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POTASSIUM-INDUCED MOTION INCREASE IN A CENTRAL NERVOUS GANGLION

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SUMMARY

1. By means of light-beating spectroscopy, a four-fold increase in the modulation of laser light scattered at right angles by a locust (*Schistocerca gregaria*, Forskål) ganglion is detected when potassium ions replace sodium ions in the Ringer solution.

2. This is interpreted as an increased level of motion of the scattering particles (size 0·3–3·0 μm, if viscosity is taken to be 0·01 poises).

3. The amplitude of the potassium-response is similar at all frequencies in the range 6·3–150 Hz and is reversible on return to normal (Na^+) Ringer.

4. Desheathing the ganglion reduces the half-time of the potassium-response by 3–4 times.

5. By means of photon-correlation spectroscopy it was estimated that less than 10% of the tissue contributes to the motion detected.

6. Cyanide (1–2 mM) typically enhances the potassium-response and renders it irreversible, suggesting that the response is thermally rather than metabolically-driven. In addition, the dependence of both the correlation function and the power spectrum on the scattering angle is in the direction predicted for a diffusive process.

7. Cobaltous ions (2–10 mM), which block calcium entry into nerve cells, depress the potassium-response.

8. It is proposed that potassium-depolarization and the resultant calcium entry into the cells causes a partial liquefaction of the cytoplasm which is detected as an increase in the level of Brownian motion. This mechanism could be used in vesicular release or in growth.

INTRODUCTION

Several optical properties of nervous tissue have been measured under a variety of physiological conditions by techniques utilizing fluorescence, ultra-violet absorption, infra-red absorption and emission, birefringence and the scattering of incoherent light (see Cohen, 1973). The bulk of these investigations have attempted to correlate structural changes in the nerve membrane with its electrical activity. Few such studies have been concerned with dynamic events within the nerve cell. A good deal of circumstantial evidence suports the view that many aspects of neuronal function, including growth, axoplasmic transport and neurotransmitter release involve the movements of vesicular

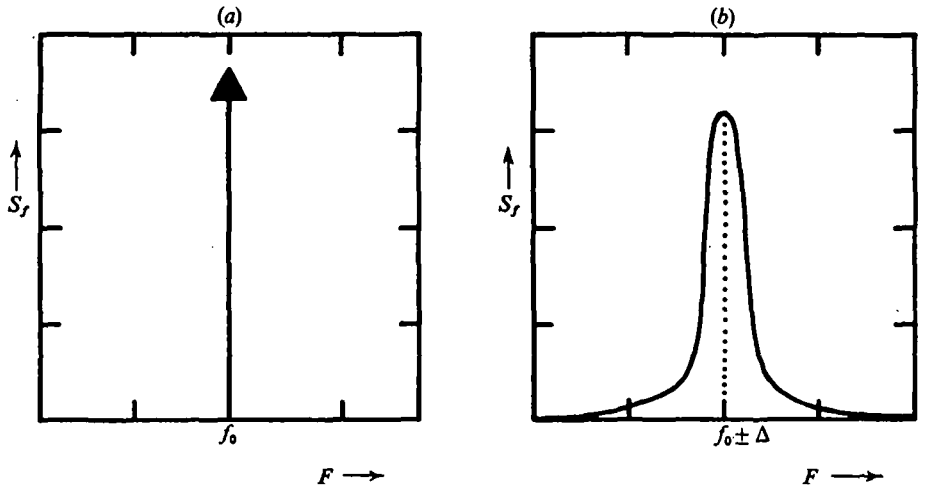

Fig. 1. Schematic optical spectra for the incident (*a*) and scattered light (*b*) in a laser light scattering experiment. The broadening in (*b*) is due to the modulating effect of uniform spherical scatterers undergoing Brownian motion. The curve in (*b*) is a Lorentzian; modulated frequencies are due to Doppler shift. Abbreviations: S_f, power per unit bandwidth; F, frequency; f_0, optical frequency of laser (*ca.* 10^{14} Hz); Δ, modulation.

organelles within the cytoplasm. Shaw & Newby (1972) attempted to measure the motion of synaptic vesicles within a ganglion using scattered laser light. This report describes the further development of this work using light-beating spectroscopy and photon-correlation spectroscopy. A brief account of some of these findings has already been reported (Piddington & Sattelle, 1975*a*); the techniques used together with reviews of other such work in biology can be found in the recent volume edited by Cummins & Pike (1974) and the article of Piddington & Sattelle (1975*b*).

In these experiments an incident carrier wave (laser light) is modulated by the movements of small particles within the beam. The spectrum of the laser can be represented as a single delta function at the optical frequency; this spectrum is broadened by diffusive particle motion (Fig. 1). The power as a function of frequency can be calculated for the scattered light by assuming that the moving particles induce a Doppler-shift to the laser light. On a non-linear detector, such as a photomultiplier, these shifted frequencies beat either with each other or with the carrier in a manner analogous to sound-beating and produce a fluctuating photocurrent, the frequency composition of which corresponds to that of the beat frequencies.

In the method of light-beats (see Cummins, 1974) a wave analyser is used to perform a frequency analysis of the photocurrent. The analyser is tuned to various frequencies in turn, and the power spectrum of the scattered light is built up (Fig. 2). This method of signal processing has a relatively poor time resolution and for experiments in which the time course of a motion change is to be studied it is necessary to operate at a single frequency.

