Dynamic Correlation of Neuronal Activity in Rat Cerebellar Cortex Modulated by Behavior

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ABSTRACT: Sprague-Dawley rats (2–4 months old) were trained to perform a reaching–grasping task while their head was fixated and multielectrode recordings were performed in the ipsilateral cerebellar hemisphere. Multiunit (MU) activity was recorded with 2–3 electrodes in the Purkinje cell layer at various depths (1.5–4.5 mm) from the pial surface while the animal either performed the reaching–grasping task or was at rest. Recording sites were visually aligned along the transverse or the sagittal axis of Crus IIa. Excess correlations of MU spike activity were calculated at 10-ms time resolution using the joint peristimulus time histogram (JPSTH), to reveal the dynamics of cross-correlations corrected for nonstationarities in spike rates. Peak correlation amplitudes and areas were calculated separately for a time period preceding the movement and for a succeeding period during which the movement occurred. Correlations were compared across different paradigms: transversal versus sagittal alignment of recordings sites and behavior versus rest. No significant differences were found between transversally and sagittally aligned recording sites. Significant differences in peak correlation amplitude and/or peak area, however, were found both between premovement and movement time, as well as between both these time periods and periods while the animal was at rest.

KEYWORDS: cerebellum; correlation dynamics; timing; parallel fibers; multielectrode recording; awake rat; reaching-grasping

INTRODUCTION

The cerebellar cortex is wired in such a way that all intrinsic excitatory fibers, that is, the parallel fibers, run in the transversal direction, whereas the inhibitory axons extend in the sagittal plane.[1] The functional significance of this arrangement, especially the effect of parallel fibers on the spike firing of Purkinje cells, is still under debate.[2–7] Cells aligned along the axis of parallel fibers receive largely overlapping

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excitatory input,[8,9] which, if sufficiently strong, could drive the cells to correlated spike firing. We investigated the temporal correlation of neuronal spike activity in the Purkinje cell layer of awake-behaving rats using multielectrode recordings and dynamic correlation analysis.[10] Multiunit (MU) spike activity was recorded simultaneously from 2–3 sites that were either aligned with the transversal axis of Crus IIa or perpendicular to that axis. The temporal correlation of spike activity was calculated at 10-ms resolution for time periods preceding the movement and during the movement, and for a control period during which the animal was at rest. Multiunit spike activities at different sites exhibited temporally loose correlations, with peak widths of cross-correlograms in the order of 100 ms. These correlations were strongly modulated during behavior, but did not differ significantly between transversally and sagittally aligned recording sites. The independence of these loose temporal correlations off the highly asymmetric cerebellar cortical anatomy suggests that they may be caused by external sources providing common input to the observed neurons.

METHODS

Surgery and Behavior

Sprague-Dawley rats (2–4 months old) were trained to reach for a small food pellet.[11] Rats were kept in a training cage with a grid wall. Food pellets were offered outside the cage in front of the grid wall. Rats learned to reach for the food pellets in one to three training sessions, each session lasting 4 h. After successful training, screws for head fixation and a recording chamber were mounted on the skull using acrylic cement. On day 3 after surgery, the animals were set in a head-fixating device, and trained to reach for and grasp food pellets during head fixation. The pellets were manually set down on a capacitance switch mounted in front of the rat (about 1 cm below the mouth). The switch was set off by the rat's paw touching the food pellet, and the status of the capacitance switch (open or closed) was recorded together with the spike data and later used for event-triggered spike-train analysis (see below). The behavioral performance was taped on video for off-line classification of reaching–grasping movements. A light-emitting diode, visible in the video recordings, was connected to the capacitance switch, and lit up when the paw touched the pellet. Only successful trials, that is, grasping of the pellet in one reach, were included in the event-triggered spik-train analysis. Because of the manual delivery of food pellets, the rats were able to predict pellet presentation by the experimenter's movement, and typically reached for the pellet as soon as it was set down on the switch.

