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RESPONSES OF VIBRATION-SENSITIVE RECEPTORS IN THE INTEROSSEOUS REGION OF THE DUCK'S HIND LIMB

BY PATRICIA K. DORWARD AND A. K. McINTYRE
From the Department of Physiology, Monash University,
Clayton, Victoria, Australia

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SUMMARY

1. Responses of receptors with fibres in the interosseous nerve of the duck's leg have been studied by recording unit discharges in filaments dissected from the sciatic nerve.

2. Seventy-two of the ninety-four units examined served highly phasic, vibration-sensitive mechanoreceptors in the interosseous region interpreted as being Herbst corpuscles. Receptor types for most of the other units could not be determined, but some were slowly adapting mechanoreceptors.

3. Rheobase threshold values for the most sensitive vibration-receptors were similar to those of mammalian Pacinian corpuscles.

4. Threshold–frequency relationships for the vibration receptors showed a wider range of low frequency cut-off values, and a greater capacity to signal high frequencies, than is the case with Pacinian corpuscles.

5. Fibres of the vibration-receptors had calculated diameters ranging from 5 to 10 μm and account for the bulk of the larger fibres in the interosseous nerve.

6. It is suggested that Herbst corpuscles in the legs of birds might act as a warning device by detecting vibratory disturbances of the ground or other supporting surface.

INTRODUCTION

Electrophysiological investigations have shown that the mammalian Pacinian corpuscle is a phasic mechanoreceptor of great sensitivity to vibration (Gray & Malcolm, 1950; Gray & Matthews, 1951; Hunt, 1961; McIntyre, 1962). However, little information has yet been published about the properties of the somewhat similar Herbst corpuscle of birds (Quilliam & Armstrong, 1961, 1963; Munger, 1971), although the behavioural studies of Schwartzkopff (1948, 1949) and a brief report by Skoglund (1960) suggest that Herbst corpuscles are vibration-sensitive, like their mammalian counterparts.

In many species of birds, an impressive array of Herbst corpuscles is found along and between the tibia and fibula (Schildmacher, 1931). This paper describes some properties of mechanoreceptor units located in this region of the duck's hind limb. The majority of the unit responses studied were considered to be those of Herbst corpuscles; like mammalian Pacinian corpuscles, they proved to be phasic in behaviour and showed striking sensitivity to vibration. Some of this work has been reported briefly in a previous communication (McIntyre, 1965).

METHODS

Preparation. Experiments were performed on thirteen domestic ducks anaesthetized with sodium pentobarbitone (Sagatal, May & Baker), the initial dose (60 mg/kg) being administered intramuscularly. Supplementary maintenance doses were administered intravenously. The animals were routinely given artificial ventilation by way of a tracheal cannula. A posterior incision in the skin of a hind limb gave access to the sciatic nerve in the thigh and the interosseous region of the leg. Exposed tissue was covered with liquid paraffin at 40° C retained by skin flaps. It was not possible actually to expose the Herbst corpuscles in the interosseous region because of the overlying posterior tibial vessels and their numerous fragile branches. The interosseous nerve supplying this region lies superficial to the vessels and was routinely exposed; its connexion with the sciatic nerve was left intact. The sciatic nerve was severed in the thigh and all branches distal to this level other than the interosseous nerve were cut. Under a dissecting microscope, the sheath was removed from the peripheral stump of the sciatic nerve, which was then subdivided until filaments were obtained containing functionally single units with axons coursing in the interosseous nerve. Responses in some filaments containing two interosseous nerve fibres were also studied, if the spike-size and other features of each unit were sufficiently characteristic to ensure its identification. In all, ninety-four interosseous nerve units were isolated and studied.

Mechanical stimulation. Stimulation of receptors with fibres travelling in the interosseous nerve was attempted in several different ways. These included tapping the leg bones, or the base plate and supports holding the animal, direct pressure on the interosseous region with a blunt probe, and manipulation of the ankle joint and foot. Controlled vibratory stimulation was applied either to the leg bones, or more discretely as close as possible to the receptor itself. In the first procedure, a Goodman vibrator (model V 47) was used, driven by an Exact function generator and current amplifier; contact with the bones of the leg was made by a light steel pin attached to the moving element.

