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Serotonin Modulation of Inferior Olivary Oscillations and Synchronicity: A Multiple-electrode Study in the Rat Cerebellum

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Abstract

Simultaneous recording of complex spikes from multiple Purkinje cells (up to 44) in the rat cerebellum was used to examine the effects of 5-hydroxytryptamine (serotonin, 5-HT) on olivocerebellar function. Microinjection into the inferior olive was found to increase the average firing rate of inferior olivary neurons while slowing their oscillation frequency and increasing the coherence of their oscillations. Indeed, while the normal rostrocaudal band of synchronous activity remained unchanged, the degree of synchrony between Purkinje cell complex spikes within this band was enhanced following the 5-HT injections. Multiple-electrode recordings obtained from crus IIa and vermal lobule VIb yielded qualitatively similar results; however, the effects on vermal activity were more pronounced. The effects of the 5-HT microinjection decayed with a time course of 75 min. The half-maximum effective concentration of 5-HT was between 10 and 100 μM. Injections of various 5-HT agonists and antagonists demonstrated that a 5-HT type-2A (5-HT_{2A}) receptor is the main mediator for the 5-HT effect, which was very similar to the effect produced by injections of harmaline. However, 5-HT and harmaline appear to have independent mechanisms since the action of harmaline was not blocked by the 5-HT_{2A} antagonist LY53857. A possible role for 5-HT, as a physiological enhancer of the timing of motor function of the olivocerebellar system, is discussed.

Introduction

Inferior olivary neurons express synchronous oscillatory activity near 10 Hz as a result of their intrinsic electroresponsiveness and their electrotonic coupling properties (Llinás *et al.*, 1974; Sotelo *et al.*, 1974; Llinás and Yarom, 1981a, b, 1986; Llinás, 1988). Activation of inferior olivary neurons drives the Purkinje cells of the cerebellar cortex via the climbing fibres (Eccles *et al.*, 1966) and the cerebellar nuclear neurons via axon collaterals, resulting in synchronous oscillatory activity throughout the olivocerebellar system (Llinás and Mühlethaler, 1988). A proposed functional corollary of this synchronous oscillation is the timing of motor sequences in motor coordination (Llinás, 1988; Llinás and Welsh, 1993; Vallbo and Wessberg, 1993).

Because of the special one-to-one relationship between a climbing fibre and a Purkinje cell, well defined complex spike bursts are generated in Purkinje cells following inferior olive activation (Eccles *et al.*, 1966). The unambiguous nature of complex spikes facilitates the implementation of a simultaneous recording paradigm using multiple microelectrodes implanted into the molecular layer of the cerebellar cortex (Sasaki *et al.*, 1989). In recent years this technique has enabled simultaneous recordings to be made from as many as 96 Purkinje cells (Fukuda *et al.*, 1987). The present study applied this technique to the investigation of certain pharmacological properties of the olivocerebellar system.

Although much is known about the inputs to the inferior olive from various regions of the central nervous system, the action of only a few transmitter substances has been investigated. The γ-aminobutyric acidergic (GABAergic) inputs from the cerebellar nuclei to the olivary glomerulus have been identified (Nelson *et al.*, 1984; Sotelo *et al.*, 1986; de Zeeuw *et al.*, 1989). The functional significance of this input in controlling the electrotonic coupling of inferior olivary neurons has been studied with multiple-electrode recordings (Lang *et al.*, 1989, 1990; Llinás and Sasaki, 1989). A strong serotoninergic projection to the olivary neurons has also been identified (Wiklund *et al.*, 1977; King *et al.*, 1984) and a high density of 5-hydroxytryptamine (5-HT, serotonin) receptors has been demonstrated using autoradiographic techniques (Pazos *et al.*, 1985). However, the direct effect of 5-HT on inferior olivary neurons is still controversial and its functional significance has not been extensively investigated. Systemic application of the 5-HT receptor type 2A (5-HT_{2A}) agonist, quipazine, resulted in increased firing rates and rhythmicity in inferior olivary neurons and induced a tremor in young

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rabbits (Barragan *et al.*, 1985). *In vitro* experiments by Llinás and Yarom (1986) have also demonstrated the excitatory effect of 5-HT on inferior olivary neurons. Other investigators, however, have suggested that 5-HT has an inhibitory effect on inferior olivary neurons (Headley *et al.*, 1976).

We combined multiple-electrode recording with local intra-olivary microinjections of 5-HT or its agonists or antagonists to examine the effects of 5-HT on (i) the activity of individual inferior olivary neurons, and (ii) the synchronicity of complex spike activity. Since the density of serotoninergic innervation to the rostrocaudal extent of the inferior olive is not uniform (Wiklund, 1977; King *et al.*, 1984), the effects of 5-HT injections on the activity of each of these areas were compared. This was accomplished by making multiple-electrode recordings from the hemisphere (crus IIa) and the vermis (lobule VIb), as these two lobules receive climbing fibre input from the rostral and caudal inferior olive respectively (Voogd and Bigaré, 1980).

Harmaline is known to exert its tremorogenic effects by enhancing the oscillatory activity of olivary neurons (de Montigny and Lamarre, 1973; Llinás and Volkind, 1973). It is generally thought that harmaline acts on serotoninergic systems (Burkard and Kettler, 1977; Sjölund *et al.*, 1977; Barragan *et al.*, 1985). However, *in vitro* studies have shown that harmaline directly affects the membrane conductances of olivary neurons (Llinás and Yarom, 1986; Yarom and Llinás, 1987). Therefore a comparison between the actions of harmaline and 5-HT on the oscillatory properties of olivary neurons was also implemented in the present study. Some of the results have appeared in abstract form (Sugihara *et al.*, 1991).

Materials and methods

Animal preparation

Twenty-seven adult female Sprague–Dawley rats (250–275 g) were used. The rats were anaesthetized with an initial intraperitoneal injection of ketamine (100 mg/kg), xylazine (8 mg/kg) and atropine (0.4 mg/kg). Supplementary doses (7 mg/kg) of ketamine were given through a femoral vein catheter every 30 min, beginning 3 h after the initial dose. Once the animal was anaesthetized its head was fixed in a stereotaxic apparatus (Model 1460, Kopf, Tujunga, CA) and its body was placed on an electrical heating pad that maintained the rectal temperature at 36–37°C. The occipital bone and the dura were removed, exposing the dorsal surface of the cerebellar hemisphere or vermis and proximal spinal cord, which was kept moist with mineral oil-soaked Gelfoam. The heart rate was monitored throughout the experiment.

Multiple-electrode recordings of spontaneous complex spikes

The multiple-electrode technique used in the present experiments has been described in detail (Sasaki *et al.*, 1989). Briefly, after removing the Gelfoam overlying the cerebellum, a silicone rubber platform was cemented on either left crus IIa or left vermal lobule VIb (Fig. 1A). The centre position of the platform was usually 3.7 and 1.0 mm lateral from the midline for crus IIa and lobule VIb recordings, respectively (Fig. 1B). Each electrode was attached to the holder by a wax droplet that could be melted by a microcoil in the shaft of the holder (Sasaki *et al.*, 1989). The holder was coupled to a joystick-controlled piezoelectric micromanipulator (Intelligent Inchworm Translator Micropositioning System, Burleigh, Fishers, NY, USA), and electrodes were lowered into the molecular layer of the cortex individually through a platform that served as a supporting system. When a complex spike was clearly recorded the electrode was released from the manipulator by melting the wax. The electrodes were inserted into a cerebellar folium in a rectangular array of 4–6 rostrocaudal columns and 6–8 mediolateral rows, with an inter-electrode distance of 250 μm. After electrode implantation, the threshold for each recording channel was individually set to detect the unitary complex spike activity. Typically, complex spike activity was simultaneously recorded from 17–30 Purkinje cells (minimum 12 cells, maximum 44 cells). Spontaneous complex spike activity was recorded for ~15 min for each experimental condition.

Drug solutions

Drugs to be injected into the inferior olive were freshly dissolved in physiological saline. Unless otherwise stated, the concentration of the drugs was as follows: 5-HT hydrochloride (Sigma), 1 mM; (±)-8-hydroxydipropyl-aminotetralin hydrobromide (8-OH-DPAT; RBI, Natick, MA, USA) 1 mM; 1-(3-chlorophenyl)piperazine dihydrochloride (m-CPP; RBI), 1 mM; tryptamine (Sigma), 1 mM; α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT; RBI), 100 μM; 1-(*m*-chlorophenyl)-biguanide hydrochloride (m-CPBG; RBI), 100 μM; harmaline hydrochloride (Sigma), 1 mM. The potency ratios (reciprocals of the ratios of the affinity values) of these agonists versus 5-HT to the specific 5-HT receptors are as follows: 1.7 (8-OH-DPAT, 5-HT_{1A}; Engel *et al.*, 1986); 0.9 (m-CPP, 5-HT_{2C}; Conn and Sanders-Bush, 1987); 0.7 (tryptamine, 5-HT_{2C}; Engel *et al.*, 1986); 0.56 (tryptamine, 5-HT_{2A}; Martin and Sanders-Bush, 1982); 23 (α-methyl-5-HT, 5-HT_{2A}; Engel *et al.*, 1986); and 10 (m-CPBG, 5-HT_3; Kilpatrick *et al.*, 1990). Thus, a concentration of 1 mM of 8-OH-DPAT, m-CPP or tryptamine is approximately equivalent to 1 mM 5-HT with respect to each receptor. Because the potency of α-methyl-5-HT and m-CPBG for the 5-HT receptor is approximately ten times that of 5-HT, lower concentrations of these drugs were used in order to be equivalent with 1 mM 5-HT. Note that 5-HT_{2A} and 5-HT_{2C} were previously called 5-HT_2 and 5-HT_{1C} respectively. The 5-HT antagonists were LY53857 (RBI), 1 mM, and spiperone hydrochloride (RBI), 0.1 mM. The low concentration of spiperone was chosen because it has been reported to be about as effective as ten times concentrated LY53857 (Elliott *et al.*, 1992). Injection pipettes were pulled from single-, double- or triple-barrel glass capillary tubes (6030, 6070, 6090; A-M Systems, Everett, WA, USA) using a vertical puller. The tips were honed to an internal single-barrel diameter of 10–20 μm. Each barrel was filled with solution and connected at its base to a length of polyethylene tubing, using fine metal tubing and a wax seal. A silver wire placed in one of the barrels monitored the electrical activity from the pipette tip.