In the method of photon-correlation (see Jakeman, 1974) a digital computer performs an autocorrelation on the photomultiplier output which in this instance consists of a pulse train, each pulse corresponding to a single photon detection. The detection of single photons enables these experiments to be carried out at lower light levels.

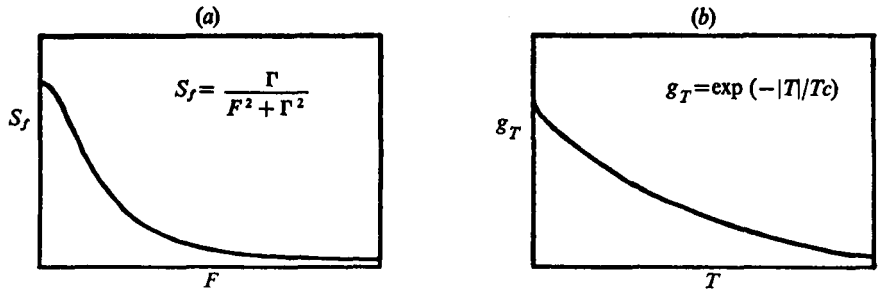

Fig. 2. Schematic representation of two ways of displaying the modulation of laser light scattered from particles undergoing Brownian motion: (*a*) power spectrum (F, frequency; S_f, power per unit bandwidth; Γ, half bandwidth); (*b*) autocorrelation function (g_T, correlation; T, time; Tc, time constant).

This power spectrum (*a*) derives from the optical spectrum (Fig. 1*b*) by light-beating. When the broadened optical signal (Fig. 1*b*) beats with unmodulated laser light (Fig. 1*a*) a beating spectrum (*a*) (which is a Lorentzian curve) of half bandwidth equal to the optical half bandwidth results (*heterodyne mixing*). When the broadened signal beats only with itself the half bandwidth is double the optical half bandwidth (*homodyne mixing*) (see Cummins & Pike, 1974). The correlation function (*b*) is an exponential and is a Fourier transform of the spectrum (*a*).

Photon emission times from a continuous laser (as used here) follow a Poisson law of probability (see Cummins & Pike, 1974). Particle movements in the laser beam result in photon arrival times at the detector which depart from the Poisson law. It is this departure or 'photon bunching' which the photon correlater detects. The bunching can be thought of as arising from a fluctuating probability superimposed on the Poisson probability: the fluctuations having the same composition in the frequency domain as the light-beating signal. The light-beating signal is the (smoothed) envelope of a large number of individual photoelectric events. The power spectrum and the autocorrelation function are a Fourier transform pair.

The overall aim of this investigation is to determine more precisely the underlying mechanism of the motion increase detected in nervous tissue in response to potassium ions (Shaw & Newby, 1972; Piddington, 1974; Piddington & Sattelle, 1975*a*). In particular, an attempt is made to test the hypothesis that the potassium-induced motion increase is the result of the liquefaction of cytoplasm (see Shaw & Newby, 1972).

MATERIALS AND METHODS

Preparation

Adult male locusts (*Schistocerca gregaria* Forskål) were used throughout these experiments. Prothoracic ganglia were dissected under normal Ringer together with the anterior and posterior interganglionic connectives. The excised preparation was pinned to a wax block, the points of attachment being close to the cut ends of the connectives, several millimetres from the ganglion. A ganglion prepared in this way was described as *intact*. In a limited number of experiments *desheathed* ganglia were prepared in which the peripheral layers of cells and the acellular connective-tissue sheath were removed using fine stainless-steel needles (cf. Callec & Sattelle, 1973). Unless otherwise stated in the text the results described in this report refer to *intact*

ganglia. The block carrying the preparation was transferred to an optical cuvette containing normal Ringer (Fig. 3). The lower end of the wax block was fixed to a perspex base which formed a close fit with the internal walls of the cuvette. Care was exercised when loading the cuvette to avoid the inclusion of air bubbles.

The normal (Na^+) Ringer used in these experiments was based on that described by Usherwood and Machili (1968) and contained (mM): NaCl, 140; KCl, 10; $CaCl_2$, 2; NaH_2PO_4, 4; Na_2HPO_4, 6 (pH 6·8, $CaCl_2$ added only on day of experiment). High-potassium (K^+) Ringer was prepared by simply replacing NaCl by KCl to maintain isotonicity. When cobaltous ions (2–10 mM) were added to the Ringer, tris replaced the phosphate buffer. All solutions were filtered using 0·45 μm grade millipore filters. Experiments were performed at room temperature.

Apparatus for light-beating spectroscopy

Incident light was provided by a 5 mW He-Ne laser (632·8 nM, Spectra-Physics model 120). The laser was supported on a small wooden table with screws for gross vertical alignment. This table was placed on an aluminium rotating arm with three adjustable feet. The arm rotated about a point in the main aluminium (6 mm plate) experimental table which was also mounted on adjustable feet. On this table were fixed a micromanipulator carrying a cuvette holder, a 50 cm light-tight tube with a 2 mm aperture at one end and a photomultiplier (EMI type 95265) mounted at the other end. A convex glass lens (f = 4·0 cm) was inserted into the light-tight tube 4 cm from the screen of the photomultiplier. At the open end of this tube a movable strip of continuously graded film enabled adjustment of the incoming light level. Light scattered by the ganglion was directed on to the surface of the photomultiplier which was operated at 500 V. The whole apparatus was shock mounted on a 30 mm thick steel plate resting on an inflated rubber tube (a large vehicle inner tube). Fig. 3 illustrates the main features of the experimental set-up.