More localized stimulation was achieved by a small glass stylus attached to the free end of a ceramic piezo-electric bender element (Gulton Industries, New Jersey), driven by the function generator and a voltage amplifier. It was necessary to stimulate through the overlying soft tissues; however, the stylus tip was placed at the point of maximum sensitivity for each unit in order to bring it as close as possible to the receptor. The amount of displacement of the tip for a given voltage was determined by direct microscopic examination, and was approximately proportional to the applied voltage up to a value of 140 V. With sinusoidal stimulation, the stylus was illuminated by a stroboscope, and displacement–voltage calibration curves were constructed for the range of frequencies used in the experiments. The actual displacements of the stimulated receptor organs could not be measured, and in most instances were probably less than the values determined from the calibration curves because of damping and attenuation of the stimulus in its passage through the soft tissues.

Conduction-velocity measurements. The action potentials recorded in the dissected filaments could of course be set up by electrical stimulation of the interosseous nerve. Conduction-velocity was determined by stimulating the nerve at a number of points along its length, and plotting latency of the unit action potential against distance; the slope of the curve gives the conduction-velocity more reliably than measurement of distance and latency to stimulation at one position only. From the conduction-velocity measurements, calculated fibre-diameter spectra were constructed for the interosseous units, assuming the conversion factor of 6 (Hursh, 1939), as a similar relationship has been demonstrated in the duck (Dorward, 1966).

Histology. The interosseous nerve was removed at the end of most experiments and fixed in 0·5 % osmic acid. Transverse sections were taken at two or three different positions along the nerve, and fibre counts made for each; from these, histograms of the distribution of fibre diameters could be constructed.

RESULTS

Of the ninety-four interosseous nerve units isolated, seventy-two were sensitive to vibratory stimulation, especially by way of the leg bones. The remaining interosseous units would not respond to vibration, and the adequate stimulus was determined for only a small number. These responded by sustained firing to localized firm pressure applied to the interosseous tissues, a procedure which readily caused bleeding from fragile blood vessels, with rapid deterioration of the preparation. For this reason, search for the adequate stimulus for most of these units was not pressed. However, the conduction-velocities of their fibres were determined.

Vibration-sensitive units

Response characteristics. These units strongly resembled Pacinian corpuscles in their behaviour, responding with bursts of discharge to quite light taps on the tibia or foot, on the massive base plate supporting the animal or on the various metal uprights and bars attached to it. The most sensitive units could also be fired by lightly stamping on the laboratory floor. A number of these units showed apparent spontaneous firing, sometimes related to respiratory rhythm, but more often irregular and unrelated to any obvious movement or other stimulus. All these units could readily be entrained by vibrating the leg bones with the Goodman device, and with strong enough stimulation and within a certain frequency range (usually 200–600 c/s) would continue responding for many seconds or even minutes to maintained vibration generated by sine-wave input. Fig. 1 illustrates the responses of a vibration-sensitive receptor stimulated by the bender-element device. The unit followed vibration at 120 c/s in one-to-one fashion, and continued to do so as frequency was increased to 800 c/s. At still higher frequencies, response became less than one-to-one, but remained regular until stimulus-frequency reached 1620 c/s. At frequencies above 800 c/s, successive impulses in the discharge tended to occur in relation to opposite phases of the sine-wave stimulus, as shown in Fig. 1 *C*. At low frequencies, many of the vibration-sensitive units responded with more than one impulse to each cycle of the stimulus, as is often the case with Pacinian corpuscles.