Electrophysiology

Multiunit neuronal activity in cerebellar cortex was recorded with 2–3 electrodes simultaneously using a multielectrode recording system (System Eckhorn, Uwe Thomas Recording, Germany). Electrodes, aligned in a row and spaced at 305 μm, were lowered into the ipsilateral hemisphere 1.5 to 4.5 mm below the pial surface (Crus 1), aiming at the paramedian lobe. Penetration depth was controlled and registered for each electrode individually with a resolution of 1 μm. Recordings from the Purkinje cell layer were accepted when complex spikes could be clearly identified.[12]

All electrodes were placed in the same lobule as confirmed by penetration depth, and the number of times the Purkinje cell layer was penetrated. The assembly of electrodes was inserted either in a transversal ($n = 14$) or a sagittal ($n = 9$) orientation. Orientation of the electrodes was adjusted visually in relation to the longitudinal axis of Crus IIa. Deviations from alignment with the parallel fibers or the perpendicular axis, however, may have occurred in cases when the electrodes were advanced more posteriorly. Multiunit activity was recorded while the animal performed the reaching–grasping task or while at rest, that is, with no food pellet presented. About 10 min of resting data were recorded at least 5 min after completion of a series of reaches. Animals were awake and alert during the resting period, but no food pellets were presented. Data obtained from four different experimental paradigms: recordings along or perpendicular to the transverse axis, while the animal was at rest or behaving, were analyzed separately. Spike data traces were bandpass filtered (0.1 Hz–5 kHz) and digitized at 20 kHz (CED 1401, Cambridge Electronic Design, UK).

Data Analysis

To calculate excess correlation in data with nonstationary rates, as typically observed in behaving animals, we used the joint-peristimulus time histogram (JPSTH)[10] (FIG. 1). With this technique, correlation values are normalized for rate modulations within trial and rate variability across trails.[10,13,14] To perform event-related correlation analysis on data from resting periods without behavioral events, artificial event triggers were generated at pseudorandom intervals (minimum interval 4.2 s; maximum interval 5 s). JPSTH analysis of the behavioral data revealed on average a high correlation 0.5 s and earlier before the touching of the food pellet, and a clearly reduced correlation around the time of the touch. We therefore calculated the average correlations separately for the time periods −1.5 to −0.5 s before and ± 0.5 s around the touch event. Since the delay between movement onset and touching of the food pellet was between 100 and 300 ms, we considered the two periods representing time before and during the reaching–grasping movement, respectively.

Average cross-correlograms were smoothed with a Gaussian ($\sigma = 20$ ms). After smoothing the peak value, the deviation of peak time from zero time lag and the area under the correlation peak were measured within the boundaries defined by the average width at half maximum.

RESULTS

Neuronal activity modulated during the reaching–grasping task was recorded at 30 different sites at various depths in three animals. Behavior-related modulation of MU firing rate was most pronounced between 3.5- and 4.5-mm penetration depth, that is, presumably in the paramedian lobe. The most common observation was an increase in firing rate around the time of the grasping movement (FIG. 1). Rate increases typically lasted several hundred milliseconds, and occurred between about 1 s before and 1 s after the paw touched the food pellet. Time of occurrence of the rate change relative to the touch varied with recording sites.