The output of the photomultiplier was led via a Tektronix 122 amplifier to a Tektronix 502A oscilloscope. Frequency analysis of the photomultiplier current was done point-by-point with a Bruel and Kjaer analyser (type 2107) and a random-noise voltmeter (type 2414). The output of the random-noise voltmeter was routinely displayed on a chart recorder. Fig. 4 shows the layout of the apparatus.

Experimental procedure for light-beating spectroscopy

The laser beam was aligned at the appropriate angle (usually 90°) to the light tube and photomultiplier. By means of the adjusting screws on the laser table the beam was aligned with the aperture of the light tube. The preparation was then placed in the laser beam (Fig. 3) and aligned using the micromanipulator. By adjusting the micromanipulator, and at the same time following the d.c. output of the photomultiplier (through a low-pass filter) on the oscilloscope, it was possible to position the ganglion so as to give the maximum signal. Once this position had been achieved the incoming light level was adjusted using the continuously graded film to give a 50 mV d.c. output. The low-pass filter was then switched out, an appropriate frequency selected on the analyser and changes in the photocurrent followed during exposure of the preparation to various physiological solutions. A solution changer comprising stainless-steel rods, attached, by flexible tubing to syringes containing test solutions, was mounted on

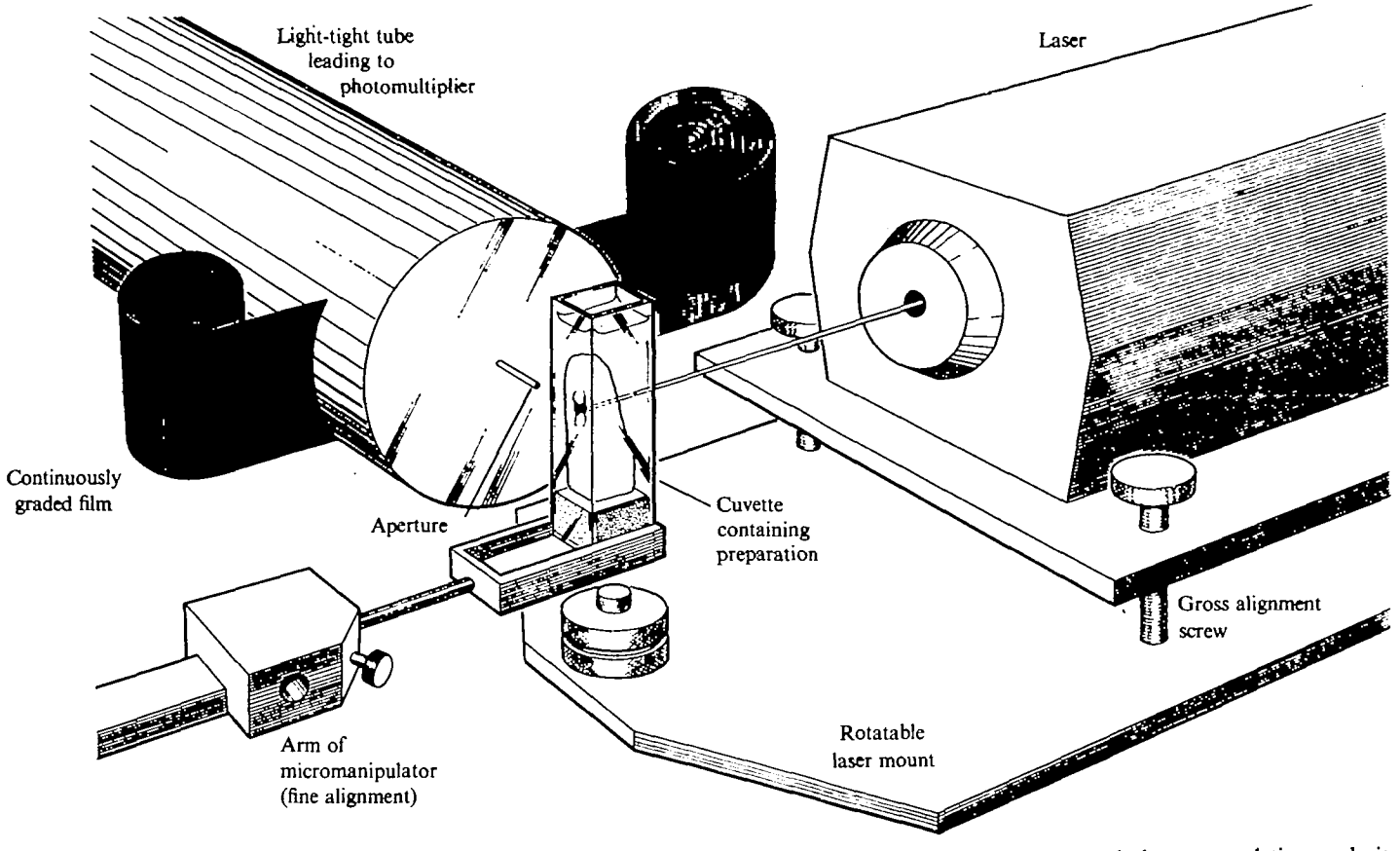

Fig. 3. Diagram of light-scattering apparatus. Laser and specimen positions are the same for both light-beating and photon-correlation analysis. For light-beating, a larger aperture to the photomultiplier is generally required; for photon-correlation, twin pinhole collimation is more usual. Both systems can also make use of lenses (see Figs. 4, 5, also Clark *et al.* 1970).