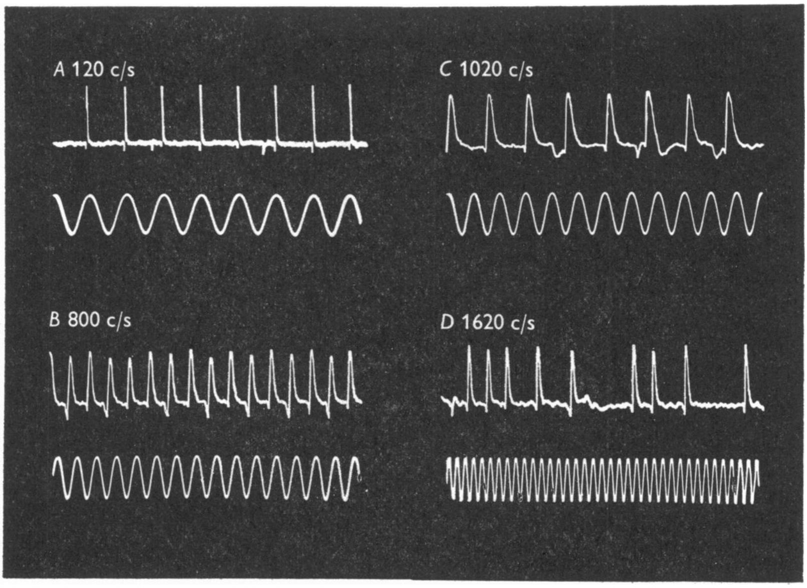

Fig. 1. Responses of an interosseous unit to sinusoidal vibration at different frequencies, recorded from a filament dissected from the sciatic nerve. *A*, *B*, *C* and *D*, different frequencies as indicated. Upper trace in each instance, discharge of the afferent fibre; lower trace, output of function generator driving the bender element, which also provides the time scale. Records retouched.

Mechanical thresholds. When stimulated by the Goodman vibrator in contact with the leg bones, there was considerable variation in the amplitude of vibration required to entrain different units, suggesting a fairly wide spread of mechanical thresholds amongst the population of receptors. This was most obvious when recording from a filament containing fibres of several vibration-sensitive units, successive recruitment of the units often taking place with progressive increase in stimulus amplitude. However, many factors could contribute towards such apparent threshold differences. Location of the receptors, their proximity and degree of attachment to vibrating skeletal structures, and the patterns of vibration of leg-tissues set up by the stimulus could significantly influence their apparent thresholds, quite apart from intrinsic differences in receptor sensitivity.

A better estimate of intrinsic thresholds could be made by localized stimulation with the bender-element device. The tip of the vibrating stylus was placed by micromanipulator at the point of maximum sensitivity in the interosseous region; when so placed, it is likely that only a small amount of tissue separated it from the actual receptor. With such localized stimulation, the rheobase threshold values for thirty-four of the vibration-sensitive units were estimated by finding the amplitude of a step-function displacement of the stylus which was just adequate to fire the receptor. The units analysed in this way were arbitrarily divided into two groups on the basis of their rheobase values, one group (fifteen units) being extremely sensitive; the responses illustrated in Fig. 1 are from one of these low-threshold receptors. Because of their great sensitivity, it was difficult to make accurate rheobase measurements for these units; all, however, appeared to be less than 1 μm, the approximate values ranging from 0·1 to 0·8 μm. The remaining nineteen units constituting the higher threshold group had rheobase values exceeding 1·0 μm, ranging from 1·3 to 10·5 μm.

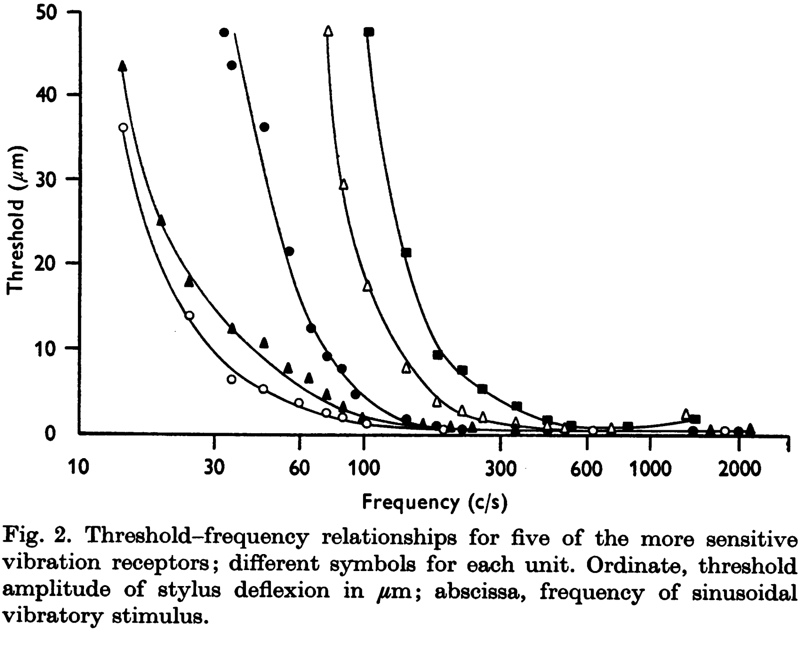

Fig. 2. Threshold–frequency relationships for five of the more sensitive vibration receptors; different symbols for each unit. Ordinate, threshold amplitude of stylus deflexion in μm; abscissa, frequency of sinusoidal vibratory stimulus.

