Recently we used this approach to examine the sensory coding
erate, increases or decreases in response amplitude, and changes in re-
sponse waveform. We used a principal component analysis (PCA) to
to determine how they contributed to the response waveform changes caused by serotonin perturbation. Such changes could be explained by new or different response components that might indicate a modification in the data processing or by a different weighting of existing components that might indicate a modification of synaptic weighting. The results were consistent with the second alternative. We found that the same underlying response components could account for both control responses and those altered by serotonin perturbations. The observed changes in waveform could be entirely accounted for by a re-weighting of response components. In particular, the changes observed after raphe stimulation could be accounted for by selective changes in the weighting of the first principal component (PC) with only minor changes of the weighting of the second PC. Because these response components were shown previously to correlate with the limb axis orientation and length trajectories respectively, the finding is consistent with the idea that limb axis length and orientation information are processed separately within the spinal circuitry.

INTRODUCTION

An ability to identify neural correlates of movement parameters can provide one key to understanding movement control. Recently we used this approach to examine the sensory coding of limb movement parameters by dorsal spinocerebellar tract (DSCT) neurons. These neurons receive converging sensory information from muscle, skin, and joint receptors, and their output is a result of spinal sensory processing that is directed to areas of the cerebellum involved in the control of posture and gait (Bloedel and Courville 1981; Osborn and Poppele 1992, 1993). We concluded that DSCT activity can be adequately accounted for by a linear relationship to limb axis kinematics during passive postures or passive limb movements (Bosco et al. 1996) and that the activity relates to global rather than to local parameters of hindlimb posture and movement. By examining the responses during continuous limb movements we also showed that specific response components related to length and orientation kinematics are expressed separately and independently in the DSCT activity (Poppele et al. 2002). This global representation accounts for most of the passive input-output behavior of the DSCT, and it seems consistent with several behavioral studies suggesting that limb axis length and orientation might represent independent control variables for posture and gait (Bianchi et al. 1998; Borghese et al. 1996; Lacquaniti et al. 1990; Maioli and Poppele 1991).

The neural correlates of limb axis length and orientation expressed by spinal neurons could result from biomechanical factors that produce a strong correlation of sensory afferent activity throughout the limb (Bosco et al. 1996) and/or from integrative mechanisms within the spinal circuitry. We examined this further here by determining whether these neural representations can be altered by acting on the central modulation of the spinal circuitry through manipulations of the serotonergic system.

Serotonergic terminals from the brain stem raphe nuclei comprise another prominent input to the DSCT circuitry (Jankowska et al. 1995, 1997b; Maxwell and Jankowska, 1996; Pearson et al. 2000). These terminals appear to synapse directly on DSCT neurons, and they exert a modulatory influence on the various sensory inputs to the DSCT (Jankowska et al. 1995, 1997a; Pearson et al. 2000). In one recent series of studies, Jankowska and colleagues (Jankowska et al. 1997a), applied serotonergic agents to DSCT neurons iontophoretically and found differential effects on their firing probability in response to electrical activation of muscle and cutaneous afferents. They also noted a differential serotonergic control of separate subgroups of spinocerebellar neurons located in the Clarke’s col-
umn and in the dorsal horn (Matsushita et al. 1979). The detailed characterization of the action of serotonin provided by these studies showed a number of specific actions of serotonin, but it did not clarify the role of serotonergic modulation in the processing of sensory information related to limb movements, for example. Their study did emphasize, however, that serotonin appears to have a wide-spread modulatory role in spinal sensory systems that includes proprioception as well as somatosensory and pain systems (Jankowska et al. 1998).

We investigated the possible role of serotonergic modulation of proprioception by examining its effects on the DSCT representations of whole-limb kinematics. Specifically, we evaluated its influence on the processing of limb axis length and orientation information by quantifying changes in DSCT responses to passive limb movements after both functional and pharmacological manipulations of the serotonergic system. Both procedures were employed as methods to perturb the system and thereby act as probes. We analyzed the results using principal component analysis (PCA), which is a powerful statistical technique for extracting common, independent components from the responses of a population of neurons. We used this approach to distinguish between two possibilities. One is that the modulation acts directly on the DSCT cells, and the other is that it acts presynaptically on inputs to the DSCT cells.

If serotonin acts postsynaptically by changing the way in which the DSCT cells respond to their inputs, we might expect the PCA to extract different response components (principal components or PCs) than those observed under control conditions. If on the other hand, serotonin acts presynaptically, the PCA might not extract different components because the serotonergic modulation may simply modify the weighting of a given set of components. But serotonin might also affect presynaptic processing, in which case there might also be different components. This case might be difficult to distinguish from the postsynaptic case, but because it is likely that the changes would be selective on only some inputs, not all the components would be affected.