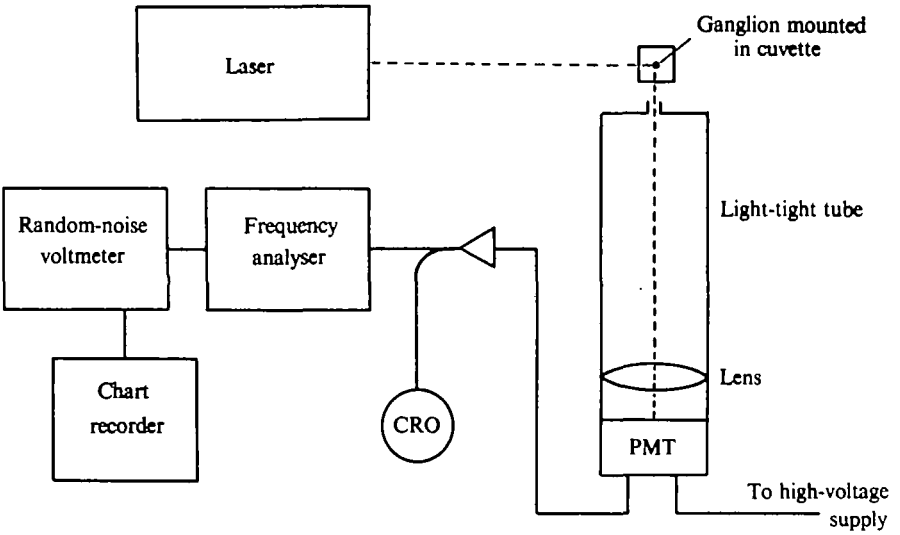

Fig. 4. Schematic layout of the instrumentation for light-beating spectroscopy. Abbreviations: CRO, oscilloscope; PMT, photomultiplier tube. The lens is located one focal length from the photocathode so as to select parallel rays. The random-noise voltmeter provides a range of time constants.

a separate micromanipulator. By this means solutions could be delivered into the cuvette with minimum disturbance to the preparation. Except where indicated all experiments were performed at a scattering angle (Θ) of 90°.

When compiling power per unit bandwidth (S_f) versus frequency (F) spectra of laser light scattered from a ganglion, a number of computations were applied to the observed meter reading on the random-noise voltmeter. Each meter reading obtained from the ganglion was squared to convert it to power units and this power term was then divided by a term (constant for a given frequency) to compensate for the percentage bandwidth (as opposed to constant bandwidth) analyser used in our experiments. Following this correction the power per unit bandwidth (G) was determined for each frequency. G includes a shot-noise component resulting from the quantal nature of the detection process (i.e. photon arrivals). A value for the shot-noise was obtained by scattering from a teflon block (in place of the ganglion) and was similarly adjusted to constant bandwidth. The shot-noise (T) was then subtracted from (G) to give the values of S_f plotted in our power versus frequency spectra (see Fig. 9). Teflon block scattering also controlled for solution clarity and laser drift. The d.c. output of the photomultiplier was set to 50 mV during the scattering experiments from which values of G and T were determined. In some cases results were presented as reciprocal plots ($1/S_f$ versus F^2). The intercept on the negative F^2 axis in such a plot is equal to the square of the half-bandwidth (Γ^2) (see Cummins & Pike, 1974).

Although a quantitative correlation of increases in S_f with increases in particle mobility cannot be attempted for a complex preparation such as a ganglion, it is reasonable to interpret an increase in S_f as an overall increase in motion in the tissue (see Cummins & Pike, 1974; Piddington & Sattelle, 1975*a*).

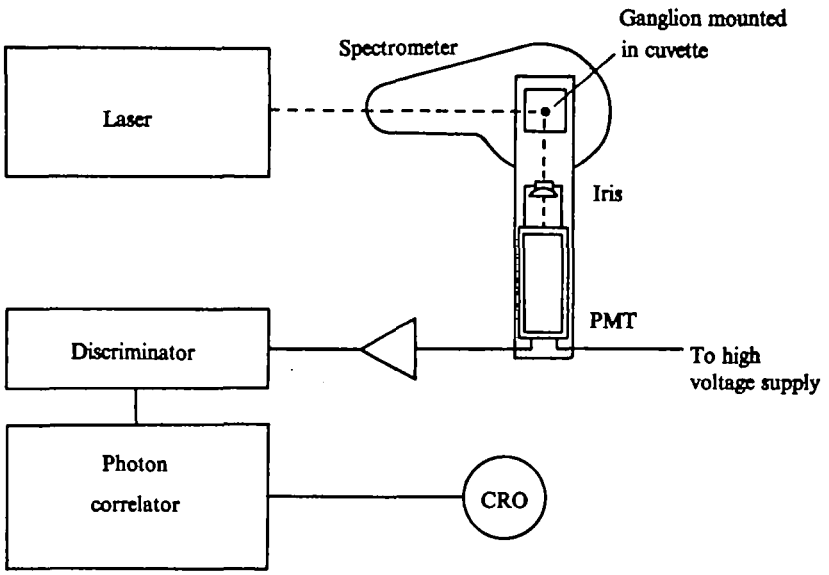

Fig. 5. Schematic layout of the instrumentation for photon-correlation spectroscopy. Abbreviations: CRO, oscilloscope: PMT, photomultiplier tube.