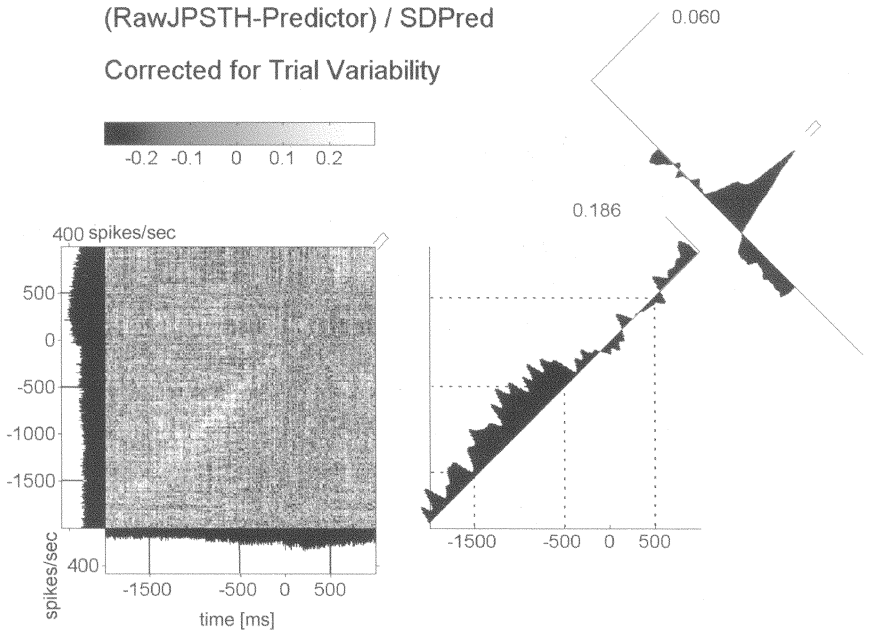

FIGURE 1. Joint-peri-stimulus-time histogram (JPSTH) reveals the dynamics of excess spike correlation after correcting for rate modulations within trial and rate variability across trials. The abscissa and ordinate each show a standard peri-stimulus-time histogram (PSTHs) of multiunit spike recordings aligned on the behavioral trigger (time zero = touching of the food pellet). Each element in the JPSTH matrix gives in gray scale the correlation value for a given time delay between the two spike trains. The elements aligned along the main diagonal (*bottom left to upper right*) hold the values for correlations around zero time delay, that is, synchronous firing. The main diagonal is shown again in histogram format to the right of the matrix. As clearly visible in the matrix as well as in the histogram of the main diagonal, excess correlation of neuronal activity was higher during the period preceding the touch than during the period around the touch. Correlation analysis was therefore performed separately on two time intervals (−1.5 to −0.5 and −0.5 to 0.5 s), as indicated by the *lines* in the two PSTHs and the *dashed lines* in the graph showing the smoothed diagonal. The *correlation histogram* on the upper right is the result of averaging over all elements of the matrix in a direction parallel to the main diagonal. It represents the average excess correlation across the entire 3 s trial duration. Its peak amplitude and the area under the peak (calculated within the limits of the full width at half maximum) were measured separately for the premovement and the movement period, and compared across experimental paradigms. Binwidth in the PSTH and JPSTH matrix is 10 ms. Binwidth in the average cross-correlogram is 5 ms. Correlation histograms were smoothed with a Gaussian (sigma = 20 ms).

Correlation Analysis

Excess correlation of multiunit activity recorded on two electrodes separated by 305 or 610 mm was calculated using the JPSTH with a binwidth of 10 ms (FIG. 1). Peak area and maximum of the smoothed average cross-correlograms were analyzed and compared across paradigms and time periods relative to the touching of the food pellet (FIG. 2). Neuronal activity was clearly correlated during both the behavioral

