkey removed its reach hand from the switch, then reappeared at a different location on the screen, 50 mm from the initial target location and repeatedly in one of eight directions (separated by 45° increments). The location on the screen of the shifted TR relative to initial TA was kept constant throughout the block but was varied pseudo-randomly among the eight directions from block to block. The de-adaptation condition consisted of 10 completed trials after the shift condition and were identical to baseline trials. A testing session thus consisted of blocks of 10 completed baseline trials, 30 completed shift trials, and 10 completed de-adaptation trials, repeated eight times for eight shift directions for a total of 400 trials with the right arm. Once reaches were completed using the right arm, the process was repeated for the left arm for as long as the monkey continued to work.

**Surgical procedures**

Two surgeries were performed on each monkey to affix an acrylic head-holder and stereotaxically place a lucite recording chamber with internal dimensions of 20 mm squared at 10 mm posterior of the interaural line and 10 mm lateral (right) of the midline. A third surgery was performed on monkey R to place 2 mm diam × 1 mm thick neoboridium magnets onto the sclera of the right eye just above and below the lateral rectus muscle using a surgical adhesive. The two magnets were separated by 90° on the surface of the eye.

**Electrophysiological recordings**

Extracellular single-unit recordings were made using high-impedance glass-coated platinum/iridium microelectrodes (Frederick Haer). The electrodes were mounted on an X-Y drive that aligned with the interaural line and the midline to generate stereotaxic coordinates for each penetration. After penetrating dura-mater with a beveled guard...
tube, electrodes were lowered into the cerebellar cortex where PCs were identified by the presence of simple spikes (SS) and CSs (Thach 1967, 1968). Based on these criteria, SSs and CSs were recorded from a total of 90 PCs (45 in monkey R and 45 in monkey T). The signals were sent to an AC-coupled differential amplifier (gain: 10,000; band-pass filter: 0.1–10 kHz). The analog waveform was sent to a CED-1401plus waveform recorder, and digitally recorded at 20 kHz on a PC. The digitally recorded waveform was converted to spike time points by template matching using Cambridge Electronic Design’s Spike2 software. Many of the PCs of which CSs were analyzed for the current manuscript were from the same neuronal population in which SSs were analyzed by Greger et al. (2004). In addition to the units previously reported, 24 additional penetrations and 15 additional PC recordings were made in monkey R prior to the analyses presented in the current manuscript.

Electromyographic and eye-movement recording

In monkey T, EOG electrooculography was recorded using fine wire electrodes, one placed subcutaneously at the lateral edge of each orbit. EOG was amplified at a gain of 10,000. In monkey R, neodymium iron boron magnets were implanted above and below the right lateral rectus muscle on the sclera. The magnetic field induced by the moving ocular magnets was measured using giant magnetoresistive field sensors (Nonvolatile Electronics). This signal was amplified using a custom-designed three op-amp instrumentation amplifier with a gain of 20. Both EOG and magnetic field signals were band-pass filtered from 0.1 to 100 Hz, sent to a CED-1401plus waveform recorder, and digitally recorded at 2 kHz on a PC.

Histology

The monkeys were killed by an overdose of intravenous pentobarbital sodium followed by an intracardiac perfusion of normal saline, heparin, and 10% formalin in phosphate buffer. The chamber coordinates were marked by stereotaxically aligning a 28-gauge probe, coated with dye, on the center of the chamber, and guiding it through the dorsal-ventral extent of the cerebellum in situ. The cerebellum was removed, sectioned in 1 mm slices, and Nissl stained.

Analyses

Behavioral data were recorded and sent to the task control PC and to a data-storage PC, which also stored simultaneously recorded spike waveforms and eye-movement data. Analysis of behavioral data was conducted off-line. Mean values were calculated for success rate, reach time, and return time and were tested across various behavioral conditions for statistical differences (i.e., right-hand reaches vs. left, correct trials vs. incorrect, and baseline condition reaches vs. shift–ANOVA P < 0.05). To measure the behavioral motor adaptation within a novel shift block (i.e., a tendency to touch closer to the target over successive trials), an adaptation coefficient (AC) was calculated (see Martin et al. 1996a for calculation). To measure behavioral motor learning (i.e., a tendency to touch closer to the target across successive blocks over days and weeks of training), we compared AC’s and the means of the first five touch/target proximities of the many shift blocks over time and training. Motor learning was defined to have occurred if there was a reduction in the AC, and/or magnitude of the mean of the first five target/touch discrepancies over shift blocks within a session and across sessions.

For both methods of measurement of eye movement, the raw voltage trace was visually scanned for sharp transients indicating that a saccade had occurred. The initial change in voltage was digitally marked as an event. Eye-movement data were time aligned on target appearance, start of movement, target reappearance and touch, then analyzed in relation to complex spike events. The second method of implanted eye magnets further gave an eye-position signal, which was calibrated prior to each test session.