Apparatus and experimental procedure for photon-correlation spectroscopy

A limited number of experiments were performed using the Precision Devices photon-correlation spectrometer (Malvern System 4300). Fig. 5 illustrates the layout of this system. A 15 mW He-Ne laser (632·8 nm, Scientifica and Cook) was used in experiments with this apparatus. The cuvette, loaded as before, was placed in the centre of the turntable unit and an appropriate scattering angle chosen on the degree and vernier scales (to an accuracy better then 0·25 degree). The photomultiplier (Type PDS 30, S20 cathode) had a low dark-current; both magnetic and electrostatic shielding being incorporated. The output pulses from the photomultiplier were shaped by a discriminator (into 30 ns wide pulses from a 50 Ω source), amplified and fed into the correlator. The autocorrelation function was displayed on an Advance Instruments OS 1000 oscilloscope. In all these experiments the constant temperature bath, normally a standard part of the apparatus, was removed to facilitate alignment of the preparation.

With this apparatus autocorrelation functions were obtained for ganglia in Na^+-Ringer and K^+-Ringer at a scattering angle (Θ) of 90°. Autocorrelation functions were also obtained at angles other than 90°. The mounting of the preparation, the system for changing solutions and the alignment procedure were similar to those described for the light-beating spectroscopy experiments.

In photon-correlation and light-beating control experiments it was shown that light scattered from moving structures within the tissue was mixed with light from fixed scatterers so as to produce *heterodyne* conditions (see Cummins & Pike, 1974). Our estimates of particle sizes (see Results section) incorporated this finding.

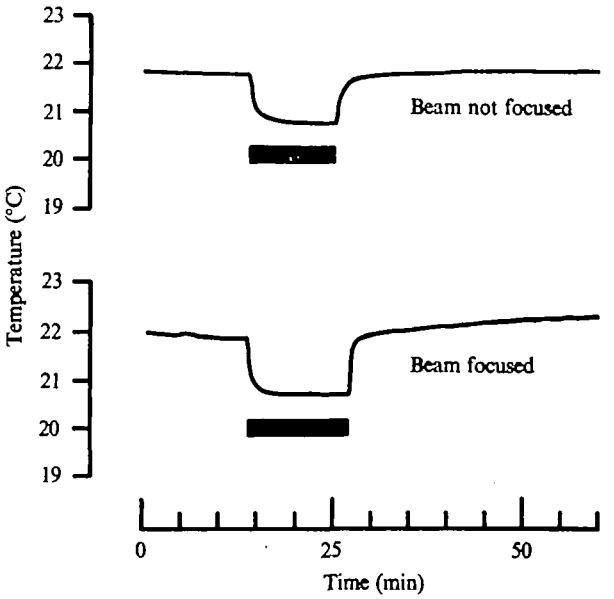

Fig. 6. Temperature changes of Bead (type THB 12) thermistor during laser illumination. Bars denote blocking off of laser beam.

Heating effects of the laser

It could be argued that some of the results reported here are attributable at least in part to laser damage. The reversibility of the phenomena argue against this. Two groups of control experiments provided evidence that laser damage did not account for the motion increase induced by potassium and reported in earlier investigations (Shaw & Newby, 1972; Piddington & Sattelle, 1975*a*). First, by focusing the beam to $\frac{1}{25}$ of the normal cross-sectional area a 25-fold increase in the intensity per unit area could be achieved. This did not affect either the amplitude of the potassium-response or its reversibility. Furthermore, equivalent results were obtained from ganglia illuminated by either 5 or 15 mW lasers. Secondly, the laser beam was directed at a bead thermistor (type TH12) mounted in the same way as the ganglion. With the beam focused a 1–2 °C elevation of temperature was noted. Using an unfocused beam smaller temperature rises were noted (Fig. 6). These values probably represented the maximum heating effect, since the black thermosensitive bead would absorb more heat than a ganglion. All experiments on ganglia reported here were done with the laser unfocused.

Pressure effects of laser light

Light carries momentum and on striking an object produces a small force on the object known as radiation pressure (Askin, 1972). For particles in the size range estimated in these experiments (see Results section), the pressure effects induced by the low-power lasers employed are too low by about five orders of magnitude to be detected with our instruments.

Estimation of particle size

For a suspension of monodisperse spheres of uniform size, which are free to move independently of one another, the frequency spectrum of the intensity of the scattered light (and therefore the frequency composition of the photocurrent) is a Lorentzian curve (see Pusey, 1974). The half bandwidth of the spectrum (Γ) depends on the diffusion coefficient (D) of the particles as follows (see Clark *et al.* 1970):

$$\Gamma(\Theta) = 2D\left(\frac{4\pi}{\lambda/n}\sin\tfrac{1}{2}\Theta\right)^2, \tag{1}$$

where Θ is the scattering angle; λ, the wavelength of the incident light; n, the refractive index of the solution.