Recordings and injections

Before insertion of the injection pipette into the inferior olive a baseline measure of spontaneous complex spike activity was recorded. A perpendicular approach from the dorsal surface of the medulla was used to reach the right inferior olive, since the head of the animal was rotated 50°, nose down (Fig. 1A). In accordance with the topography of the olivocerebellar projection (Voogd and Bigaré, 1980), the pipette was aimed at the rostral or caudal inferior olive depending on the recording site in the cerebellum. For the hemispheric crus IIa recordings, the pipette was inserted at a point 0.5 mm lateral from the midline, just posterior to the caudal edge of the cerebellum. For the vermal lobule VIb recordings the pipette was inserted 0.4 mm caudal to the former point. The electrical signal from the pipette tip was monitored acoustically. The inferior olive was identified by the presence of typical 8–12 Hz rhythmic activity, which was correlated with the complex spike activity monitored by an LED

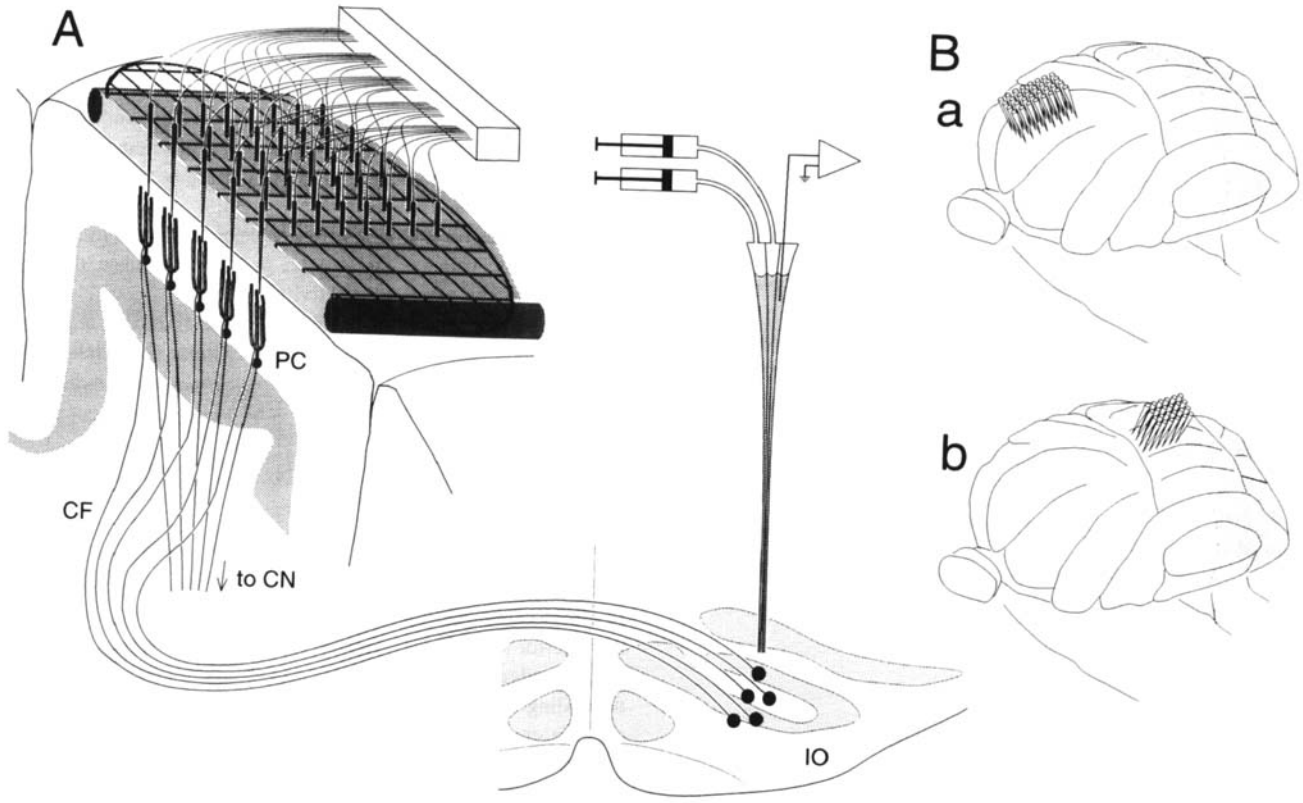

FIG. 1. (A) Schematic of multiple electrode recording from Purkinje cells in the molecular layer of the cerebellar cortex and injection of solutions into the inferior olive. (B) Schematic illustration of multiple-electrode recording from the hemispheric crus IIa (a) and from the vermal lobule VIb (b). IO, inferior olive; CF, climbing fibre; CN cerebellar nucleus.

panel display system (Sasaki *et al.*, 1989). Before any injections were made, the spontaneous complex spike activity was recorded a second time. This recording period was used as the control baseline activity, unless otherwise stated. The drug solution was pressure-injected using an air-filled syringe connected directly to the injection pipette through polyethylene tubing (Fig. 1A). A volume of 1 µl was injected at a rate of 0.20 µl/min, as measured by the movement of the meniscus (1.8 mm for single-barrel and 3.8 mm for double- and triple-barrel pipettes). Complex spike activity was recorded for ~15 min, starting 2 min after the onset of the injection. If more than three solutions were used, the injection pipette was removed and a second pipette was lowered into approximately the same position. In the dose–response experiments the solution inside the pipette was exchanged for one of a higher concentration without removing the pipette.

After the completion of the recording sessions, 0.1–0.2 µl of alcian blue (10 mg/ml in saline) was injected from one barrel of the injection pipette to mark the tip position. The animal was then perfused intracardially with saline followed by 10% formalin. The dissected brain was immersed in 10% formalin overnight and then in 30% sucrose plus 10% formalin for 2 days. Parasagittal or coronal frozen sections of the medulla and cerebellum were stained with cresyl violet.

Multichannel recording system

The multichannel recording system was similar to that described before (Sasaki *et al.*, 1989; Sugihara *et al.*, 1993). Complex spike signals from all recording channels were digitized, stored on VCR tape, and directly transferred onto a 80386-based personal computer with a 1 ms intersampling period. The file containing the digital signals was transferred to a minicomputer (MicroVAX 3100; Digital Equipment, Acton, MA, USA) and analysed using FORTRAN programs.

Synchronicity calculation

To measure the degree of synchrony, a cross-correlation coefficient (Gerstein and Kiang, 1960; Sasaki *et al.*, 1989) was calculated from the spike train of a pair of Purkinje cells (a 'master' cell and a compared cell) as described previously (Sasaki *et al.*, 1989; Sugihara *et al.*, 1993). Unless mentioned otherwise, a 3 ms time bin was used to define synchronicity. The zero-time cross-correlation coefficient was used as a measure of the degree of synchrony. In the text below, 'synchronicity' or 'degree of synchrony' will be used in place of 'zero-time cross-correlation coefficient'.

The significance level for synchrony was determined by simulating random independent spike trains on a computer (MicroVAX 3100), generating 30 000 pairs of randomly firing spike trains with average firing rates of 0.5–10 Hz and a duration of 10 min. The zero-time cross-correlation coefficient was calculated from each pair with a bin width of 3 ms. Under these conditions, 99% of the zero-time cross-correlation coefficients calculated from the random data were smaller than 0.0063 for any of the firing rates. We took this value to be the 99% significance level of synchronicity in this study.

Analysis of oscillation rhythm

Autocorrelation histograms were plotted for complex spike trains recorded from single Purkinje cells with a time bin of 10 ms. The peaks and valleys were determined using the following mathematical

criteria. The baseline level in the autocorrelogram was obtained by the following formula:

$$\text{baseline level} = (\text{total firing number})^2/\{(\text{recording time})/(\text{bin width})\} \quad (1)$$

The random fluctuation about the baseline was measured at time lags of −1280 to −1000 and 1000 to 1280 ms. Peaks and valleys were recognized if their heights and depths exceeded the mean baseline level ± SD of the fluctuation, or if the difference between a peak and the next valley exceeded twice the SD of the spontaneous fluctuation. The first peak was defined as the highest bin within a time lag of 50–250 ms while the first valley was defined as the shortest bin within 100–500 ms. If the nth peak and valley were recognized, the next peak and valley were searched for within a time range of

$$t_n + t_{(\text{peak 1})} \pm t_{(\text{peak 1})}/2$$

where t_n is the time lag of the nth peak or valley and $t_{(\text{peak 1})}$ is the time lag of the first peak.

The oscillation frequency was defined as the reciprocal of the time lag of the first peak (Fig. 2). The rhythm index was defined by the following formula:

$$\text{rhythm index} = a_1/z + b_1/z + a_2/z + b_2/z + \quad (2)$$

in which a_i (i = 1,2,...) is the difference between the height of the ith peak and baseline level in the autocorrelogram, b_i (i = 1,2,...) is the difference between the depth of the ith valley and baseline level, and z is the difference between the height of the zero-time bin, which indicates the total number of spikes, and the baseline level (Fig. 2). The terms of the rhythm index (equation 2) are identical to the absolute values of autocorrelation coefficients at the time lags of the peaks and the valleys. The greater the rhythm index, the tighter or stronger was the oscillatory activity. In those autocorrelograms with no recognizable peaks and valleys, a value of zero was given to the rhythm index. In these cases as well as those in which the rhythm index was <0.01, the autocorrelation was regarded as non-oscillatory, and oscillation frequency was not determined.