Stored spike data were analyzed off-line using Spike2 (Cambridge Electronic Design), and MATLAB. All cells from which at least six of eight shift directions were completed were for significant modulation in relation to the task performance. Spike data were independently placed in 20-ms bins and time aligned on target appearance, start of movement, target reappearance and touch. A baseline, non-task-related firing rate was calculated using an interval of 500–200 ms prior to initial target appearance. Complex spike discharge was analyzed independently for baseline trials and shift trials. Modulation of the complex spike firing was determined to occur if the response significantly changed from the baseline firing rate (Student’s t-test). For all shift and de-adaptation blocks, to determine the CS’s role specific to adaptation, we tested the likelihood that the distribution of CSs within each shift block was uniform using the Kolmogorov-Smirnov test. To distinguish whether deviation from a uniform distribution was random or not, we conducted a runs test.

For PCs demonstrating significant modulation, (x,y) data for initial target location, touch position, target reappearance position, touch relative to initial target appearance, and touch relative to target was plotted for all trials, then overlaid with plots of similar data for trials in which a complex spike occurred within a 100-ms window centered on the peak of CS modulation. Plots were divided into quadrants based on the center of distribution for (x,y) coordinates of all trials. A χ2 statistic was used to test whether the distribution of complex spike data were even in each of the plots. χ2 values with P < 0.05 were considered significantly uneven (df = 3), and the quadrant with the largest value was considered the preferred direction. Preferred direction was further specified by subtracting the (x,y) center of mass for those trials in which a CS occurred following the specified task event from the (x,y) coordinates for the center of mass of all trials.

In addition to detecting increases in CS discharge frequency, we attempted to detect potential decreases in CS activity or any directionally tuned canceling effects (Kitazawa et al. 1998; in intermediate cerebellum) due to bidirectional plasticity in cerebellar cortex (Jorn- tell and Ekerot 2002; Medina et al. 2002). Such analysis was used to detect information about endpoint errors, where we set a time window (which was systematically shifted and varied in time) following touch and aligned the CS data at touch on the screen, plotted (x,y) position relative to TR position separately for trials with CSs and for all trials, and calculated the chi-squared statistic concerning the endpoint errors relative to the final target for trials with CSs. χ2 P values were then used to judge if significant information about endpoint errors existed (P < 0.05). In addition, this analysis was completed having aligned touch position data on the occurrence of visual feedback of touch.

For PCs that demonstrated an uneven distribution of trials in which complex spikes occurred, the predictive information associated with the occurrence of complex spikes (ICS) was quantified (Kitazawa et al. 1998). ICS measures any predictive information associated with occurrence of a complex spike and is quantified as a decrease in entropy in absolute touch position

\[
I_s = \sum_{i=1}^{4} \frac{(a/N) \log(a/N) - \sum_{i=1}^{4} (m/M) \log(m/M)}
\]

where \( n_i \) and \( m_i \) represent the number of all trials and only trials in which a CS occurred in the \( i \)th quadrant, and \( N \) and \( M \) represent the total number of all trials and only trials in which CSs occurred. If the trials in which CSs occurred were all confined to a particular quadrant, \( I_{CS} \) would be equal to 2, and if they were evenly distributed, \( I_{CS} \) would equal 0.
RESULTS

Behavior

Baseline. In the baseline condition, monkey T performed at a 58 ± 13% (mean ± SD; right hand) and 52 ± 13% (left hand) success rate, whereas monkey R successfully touched the target 82 ± 8% (right hand) and 44 ± 12% (left hand) of the trials. For monkey T, reach time was 236 ± 16 (SD) ms and return time was 311 ± 22 ms. For monkey R, reach time was 218 ± 9 ms and return time was 246 ± 13 ms. For both monkeys, there was no significant difference in reach time between correct and incorrect trials (t-test, P > 0.05). Mean reach time for the left hand was not significantly greater than that of the right hand, but the return time of the left hand was significantly greater than the right hand (t-test, P < 0.05) in both monkeys.

Shift. In the shift condition, monkey T performed at a 23.6 ± 14% success rate (right hand) and 21.3 ± 12% (left hand), whereas monkey R successfully touched the target 45.9 ± 15.9% (right hand) and 36.3 ± 17.3% (left hand). For monkey T, reach time was 245 ± 14 ms and return time was 320 ± 30 ms; for monkey R, reach time was 253 ± 10 ms and return time was 241 ± 9 ms. For both monkeys, there was no significant difference in reach or return time for correct versus incorrect trials (t-test, P > 0.05). A significant difference in mean reach time and mean return time between baseline and shift conditions was present in monkey R (t-test, P < 0.05) but not in monkey T.