We found that the responses to robot-controlled limb movements can be modified by serotonin, but the components of the responses were not. This result implies that serotonin modifies the weightings of inputs to the DSCT cells. In the accompanying paper (Bosco and Poppele 2003), we compare this result with the effect of perturbing the biomechanical properties of the limb to change the pattern of sensory input during the same limb movement.

**METHODS**

Experiments were done with eight adult, barbiturate-anesthetized cats [pentobarbital sodium (Nembutal), Abbott Pharmaceuticals; 35 mg/kg ip supplemented by intravenous administration to maintain a surgical level of anesthesia throughout the experiment]. The animals were placed in a stereotaxic frame with the pelvis fixed in position by pins inserted in the iliac crests. The left hindfoot was attached to a small platform connected to a computer controlled robot arm (Microbot Alphal II+, Questech, Farmington Hills, MI) that moved the limb passively through preprogrammed foot trajectories (see also Poppele et al. 2002).

The animals rested on a sling lined with a thermocautically controlled, heated water jacket, and body core temperature was monitored continuously. We also monitored end-tidal CO2 and breathing rate (Capnometer Mod 2200, Traverse Medical Monitors). The blood pressure and heart rate were monitored from a catheter placed in the femoral artery contralateral to the side of the recording and limb movements. All readings were automatically logged with a time stamp approximately every 1–5 min throughout the experiment.

**Kinematic measurements**

We recorded limb kinematics using a video camera (Javelin Model 7242 CCD camera; 60 frames/s), and we digitized the result off-line with a motion analysis system (Motion Analysis, Santa Rosa, CA, model VP110) (see also Bosco et al. 2000). In summary, we placed reflective markers (~6 mm diam) on the skin over the hip, knee, ankle, and lateral metatarsal-phalangeal joint of the foot. We corrected the digitized positions of the markers in an image plane approximately parallel to the plane formed by the hip, knee, and ankle markers for skin slippage at the knee and also for out-of-plane movements using an algorithm described in detail in Eian and Poppele (2002) and in Bosco et al. (2000). Finally, we resampled the kinematic data at 30/s to correspond to the 33.3-ms bin width of the neural data (see following text).

We represented the limb kinematics in the coordinates of the limb axis, that is, the segment joining the hip joint and the foot. The limb axis defines the position of the foot in polar coordinates by its orientation angle O, measured clockwise from the horizontal to the axis, and its length L, in centimeters (Fig. 1A). The rationale for using this type of representation was the robust correlation we have found between DSCT activity and the waveforms of the limb axis length and orientation trajectories (Poppele et al. 2002).

The kinematics of the passive movements we used are illustrated in Fig. 1, B and C. The robot moved the limb through a basic footprint modeled on a step cycle with a velocity profile comparable to that of slow walking. We used two basic foot trajectories based on slightly different computer algorithms. They are illustrated in Fig. 1, B and C, respectively. Moreover we used two different speeds for the trajectory illustrated in 1C, a slow trajectory with a 4.82-s duration and a faster one with a 2.83-s duration. However, as we point out later, these small differences did not have any further implications for data analysis and interpretation.

**Raphe nuclei stimulation**

It has been well documented by microdialysis studies that electrical stimulation of the raphe complex in the brain stem is an effective technique for releasing serotonin in the target sites of the raphe nuclei projection (McQuade and Sharp 1995). A single 2- to 4-min period of raphe stimulation has been shown to increase serotonin (5HT) levels in the spinal cord for a period of 10–15 min.

We employed this technique in four experiments to study the effect on DSCT movement-related activity. We introduced bipolar stimulating electrodes (FHC, Brunswick, ME) in the raphe area of the caudal brain stem using the following stereotaxic coordinates from an atlas of the cat brain (Berman 1968): P = 6.0–11.0; L = 0–0.5; H = 8 to –10 for raphe magnus (Rma) and P 8.0–10.0; L = 0–0.5; H = 9.0–1.0 for the raphe pallidus (Rpa). These two nuclei represent the major source of the serotonergic projections to the intermediate gray of the lumbar spinal cord where most DSCT neurons are located (Willis 1984). Stimulation parameters were: pulse width, 0.5 ms; frequency, 5–30 Hz; intensity, 50 μA, duration, 2–4 min. We verified the location of the stimulation sites by passing DC current (20 μA for 60 s) through the stimulating electrodes at the end of each experiment to mark the electrode position.

In one animal (cat 1) we used only one stimulation site, which was located in the area of the Rpa. In the remaining three experiments, we positioned stimulating electrodes in both the Rma and the Rpa areas. The locations of seven stimulation sites reconstructed from histological sections of the cat brain stem are plotted in Fig. 2 on a midline...
was to perturb serotonergic transmission, in some cases, we also administered intravenous bolus injections of the 5HT2 agonist (±)-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) (initial dose: 0.01 mg/kg, up to a maximal dose of 0.1 mg/kg). In a smaller number of cases, we also administered the 5HT1a receptor agonist (±)-8-hydroxy-dipropylaminotetralin HBr (8-OH DPAT) (0.2–0.4 mg/kg) or the 5HT2 receptor antagonist spiperone (0.3–1.0 mg/kg).