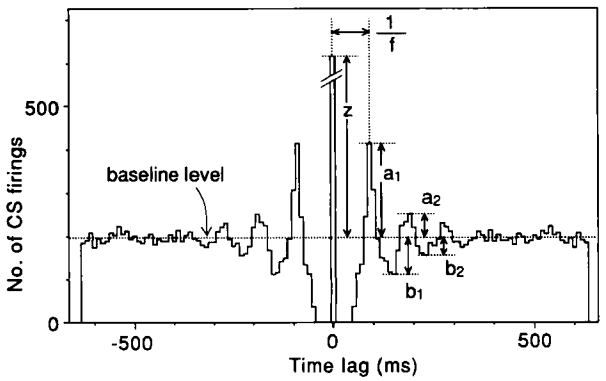

FIG. 2. Complex spike autocorrelogram detailing the parameters used in the rhythm index calculation. See text for explanation.

Results

In 24 of 27 animals the injection site was histologically confirmed. In all 24 cases, the injection sites (Fig. 3) were either located within the inferior olive (0.2–1 mm lateral to the midline) or at its dorsal or ventral edges. The injection was located rostrally or caudally depending on whether the complex spike activity was recorded from crus IIa or vermis, respectively.

Local injections of 5-HT into the inferior olive produced increases in the average firing rate of complex spikes, rhythmicity, and synchronicity of complex spike activity within a few minutes after the onset of the injection. These increases were sustained for the duration of the drug injection (5 min) and for at least 10 min after the completion of the injection. Subsequently the firing rates decreased slowly to control levels (see section headed Time course of 5-HT injections). Thus, a recording period of ~15 min following an injection was usually sufficient to calculate reliable auto- and cross-correlations between the complex spikes of individual Purkinje cells.

In each experiment, electrodes covered a 1.5–2.0 mm expanse of cerebellar cortex in the mediolateral direction. Thus, climbing fibre input to the Purkinje cells arose from a wide region of the inferior olive. An injection volume of 1 µl of 5-HT solution resulted in increases in complex spike activity in most or all Purkinje cells.

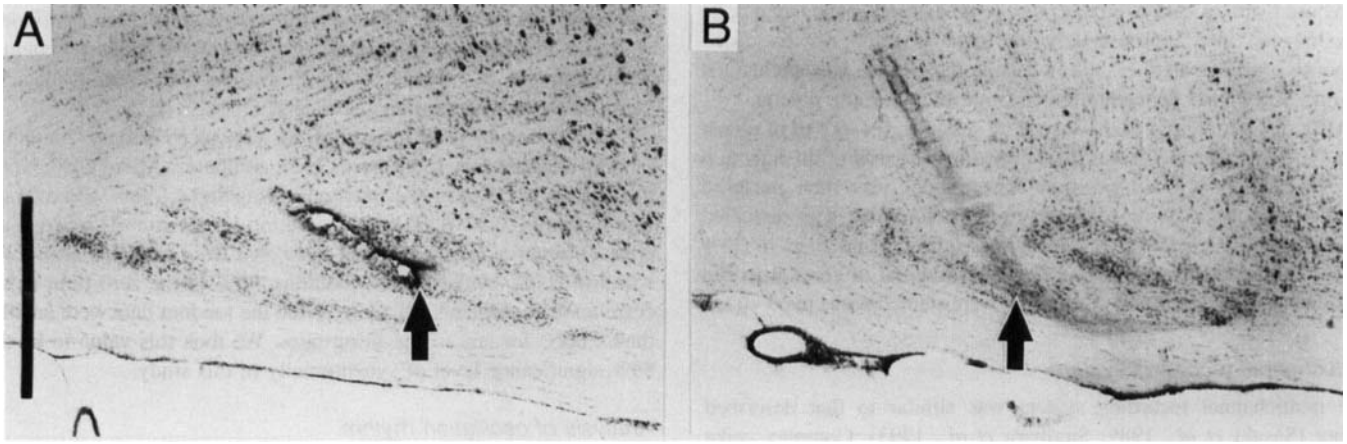

FIG. 3. Injection sites and electrode tracts marked by alcian blue. Parasagittal sections (60 µm) were stained with cresyl violet. Injection sites (arrows) were rostral (A) and caudal (B) relative to the centre of the olive for hemispheric and vermal recordings respectively. Rostral is to the right and dorsal to the top in the figure. Calibration bar, 1 mm.

5-HT effects on spatial pattern of synchronicity

Spontaneous complex spikes in different Purkinje cells have been shown to be well correlated in time, particularly if the Purkinje cells are located in the same parasagittal plane (Bell and Kawasaki, 1972; Sasaki *et al.*, 1989). The spatial pattern of this synchronicity has been well defined by using the technique of multiple-electrode recording (Llinás and Sasaki, 1989; Sasaki *et al.*, 1989). The spatial pattern of complex spike synchrony in crus IIa Purkinje cells before and after 5-HT is shown in Figure 4A and B for two master Purkinje cells. In this experiment the complex spike activity was recorded from 44 Purkinje cells; the electrode positions in the cerebellar cortex are given by the positions of the circles in the figure, while the size of each circle indicates the degree of synchrony of each cell with the master cell. The complex spike activity of cells near the master cell generally had a high degree of synchrony (>0.0063; see Materials and methods) and were often organized in a rostrocaudal band that was 250–500 μm across (upper panels of Figure 4A and B). The slight deviation (~20° clockwise) from the vertical, or rostrocaudal, orientation is due to slight misalignment between the axis of the electrode array and the rostrocaudal axis of the cerebellar lobule. After 5-HT injection (lower panels of Figure 4A and B), the basic rostrocaudal banding remained. However, the synchronization of the activity of cells within the same band increased, as indicated by the increased size of the circles. The activity of the majority of cells outside the original rostrocaudal bands remained unsynchronized with the master cells.

The patterns of complex spike activity recorded from vermal lobule VIb were similar to those found in crus IIa with rostrocaudal bands of high synchrony (upper panels of Figure 4C and D). Although present, the bands appeared to be somewhat wider and less sharply defined than those usually seen in crus IIa. The effects of olivary injections of 5-HT are also similar to those seen in crus IIa Purkinje cells, i.e. there was an increase in synchronicity while the basic rostrocaudal banding pattern remained intact (Fig. 4C and D).

Similar effects of 5-HT on synchronicity were seen for any selected master cell in each experiment. This is demonstrated in Figure 4E, where all Purkinje cell pairs from the experiment depicted in Figure 4C and D were sorted according to the mediolateral separation of the cells, and the mean and SE of synchrony were plotted. The effect of 5-HT was more pronounced in Purkinje cell pairs with a small (≤0.5 mm) mediolateral separation than for Purkinje cell pairs with a larger separation (>1.0 mm) (Fig. 4E). This further demonstrated that olivary injections of 5-HT increased the synchronization of complex spike activity without disrupting its fundamental banding organization. The effects of 5-HT on the synchronicity of complex spike activity were similar to those elicited by systemic or inferior olivary injections of harmaline (Llinás and Sasaki, 1989).

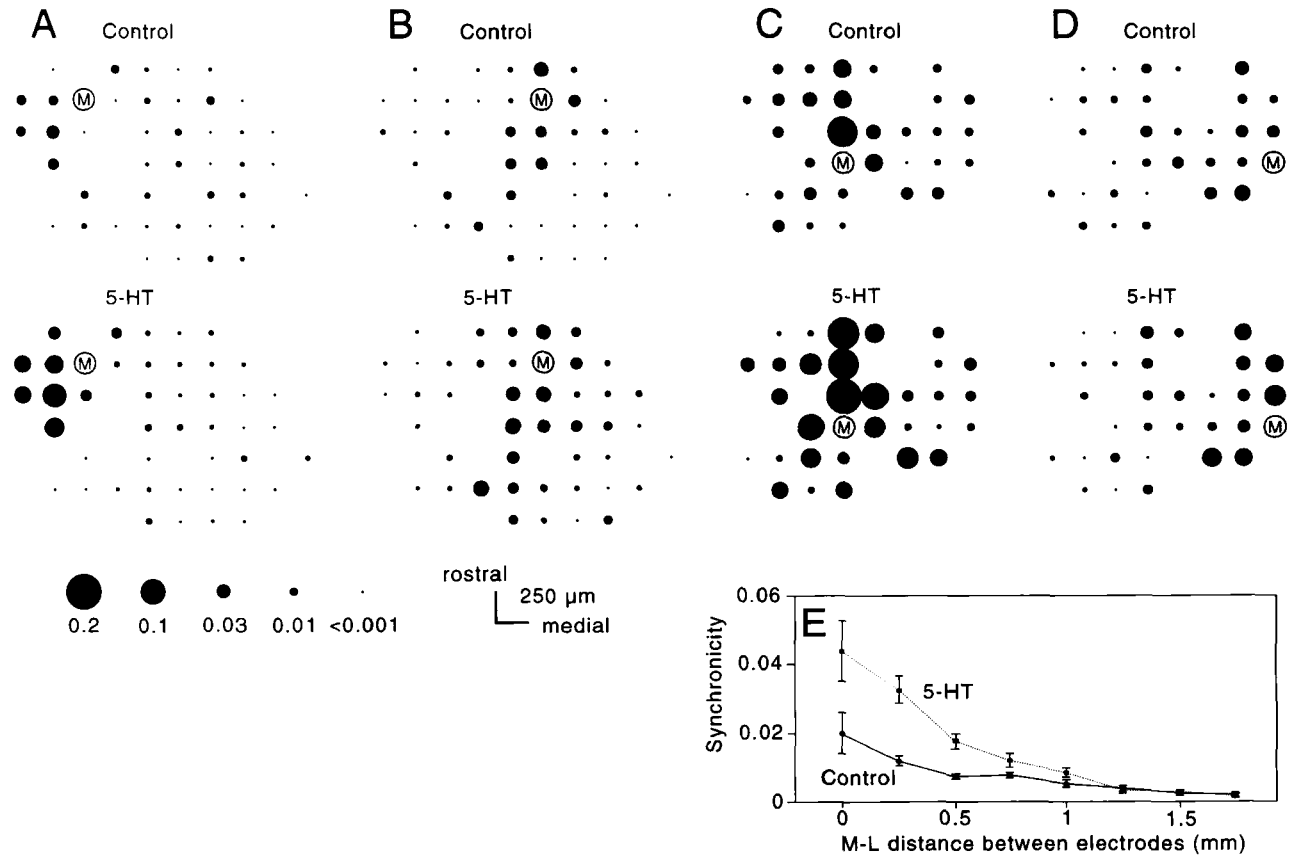

FIG. 4. Effects of 5-HT on spontaneous complex spike synchronicity. (A, B) The spatial distribution of synchronicity for control (top) and 5-HT (bottom) conditions in a recording from 44 crus IIa Purkinje cells. The master cell (M) for cross-correlation analysis is different in A and B. The total number of complex spikes was 37 374 (15.0 min) in A and 60 446 (16.1 min) in B. (C, D) Results of a recording from 32 Purkinje cells in vermal lobule VIb. The total number of complex spikes was 32 962 (24.3 min) in C and 37 468 (21.0 min) in D. (E) Comparison of synchronicity between control and 5-HT conditions for the experiment depicted in C and D. Electrode pairs were sorted according to the mediolateral distance between the cells, and the mean and SE of the synchronicity value (cross-correlation coefficient at time zero) were plotted. Bin width = 2 ms in A–E.