In the shift condition, a number of trials were required to successfully reach to the shifted target position (“adaptation”) (Martin et al. 1996a). An adaptation coefficient was calculated using the equation \( y = a - b \times e^{-ct} \), where \( a \) is the final value that the exponential decay function approaches, \( b \) is the magnitude of the adaptation required from the first throw to the value \( c \) (the exponential decay constant) represents the rate at which adaptation takes place (AC), and \( t \) is the trial number (Martin et al. 1996a). AC represents the number of trials required to reach \( 1 - e^{-c} \) (~63.2%) of the full adaptation to the shifted target position. Over time and practice across shift blocks, days and weeks, the monkey decreased the number of trials required to adapt to the shifted target and hence lowered this AC score. We have previously called this longer-term motor “learning” to distinguish it from the short-term within-block adaptation (Martin et al. 1996b). In an effort to require the monkey to continuously adapt from block to block and session to session, we increased the latency between TA disappearance and TR and by increasing the delay of visual feedback following touch. Both procedures resulted in an increasing number of trials to adapt (cf. Kitazawa and Yin 2002). Mean AC values calculated across all electrophysiological recording sessions were 5.4 for monkey T and 1.8 for monkey R. For monkey T, AC values ranged from a mean of 7.6 for the first week to 4.4 for the last week of performance; and for monkey R, from a mean of 5.4 for the first week to a mean of 1.2 for the last week of performance. Mean \( a \) and \( b \) values calculated across all sessions were 3.3 and 35.3 mm, respectively, for monkey T and 2.1 and 37.5 mm for monkey R. For monkey T, \( a \) and \( b \) values ranged from a mean of 4.2 and 39.1 mm for the first week to 1.7 and 33.3 mm for the last week of performance and for monkey R, from a mean of 3.9 and 38.3 mm for the first week to 2.7 and 36.2 mm for the last week.

In the de-adaptation condition, Mean AC values calculated across all electrophysiological recording sessions were 4.7 for monkey T and 2.1 for monkey R. For monkey T, AC values ranged from a mean of 6.1 for the first week to 3.8 for the last week of performance and for monkey R, from a mean of 5.1 for the first week to a mean of 1.7 for the last week of performance. Mean \( a \) and \( b \) values calculated across all sessions were ~4.1 and 37.2 mm, respectively, for monkey T and 1.3 and 34.3 mm for monkey R. For monkey T, \( a \) and \( b \) values ranged from a mean of ~3.3 and 40.2 mm for the first week to ~4.3 and 27.0 mm for the last week of performance and for monkey R, from a mean of ~0.1 and 38.7 mm for the first week to 1.6 and 24.8 mm for the last week.

CS activity

Figure 2 shows separate raster plots and average discharge frequencies for the baseline (Fig. 2A), shift (B), and de-adaptation (C) conditions of a single recorded PC. As observed in all three conditions, a peak in CS activity (T1 CS) occurs ~150 ms after initial target appearance (TA). Rasters shown for all three conditions demonstrate an even distribution of spikes across sweeps at time T1 and that T1 CS discharge during the baseline condition is comparable to that of the de-adaptation condition.

A second peak in CS activity (T2 CS) occurs only in the shift condition, ~150 ms following the target reappearance (TR). The rasters in Fig. 2C demonstrate highly clustered neuronal activity at time T2 during trials in which the target shifted a specific direction and location (i.e., 115° is highly clustered with CS activity, while the complement angle of 295° has little CS activity at T21). T1 CS after TA. For the same cell portrayed in Fig. 2, Fig. 3, A and B, shows the (x,y) position of initial target appearance, target reappearance, and touch relative to initial target position for all trials (small black crosses), trials with T1 CSs (large red squares), and trials with T2 CSs (large green squares). T1 CS baseline data are shown separate from T1 CS shift data, whereas T2 CS shift data are shown alone because no significant T2 CS peaks were observed during baseline conditions.

Figure 3Aa shows that T1 CSs for this particular PC demonstrate a locational preference for TA in the upper left quadrant of the screen during baseline trials. Recall that T1 CSs occur following TA by a fixed interval (~150 ms) but occur prior to the beginning of movement and TR (Fig. 1, timeline). While T1 CSs clearly demonstrated directional preference for the TA that had already passed, we plotted TR and touch relative to initial target (x,y) data for T1 CSs to test for predictive information contained in the T1 CS. While in the baseline condition, TR occurred at the same position of TA. Figure 3Ab demonstrates that T1 CS TR locations match that of TA. However, there was no directional preference for T1 CSs in predicting relative touch errors as demonstrated in Fig. 3Ac. Having just examined the baseline condition, we are unable to determine whether a T1 CS is a result of TA location or

\[1\] All angles discussed in this manuscript are based on the polar coordinate system, such that 0° is directed north, 90° toward the top, 180° toward the left, 270° toward the bottom, and 360° toward the right again, thus moving in a counterclockwise direction from 0 to 360°. This is not the same as coordinates defined on a conventional compass, where 0° is directed north (toward the top) and moves clockwise from 0 to 360°.

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predictive of TR and/or touch location because they are all in the same location.

Consistent with the baseline condition, Fig. 3Ad shows that T1 CSs for the same Pc demonstrate a locational preference for TA in the upper left quadrant of the screen during shift trials. Testing for predictive information in the T1 CS, we plotted the later TR event data (Fig. 3Ae) and observed that it was evenly dispersed around the TA distribution. This can be explained by the fact that T1 CSs were evenly distributed across all eight shift directions as shown in Fig. 3Af and contained no preference for reach direction relative to TA. Thus the T1 CS peak did not convey predictive information regarding target shift/touch position.

T2 CS AFTER TR. While recalling that T2 CSs occur at varied intervals after TA, T1 CS, and movement initiation but at a fixed interval after TR (\sim 150 ms; Fig. 1, timeline), one might suspect that T2 CSs would be independent of TA. Figure 3Ba demonstrates this: based on TA, T2 CSs were evenly distributed across the screen. However, Fig. 3Bb demonstrates that T2 CSs (occurring at a fixed latency from TR) showed directional preference for TR in the upper left quadrant. The directional preference of the T2 CSs was similar to that of the T1 CSs for this same Pc. Figure 3Bc shows that T2 CSs preferentially discharged during trials in which the monkey touched a target shifting up and left of TA.