Neuronal recording and data analysis

We recorded DSCT unit activity extra-axionally from the dorsolateral funiculus at the T10–T12 level of the spinal cord by means of insulated tungsten electrodes (5 MΩ, FHC, Brunswick, ME). We identified units as spinocerebellar on the basis of their antidromic response to electrical stimulation of the inferior cerebellar peduncle or the white matter of the cerebellum. Activity recorded continuously during series of 10–15 passive step-like movement cycles was aligned to the reference starting point at the beginning of the forward part of the trajectory (black arrows in the footpaths depicted in Fig. 1, B and C). Cycle histograms were computed using aligned, binned activity (bin width: 33.3 ms) from ≥10 consecutive cycles (Poppele et al. 2002).

We used two different step cycle speeds in the pharmacological series of experiments. Therefore to compare activity pattern across neurons recorded with different movement speeds, we normalized the activity histograms to the cycle length. This procedure was justified by our earlier finding of a low dependence of DSCT responses to the movement speed (Bosco and Poppele 1999), and it allowed us to pool together responses obtained with different movement speeds.

Typically, we recorded one or two series of 10–15 movement cycles before raphe stimulation or drug administration (control condition). Then we followed the time course of the effects of altering the serotonergic system by recording unit activity during successive series of 10–15 step cycles per series until the activity appeared to return to the control levels. In each case, we determined whether the control and poststimulus responses differed by applying the two-sample non-parametric Kolmogorov-Smirnov (KS) test to pairs of cycle histograms. Differences were considered statistically significant at the 0.05 level.

Any differences between control and poststimulus responses were assessed for significance by comparing the histograms with a KS test.

![Figure 1](https://example.com/fig1.png)  
**FIG. 1.** Experimental setup and passive limb kinematics. A: representation of the stimulation and recording setup for these experiments. The left (ipsilateral) hindlimb is kinematically represented by the trajectory of the limb axis, which is the gray line connecting the hip joint to the foot (metatarsalphalangial joint). The limb axis is defined by its orientation angle with respect to horizontal, measured counter-clockwise, and its length. B and C: trajectories imposed to the foot by the robot. Top: the foot trajectory in a parasagittal plane in Cartesian coordinates. The forward foot trajectory (FT) is marked for each sample frame with black dots and the backward trajectory (BT) with gray dots. Middle and bottom: the same trajectories in the coordinates of the limb axis, orientation on the left and length on the right. Bottom: the respective velocities. Different foot trajectories were programmed for the Raphe stimulation (B) and serotonergic drug (C) experiments.

**Pharmacological procedures**

In another series of experiments (4 cats), we manipulated the serotonergic system by means of various pharmacological agents administered intravenously through a catheter in the radial vein (see Fig. 1A). These experiments were designed to simply perturb serotonergic transmission rather than to mimic any physiological actions. Moreover, the experimental design required that we observe the effects on the behavior of many cells rather than make a detailed study of any one cell. Thus any pharmacological assessments, such dose-response relationships or receptor type identification were beyond the purpose of this study.

The primary pharmacological agent we used was the serotonin antagonist ketanserin, a selective blocker of 5HT2 receptors. We administered intravenous bolus injections of a saline solution containing a 0.01 mg/kg dose of ketanserin. If the first dose of ketanserin did not have a significant effect on DSCT firing activity, we repeated the bolus injections up to a maximal cumulative dose of 0.1 mg/kg. Although the main purpose of these pharmacological manipulations was to perturb serotonergic transmission, in some cases, we also assessed the specificity of ketanserin effects on DSCT activity by blocking the ketanserin action with bolus injections of the 5HT2 agonist (±)-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) (initial dose: 0.01 mg/kg, up to a maximal dose of 0.1 mg/kg).
quantified by comparing the response amplitudes (based on the difference between maximum and minimum firing rates), the mean level of firing over a movement cycle, and the standardized responses (normalized for a single cycle by subtracting the mean activity level and dividing by the SD). Any significant difference between standardized control and poststimulus responses was considered to indicate a difference in response waveform. Differences in amplitude of ≥5 imp/s were also considered significant.

**Principal component analysis**

To describe the variety of response waveform patterns recorded during these movement cycles and to quantify further the changes occurring as a result of serotonergic manipulations, we used principal components analysis (PCA). This is a commonly used statistical technique to extract from a large data set a small number of linearly independent components (basis functions or principal components) accounting for most of the original data set variance. Because we extracted the principal components (PCs) from a correlation matrix of the response histograms data set (equivalent to normalizing response amplitudes), this analysis examined only the response waveform rather than any changes in response amplitude.