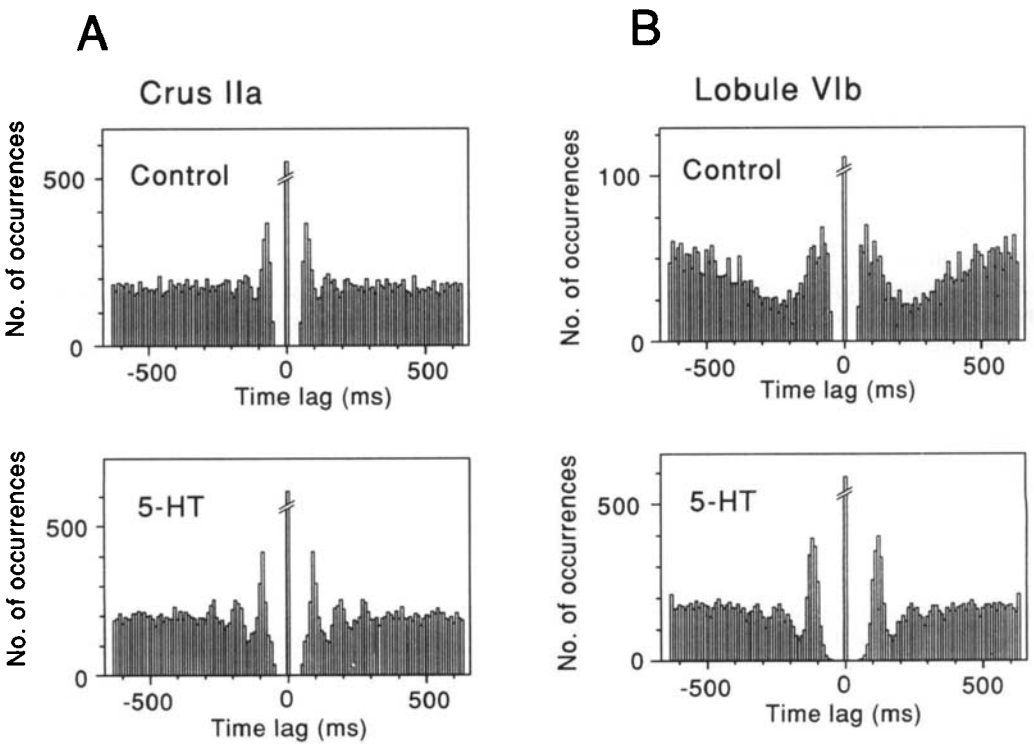

FIG. 5. Effects of 5-HT on the rhythmicity of spontaneous complex spike activity. (A) Autocorrelogram of the complex spike activity from a crus IIa Purkinje cell in control (top) and 5-HT conditions (bottom). The oscillation frequency was 14.3 Hz and the rhythm index 0.063 in control, and 11.1 Hz and 0.14 respectively after injection of 5-HT. The total number of complex spikes was 5297 (control, 27.0 min) and 3850 (5-HT, 16.1 min). (B) Autocorrelogram of complex spike activity in control (top) and 5-HT conditions (bottom) from a Purkinje cell in vermal lobule VIb. The oscillation frequency was 12.5 Hz and the rhythm index 0.027 in control, and 7.7 Hz and 0.16 respectively after the 5-HT injection. The total number of complex spikes was 1836 (control, 24 min) and 2888 (5-HT condition, 21 min).

5-HT effects on the oscillation rhythm

Spontaneous complex spike activity routinely displayed an oscillatory rhythm near 10 Hz, consistent with previous reports (Bell and Kawasaki, 1972; Sasaki *et al.*, 1989). The autocorrelogram of the complex spike activity from a crus IIa Purkinje cell under control conditions (Fig. 5A, top) peaked at 70 and 140 ms, showing that the complex spike activity of this Purkinje cell was rhythmic, with a dominant frequency of 14.3 Hz. Injection of 5-HT into the inferior olive (Fig. 5A, bottom) resulted in a shift of the peaks in the autocorrelogram to 90 and 180 ms and the appearance of a third peak at 270 ms. The shift in the time lag of the peaks shows that the injection of 5-HT slowed the oscillation frequency to 11.1 Hz. In addition to lowering the oscillation frequency, 5-HT also increased the coherence of the rhythm, as indicated by changes in the rhythm index (see Materials and methods). For the Purkinje cell shown in Figure 5A, the rhythm index increased from 0.063 to 0.14. Thus, injection of 5-HT resulted in a 120% increase in the rhythmicity of the complex spike activity. The appearance of a third peak in the autocorrelogram also indicated that 5-HT enhanced the degree of rhythmicity of the complex spike activity (Fig. 5A, bottom). The average complex spike firing rate of this Purkinje cell increased from 3.3 to 4.0 per second.

Similar changes in the oscillation frequency and coherence also occurred in the vermal lobule VIb, in response to olivary injections of 5-HT (Fig. 5B). In this case 5-HT decreased the oscillation frequency of the complex spike activity from 12.5 to 7.7 Hz and increased the rhythm index from 0.027 to 0.16 (Fig. 5B). The average complex spike firing rate of this Purkinje cell increased from 1.3 to 2.3 per second.

5-HT effects in the vermis compared with those in the hemisphere

The results from five crus IIa experiments (148 Purkinje cells) and four lobule VIb experiments (85 Purkinje cells) are summarized in Figure 6. Here, the mean and SE of the average firing rate, synchronicity, oscillation frequency and rhythm index are plotted for all Purkinje cells under control conditions and following the injection of 5-HT into the inferior olive. Note that the oscillation frequency in the ordinate of Figure 6C is labelled opposite in direction from that of other plots. The injections produced significant changes in all four measured parameters of the inferior olive activity in both crus IIa and lobule VIb recordings, with P values ≤ 0.01 (two-sided t-test) in all cases except for the increase in the average firing rate in crus IIa ($P < 0.05$; Fig. 6). Thus the changes described in the preceding paragraphs for single cells, i.e. increase in average firing rate, increase in synchronicity, slowing of the oscillation rhythm and improvement of coherence of the oscillation, were observed in the great majority of Purkinje cells.

The relative effectiveness of 5-HT in modifying complex spike activity for different regions of the cerebellum is also illustrated in Figure 6. Thus, the relative increase in complex spike average firing rate from control was larger for the vermal Purkinje cells (43.9%) than for crus IIa Purkinje cells (21.5%; Fig. 6A). From a synchronicity point of view, the 5-HT injections had a stronger effect on

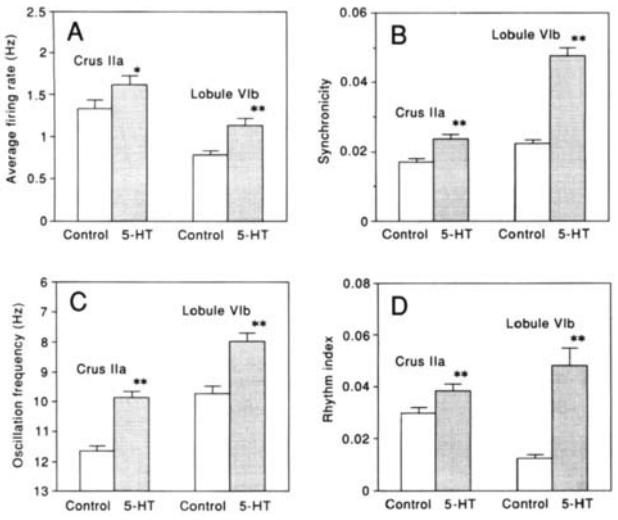

FIG. 6. Comparison of 5-HT effects in the hemisphere and in the vermis. Mean and SE bar of the average firing rate (A), synchronicity (B), oscillation frequency (C) and rhythm index (D) of all Purkinje cells (or all pairs of complex spikes in B) in the control and 5-HT conditions of five crus IIa (left two columns in each set) and four vermal lobule VIb (right two columns in each set) experiments. Recordings were obtained from a total of 148 crus IIa and 85 vermal Purkinje cell. The total number of Purkinje cell pairs was 2415 and 897 respectively. The total number of complex spikes in the control condition was 115 714 and 58 134, and in the 5-HT condition it was 58 134 and 87 304, for the hemispheric and vermal experiments respectively. In C, oscillation frequency was measured in 129 (87%), 131 (89%), 53 (64%) and 61 (75%) of Purkinje cells in crus IIa control, crus IIa 5-HT, lobule VIb control and lobule VIb 5-HT conditions respectively. Single ($P < 0.05$) and double ($P < 0.01$) asterisks indicate statistical significance by two-sided t-test compared to the control level.

vermal (110.8%) than on crus IIa complex spike activity (37.8%; Fig. 6B). Similarly, the magnitude of the increase in the rhythm index was significantly larger for the vermal cells (288 versus 20%; Fig. 6D). Only with regard to the lowering of the oscillation frequency did 5-HT have a similar degree of effect on vermal (–15%) and on crus IIa Purkinje cells (–18%; Fig. 6C).