Population data

A total of 156 penetrations were made, and a total of 114 recordings were stored and analyzed. Of the 114 recordings, CSs were isolated in 90 Pcs, and 42/90 (47%) demonstrated a significant T1 CS peak. This peak was present in both baseline and shift conditions in all Pcs, and the firing rate did not differ statistically between the baseline and shift condition in 37/42 Pcs (Wilcoxon rank-sum, \( P_{H11022} 0.05 \)). A total of 31/90 Pcs (34%) demonstrated a significant T2 CS peak during the shift condition, and none of these demonstrated a significant peak in T2 CS activity during the baseline condition. Twenty-six Pcs significantly contributed to both T1 CS and T2 CS peaks. Figure 4A shows the CS firing rate for the 26 Pcs that significantly contributed to both T1 CS and T2 CS peaks. Penetration sites for all 26 Pcs that significantly contributed to T1 CS and T2 CS peaks are shown in Fig. 4B.

Of the 26 Pcs that contributed to T1 CS and T2 CS peaks, 19 demonstrated locational preference as measured by an uneven \( \chi^2 \) distribution across the four quadrants of the screen. For each of these Pcs, T1 CS directional tuning was more specifically defined as the center of mass of TA \((x,y)\) location for trials in which a CS occurred, subtracted from the center of mass of TA \((x,y)\) location for all trials. The magnitude and directional component of this measured center of mass is displayed for each Pc in Fig. 4C relative to the center of the screen. T2 CS directional tuning was calculated in a similar manner but for \((x,y)\) TR location relative to TA. For the entire cell population, we were unable to detect any topographical representation within the cerebellar cortex for the preferred direction of CS firing. All Pcs that demonstrated T1 CS and T2 CS peaks consistently demonstrated the same locational preference for both peaks, as demonstrated in Fig. 5A (linear fit, \( r^2 = 0.8366 \)).

### FIG. 2. A: complex spike activity for baseline trials in a single Pc. Data in the upper raster shows CS discharge for all baseline trials. The PSTH is aligned on Target Appearance (TA) for data to the left of the solid vertical line. On the right, complex spike activity is aligned on Target Reappearance (TR). B: the same plots as in A, but for complex spike activity occurring in shift trials for the same Pc. Shift angles are depicted on the right of the raster. C: the same as for A and B, but for complex spike activity occurring in deadaptation trials. Task events are defined as TA: target appears, M: begin movement, TR: target reappear, S: screen touched, R: hand returned, T1: T1 CS peak, T2: T2 CS peak.
To test whether the CSs role was specific to adaptation and changed over trials, we tested the likelihood that the distribution of CSs within each shift block was uniform using the Kolmogrov-Smirnov test. Of 26 PCs that contained a significant peak in both T1 CS and T2 CS activity, 4 demonstrated a significant \( (P < 0.05) \) deviation from the uniform distribution during at least one block of shift trials. To distinguish whether deviation from the uniform distribution was random or not, we conducted a runs test. The runs test demonstrated that two shift blocks contained an increased CS firing rate at the onset of the block \( (P < 0.05) \). The same analyses were run for blocks of de-adaptation trials, and four blocks (in 4 separate PCs) were found similarly to have significantly increased CS firing at the onset of the block \( (P < 0.05, \text{runs test}) \).

**A** T1

1. **Target Appear**
2. **Target Reappear**
3. **Touch Relative to TA**

**B** T2

1. **Target Appear**
2. **Target Reappear**
3. **Touch Relative to TA**

**C** Touch

1. **Baseline**
2. **Shift**

**FIG. 3.** A: \((x,y)\) data plotted for trials in which a T1 CS occurred (red squares), and for all trials (crosses). \(a, b, \) and \(c\) show \((x,y)\) screen location for target appear, target reappear, and touch relative to initial target respectively for baseline trials. \(d, e, \) and \(f\) show \((x,y)\) screen locations for shift trials. B: \((x,y)\) data plotted for trials in which a T2 CS occurred (green squares), and for all trials (crosses). \(a, b, \) and \(c\) show \((x,y)\) screen location for target appear, target reappear, and touch relative to initial target for shift trials. C: \((x,y)\) data plotted for trials in which a CS occurred following touch (blue squares), and for all trials (crosses) during the baseline (left) and shift (right) conditions.