In this study, we applied the PCA to each of two separate sets of DSCT response histograms, recorded either before (control) or after serotonergic manipulations. The control response waveforms were compared with the responses showing maximal effects of the serotonergic manipulations. The maximum effect was evaluated from the root mean square differences between control and poststimulus responses. We showed previously that the PCA reveals response components that are specifically related to the limb axis trajectory (Poppele et al. 2002). Because the two experimental data sets were acquired here using slightly different movement trajectories, it was important to determine that the PCA analysis was nevertheless equivalent for both data sets. We did this by examining the relationship between the waveforms of first two PCs and the limb axis trajectory by using the limb orientation parameters to predict the first PC and the limb axis length parameters to predict the second PC. In both cases, the limb axis kinematics explained >93% of the variance in PC1 and ≥73.8% of the variance in PC2. Moreover, there was no difference between PCs obtained separately for the faster and slower movement trajectories. (The results are depicted below in Figs. 5 and 8, green traces)

We also evaluated the PC weighting coefficients, that is, Pearson product-moment correlations between each response and the principal components. These coefficients quantify the relationship between the response histograms and each of the PC waveforms. For example, a weighting coefficient equal to 1 indicates that the original activity histogram is entirely accounted for by a given PC (a negative sign indicates that the two waveforms are mirror images), whereas a weighing coefficient equal to 0 occurs when the activity histogram and a given PC are unrelated. All statistical analyses were performed using SYSTAT (Wilkinson 1990).

**RESULTS**

We investigated the role of serotonergic modulation in the DSCT circuitry by recording the activity of 72 DSCT neurons during passive hindlimb movements before and after perturbing the spinal serotonergic system. In one series of experiments, we stimulated the raphe areas in the brain stem that have serotonergic projections to the spinal cord, and in another series, we manipulated the serotonergic system pharmacologically.

**Raphe stimulation**

Electrical stimulation of the raphe areas at 5–30 pps (see methods) modified the limb movement responses in ~70% (29/42) of the DSCT cells tested, beginning ~1 min after a 2- 4-min period of stimulation. The effects were quite variable across cells, and they could involve changes in response amplitude, mean firing rate, and response waveform. Most of the changes involved response amplitudes (62%, 18/29; 9 responses increased and 9 decreased) and/or significant waveform changes (also 62%, KS test, P < 0.05). Mean firing rate changed by more than 5 imp/s in 38% (11/29) of the cells.

Response changes usually reached a maximum within 5 min after stimulation ended (average: 5.6 min, range: 3–17 min) and returned to control levels after 15–20 min (average: 17.5 min, range: 6–39 min). We also observed moderate changes in blood pressure related to the stimulation. However, the pressure changes were inconsistent and did not correlate overall with the occurrence of response changes (Wilcoxon pairs test, P = 0.55).

A typical time course of the stimulation effect for one of these neurons is illustrated in Fig. 3. It shows three response histograms recorded from cell 2409 at successive times after stimulation [5 min, 7 min (red traces) and 10 min (gray)] together with the response recorded prior to the stimulation (black trace). The stimulation enhanced the peak of this neuron’s activity occurring during the forward movement trajectory. The effect was maximal within 5 min and gradually returned to the control level after 10 min. The standardized histogram (right) shows how the response waveform was modified (see Methods).

The examples in Fig. 3 also include changes in response amplitude (cell 2396), in mean firing level (cell 2357) and changes in response waveform (cells 2409, 2285, and 2382). Some of the changes seemed quite small (e.g., cell 2396), but they were nevertheless significant because the responses were highly stable and reproducible (Bosco and Poppele 1999). The waveform changes are illustrated in standardized histograms. In many cases such as cells 2285 and 2382, the changes could occur in specific parts of the trajectory and the effects could even be opposite in different parts of the trajectory (cell 2409). Two of the examples illustrated show changes that were confined to the forward trajectory (cells 2382 and 2396).

Typically Rma and Rpa stimulation evoked similar activity changes, as exemplified by cell 2396 (compare red and green traces). There were also exceptions, such as the effects on cell 2382. This cell was recorded in the same experiment as cell 2396, yet it did not respond to both stimuli even though the stimulus parameters were the same for both cells.

The effects were generally distributed throughout the movement trajectory with no predominance in the forward or backward trajectories. This is illustrated by the average of the activity differences between pre- and postraphe stimulation responses, which were essentially uniform over the movement cycle (Fig. 3, average difference).