Time course of 5-HT injections

The time courses of the effects of 5-HT were investigated by determining the average firing rate, oscillation frequency and rhythm index from all cells and the synchronicity from all cell pairs over seven successive 15 min periods surrounding the 5-HT injections in one experiment (Fig. 7). The characteristics of the complex spike activity remained essentially unchanged by the insertion of the injection pipette into the inferior olive, as indicated by the first two points in each graph of Figure 7. However, injection of 5-HT led to dramatic changes in the average firing rate, synchronicity, oscillation frequency and oscillation rhythm index (Fig. 7A, B, C and D respectively). The effects of the 5-HT injection decayed over 75 min by at least 85%, and a second injection of 5-HT had similar effects to the first. Throughout the course of this recording, average firing rate, synchronicity, oscillation frequency and oscillation rhythm index of complex spike activity showed roughly parallel changes. Effects on synchronicity may be somewhat longer lasting, as it was still high 25 min after the injection (Fig. 7B).

Dose-dependency of 5-HT effects

Dose–response curves were obtained for the effect of 5-HT on the average firing rate and synchronicity of complex spike activity. Successive 1 µl injections of increasingly concentrated 5-HT solutions were made into the inferior olive every 15–20 min in two separate experiments. The mean firing rate from all cells and the mean degree of synchrony from all cell pairs following each injection is shown in Figure 8 for the two experiments. No significant increases in average firing rate were observed with concentrations of 10 µM or less. Indeed, while the increase in synchronicity seen with 0.1 and 1 µM concentrations was very slight, the 10 µM solution produced a clear increase. The slopes of the dose–response curves were steepest between 10 and 100 µM, further increases in 5-HT concentration producing smaller increases in both the average complex spike firing rate and synchrony. Thus, the 1 mM concentration used in the other sections of this study produced near-maximal effects. The sigmoidal dose–response curves for the average firing rate and synchronicity were nearly identical and had half-effective doses between 10 and 100 µM.

In *in vitro* preparations, 10–300 µM 5-HT was effective in producing changes in the activity of olivary neurons (Llinás and Yarom, 1986). Similar results were obtained in other cells, such as nucleus reticularis thalami neurons (McCormick and Wang, 1991), facial nucleus neurons (Larkman *et al.*, 1989) and spinal motoneurons (Elliott and Wallis, 1992). Although a similar range of concentrations was effective, a higher concentration (1 mM) of 5-HT could elicit a further effect in the present *in vivo* experiments. This may have been due to a lower effective 5-HT concentration at the receptor site in the *in vivo* experiments because of the presence of enzymatic deactivation and reuptake mechanisms in the inferior olive and because of the dilution of the 5-HT solution as it diffuses away from the injection site.

Effects of 5-HT agonists and antagonists on complex spike activity

Several different types of 5-HT receptors have been identified (Zifa and Fillion, 1992). To identify the 5-HT receptor types in the inferior olive, specific serotoninergic agonists or antagonists were injected into the nucleus. The concentrations of the agonists used here were approximately equivalent to 1 mM of 5-HT (see Materials and methods). After recording the complex spike activity following an agonist injection, 5-HT itself was injected into the inferior olive through a second barrel in the pipette. The average firing rate from all cells and synchronicity from all pairs of cells was calculated for the 15 min period following each injection (Fig. 9). These experiments were carried out in eight animals, each drug being tested in one or two animals. Injections of solutions containing the 5-HT_{1A} agonist 8-OH-DPAT (Zifa and Fillion, 1992) or the 5-HT_{2C} and 5-HT_{1B} agonist m-CPP (Sills *et al.*, 1984; Conn and Sanders-Bush, 1987) did not elicit any change in complex spike firing rate or synchronicity (Fig. 9A, B). However, injections of tryptamine, a 5-HT_{2C} and 5-HT_{2A} receptor agonist (Engel *et al.*, 1986; Zifa and Fillion, 1992), resulted in changes in complex spike activity comparable to those produced by 5-HT (Fig. 9C). An injection of α-methyl-5-HT, a 5-HT_{2A} receptor agonist and weaker 5-HT_1 receptor agonist (Engel *et al.*, 1986; Ismaiel *et al.*, 1990), also resulted in significant increases in complex spike activity (Fig. 9D). In the case of α-methyl-5-HT, the subsequent injection of 5-HT produced a greater increase in complex spike activity (Fig. 9D). This was probably because a ten-fold less concentrated solution of α-methyl-5-HT (0.1 mM) was used here, although the potency of α-methyl-5-HT for the 5-HT_{2A} receptor was only about 1.1 times greater than that of 5-HT given in a recent report (Ismaiel *et al.*, 1990). Olivary injections of m-CPBG, a 5-HT_3 agonist (Kilpatrick *et al.*, 1990; Maricq *et al.*, 1991), produced a slight increase in complex spike synchronicity without any significant

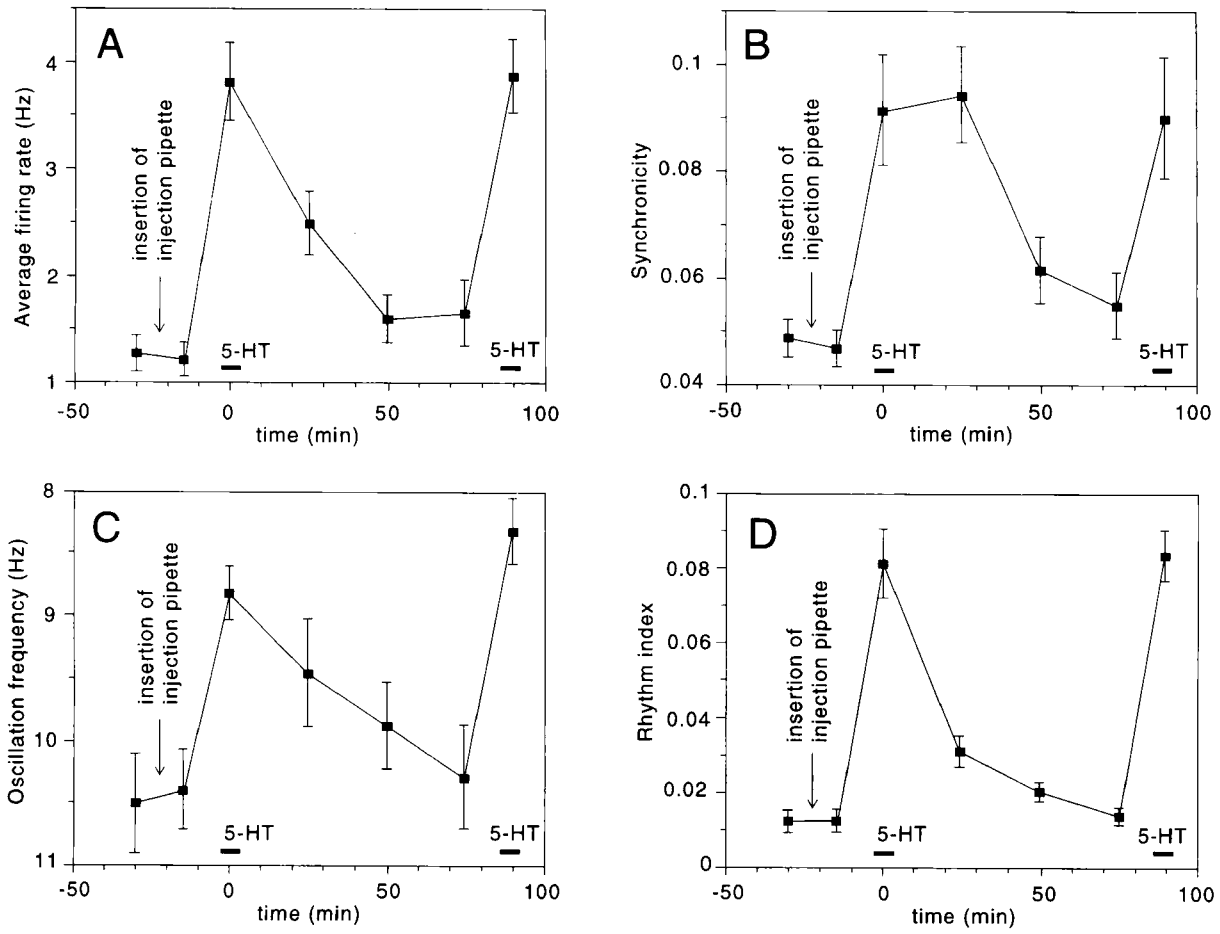

FIG. 7. Time course of 5-HT action. In this animal, complex spikes were analysed from seven 15-min periods: (1) before and (2) after inserting the injection pipette, (3) starting during the first 5-HT injection, (4) 25 min, (5) 50 min and (6) 75 min after the first injection, and (7) starting during the second injection of 5-HT. The mean and SE of the average firing rate of all cells (A), synchronicity of all pairs (B), frequency of the oscillation rhythm (C) and the rhythm index of the oscillation (D) are shown. Recordings were made from vermal lobule VIb. Number of Purkinje cells, 13.

changes in average firing rate (Fig. 9E). A ten-fold lower concentration of m-CPBG (10 μm) produced no significant changes (not shown). The present results therefore suggest that the effects of 5-HT on olivary neurons are mediated primarily by the 5-HT_{2A} receptor, although the 5-HT_3 receptor may participate to a small extent.