**RIGHT HAND VERSUS LEFT HAND REACHES.** Of 23 neurons in which a T1 CS peak occurred for the right arm and reaches were made using the left arm, significant T1 CS peaks were observed in 20 PCs for reaches made with the left arm. Comparing reaches made with the right versus the left arm, the CS firing rate did not significantly differ in 16/20 PCs (Wilcoxon rank-sum, \(P > 0.05\)), and the mean time in which a T1 CS occurred after TA did not statistically differ \((t\text{-test}, P > 0.05)\). Quadrant analysis demonstrated that the quadrant in which a CS occurred in relation to TA did not differ between arms in 15/16 PCs. In addition, when the center of mass of TA \((x,y)\) location for trials in which CSs occurred [subtracted from the center of mass of TA \((x,y)\) location for all trials] was plotted for the right arm versus the left arm, a linear fit produced an \(r^2\) value of 0.9221 (Fig. 5B).
For only three recorded PCs did the monkeys complete all shift angles using both arms for which a significant T1 CS peak occurred. In these, two PCs demonstrated a significant T1 CS and T2 CS peak. The T2 CS peak firing rate did not differ significantly from right arm to left, and the timing of both T1 CS and T2 CS peaks was not significantly different when compared across the arms (t-test, \( P > 0.05 \)). Using quadrant analysis, T2 CSs were directionally tuned to the same quadrant of the dashed line is aligned on TA, and data to the right of the dashed line is aligned on TR. Task events are defined as TA: target appears, T1: T1 CS peak, TR: target reappear, and T2: T2 CS peak. B: the location of electrode penetrations (in both monkeys) for the 26 PCs that contained both significant CS peaks at T1 and T2. C: Magnitude and angular direction of the center of mass for trials in which a CS occurred following TA. Only PCs that demonstrated significant directionality are shown, and letters correspond to PCs in Fig. 4B.

**Eye movement and CSs**

Because calibration of eye position required many trials and was necessary prior to each experimental session in which eye position was recorded, we were seldom capable of motivating the monkey to complete an entire reach session when recording eye position. Thus eye position was recorded during 12 sessions but only recorded simultaneously with five PCs that exhibited significant peaks in both T1 CS and T2 CS firing in *monkey R*. Of these five PCs, reaches were completed in all shift directions for the right hand, but for only one PC were all eight shift angles completed with the left hand. In an additional PC, 200 trials were completed across four shift directions of which both T1 CS and T2 CS peaks were observed. A total of 2,200 trials were analyzed for eye position in *monkey R*.

Saccades were found to occur following both TA and TR. The mean latency between TA and a saccade was 191 ± 45 ms.
ms. The mean latency between TR and a saccade was 184 ± 49 ms. Saccades were found to occur after T1 CS or T2 CS activity (Fig. 7A) by a mean of 46 ± 7 and 41 ± 6 ms, respectively. Whether or not a CS and a saccade occurred after TA was highly dependent on the direction of gaze at the time of TA. When gaze was initially directed away from TA, there was a CS followed by a saccade to the target when the target appeared in the preferred quadrant (91% of 403 trials with gaze away from TA and a saccade). When the gaze happened less commonly to be foveated on the screen on or near TA in a preferred quadrant, the T1 CS tended not to occur, and there was no saccade (84% of 49 trials with gaze toward TA and no saccade). Of 42 trials in which gaze was initially directed away from a TA located in a preferred quadrant and no saccade (or reach) followed, only 3 (7%) of trials resulted in a T1 CS. Because eye position was continuously recorded, we were able to record 141 instances outside the bounds of a trial (500 ms after reward until 200 ms prior to TA) in which the monkey’s gaze moved from the null quadrant into the preferred quadrant without a TA. Of these 141 gaze shifts into the preferred direction without TA, we recorded 35 (25%) CSs that preceded the saccade by <100 ms.

Figure 7B shows the position of TA (left) and TR (right) in terms of the position of gaze in monkey R for data collected in a single recording session. The $I_c$ value of trials with T1 CSs about the off-gaze position for TA in Fig. 5B ($I_c = 0.59$) was greater than the $I_c$ value for trials with T1 CSs about absolute position on the screen in TA ($I_c = 0.46$). In accordance, the $I_c$ value of trials with T2 CSs about the off-gaze position for TR in Fig. 5B ($I_c = 0.64$) was greater than the $I_c$ value of trials with T2 CSs about absolute position on the screen in TR ($I_c = 0.53$). For each of the four PCs in which both a significant T1 CS and T2 CS peak occurred and eye position was recorded, the $I_c$ values for both TA and TR in terms of gaze position were greater than $I_c$ values for TA and TR in terms of absolute screen location. Of these PCs, there was very little evidence of saccadic adaptation to TR within shift blocks. When analyzing

**Fig. 6.** A: (x,y) data plotted for trials in which a T1 CS occurred (red squares), and for all trials (crosses) following TA for trials in which a reach ensued (left), and trials in which no reach followed TA. B: simple spike rasters and PSTHs for the same PC as shown in A. The data are aligned on Target Appearance (TA). The top plot shows trials in which a reach followed TA and the lower plot shows trials in which no reach followed TA.