Under the conditions outlined above, the diffusion coefficient (D) is related to the particle size by the Stokes–Einstein relationship:

$$D = \frac{k_B T}{6\pi\eta a}, \tag{2}$$

where k_B is Boltzmann's constant; T, absolute temperature; η, viscosity; a, radius of the particle. Applying equations (1) and (2) to spectral half bandwidths (Γ) derived from ganglia, it is possible to calculate the approximate size range of the moving particles.

RESULTS

Potassium-induced motion increase in intact and desheathed ganglia

In light-beating experiments, scattered light from a ganglion in normal (Na^+) Ringer consistently gave rise to a photocurrent modulated by low frequency fluctuations, the peak-to peak value of which was approximately one fifth of the average scattered intensity (d.c. output of photomultiplier). We estimated that less than 10% of the tissue was responsible for these fluctuations, the remainder acting as a fixed solid scatterer. The fluctuations detected in light-beating studies increased by approximately four-fold in K^+-Ringer (see also next section). Such changes were followed with time for a number of preparations using a 40 Hz setting on the wave analyser. The effects of potassium were reversible and were not accompanied by a change in the average scattered intensity. The result of a typical experiment using an *intact* ganglion is shown in Fig. 7. For such preparations the time taken to reach a steady, elevated amplitude for the photocurrent was 15–30 min. In nine experiments on *intact* ganglia the mean half-rise time ($t_{0.5}$) for the onset of the potassium-response was 8·1 (S.E. ± 1·0) min; the mean $t_{0.5}$ for recovery in normal Ringer was 7·0 (S.E. ± 0·9) min. Repeated potassium-responses each of very similar magnitude were readily obtained from a single *intact* ganglion, by successive exposure to 20 min. potassium-pulses followed by recovery in Na^+-Ringer (see Piddington & Sattelle, 1975*a*). A ganglion left in K^+-Ringer for 24 h showed an unchanged elevated value for S_f. Both short-term and long-term potassium-responses are interpreted as an increased level of motion (see Methods, also Piddington & Sattelle, 1975*a*).

In experiments with *desheathed* ganglia the potassium-response was completed within 2–5 min (Fig. 8). For three experiments (on two desheathed ganglia) the mean

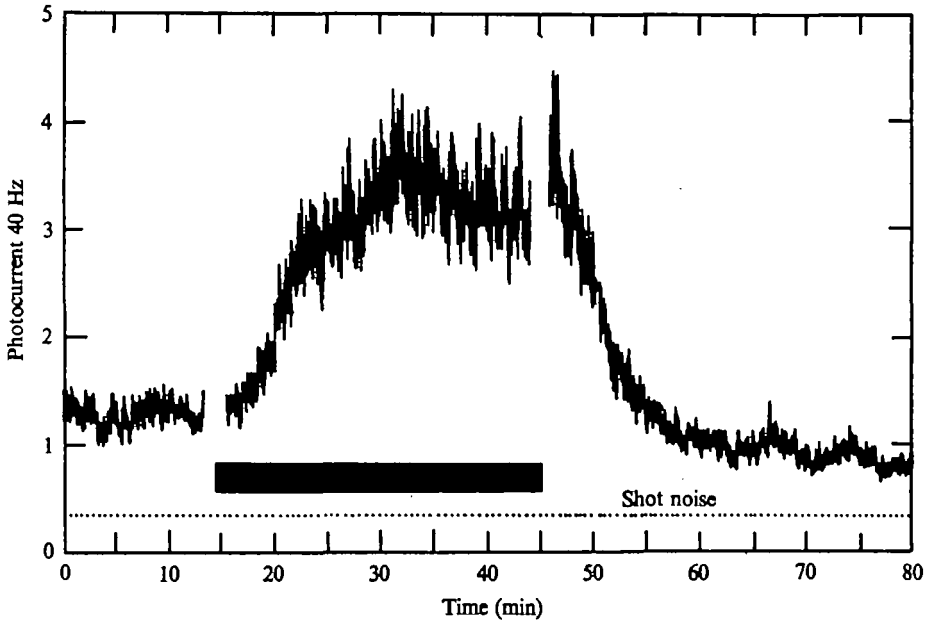

Fig. 7. Potassium-induced motion-response. Changes in photocurrent (arbitrary units) at 40 Hz recorded from an *intact* ganglion. Black bar denotes exposure to K^+-Ringer; for the rest of the experiment the preparation is bathed in normal (Na^+) Ringer. (Time constant of random-noise voltmeter = 30 sec.)

$t_{0.5}$ for the onset of the potassium-response was only 1·8 (S.E. ± 0·8) min; the mean $t_{0.5}$ for recovery was 2·2 (S.E. ± 1·2) min. In another experiment, exposure of a *desheathed* preparation to K^+-Ringer for a period of 25 min prevented recovery of the potassium-response on subsequent re-exposure to Na^+-Ringer. It was clear that desheathing considerably reduced the time taken for both the onset and the recovery of the potassium-response in the ganglion.