To examine further the hypothesis that the 5-HT_{2A} receptor mediates the response of olivary neurons to 5-HT, we attempted to block the action of 5-HT with LY53857, a 5-HT_{2A} receptor antagonist (Cohen *et al.*, 1983). In three experiments LY53857 was injected into the inferior olive, followed by a solution containing 5-HT and LY53857. The injection of LY53857 alone or in combination with 5-HT produced no significant changes in complex spike average firing rate, synchronicity or oscillation rhythm (Fig. 10). However, since LY53857 is both an antagonist of both 5-HT_{2A} and 5-HT_{2C} (Zifa and Fillion, 1992), we repeated the antagonist experiment substituting spiperone for LY53857. Spiperone is a 5-HT_{2A} and 5-HT_{1A} antagonist, but its affinity to 5-HT_{2C} receptors is low (Pazos *et al.*, 1985; Elliott and Wallis, 1992). Injection of spiperone alone or in combination with 5-HT did not produce any significant changes in complex spike activity [average firing rate (Hz) and synchronicity values, and conditions were as follows: 1.64 ± 0.20 and 0.091 ± 0.013 for control; 1.64 ± 0.19 and 0.093 ± 0.013 for spiperone; and 1.52 ± 0.15 and 0.090 ± 0.013 for spiperone plus 5-HT; n = 15 cells and 103 cell pairs, 1 animal]. The combined results of the

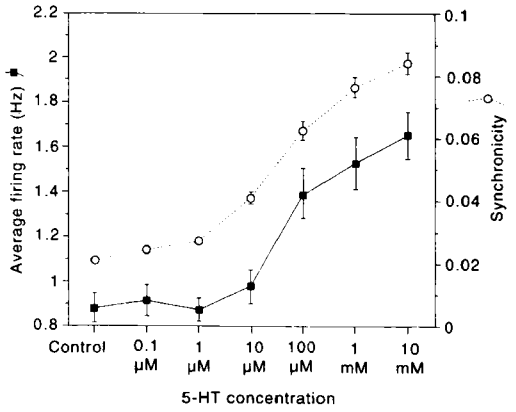

FIG. 8. Dose–response relationship of 5-HT effects. In two animals, solutions of increasing 5-HT concentrations were injected sequentially. Injection amount was 1 μl for all concentrations. The mean and SE of the average firing rate (filled squares) and synchronicity (open circles) for all cells and for all cell pairs obtained in two experiments are shown. Recordings were made from vermal lobule VIb. Number of Purkinje cells, 45 (18 + 27).

antagonist injections further suggest that the changes in complex spike activity were mediated by the 5-HT_{2A} receptor. The fact that the antagonist did not cause any significant changes in complex spike

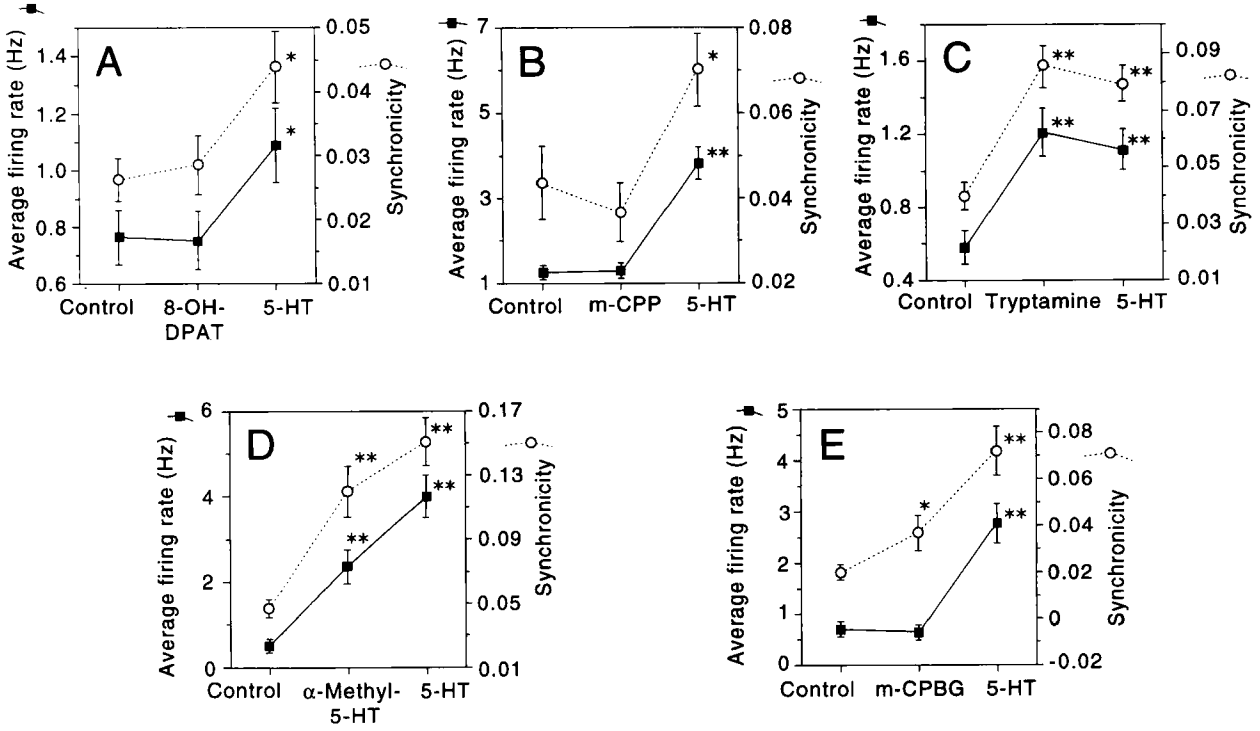

FIG. 9. Effects of different 5-HT agonists, 8-OH-DPAT (A), m-CPP (B), tryptamine (C), α-methyl-5-HT (D) and m-CPBG (E), on the average firing rate and synchronicity of complex spikes. Each figure was generated from a single animal. 5-HT was injected after the agonist injection from the same multibarrel injection pipette. The average and SE of the average firing rate of all cells (filled squares) and synchronicity of all pairs (open circles) are shown. Number of Purkinje cells: A, 19; B, 12; C, 17; D, 15; E, 25. Single ($P < 0.05$) and double ($P < 0.01$) asterisks indicate statistical significance by two-sided t-test compared to the control level.

activity may be interpreted in two ways. Either basal levels of olivary 5-HT are low in ketamine-anaesthetized rats, or some effects of 5-HT are long-lasting and are therefore not blocked initially by the antagonist injection.

5-HT and harmaline

The effects of olivary injections of 5-HT on complex spike activity reported here were very similar to those produced by systemic harmaline administration (de Montigny and Lamarre, 1973; Llinás and Volkind, 1973; Llinás and Sasaki, 1989; Sasaki *et al.*, 1989). Several experiments were performed to determine whether the effects of 5-HT and harmaline are mediated by the same pathway.

In the first set of experiments, the two drugs were injected within a 15 min interval of each other to determine if their action was additive. Whether 5-HT was injected first and harmaline second (Fig. 11A) or vice versa (Fig. 11B), the effects of two substances on the oscillatory complex spike activity were very similar. The oscillation frequency and rhythm index changed as follows: from 11.1 Hz and 0.012 (control) to 9.1 Hz and 0.09 (5-HT), and then to 6.7 Hz and 0.22 (harmaline) (Fig. 11A); and from 9.1 Hz and 0.017 (control) to 6.7 Hz and 0.15 (harmaline), and then to 6.3 Hz and 0.25 (5-HT) (Fig. 11B). Thus, the second injections produced a further enhancement of the coherence of the oscillation rhythm, suggesting that the effects of 5-HT and harmaline are indeed additive.

In a second set of experiments, a solution of LY53857 and harmaline was injected to see if the effect of harmaline was blocked by this specific 5-HT_{2A} antagonist. Although the action of 5-HT on olivary neurons was completely blocked by LY53857, when it was added to harmaline no blockade was observed on the average firing rate (Fig. 10C), on synchronicity (Fig. 10A and C) or on oscillation rhythm of complex spikes (Fig. 10B). These results demonstrate that the mechanism through which harmaline exerts its effects is functionally independent of the serotoninergic system.

The effects of olivary injections of LY53857 ($n = 4$ experiments), LY53857 plus 5-HT ($n = 3$), LY53857 plus harmaline ($n = 4$), 5-HT alone ($n = 4$) and harmaline alone ($n = 2$) on the average firing rate and synchronicity are summarized and compared in Figure 12. Because the data were grouped from several different animals, increases in the indices of complex spike activity in the experimental conditions, compared with the control values, were calculated for each Purkinje cell or each pair of Purkinje cells and normalized. Thus, a value of 1 indicates no change from the control value (dotted line in Fig. 12A and B). The effects of 5-HT on olivary neurons were almost completely blocked by LY53857, as indicated by the absence of changes from control levels. However, the increases produced by the injections of harmaline plus LY53857 were only slightly less than those produced by injections of harmaline alone.

Discussion

Functional significance of 5-HT in the inferior olive

Although serotoninergic neurons are located only in restricted regions of the brainstem, mainly the raphe nuclei, their axon terminals are widely distributed throughout the central nervous system. Pharmacological manipulations as well as behavioural and clinical studies have implicated the serotoninergic system in various functions such as sleep, learning, pain sensation, aggressiveness, sexual behaviour,