**Fig. 7.** A: Top: histograms showing the number of saccades for a single recording session. Only saccades for the shift condition are shown. The histogram on the left is aligned on TA, and the histogram on the right is aligned on TR. Bottom: plots showing the CS firing rate for the T1 CS peak (left), aligned on the saccade following TA, and for the T2 peak (right) aligned on the saccade following TR. B: gaze position is at the origin (0,0) in both plots, and (x,y) data are plotted for TA (left) and TR (right) relative to gaze. Trials in which a T1 CS occurred are plotted as red squares, and trials in which a T2 CS occurred are plotted as green squares. Remaining trials are plotted as crosses.
the eye position after an initial sharp transient after TR we discovered that the eye position was almost always within a 10-mm radius of TR location. Exceptions were in the first trial of the 270 and 315° shifts during 2/4 recordings with TR delay times of 125 and 150 ms, respectively, where the monkey did not change gaze position from the TA position until well beyond the completion of the reach. These two Pcs were recorded with TR delay times of 125 and 150 ms, respectively. Thus we speculate that the arm may have impeded the monkey's vision of TR during these two shift directions such that TR was not visible to the monkey until the monkey returned its hand to the switch. In addition to the eye foveating TR on the initial trial, we were unable to fit exponential decay adaptation curves to the magnitude of deviation of eye position relative to TR after the initial saccade. Thus we conclude that adaptation of eye movements to TR was not robust.

CS relationship to SSs

Previously, we reported on Pc SS modulation during a period of 200 ms before to 300 ms after the start of movement (Greger et al. 2004). Many of the Pcs analyzed and described in that study are the same for which CSs are analyzed in the current study. Sixteen of the 26 Pcs with significant T1 CS and T2 CS peaks in the current study were the same as those previously reported to have modulation in SS firing during reaches made irrespective of the effector limb. Of the remaining 10 Pcs that contained significant T1 CS and T2 CS peaks, 6 were recorded after completion of the previous study.

For the 19 Pcs that exhibited T1 CS and T2 CS directional tuning, during the 100-ms time windows corresponding with T1 CSs and T2 CSs, the SS discharge (in relation to TA or TR location and time) showed short periods of decreased firing rate. SS discharge was found to be inversely related to T1 CS peaks in 18 of 19 Pcs that exhibited T1 CS directional tuning. In 17 of the same 19 Pcs, SS discharge was inversely related to T2 CS peaks as well. These pauses in SS firing that accompany CS activity probably represent the transient inactivation period that follows a CS as first described by Granit and Phillips (1956).

Beyond this early 100- to 200-ms post TA/TR period for which the SS modulated inversely in relation to CS occurrences, other later differences were noted in the trial modulation of SSs across Pcs. Figure 8A demonstrates differences in SS modulation within trials across five different Pcs. While each of these Pcs had decreased SS firing rate...
immediately after the CS (~150 ms after TA and ~150 ms after TR), they all had very different SS firing patterns outside this time window. In fact, this prolonged SS modulation occurred in trials for which a T1 CS and/or T2 CS did not occur (Fig. 8B). Moreover, visual comparison of SS rasters and peristimulus time histograms across quadrants for the same Pcs demonstrated that this prolonged (>700 ms) SS modulation, long past the CS occurrences, was indeed also dependent on the location of TA or TR. Peak detection (Matlab) demonstrated that when SS data were aligned on movement onset, TA, TR, T1 saccade, T2 saccade, or touch, there was no consistent temporal relation of the late prolonged SS peak firing rate across Pcs. Furthermore, there were significant differences (t-test, P < 0.05) in SS firing rate when trials in which a T1 CS and/or T2 CS and saccade occurred were compared with trials in which no T1 CS or T2 CS and saccade occurred. Also, for all Pcs, significant SS modulation (Wilcoxon rank sum, P < 0.05) occurred throughout the trial in which no T1 CS or T2 CS or saccade occurred when compared with baseline firing rate (Fig. 8C).

Finally, analysis was completed for Ss of the 25 Pcs for which the target appeared, but no arm movement ensued (Pcs analyzed contained a minimum of 25 nonreach trials and were the same as those previously analyzed with respect to Cs). SSs did significantly modulate firing rate after TA during nonreach trials (Wilcoxon rank sum, P < 0.05). Figure 6A shows T1 CS directional tuning after TA for trials completed (left) and trials in which no reach ensued (right). Figure 6B shows SS firing in the same Pcs for trials completed (top), and trials in which no reach ensued (bottom).

Because the late SS modulation was not consistent across Pcs, we visually determined the point of greatest difference in firing rate across quadrants for data aligned on TR then quantified significance as a difference in firing rate across quadrants during a 200-ms window centered on the peak or trough. Differences in the late prolonged SS firing were significantly different across quadrants in 23/26 Pcs (ANOVA: P < 0.05). For any one Pcs, the late prolonged change in SS firing rate from baseline (the period 500–200 ms prior to TA) was always greatest in the preferred quadrant for the CS of that Pcs. The late prolonged SS change was reduced in 19/23 Pcs, and increased in 4/23 Pcs. This difference in SS modulation based on TA/TR location across quadrants is shown in Fig. 8D.

**Discussion**

Results of the current study demonstrate that Pc activity in the posterior-lateral cerebellum is related to visual target location during a visually guided reach-touch. A peak in complex spike activity (T1 CS) after initial TA was significantly modulated in relation to where the initial target appeared on the screen. If the target shifted location (TR) after initiation of reach, a second peak in complex spike activity (T2 CS) was observed, only for trials in which the target shifted into the same screen location that induced T1 Cs. T1 Cs were found to occur when reaches were made with both the ipsilateral and contralateral arm and on trials in which the target appeared but no arm reach ensued. Both T1 CS and T2 Cs and oculomotor saccades seemed highly dependent not only on the visual target location but also on whether the gaze was off or on target. Transient decreases in SS firing immediately followed T1 CS and T2 CSs and most likely represent the CS Pc inactivation responses previously described. In addition, late prolonged SS modulation extended temporally beyond the T1 CS and T2 CS occurrences and was also affected by the location of target appearance or target reappearance on the screen. In sum, separation of initial gaze location and final gaze location/absolute touch location allowed us to observe striking associations of visual target location, direction of gaze, saccades to target, and occurrence of Cs. We also observed SS modulation in relation to these same sensorimotor parameters that occurred independent of T1 CS and T2 CSs and differed across Pcs.