Threshold-frequency relationships. The threshold amplitude of sine-wave vibratory stimulation at different frequencies was determined for the thirty-four units subjected to rheobase measurements, making use of the bender-element stimulator and its amplitude–frequency calibration curve. The criterion for response used in these measurements was a regular but not necessarily one-to-one discharge, maintained for at least a few seconds. Because the rheobase values for the fifteen low-threshold receptors could only be approximately estimated, threshold–frequency curves for these units were plotted using absolute threshold values for sinusoidal stimulation, rather than relative thresholds (sine-wave threshold/rheobase). In Fig. 2, the threshold–frequency relationships for five of these units are presented, and illustrate the variation occurring in this population. Minimum threshold values were only slightly higher than the rheobase thresholds, ranging from 0·25 to 1·2 μm. These minimum values were maintained for fairly wide frequency ranges, and there appeared to be little if any increase in threshold at the higher frequencies; most were extremely responsive between 200 and 2000 c/s. At low frequencies, thresholds increased with decreasing frequency up to the maximum displacement the vibrator could produce (48 μm); at still lower frequencies, the receptors failed to respond. The frequency range over which this increase in threshold occurred varied considerably between units, as shown by the spread of the ascending limbs of the curves in Fig. 2. The lowest frequency which could drive individual members of the low-threshold group varied from 14 to just over 100 c/s.

Threshold–frequency curves for the higher threshold group of receptors were constructed using the method of Sato (1961), in which relative thresholds (sine-wave threshold/rheobase) were plotted against frequency. Fig. 3 shows typical results for five of these units. The curves more nearly resemble those of Sato (1961) for Pacinian corpuscles, with obvious increase in relative thresholds at the higher as well as at the lower frequencies. As compared with the low-threshold units, the frequency range for minimum thresholds in this group was narrower (about 300–800 c/s), and there was less variation between units in the low-frequency limitation of responsiveness.

Adaptation rates. The phasic nature of all the vibration-sensitive receptors was shown by their responses to step-function mechanical displacements. Usually one, or at most two or three, impulses appeared at the 'make' and 'break' of a square wave mechanical stimulus, but rarely did any appear during the phase of maintained displacement. The rise in threshold with reduction of frequency also indicates that a critical rate of displacement is required to elicit discharge. However, a considerable range in the adaptive properties of individual units, especially those of lowest threshold, is suggested by differences in their low frequency cut-off values (Fig. 2). The possible influence of factors such as receptor-location or in coupling via intervening tissues with the vibrating stylus must, however be borne in mind in interpreting these differences.

Conduction velocities

Conduction velocities of sixty-two of the interosseous nerve units were measured, and the fibre diameters estimated from these using a conversion factor of 6 (see Methods). Forty of these units were vibration-sensitive, and