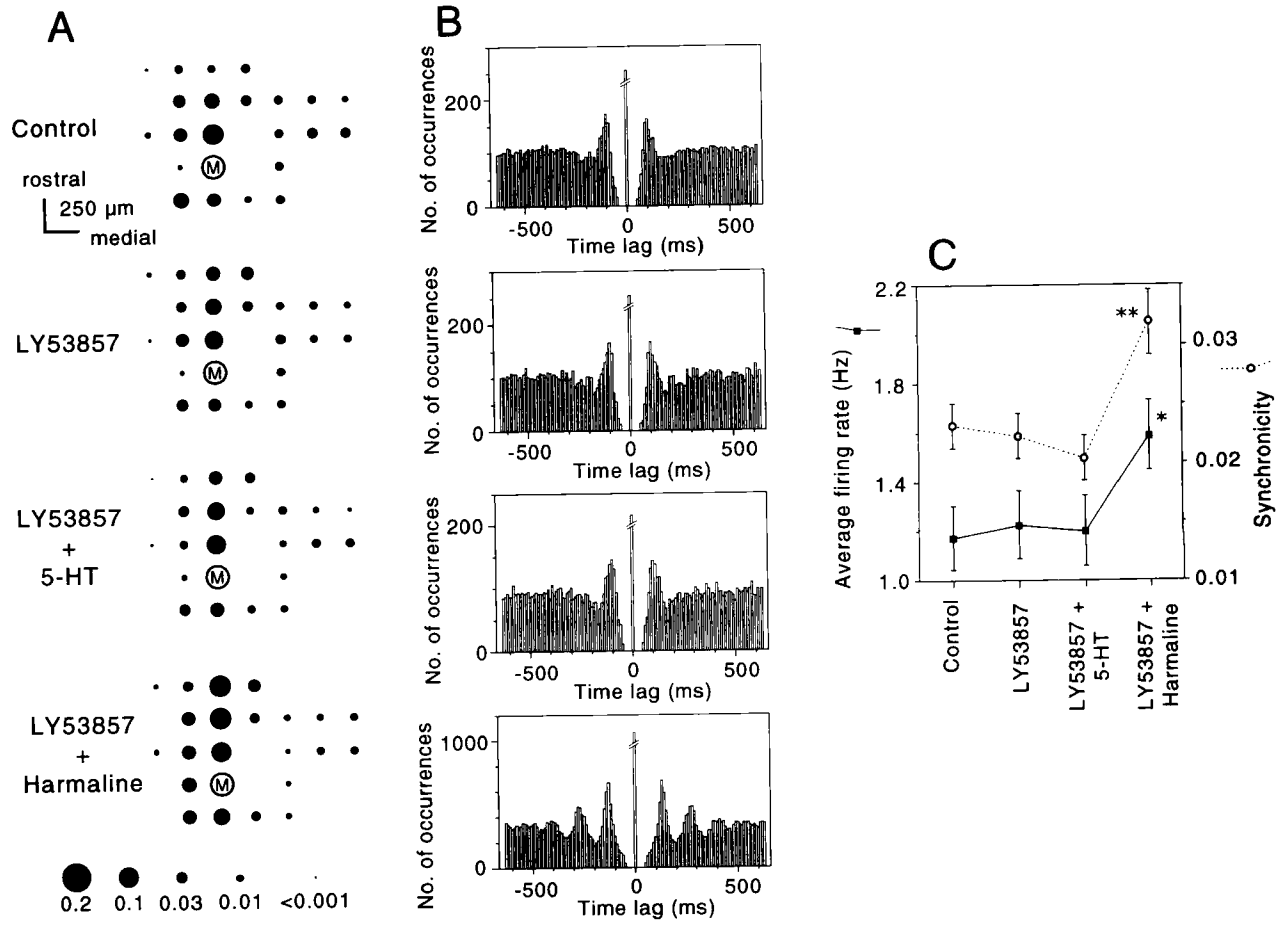

FIG. 10. Effects of 5-HT_2 antagonist LY53857 on the action of 5-HT and harmaline. Spatial distribution of synchronicity of complex spikes (A) and autocorrelogram of complex spike activity from the same Purkinje cell (B) in control period, after LY53857 injection, after LY53857 + 5-HT injection and after LY53857 + harmaline injection are shown. (C) The mean and SE of the average firing rate of all cells (filled squares) and synchronicity of all pairs (open circles) were plotted for these four periods from the same experiment. Number of Purkinje cells, 20. The recording was from vermal lobule VIb. The ordinate of B represents the number of occurrences of complex spikes. Single ($P < 0.05$) and double ($P < 0.01$) asterisks in C indicate statistical significance by two-sided t-test compared to the control level.

feeding, motor activity, neuroendocrine regulation and biological rhythms, and in psychiatric and neurodegenerative disorders (Spoont, 1992; Zifa and Fillion, 1992). Recently, recordings from 5-HT neurons in the raphe nuclei have been obtained in awake cats (Trulsion and Jacobs, 1979; Jacobs and Fornal, 1993; Veasey *et al.*, 1993) and rats (Kayama *et al.*, 1992). The activity of these neurons was not affected by a variety of external stimuli, but instead was mostly related to the motor behaviour of the animal. On this basis it has been proposed that the primary function of these 5-HT neurons is to facilitate motor output by exciting central pattern generators and alpha motor neurons (Jacobs and Fornal, 1993).

The serotoninergic projection to the inferior olive arises from the nucleus paragigantocellularis in the medullar reticular formation (Bishop and Ho, 1986) and the nucleus raphe obscurus and pallidus (Compoint and Buisseret-Delmas, 1988). Although the activity of 5-HT neurons in the nucleus paragigantocellularis has yet to be investigated, the activity of neurons in the raphe pallidus and obscurus has been reported to fire regularly near 3 Hz when the animal is awake but not moving (Trulson and Jacobs 1979; Kayama *et al.* 1992; Jacobs and Fornal, 1993; Veasey *et al.*, 1993). During treadmill walking, their firing frequency is directly related to the speed of locomotion (Jacobs and Formal, 1993; Veasey *et al.* 1993). Therefore, a reasonable inference is that during movements the activation of the serotoninergic afferents to the inferior olive results in an enhancement of the average firing rate, synchronicity, and rhythmicity of complex spike activity.

This inference strongly suggests that the synchronized oscillatory activity of the inferior olivary has a role in the proper execution of movements. It has been proposed that the synchronized oscillatory standing waves generated by inferior olivary neurons may serve as a timing signal for other motor systems to use to coordinate their output (Llinás, 1981, 1988; Llinás and Welsh, 1993). From this point of view, 5-HT enhancement of the coherence of the synchronous oscillatory activity can serve as a decisive modulator of the motor timing properties of the inferior olive.

Indeed, there are behavioural responses that may be explained by the activation of serotoninergic afferents to the inferior olive. For example, systemic injection of quipazine, a 5-HT_{2A} agonist, can produce tremor and activate rhythmic firing of inferior olive neurons at up to 8–10 Hz in young rabbits (Barragan *et al.*, 1985), suggesting that activation of olivary neurons as demonstrated in the present study would be able to modulate motor output. A similar tremorogenic

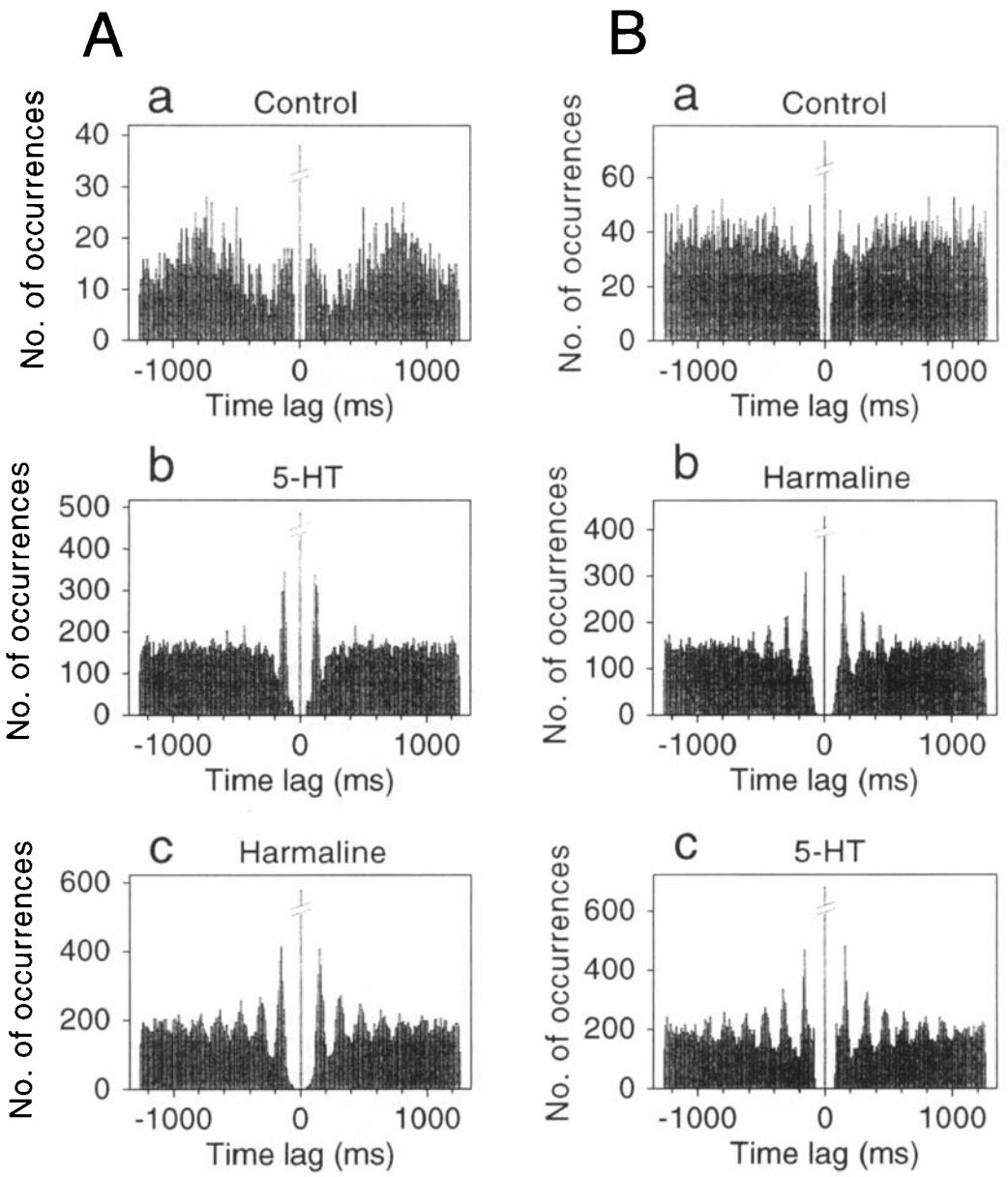

FIG. 11. Autocorrelograms of spontaneous complex spike activity from single vermal lobule VIb Purkinje cells from two multiple electrode experiments. (A) After recording the control condition (a), 5-HT was injected (b) followed by harmaline (c). (B) After recording in the control condition (a), harmaline was injected (b) followed by 5-HT (c). The ordinate represents the number of occurrences of complex spikes. The total number of complex spikes was 1183 (14.7 min), 3179 (10.6 min), 4020 (15.2 min), 1192 (19.2 min), 4115 (21.1 min) and 4024 (14.9 min) in Aa, Ab, Ac, Ba, Bb and Bc respectively.

effect of systemic quipazine injection has also been reported in young rats (Michela *et al.*, 1990). Furthermore, an impairment of the 5-HT system in the cerebellum as well as in the inferior olive has been hypothesized in patients with cerebellar ataxia, given that the administration of 5-hydroxytryptophan, a precursor of 5-HT, can induce a significant regression of the symptoms (Trouillas, 1993).