*Do the complex spikes relate to sensory (visual) parameters alone?*

During the baseline condition, we observed a single T1 CS peak after TA. This peak was tuned to TA location on the screen. As the monkey reached toward the initial gaze-target direction in this condition, there was a correlation between visual stimulus location, gaze direction, reach direction, CS occurrence, and ocular saccade. If gaze was off target, as was common, then a target at a preferred location would trigger both a CS and a saccade to the target in 91% of such trials. While absolute reach direction matched that of gaze direction in baseline trials, trials in which no saccade occurred allowed us to dissociate Cs signaling absolute reach direction versus gaze direction. If gaze happened by chance to be on or near a TA in the preferred location, then there was no T1 CS or saccade in 84% of such trials, although the absolute reach direction remained the same as that in trials that triggered Cs. Furthermore, in the shift condition, the monkey was required to reach to an area of the screen (TR) spatially distinct from the location of the initial target (TA). While T1 Cs maintained directional preference for TA in the shift condition, target shifts were distributed in all directions away from the initial target during a single day’s recording session. It was of considerable interest that, after TR, the T2 CS preferred screen location of shift trials was the same as the preferred location of T1 CS peaks after TA in baseline trials. Furthermore, T2 Cs only occurred in shift directions that relocated the TR to the preferred screen location. It is also important to note that regardless of increased delay times from movement to target reappearance (50–150 ms), the T2 peak in Cs activity consistently followed TR by ~150 ms but failed to maintain a consistent latency after initiation of arm reach.

While a saccade to the preferred location was necessary to trigger a CS, it may be that the condition of *not* requiring movement of the eye or arm detracts from the phenomenon of visual preferred location. However, it would also fit that movement of the eye or arm would not occur if the target were not recognized in the visual field, thus no response would follow. Finally, the issue is complicated by the fact that Cs preceded 25% of those saccades in a preferred direction when there was no visual target.
Does the complex spike contribute to error feedback?

Given our preceding analysis of visual preferred location, gaze direction, CS, and ocular saccade occurrence, it seems likely that the information encoded by the complex spike may be a retino-topic representation of the workspace, signifying errors of magnitude and direction between actual gaze and desired gaze. The CS may also initiate or specifically control precise muscle activity to guide the eye to the new visual stimulus location. The information processed by the complex spike would seem to be important to recognize and process the visual error and must be necessary to correct for the sensory discrepancy. Thus it seems proper to argue that the complex spike contributes to on-line sensory-motor error feedback and/or to the correction of visually detected error. The complex spikes observed in the current study may contribute to on-line visual mediation and error feedback control.

Work by Kitazawa et al. (1998) suggests that the complex spike may encode motor errors relative to the target in a multi-jointed reach movement. This group concludes that complex spikes occurring early in a reach movement encode absolute destination of the arm, while complex spikes occurring late in the movement encode relative endpoint errors of the reach. While we demonstrate that complex spikes in the posterolateral cerebellum are related to visual stimuli, perhaps leading to the absolute destination of the arm, we agree that these signals were said to provide “real-time control and learning of movements” in a task requiring a reach to terminating at the location of initial target appearance.

The fact that we were unable to detect CSs conveying information regarding endpoint reach-touch error was surprising when compared with results presented by Kitazawa et al. (1998). However, Pc recordings made by this group were located in intermediate zones of cerebellar cortex, primarily in lobules IV–VI. As noted by Snider and Eldred (1952), these areas in monkeys somatotopically represent regions of the arm. Data collected in our current study were from Pcs located in CRUS I and II, more posterior and lateral to those previously reported by Kitazawa et al. Other differences between the studies existed in the task setup (i.e., a shutter prevented the monkey from viewing any error feedback until a specified time in the Kitazawa study, whereas continuous error feedback was present in the form of hand position in the current). Perhaps such information could serve as a clue as to how the distinct temporal presentation of error feedback in the Kitazawa study was important to evoke the CS, whereas continuous feedback of arm position may override the distinct touch feedback in the current study. Further studies must be completed to compare the effects of temporally altering error feedback in relation to CS firing in the posterolateral cerebellum and to compare this Pcs activity with Pcs in areas more anterior-medial.

Do these complex spike firing patterns fit a learning hypothesis?