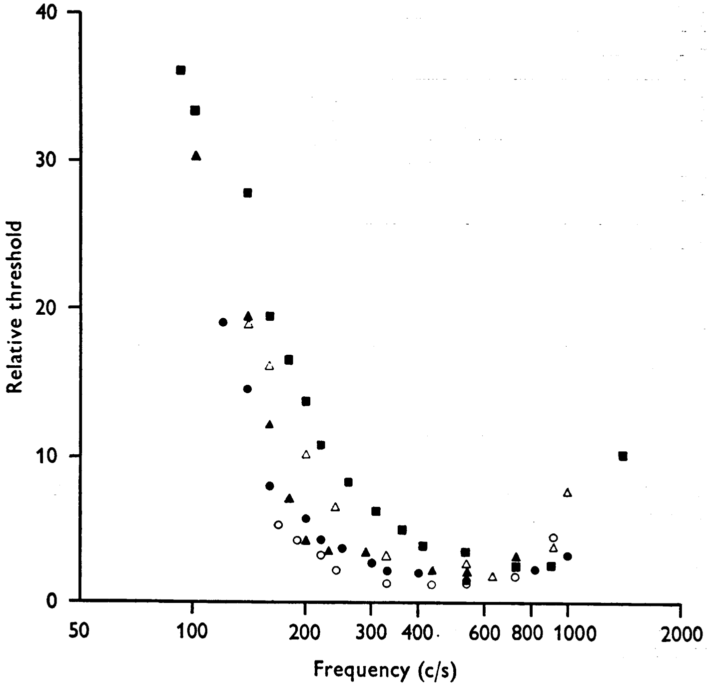

Fig. 3. Threshold–frequency relationships for five of the less sensitive vibration receptors, plotted as in Fig. 2 except that the ordinate gives relative threshold, i.e. ratio of sinusoidal vibration–threshold to step-function rheobase threshold.

the remainder were not; as noted previously the receptor types associated with the latter were not determined in most instances. Fig. 4 displays histograms of the conduction velocities and calculated diameters of the whole population (*A*), the fibres of vibration-sensitive receptors (*B*) and of the other fibres isolated as units (*C*). For comparison, a histogram showing the distribution of myelinated fibre diameters of the interosseous nerve determined histologically is presented (*D*). Despite the relative smallness of the conduction-velocity sample, there is reasonable agreement between the range of calculated diameters and the histological spectrum. The fibres of the vibration-sensitive receptors are distributed unimodally, their calculated diameters ranging from 5 to 10 μm, whereas only two of the other interosseous units had diameters greater than 6 μm. It thus appears that the vibration-sensitive receptors contribute the bulk of the coarser fibres in the nerve.

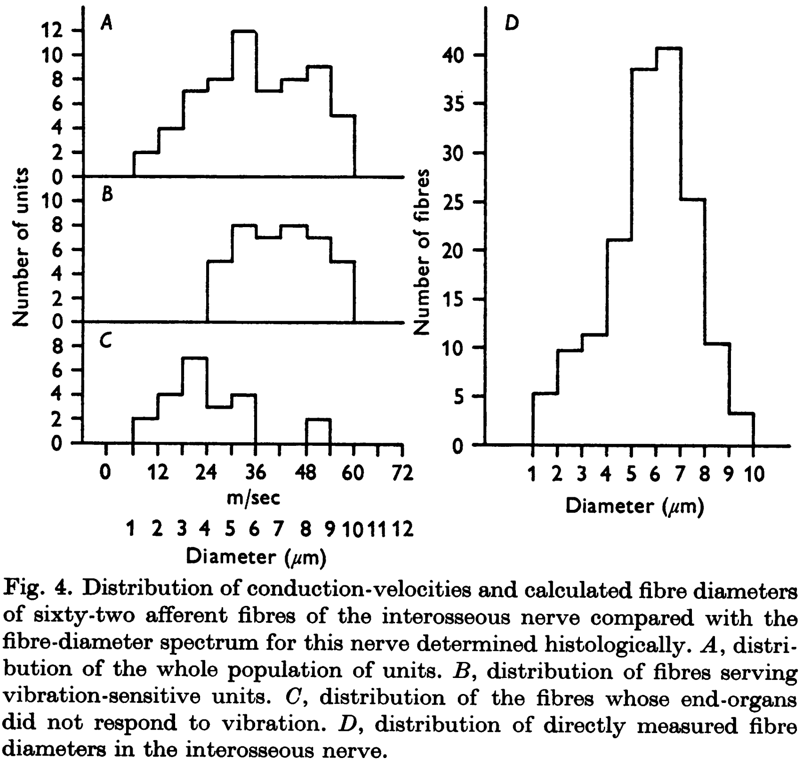

Fig. 4. Distribution of conduction-velocities and calculated fibre diameters of sixty-two afferent fibres of the interosseous nerve compared with the fibre-diameter spectrum for this nerve determined histologically. *A*, distribution of the whole population of units. *B*, distribution of fibres serving vibration-sensitive units. *C*, distribution of the fibres whose end-organs did not respond to vibration. *D*, distribution of directly measured fibre diameters in the interosseous nerve.