Power spectra of scattered light from ganglia

By tuning the wave analyser through about thirty different frequency settings between 6·3 and 150 Hz, it was possible to construct a power (S_f) versus frequency (F) curve for the light scattered by a ganglion (Fig. 9). Replacing the sodium level of normal Ringer by potassium for four separate *intact* ganglia resulted in a mean increase in S_f by a factor of 3·8 (S.E. ± 0·94); this value was calculated using the data points at all frequencies measured. No consistent differences were noted in the magnitude of the change in S_f by comparing low and high frequency data (in the range 6·3–150 Hz). Also the spectral shapes observed from ganglia in Na^+-Ringer and K^+-Ringer appeared very similar (Fig. 9). The reversibility of the potassium-response at all frequencies can be seen by a comparison of spectra labelled Na_1, K and Na_2 (Fig. 9).

The spectra obtained in Na^+-Ringer and K^+-Ringer deviated from a single Lorentzian. The reciprocal plots ($1/S_f$ versus F^2) departed from a straight-line, being consistently convex upwards, as illustrated in Fig. 10 for a ganglion in K^+-Ringer. An approximate range of particle sizes was estimated by fitting straight lines to both low and high frequency data in the $1/S_f$ versus F^2 plots and assuming heterodyning (see Methods section). Spectral data in the frequency range 6·3–150 Hz obtained from

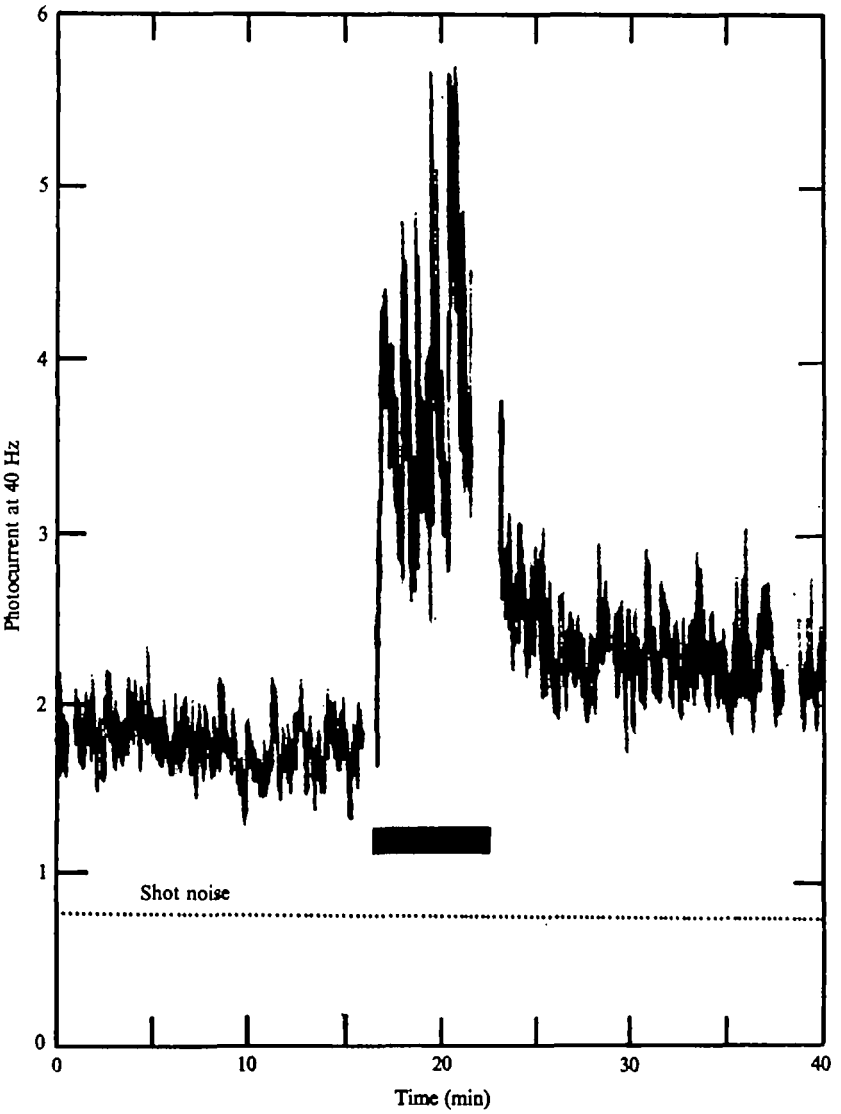

Fig. 8. Effect of desheathing on the potassium-response. Changes in photocurrent (arbitrary units) at 40 Hz recorded from a *desheathed* ganglion. Black bar denotes exposure to K^+-Ringer; for the rest of the experiment the preparation is bathed in normal (Na^+) Ringer. (Time constant of random-noise voltmeter = 10 sec.)

four ganglia gave a range of half-bandwidths from 7·5 to 86 Hz corresponding to a range of particle sizes of 0·3–3 μm diameter. This estimation assumed a viscosity equal to that of water. If the viscosity of nerve cytoplasm is taken to be 0·06 poises (Rieser, 1949), a particle size range of 0·05–0·5 μm diameter is obtained (see also Piddington & Sattelle, 1975*a*).