Marr (1969), Albus (1971), Ito et al. (1982), and Gilbert (1993) have argued that for the cerebellar circuitry to properly correct ongoing movement, a signal context (via the mossy fibers), and a learning enabler (via the climbing fibers) are required to effectively change the parallel fiber-Purkinje cell synapses. According to this theory, one would suspect that complex spike activity would fire at high frequency early in adaptation phases, and as adaptation progresses and performance error is reduced, the complex spike frequency would diminish. Indeed, this pattern has been seen previously (Gilbert and Thach 1977). In the present experiment, we rarely saw a reduction of the complex spike firing within blocks of shift trials or de-adaptation trials. The fact that we did not observe this reduction may have been for several reasons. 1) The blocks of shift and de-adaptation trials were kept short (30 and 10 trials, respectively), and in the previous experiment, Gilbert and Thach observed that it commonly took >100 trials before performance improved and the complex spike frequency diminished. 2) In the current experiment, the attempt was made by delaying error feedback to prevent motor adaptation and learning from occurring too quickly. 3) Each trial was always to an extent novel because of TA occurred at random locations over the screen. This also could be expected to prolong motor adaptation and learning. Thus we do not feel that not seeing a consistent reduction in complex spike firing frequency across the shift condition block militates against the Marr-Albus-Ito-Gilbert hypothesis.

In this paper, we show CS firing to be triggered by a visual stimulus in a specific location off the fovea when the stimulus is followed by a saccade to the target location. The reach-touch of the arm was ultimately in the final direction of gaze, although gaze often shifted late reach or even after touch. In fact, Fig. 5B (right) demonstrates that in the shift condition the eyes almost never deviated from TA prior to TR, although TR occurred after the onset of reach movement. We believe that we may have trained the monkeys to adapt or learn novel gaze-reach calibrations as observed in other experiments using laterally displacing prisms (Martin et al. 1996b). In such experiments, humans and monkeys were required both to adapt and to learn novel gaze-reach calibrations and were impaired in gaze-reach adjustment by lesions of this part of cerebellar cortex (Baizer et al. 1999; Martin et al. 1996a). In the current experimental paradigm, we did not observe CSs firing in relation to the adaptation or learning of the suspected multiple gaze-reach calibrations. However, it is possible that the CS might provide error feedback necessary for visual adaptation alone. In the current study, lack of adaptive eye movement, the CS’s relationship to visual error, and the rarity of changes in CS firing within a shift block all support the Marr-Albus-Ito-Gilbert learning hypothesis with regard to motor learning of eye movement. However, having not measured CS modulation in relation to adaptive eye movement, we are unable to conclude on the potential role of the CS of the posterolateral cerebellum in visual adaptation alone.

CS and SS correlation

Many of the CSs currently described are from the same population of Pcs in which SSs were previously described to represent movement parameters alone (Greger et al. 2004). However, clues from CS behavior allow us to further interpret SS behavior. Temporal specificity of T1 CS and T2 CS peaks in the current study allowed us to reevaluate SS firing in relation to these time periods. Reanalysis of the SSs during T1 CS and T2 CSs (rather than from 200 ms before TA to 300 ms after the start of movement) (Greger et al. 2004) demonstrates that SS firing transiently decreases immediately after a CS. As noted in RESULTS, this observation is consistent with the previously described physiological inactivation period after CS discharge (Granit and Phillips 1956).
Beyond the inverted SS discharge temporally locked with the CS, late prolonged SS modulation is shown in Fig. 8 to occur regardless of T1 CS or T2 CS occurrence and regardless of a TA and TR saccade. The magnitude of SS modulation was dependent on target location. These findings fit with our previous interpretation that SS modulation (at least in part) represents arm movement parameters (Greger et al. 2004) and makes it difficult to argue for a parallel role to the current findings that the CS response to visual error. However, the fact that the late SS modulation occurred independent of reach suggests a role in parameters outside of arm movement alone (cf. Liu et al. 2003). Evidence that the SS may play a role independent of arm movement supports previous reports that SS firing of PCs in the posterior lateral cerebellum may be saccade related (Mano et al. 1991, 1996). We were able to demonstrate that the CS fires in relation to a retinotopic representation of target position or represents an error signal controlling a specific eye movement. Because of difficulty isolating the SSs role in relation to the CS during this task, we are unable to conclude the SSs role in sensory versus motor parameters. Evidence in the current study suggests a possible role in both. However, the SS may also modulate in response to undetected, task-related, attempts at postural adjustment in any of the head, neck, and trunk, which in the head-restrained animal may be involved in controlling gaze.

Beyond information potentially conveyed during the inactivation period after a CS, SSS may contain neural coding that is not represented in the CS. Further analysis and studies are necessary to determine the ultimate relationship between the CS and the SS in cerebellar PCs. However, the current study demonstrates that the low-frequency occurrence of the CS may help to further narrow the search for specific parameters controlled by neural firing of PCs in the posterolateral cerebellum and their role in visual error.

Conclusions

Our results suggest the role of the Pc CS in signaling visual/gaze error and a corrective saccade during a visually guided reach-touch movement. We also here report that the task-related changes in PC SS firing correlated with target position and/or a corrective saccade and that the patterns were the same independent of whether the ipsilateral or the contralateral arm was used to reach and touch the screen. Although lesions of this part of cerebellar cortex have been shown in humans and macaques to disrupt the adaptation and learning of visually targeted gaze-arm reaching and throwing, it remains to be seen how the arm is coupled to the gaze by the cerebellum.

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