DISCUSSION

The results demonstrate the presence of a considerable population of highly phasic, vibration-sensitive receptors in the tibio-fibular region of the duck's leg, with axons travelling centrally by way of the interosseous nerve, together with other mostly unidentified receptors which do not readily respond to vibratory stimulation. That the vibration-sensitive endings are Herbst corpuscles is strongly suggested by their predominance in the population of interosseous nerve units studied, and the abundance of such corpuscles in the leg region supplied by this nerve. Furthermore, similar vibration-sensitive receptors are present in the duck's bill in which the Herbst corpuscle is the predominant organized sensory structure (Gregory, 1968). Although it has not been possible to expose and directly stimulate individual corpuscles in the leg, this has been achieved in the duck's wing (Dorward, 1970), where selective stimulation of single exposed Herbst corpuscles has demonstrated properties which match those of vibration-sensitive units with fibres in the interosseous nerve. It thus seems justified to conclude that the sensory endings of the latter units are indeed Herbst corpuscles.

Thresholds. The range of thresholds shown by the receptors, both to step-function and vibratory stimulation, is likely to arise from a number of factors. As appears to be the case with Pacinian corpuscles, intrinsic differences in sensitivity probably occur in a population of Herbst end-organs which could be related to structural variations, such as size of corpuscle, precise coupling arrangements between capsular cells and nerve terminal, or differences in sensitivity of the terminal membrane. However, under the circumstances of the present experiments, other factors must contribute to the observed threshold differences. Of these, the situation and orientation of the corpuscles within the leg and in relation to the site of stimulation, as well as their degree of attachment to bony resonating structures, seem likely to be important. With sinusoidal stimulation, the patterns of vibratory disturbance in the leg tissues must be extremely complex, and would be expected to have regions of greater or lesser amplitude and to show a variety of spatial and temporal distortions, especially when set up by the Goodman vibrator. When using the bender element stimulator, the spatial spread of vibration would be much more restricted, but some distortion would also be inevitable. Nevertheless, under natural conditions when vibratory disturbance would most likely be conveyed from the ground through the feet, threshold differences as observed in this study would still provide a means of coding the intensity of the stimulus by engaging a greater or lesser number of units.

Responsiveness to different frequencies. Although there were considerable differences in threshold and in range of frequency–sensitivity to vibratory stimulation amongst the units studied, the population as a whole would be capable of signalling frequencies as low as 14 c/s and as high as 2000 c/s, at least when stimulated by localized vibration, and with steady discharge maintained for a few seconds as the criterion of response. At the lower frequencies, unit responses often exceeded a one-to-one relationship, one or more impulses appearing during opposite phases of the oscillatory stimulus, as commonly observed with other highly phasic receptors. One-to-one following was usual with frequencies between 100 and 600 c/s, but at higher frequencies, responses, though regular, were usually less than one-to-one. Some of the most sensitive units, would, however, respond once per cycle to frequencies as high as 1000 c/s, even when the vibration was applied to the leg bones rather than to the immediate vicinity of the receptor. Should the population examined in this study be a sufficiently representative sample, it would appear that a duck under natural conditions could receive input from these receptors coding information related to frequency as well as amplitude of vibratory disturbances affecting the lower limbs.

In general the characteristics of the Herbst corpuscles resemble those of Pacinian corpuscles, though some differences are apparent. The rheobase threshold values for the most sensitive units are comparable, though slightly lower, than that quoted by Gray & Malcolm (1950) for the isolated Pacinian corpuscle (0·5 μm). However, it is probably not valid to compare measurements carried out under such different conditions. It is, nevertheless, clear that the two end-organs are of a similar order of sensitivity. There appears to be a greater spread of the lowest frequencies to which different Herbst corpuscles may respond than is the case for their mammalian counterparts, and a significantly greater ability to follow high frequencies. For example, Sato (1961) reported that Pacinian corpuscles may respond with steady, but not one-to-one, discharge up to a frequency of 1000 c/s, whereas in the duck some units gave one-to-one responses to stimulation at 1000 c/s and continued to give steady responses at about 2000 c/s. Again, with the same method of stimulation by way of the leg-bones, one-to-one following up to 700 c/s for cat Pacinian corpuscles was observed by Hunt & McIntyre (1960), in contrast with our finding of some units in the duck which would similarly follow a frequency of 1000 c/s.