Excitatory effects of 5-HT on the inferior olivary neurons

In vitro studies have demonstrated that application of 5-HT enhances the intrinsic oscillations and decreases the K^+ conductance of olivary neurons (Llinás and Yarom, 1986). The increased complex spike firing rate following olivary injections of 5-HT observed here is consistent with an increase in the excitability of olivary neurons that should result from a reduction in K^+ conductance. Furthermore, the increase in complex spike rhythmicity produced by olivary injections of 5-HT is consistent with the ability of 5-HT to increase the amplitude of the membrane potential oscillations in olivary neurons *in vitro* (Llinás and Yarom, 1986). The observed decrease in oscillatory frequency of complex spike activity following 5-HT injections is to be expected given the decrease in K^+ conductance, which, by increasing the input resistance, would increase the Ca^{2+} influx and elongate the Ca^{2+}-dependent afterhyperpolarization.

In addition to extending the *in vitro* results to an *in vivo* preparation, this study has provided another aspect of the role of 5-HT in complex

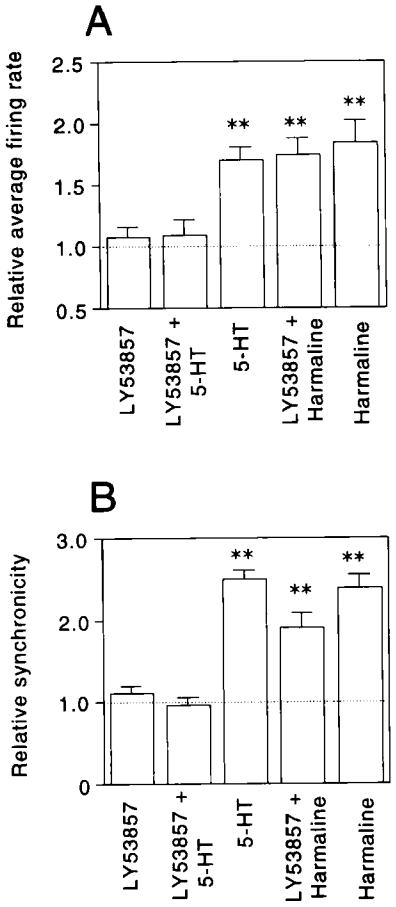

FIG. 12. Comparison of the effects of injections of LY53857, LY53857 + 5-HT, 5-HT alone, LY53857 + harmaline, and harmaline alone. The average firing rate (A) of each cell and the synchrony value (B) of each cell pair in each of the conditions was normalized against the value in the control condition, which was taken as one, then the mean and SE of the relative changes were calculated. Data were from three experiments using successive injections of LY53857, LY53857 + 5-HT and LY53857 + harmaline, one experiment using successive injections of LY53857 and LY53857 + harmaline, four experiments using 5-HT injection and two experiments using harmaline injection. The total number of cells was 72 for LY53857 and LY53857 + harmaline, 58 for LY53857 + 5-HT, 85 for 5-HT and 33 for harmaline. All recordings were from vermal lobule VIb. Double asterisks indicate statistical significance ($P < 0.01$) by two-sided t-test compared to the control level.

spike activity. The synchronous firing of olivary neurons can be observed with the multiple-electrode technique, as simultaneous complex spike activity results from electrotonic coupling between adjacent olivary neurons by gap junctions between dendritic spines (Llinás *et al.*, 1974; Sotelo *et al.*, 1974; Llinás and Yarom, 1981a). GABAergic terminals that synapse with the spines adjacent to the gap junctions (Nelson *et al.*, 1984; Sotelo *et al.*, 1986; de Zeeuw *et al.*, 1989) have been demonstrated to modulate the effectiveness of electrotonic coupling (Llinás and Sasaki, 1989; Lang *et al.*, 1989, 1990). However, action potentials in olivary neurons are often triggered by the somatic low-threshold Ca^{2+} current (Llinás and Yarom, 1986), which suggests that a significant number of electrotonic currents must reach the cell body to initiate the simultaneous firing of a second neuron. Thus, membrane conductances in the extraglomerular proximal dendrite, to which most 5-HT terminals synapse (King *et al.*, 1984), should also influence the degree of complex spike synchronicity. Also, a small percentage (7%) of the 5-HT terminals synapse with dendritic spines (King *et al.*, 1984), most of which are presumably located within olivary glomeruli (de Zeeuw *et al.*, 1990). Our results are consistent with these anatomical results and argue for a secondary role for 5-HT in modulating complex spike synchronicity. That is, injections of 5-HT led to an increase in complex spike synchrony, but did not alter the basic rostrocaudal organization of complex spike synchronicity. This is in contrast to the dramatic reorganization of the spatial pattern of complex spike synchronicity following administration of GABAergic antagonists (Lang *et al.*, 1989; Llinás and Sasaki, 1989).

Effects of 5-HT on olivary neurons are mediated primarily by the 5-HT$_{2A}$ receptor

The effect of 5-HT mediated through 5-HT$_{2A}$ receptor has been often demonstrated to be, with few exceptions, excitatory in many nervous system neurons. A decrease in K^+ conductance mediated by the 5-HT$_{2A}$ receptor has been reported in facial motoneurons (Larkman *et al.*, 1989), nucleus reticularis thalami neurons (McCormic and Wang, 1991) and in spinal motoneurons (Elliott and Wallis, 1992). Our results suggest that the excitatory effects of 5-HT on olivary neurons are likewise mediated by the 5-HT$_{2A}$ receptor. This is consistent with the report of activation of inferior olivary neurons by systemic application of a 5-HT$_{2A}$ agonist (Barragan *et al.*, 1985). While G proteins are activated by the 5-HT$_{2A}$ receptor, the 5-HT$_3$ receptor is a ligand-gated non-selective cation channel that causes depolarizing responses (Maricq *et al.*, 1991). Although the 5-HT$_3$ receptor has been identified in the inferior olive by autoradiography, its density in the inferior olive is much lower than in other 5-HT$_3$-specific areas in the brainstem (Barnes *et al.*, 1990). Similarly, the effects caused by 5-HT$_3$ agonists were much weaker than those caused by 5-HT and 5-HT$_{2A}$ agonists in the present study.

Harmaline and 5-HT

Harmaline, injected systemically, causes a continuous body tremor by exciting olivary neurons (de Montigny and Lamarre, 1973; Llinás and Volkind, 1973). This drug acts by enhancing the rhythmicity and synchronicity of the inferior olivary neurons (Llinás and Volkind, 1973; Llinás and Sasaki, 1989). At the cellular electrophysiological level, it has been demonstrated to have several direct effects on these cells (Llinás and Yarom, 1986; Yarom and Llinás, 1987). The effect of harmaline has been shown to be closely related to the serotoninergic afferent to the inferior olive (Sjölund *et al.*, 1977). At the pharmacological level, however, the effects of harmaline are not fully understood. Harmaline has been shown to be a type-A monoamine oxidase inhibitor (A-MAOI) (Burkard and Kettler, 1977) and an inhibitor of Na^+–HCO_3^- cotransport (Newman, 1991). In addition, it has been found to bind to the benzodiazepine, opiate, muscarinic, dopamine (type 2), 5-HT (type 1), GABA and glycine receptors (Pranzatelli and Snodgrass, 1987).

While the A-MAOI action of harmaline would increase the olivary 5-HT levels, and 5-HT and harmaline injections had similar effects on inferior olivary neurons, the failure of 5-HT$_{2A}$ antagonists to block the effects of harmaline in the present study suggests that the two drugs act independently. Furthermore, *in vitro* electrophysiological studies have shown that 5-HT reduces the K^+ conductance of olivary neurons, generating a depolarization of their membrane potential, while harmaline causes a hyperpolarization, shifts the inactivation curve of the somal low-threshold calcium current towards less negative values, and blocks the time-dependent component of anomalous-rectification K^+ current (Llinás and Yarom, 1986; Yarom and Llinás,

1987). Some behavioural and biochemical studies have also indicated that the excitatory effect of harmaline on inferior olive neurons and the consequent tremorogenic effect and the increase of cyclic guanosine monophosphate level in the cerebellum are not parallel to type A monoamine oxidase (A-MAO) inhibition when harmaline was compared with more potent or specific A-MAO inhibitors (Burkard and Kettler, 1977; Pranzatelli and Snodgrass, 1987). In conclusion, it appears that in the inferior olive harmaline acts neither by increasing 5-HT levels nor by binding to the same receptor as 5-HT.

It is nevertheless quite significant that both 5-HT and harmaline produce similar changes in inferior olivary neuron activity and that the effects of the two substances are mutually reinforcing (Fig. 11). A cooperative effect of harmaline and 5-HT (its precursor, 5-hydroxytryptophan, was used in this case) in producing tremor and other abnormal movements has already been described in a behavioural study (Pranzatelli and Snodgrass, 1987). The cooperation of 5-HT and harmaline presumably takes place at the level of the inferior olivary neuron. However, further study would be necessary to elucidate the mechanisms by which 5-HT, harmaline, GABA and other potential olivary neurotransmitters could interact to modulate the activity of olivary neurons. Understanding these interactions is a necessary step towards the further definition of inferior olive function in motor coordination.

Acknowledgements

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Abbreviations

A-MAO	type A monoamine oxidase
A-MAOI	type A monoamine oxidase inhibitor
GABA	γ-aminobutyric acid
5-HT	5-hydroxytryptamine
5-HT_2	5-hydroxytryptamine receptor type 2
m-CPBG	1-(*m*-chlorophenyl)-biguanide hydrochloride
α-methyl-5-HT	α-methyl-5-hydroxytryptamine maleate
m-CPP	1-(3-chlorophenyl)piperazine dihydrochloride
8-OH-DPAT	(±)-8-hydroxydipropylaminotetralin hydrobromide

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