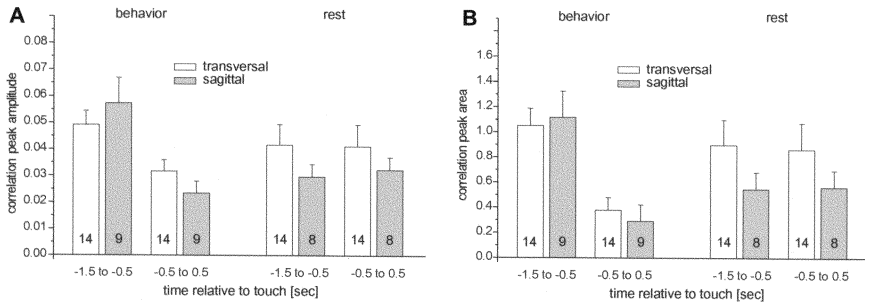

FIGURE 2. Correlation of neuronal activity recorded from sites aligned with the parallel fibers (transversal) or perpendicular to that axis (sagittal). Data were analyzed separately for the period preceding the reaching for the food pellet (−1.5 to −0.5 s) and the time around the touch (−0.5 to 0.5 s). Event-triggered analysis of data recorded during rest was based on pseudorandom event triggers. Average excess correlations for the two time periods were calculated using the JPSTH (cf. FIG. 1). After smoothing the correlation histogram, the (**A**) peak amplitude and (**B**) the area under the peak were measured. No significant differences (Wilcoxon) in peak amplitude or peak area could be found comparing transversally with sagittally aligned recording sites. Numbers of measurements are shown in each *bar*. *Error bars* show S.E.M.

and the resting periods. The temporal precision of the correlation, however, was rather low, with an average width of the correlation peak at half maximum of 204 ± 105 ms.

Neuronal correlation was strongly modulated during behavior. The delay between movement onset and the touching of the food pellet was about 100 to 300 ms. Visual inspection of the JPSTH results revealed a markedly stronger correlation of MU spike activity during the period −1.5 to −0.5 s before the rat touched the food pellet (premovement) than during the period −0.5 to 0.5 s around the touch (during the reaching–grasping movement) (FIG. 1). Therefore, all correlation analyses were performed separately on data obtained during the premovement and the movement intervals.

We measured the correlation peak amplitude (FIG. 2A) and the correlation peak area (FIG. 2B) for sagittally and transversally aligned recording sites and compared the values across three different sets of data: (1) data recorded before the reaching movement, (2) during the reaching–grasping movement, and (3) data recorded while the animal was at rest, but awake. No significant differences between transversal or sagittal alignment of recording sites were found (FIG. 2A and 2B). We also tested for a dependence of the correlation on distance between recording sites. Neither the peak amplitude nor the peak area were significantly different when comparing recording sites separated by 305 or 610 μm (data not shown).

Highly significant differences, however, in both correlation peak area and correlation peak amplitude were found when comparing the periods before and during the reach (FIG. 3A and 3B). Both correlation measures were significantly smaller ($p < 0.01$) during the reaching–grasping movement than during the time immediately preceding the movement.

A comparison of behavioral and control (rest) data revealed that neuronal activity recorded during the resting period was on average less correlated than during the pe-

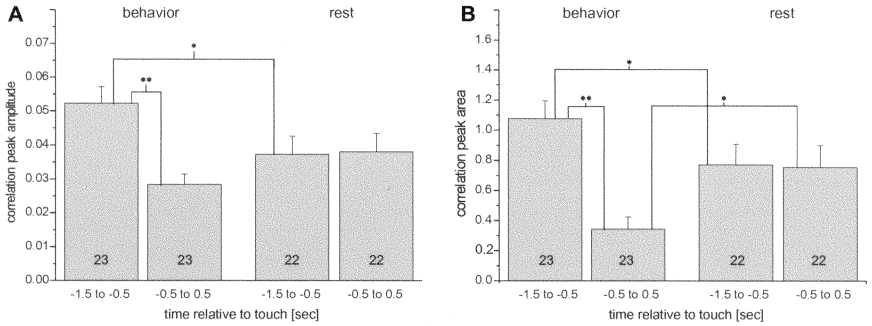

FIGURE 3. Comparison of the (**A**) excess correlation peak amplitude and (**B**) correlation peak area of neuronal activity measured during the time periods preceding and during the reaching–grasping movement, with the correlation during rest. In the behavioral paradigm, excess correlation peak amplitude as well as peak area were significantly higher during the period preceding the movement (−1.5 to −0.5 s) than during movement (−0.5 to 0.5 s). Peak correlation preceding the touch was significantly higher than the peak correlation during a period of identical duration at rest. Compared to the control situation at rest, correlation peak area was significantly higher during the period preceding the touch and significantly lower during the period around the touch (**$p < 0.01$; *$p < 0.05$). Numbers of measurements are shown in each *bar*. *Error bars* show S.E.M.

riod preceding the reaching–grasping movement in the behavioral paradigm. Both the mean correlation peak area and correlation peak amplitude were significantly higher in the period preceding the reach than during rest ($p < 0.05$; FIG. 3A and 3B). On the other hand, the correlation during the reaching movement was significantly lower than during rest. This difference, however, was significant only for the correlation peak area ($p < 0.05$; FIG. 3B), but not significant when comparing the correlation peak amplitude (FIG. 3A).