**Pharmacological experiments**

We also recorded the responses of 30 neurons before and after intravenous administration of various serotonin agonists.
and antagonists. We used mainly the 5HT2 antagonist ketanserin (dosage: 0.01–0.1 mg/kg) and to a lesser extent DOI (a 5HT2 agonist), 8-OH DPAT (a 5HT1a agonist), and spiperone (another 5HT2 antagonist) to perturb serotonergic transmission in the spinal cord. Intravenous administration of serotonergic drugs, however, is known to produce strong effects on the circulatory system (van Zwieten et al. 1992). These effects appeared to be minimal, however, when ketanserin was administered in doses that were effective in modifying DSCT activity. Even the moderate changes in blood pressure we did observe after ketanserin injections did not correlate with the presence of an effect on the DSCT responses (Wilcoxon pairs test, $P = 0.099$). We also reported previously that ketanserin has more specific effects on DSCT movement responses than did the other serotonergic agents we tried (Rankin et al. 1998). Consequently, we used ketanserin as the primary means of pharmacological manipulation for this current series of experiments.

Overall, we found that the responses to the limb movements were altered in 73% (22/30) of the DSCT neurons tested with one or more of these pharmacological manipulations (KS test, $P < 0.05$). The drugs also had a variety of effects on the responses that again included changes in activity levels as well as changes in response waveform. We observed the effect of more than one drug in 7 of the affected cells for a total of 28 effects in the 22 affected cells. Amplitude changes were again a major effect (68%; 19/28 responses), but in this case, they were almost entirely amplitude decreases; only one response showed an increase. About 40% of the changes (11/28) were accompanied by changes in mean firing rate of $>5$ imp/s, and the response waveform was significantly altered in 50% (14/28) of the responses.

The effects occurred more slowly than those evoked by raphe stimulation, reaching a maximum in $\sim$15 min (average $= 16.4$ min, range: 3–40 min) and lasting for $\sim$45 min (average $= 45.1$ min, range: 26–87 min) after drug administration. Some specific examples of the effects are illustrated in Fig. 4.

The effect of ketanserin administration on the firing pattern of cell 2398 consisted of a reduction in the peak of activity occurring during the forward trajectory (red trace, black trace is control). This effect reached its maximum in $\sim$30 min and persisted at the same level for another 35 min. The response then returned to control within 15 min after the administration of the 5HT2 agonist DOI (0.01–0.1 mg/kg iv; green trace). Thus the effect on this cell seemed to be receptor specific.

Ketanserin had stronger effect on the responses of cell 2615, where activity levels were again decreased during the forward trajectory and also increased in the backward trajectory. The maximal effect (lowest red trace) was observed 14 min after ketanserin administration, and it fully recovered to control levels after 29 min (gray trace). The standardized histograms show that the effect during the forward trajectory was mainly a reduction in the response amplitude, although there were changes in the waveform during the backward trajectory.

A typical effect of ketanserin administration was an overall attenuation of the movement responses that may have been accompanied by some changes in the response waveform (cells 2615, 2607, and 2398). A similar response attenuation was also observed after administration of spiperone, another 5HT2 an-
agonist (cell 2594, blue trace). Administration of 8-OH DPAT, a 5HTP1a agonist, produced an even more dramatic attenuation in cell 2609 (green trace).

The changes in responses induced by the drugs did not appear to favor any particular part of the movement trajectory, although there may have been a slight tendency for greater changes at the beginning of the forward trajectory (Fig. 4, average difference).

Population behavior

We characterized the response waveforms across neurons more systematically with a PCA (see METHODS). The motivation for using this analytical approach was to determine whether the modified response waveforms were the result of new or different response components contributing to the DSCT activity. We analyzed the responses of the neurons in the raphe study separately from those recorded in the pharmacological study because we used different limb movement trajectories for the two experimental series. Because the PCs have been shown to be strongly correlated with the movement trajectory, we expected different PCs for the two sets of responses (see METHODS). We extracted separate PC waveforms from responses recorded in the control condition and from those showing significant changes after serotonergic manipulations for each subgroup.

The PCA results for the raphe data are illustrated in Fig. 5 where the black and red curves represent the PC waveforms extracted from control and post raphe-stimulation data sets, respectively. The two sets of PCs were highly correlated and superimposed almost exactly. The coefficients of correlation for the first three PCs were >0.97 and >0.85 for the next two PCs. This result implies that modified weightings of the same set of response components could account for the altered waveforms.

These PC waveforms were slightly different from those documented previously for a slightly different movement trajectory (Poppele et al. 2002). Nevertheless, they were as well predicted by the kinematics of the foot trajectory (green traces, Fig. 4).
PC 1 and PC 2). The predictions were made using limb axis orientation predictors for PC 1 and length predictors for PC 2.

A closer examination of the PC weightings showed that the response changes were accompanied primarily in the weightings of PC 1 and PC 3. PC 1 accounted for less of the total variance after stimulation (47% vs. 55%) and PC 3 accounted for more (~3 times more). The second PC accounted for the same fraction of the total variance (23%) in both conditions. Histograms of weighting coefficient differences illustrate this difference on a cell-by-cell basis for the first four PCs (which accounted for >90% of the total data set variance; Fig. 6). Although the four distributions were all centered about zero, the spread was larger for PCs 1, 3, and 4 compared with PC 2, where the differences were mostly confined between -0.1 and 0.1. The difference between histograms was significant at the $P < 0.05$ level (2-sample variance F test).