It is interesting to note that most of the larger diameter fibres in the interosseous nerve appear to be those serving Herbst corpuscles. Dorward (1966) has shown that the central extensions of these larger fibres extend throughout the dorsal columns, and it has also been shown that weak, brief mechanical stimulation of the tibia evokes responses in the forebrain by way of the interosseous nerve (J. E. Gregory & A. K. McIntyre, in preparation). It thus appears likely that signals from these Herbst corpuscles are of behavioural significance, as postulated by Schwartzkopff (1948, 1949) for the bullfinch. Schwartzkopff was able to establish conditioned reflexes to vibratory stimulation of the perch at frequencies of 100–1600 c/s, and his threshold–frequency relationship is similar to those of low-threshold units in the present series with intermediate adaptive properties. In Schwartzkopff's experiments, the bullfinch's ability to discriminate between frequencies was poor. The lack of one-to-one following by many of our units over certain frequency-ranges, with either greater than or less than one-to-one relationships, may be relevant to this poor frequency discrimination. However, a highly developed capacity for discriminating between different frequencies need not be expected. Under natural conditions, weak and relatively brief vibratory disturbances of the supporting surface would probably be the most behaviourally significant stimuli. Our experiments support the notion that one function of the large number of Herbst corpuscles in the lower limbs of birds could be to act as a seismographic device giving warning of the approach of predators, through signalling weak vibrations of the ground or perch.

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REFERENCES

DORWARD, P. K. (1966). Responses of mechanoreceptors and intraspinal projection of their afferent fibres in the domestic duck. Ph.D. Thesis, Monash University, Australia.

DORWARD, P. K. (1970). Response patterns of cutaneous mechanoreceptors in the domestic duck. *Comp. Biochem. Physiol.* **35**, 729–735.

GRAY, J. A. B. & MALCOLM, J. L. (1950). The initiation of nerve impulses by mesenteric Pacinian corpuscles. *Proc. R. Soc.* B **137**, 96–114.

GRAY, J. A. B. & MATTHEWS, P. B. C. (1951). Response of Pacinian corpuscles in the cat's toe. *J. Physiol.* **113**, 475–482.

GREGORY, J. E. (1968). Properties of receptors in the duck's bill. *Aust. J. exp. Biol. med. Sci.* **46**, 4.

HUNT, C. C. (1961). On the nature of vibration receptors in the hind limb of the cat. *J. Physiol.* **155**, 175–186.

HUNT, C. C. & MCINTYRE, A. K. (1960). Characteristics of responses from receptors from the flexor longus digitorum muscle and the adjoining interosseous region of the cat. *J. Physiol.* **153**, 74–87.

HURSH, J. B. (1939). Conduction-velocity and diameter of nerve fibres. *Am. J. Physiol.* **127**, 131–139.

MCINTYRE, A. K. (1962). Cortical projection of impulses in the interosseous nerve of the cat's hind limb. *J. Physiol.* **163**, 46–60.

MCINTYRE, A. K. (1965). Perception of vibration. *Proc. Aust. Ass. Neurol.* **3**, 71–76.

MUNGER, B. L. (1971). Patterns of organization in peripheral sensory receptors. In *Handbook of Sensory Physiology, vol.* 1, *Principles of Receptor Physiology*, ed. LOEWENSTEIN, W. R. Berlin: Springer-Verlag.

QUILLIAM, T. A. & ARMSTRONG, J. (1961). Structural and denervation studies of the Herbst corpuscle. In *Cytology of Nervous Tissue*, pp. 33–38. London: Taylor and Francis.

QUILLIAM, T. A. & ARMSTRONG, J. (1963). Mechanoreceptors. *Endeavour* **22**, 55–60.

SATO, M. (1961). Response of Pacinian corpuscles to sinusoidal vibration. *J. Physiol.* **159**, 391–409.

SCHILDMACHER, VON H. (1931). Untersuchungen über die Funktion der Herbstschen Körperchen. *J. Ornithol.* **79**, 374–415.

SCHWARTZKOPFF, J. (1948). Der Vibrationssinn der Vögel. *Naturwissenschaften* **35**, 318–319.

SCHWARTZKOPFF, J. (1949). Über den Zusammenhang von Gehör und Vibrationssinn bei Vögeln. *Experientia* **51**, 159–161.

SKOGLUND, C. R. (1960). Properties of Pacinian corpuscles of ulnar and tibial location in cat and fowl. *Acta physiol. scand.* **50**, 385–386.