DISCUSSION

We have shown here that the correlation of neuronal activity in the Purkinje cell layer of the cerebellum undergoes dynamic changes during a reaching–grasping task. Comparing the excess spike correlation during behavior with data obtained during rest, we found a higher than resting correlation during the time immediately preceding the movement and a lower than resting correlation during the actual movement.

In our experiments we used electrodes spaced 305 μm apart to record simultaneously from 2–3 sites along the transverse axis, that is, in line with the parallel fibers or along the sagittal axis, that is, aligned with the inhibitory axons. Typically, neurons in the Purkinje cell layer mostly increased their firing rate in relation to the reaching–grasping movement. Other than expected from a "beam"-like organization,[15] where lateral inhibition would occur between neighboring areas in the sagittal direction, there was no obvious difference in rate change patterns between transversal and sagittal recordings.[11]

In the present study we have shown that the temporal correlation of neuronal activity at a 10-ms time resolution during rest or behavior was not different when comparing recording sites aligned along the transversal or along the sagittal axis. The precision, however, with which we could align the electrodes with the anatomical axes of the cerebellar cortex was limited, especially in deep recordings. Vos and colleagues[16] have reported differences in the correlation of Golgi cell activity (expressed in *z*-scores[17]) when comparing recordings along the transversal and the sagittal axis. They recorded from anaesthetized animals and verified the alignment of their recording sites anatomically. Using the same *z*-score analysis as Vos *et al*. on our data, however, we also found significantly higher peak correlations along the transversal compared to the sagittal axis, both during behavior and during the resting period. This difference was no longer significant, however, when we calculated excess correlation using the joint-PSTH correcting for rate nonstationarities in the data (both rate modulations within trial and rate variability across trials). It is thus possible that nonstationarities of rates are responsible for the differences between the transversal and sagittal recordings reported by Vos *et al*.

The excess correlation of multiunit activity was strongly modulated during the reaching–grasping movement. The movement, that is, lifting the paw off the ground, starts between 100 and 300 ms before the touch occurs. The correlation of neuronal activity was significantly higher immediately before than during the reaching–grasping movement. The correlation during the premovement period was also significantly higher than during the control or resting period, indicating that the time shortly before the movement was not equivalent to a resting state. The increased correlation measured during the premovement time may reflect a state of anticipation, similar to what has been observed by Riehle *et al.*[18] in the primary motor cortex of behaving monkeys. We supplied the food pellets manually. Therefore the experimenters' movements were most likely an early preparatory signal (see methods). During the actual reaching–grasping movement, excess correlation was significantly reduced, both compared to the correlation measured in the premovement period and also compared to the resting state.

SUMMARY

Neurons in the Purkinje cell layer of awake rats showed a behavior-related modulation of spike correlation at a 10-ms time resolution. The dynamics of this correlation were independent of the alignment of the recording sites in the transverse or sagittal direction, and were also independent of the distance between recording sites. The rather loose temporal correlations observed in this study could be brought about either by intrinsic cerebellar cortical connections, equally affecting sagittal and transversal recording sites, or by external sources. However, given the highly asymmetric wiring of the cerebellar cortex, the first possibility appears unlikely. We therefore suggest that the observed correlations and their dynamics are due to activity in the cerebellar afferents, that is, the mossy-fiber system, possibly affecting loose spike correlation through the rising parts of granule cell axons.[2,3] We have, however, also observed temporal correlations in cerebellar activity occurring at submillisecond precision.[19] The dependence of these highly precise correlations on recording-site alignment and their dynamics during behavior are to be investigated.

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