The larger changes occurring in the weighting of PC 1 relative to PC 2 are more evident on a scatter-plot of the first two PC weighting coefficients (Fig. 7A). Most of the neurons with significant weighting coefficient differences in the poststimulation responses (filled symbols) showed larger changes along the PC 1 axis compared with PC 2. The effects are further illustrated by 2 examples (cells 2382 and 2409, see also Fig. 3) in which we compare the standardized responses with their respective reconstructions from the PCs and the weighting coefficients (Fig. 7A).

We quantified this result further by computing the direction angle of the vectors formed by connecting the control and poststimulus responses in the two-dimensional PC space. For vectors having lengths >0.1, we found that the corresponding circular distribution ($n = 20$) had a significant bimodal trend with major axis oriented at $\sim 176^\circ$, that is parallel to the PC 1 axis (Fig. 7B; Rayleigh test, $P < 0.05$).

The changes in response waveform evoked by pharmacological perturbations of the serotonergic system seemed to account for less of the overall effect than they did with raphe stimulation. Once again though, the first five PCs determined separately for the control and postdrug responses were essentially identical (Fig. 8). The correlation coefficients were $>0.91$ for the first three PCs and $>0.65$ for PC 4. In this case, PC 5, which accounted for $\sim 2\%$ of the total response variance, was somewhat different in the two conditions (Fig. 8F).

In this study we were concerned that the relatively small sample size of <30 responses may have introduced a bias in the results [we did show previously that a sample size of around 40 responses did not appear to do so (Poppele et al. 2002).] We checked this by comparing the drug study PCs with those obtained with a much larger number of responses from a different study in which we applied the same foot movements. The two sets of PCs (Fig. 8, black and gray traces) were essentially identical, indicating that sample we used was basi-

![FIG. 6. Distribution of weighting coefficient differences. The weighting coefficient (loading) with respect to each of the PCs for the poststimulus response was subtracted from the respective coefficient for the control response for each response that was altered by raphe stimulation. Differences are plotted as histograms in A–D for PCs 1–4.](http://jn.physiology.org/)

![FIG. 7. Raphe induced changes in response waveform represented by PC weighting. A: each response altered by raphe stimulation is represented in a plot of its weighting coefficients for PC 1 and PC 2. Control responses (open symbols) are connected by vectors to the respective poststimulus response (filled symbols). Insets: 2 pairs of responses are also illustrated by the standardized cycle histograms (thick traces) from Fig. 3 for cells 2382 (left) and 2409 (right). Thin traces show the respective reconstructions of the responses from the PCs and the weighting coefficients. B: histogram of vector direction for the vectors in A having a length >0.1. The plot indicates only directions, so each vector is treated as unit vector for this purpose; the units on the histogram radii indicate the number of unit vectors. The histogram shows a bimodal directional bias with a major axis oriented at $176^\circ$.](http://jn.physiology.org/)
and postdrug responses (60%), whereas PC 2 accounted for ~20% less variance in the postdrug responses (14 vs. 17.5%). The histograms of weighting coefficient differences computed cell-by-cell for the first four PCs are illustrated in Fig. 9. The difference distribution was significantly broader for PC 3 than for the other PCs (2-sample variances F test, P < 0.05), whereas there was no significant difference between the distributions for PCs 1 and 2. This point is also evident in Fig. 9, E and F, where many of the vectors connecting pre- (open symbols) and postdrug (filled symbols) responses had comparable changes in PCs 1 and 2 weighting. This resulted in a more uniform circular distribution (Rayleigh test of uniformity; P > 0.05).

DISCUSSION

In this study, we showed that descending serotonergic signals can modulate the responsiveness of DSCT neurons to passive movements of the hindlimb, thereby potentially modifying the content of sensory information relayed to the cerebellum. Serotonergic modulation of DSCT activity consisted of changes in the response amplitudes, response waveforms, and/or overall firing levels.

The distribution of serotonergic innervation in the intermediate spinal cord (Jankowska et al. 1995, 1997b; Maxwell and Jankowska 1996; Pearson et al. 2000) suggests that descending serotonergic signals could act on the DSCT circuitry through several mechanisms (Fig. 10). They could have direct postsynaptic effects mediated via serotonergic terminals on the DSCT neurons (Jankowska et al. 1997a). Changes in the response waveforms instead may be more difficult to classify according to a pre- or postsynaptic origin. For this purpose, we used PCA to analyze the waveform changes. If the change in response waveform was due to postsynaptic modulation of the DSCT neurons, we would expect this to be reflected in the PCs. That is, changes that affect all inputs to the DSCT will give rise to PCs that are different from those of the control responses. If instead the waveform changes are due to presynaptic modulation of only certain inputs to the DSCT, then some or all of the PCs will be the same as the control. We would expect all the PCs to be same if the modulation affected only the relative weighting of inputs. This is illustrated diagrammatically in Fig. 10. A presynaptic effect could alter the gain or waveform of some component of the response, while a postsynaptic effect would alter the overall gain or waveform.

The robust finding of this study was that serotonergic manipulations did not change the basic PC waveforms even when

![FIG. 9. Drug induced changes in response waveform represented by PC weighting. A–D: same format as Fig. 6. E and F: same format as Fig. 7. In this case, there is no significant directional bias.](http://jn.physiology.org/)

![FIG. 8. Principal component analysis of drug data. Principal components obtained from control responses (black) and altered poststimulus responses (red). PCs obtained from a separate sample of 82 cells from a different study using the same limb movements (gray) are basically the same as those obtained from the 30 control responses. PC 1 and PC2 are also compared with the waveform predicted using the limb axis kinematics as predictors (green); orientation and orientation velocity for PC 1 and length and length velocity for PC 2. Cumulative percentage of the total waveform variance explained by the 1st 10 PCs.](http://jn.physiology.org/)
Raphe stimulation produced two predominant effects. One was to change response amplitudes. This was not always accompanied by changes in response waveform (e.g., cell 2396), suggesting either an overall change in synaptic efficacy or in cell excitability. It is noteworthy though that there were about equal numbers of increases and decreases in response amplitude. These effects were consistent however with those described by Jankowska and colleagues (1997a), who found that serotonin mediates a decreased DSCT sensitivity to group II afferent input in a subset of DSCT cells referred to as dorsal horn DSCT neurons. They also found increased sensitivity to both group I and group II inputs to the Clarke’s column DSCT cells. Although we were unable to determine which type of DSCT cells we recorded from, it is likely that we recorded from both types because their axons both traverse the dorsolateral funiculus in the thoracic spinal cord, which could explain why both increases and decreases were seen.

The other major raphe effect was to change the response waveforms that were associated with changes in the specific weightings of response components represented by the PCs. The changes modified the weighting of PC 1 and also of PCs 3 and 4. Previously we showed PC 1 to be associated with the trajectory of the limb axis orientation, while the PCs 3–5 were associated with specific dynamic combinations of position and velocity sensitivity (Poppele et al. 2002). The waveforms of PCs 1 and 2 were found to be consistent with the dynamics of the muscle spindle primary ending, and other dynamics associated with a greater or lesser position or velocity sensitivity could be accounted for by various weightings of the higher-order PCs.

This interpretation of the PCA results leads to the following interpretations of the raphe effects. The first is that the inputs to DSCT neurons responsible for the two response components represented by PCs 1 and 2 are independently modifiable. While their independence was already implied by the PCA, the current result implies further that the independence is not simply a statistical property of the response data or of the sensory input but rather has an anatomical basis within the neural circuitry where serotonergic modulation can occur. The independence also suggests that the site of modulation is presynaptic to the DSCT neurons, as we noted in the preceding text. Moreover the selective effect of raphe activation on the weighting of PC 1 and not PC 2 suggests the existence of separate circuit elements representing limb orientation, perhaps driven by proximal limb receptors.

The previous finding of a differential modulation of the relative strengths of group I and II spindle inputs to the DSCT by serotonin may also be consistent with the PCA. Changes in the weightings of PCs 3 and 4 could be associated with this type of modulation because the group II afferents have a greater position sensitivity than the velocity-sensitive group I afferents. Thus a change in the relative weighting of these inputs would also be consistent with a change in the relative position and velocity sensitivities.

The reduced effect on the weighting of PC 2 may be due to a lack of modulation in some other specific input pathway to the DSCT cells. Perhaps this input originates in a population of cells less affected by serotonin released from raphe terminals. The effect might also be due to certain synaptic sites on the DSCT neurons that are not modulated by serotonin. We prefer the former explanation because the inputs responsible for PC 2

The response waveforms themselves were modified. This tends to rule out the possibility that serotonin altered the way DSCT neurons process synaptic input by changing their basic excitability dynamics, and it is consistent with a differential presynaptic modulation that affects only the input gain or sensitivity.

This finding suggests that the PCs represent stable properties of the DSCT circuitry that are not affected by serotonin. It also implies that serotonin does not unmask additional synaptic inputs that are not active or do not have a significant effect on DSCT circuitry in the control state. The most likely explanation for the finding is that the weightings of independent response components, presumably originating within different presynaptic sources, are modulated or altered separately in some way by serotonin.

There are at least two possible mechanisms whereby this might occur. One is a separate modulation of specific synaptic inputs to the DSCT [e.g., specific presynaptic modulation of afferent input as proposed by Jankowska et al. (1997a) and by Quevedo et al. (1995)]. Such modulation, mediated via presynaptic inhibition can also be highly specific for different types of sensory input. The other is a separate modulation of specific components of the presynaptic circuitry. However, these possibilities are operationally indistinguishable with the experimental approach we used.

Raphe stimulation produced two predominant effects. One was to change response amplitudes. This was not always accompanied by changes in response waveform (e.g., cell 2396), suggesting either an overall change in synaptic efficacy or in cell excitability. It is noteworthy though that there were about equal numbers of increases and decreases in response amplitude. These effects were consistent however with those described by Jankowska and colleagues (1997a), who found that serotonin mediates a decreased DSCT sensitivity to group II afferent input in a subset of DSCT cells referred to as dorsal horn DSCT neurons. They also found increased sensitivity to both group I and group II inputs to the Clarke’s column DSCT cells. Although we were unable to determine which type of DSCT cells we recorded from, it is likely that we recorded from both types because their axons both traverse the dorsolateral funiculus in the thoracic spinal cord, which could explain why both increases and decreases were seen.

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have been shown to be related to a specific global aspect of the limb movement. This input component, which correlates with the limb axis length trajectory, is likely to be more complex than could be accounted for by a specific type of afferent, and it probably involves a presynaptic circuitry. Furthermore, our studies have shown that this component is present in the responses of nearly all DSCT cells and that it occurs independently from the inputs responsible for PC 1. Thus it is not characteristic of a distinct DSCT subpopulation.

**SEROTONERGIC DRUGS.** The serotonergic drugs we used affected the responses of a similar fraction of the DSCT cells tested as did the raphe stimulation (~70% in both cases), and many of the effects were quite similar. There were some specific differences however. The serotonergic drugs consistently decreased response amplitudes, and they had less overall effect on response waveforms. One straightforward explanation for the differences would be that raphe stimulation, through synaptic release of serotonin, physiologically activated the variety of receptor types expressed by spinal neurons, whereas ketanserin, the principal drug we used, blocked only the 5HT2 receptor. Thus the predominance of response amplitude decreases we observed with the drugs might represent the effect of blocking specific receptor subtypes. Another factor that may contribute to this different behavior is the well established fact that serotonergic raphe-spinal terminals co-release different types of neuropeptides depending on their area of termination within the spinal gray (Cullheim and Arvidsson 1995; Hokfelt et al. 2000; Maxwell et al. 1996; Wassendorn and Elde 1987; Wu et al. 1993). The action of neuropeptides co-released with serotonin might in fact interact with the putative effects of serotonin, a phenomenon known as meta-modulation (Katz and Edwards 1999; Svensson et al. 2001) that is not accounted for when directly applying specific serotonergic agonist or antagonist drugs. However, we did not test this suggestion any further by infusing ketanserin together with raphe stimulation, for example, or by interfering directly with the action of the neuropeptides.

The effects seen with serotonergic blockers like ketanserin might also be associated with any number of systemic effects. One of the most pronounced effects of serotonergic drugs is on blood flow (van Zwieten et al. 1992) We monitored arterial blood pressure continuously, and we did observe changes that were induced by drug infusion. However because those changes were minimal with the doses of ketanserin we used and because we could not demonstrate a correlation between the blood pressure changes and the effects on DSCT responses, we concluded that blood flow changes were unlikely to be the cause of the decreased response amplitudes. It seems more likely that DSCT responsiveness depends normally on basal endogenous levels of serotonin, the action of which is then suppressed by pharmacological antagonists. However, with our protocol, it is impossible to localize the source or sources of the effects on cell behavior, and we cannot entirely rule out effects due to local changes of blood flow in the spinal cord, for example.

Although the cell-by-cell activity changes caused by the drugs were qualitatively different from those observed with raphe stimulation, the basic population response components were similarly unaltered, reinforcing the view that the PC waveforms might represent stable presynaptic components of DSCT circuitry. However, in this case the relative weightings of PCs 1 and 2 were altered almost equally. Given the relatively modest effects on response waveform we observed, and the small number of cells involved, our analysis may be at the limits of its capability to make any clear distinctions with this approach. It should be noted, however, that although the changes may have been modest, they were nevertheless significant, given the reproducibility of the control responses and the return to the control waveforms during recovery.

We might conclude from these results that the changes induced by systemic manipulation of the serotonergic system were qualitatively similar to, but somewhat less specific than, the changes induced by raphe stimulation. Thus it seems likely that we did observe the effects of serotonergic perturbations in both cases. The pharmacological manipulations showed that both of the major DSCT response components could be modulated independently by serotonin. The raphe stimulation shows that this system is capable of selectively modulating specific inputs to the DSCT during passive limb movements.

The results provide additional support for our interpretation of the independent components of DSCT responses to limb movement, namely that they are separately represented within the spinal cord circuitry.

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**DISCLOSURES**

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**REFERENCES**