The constancy of the spectral shape derived from ganglia in both Na^+ and K^+ Ringers eliminated a simple decrease in the viscosity throughout the cytoplasm as an explanation of the motion increase. Any such overall change in viscosity *per se* would

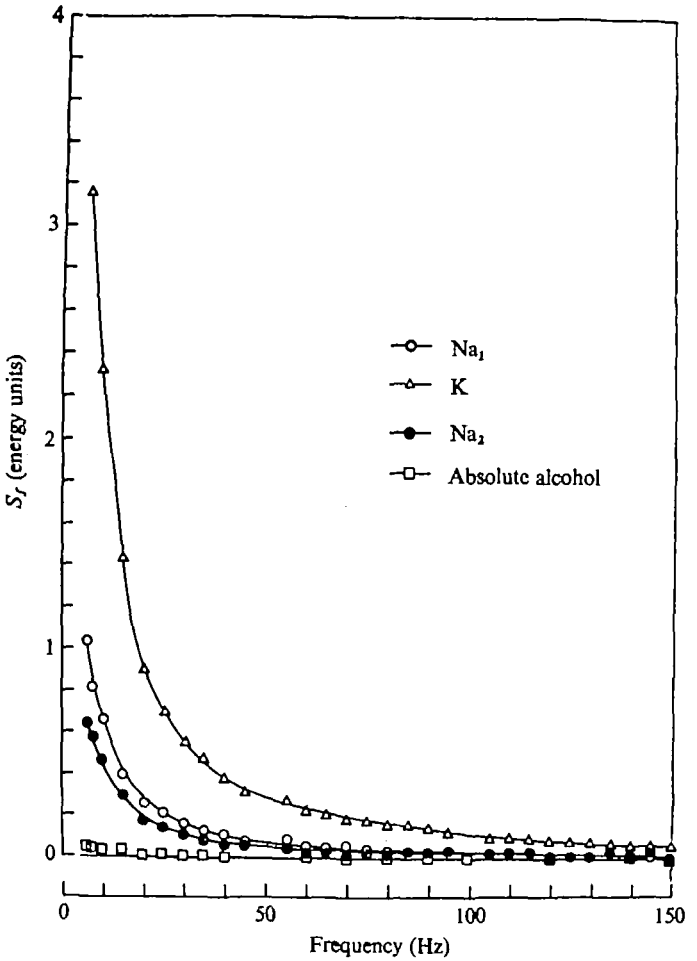

Fig. 9. Spectra from a ganglion showing motion response. Na_1 in normal Ringer initially; K, in K^+-Ringer; Na_2, in normal Ringer following potassium-pulse; Fixation (absolute alcohol) abolishes motion. Each point represents the average value of S_f (power per unit bandwidth) at a chosen frequency for a 1 min sample period following equilibration to the appropriate physiological solution. Curves do not fit single Lorentzians.

be detected as a change in Γ (see equations 1 and 2; also Cummins, 1974). The similarity in frequency composition noted for scattered light from ganglia treated with either Na^+ or K^+ indicated that the particle-size distribution of the scatterers remained constant. The increase in S_f (K^+ compared to Na^+) could, therefore, be explained as an increase in the average displacement of a population of particles which were hitherto more constrained. In the polarized nerve cell the cytoplasm probably exists as a gel (Bear, Schmitt & Young, 1937; Hodgkin & Katz, 1959; Gilbert, 1972). When the cell is depolarized a proportion of the cytoplasm may change to a sol due to calcium entry and this would result in an increased displacement of all particles (see also Shaw & Newby, 1972; Piddington & Sattelle, 1975*a*, *b*).

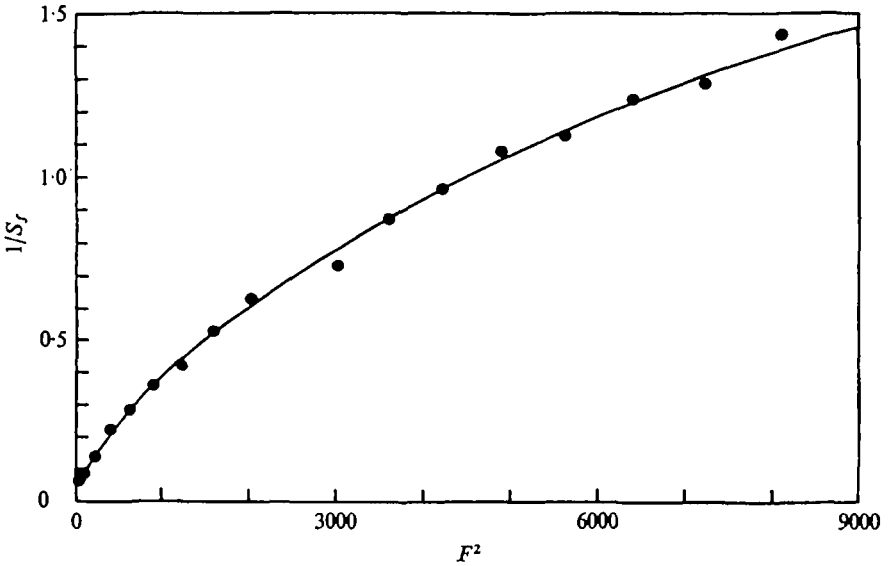

Fig. 10. Plot of reciprocal power($1/S_f$) versus frequency squared (F^2) for a ganglion in K^+-Ringer. For uniform spheres undergoing Brownian motion this plot would be a straight line. This non-fit would be expected from a heterogeneous scatterer such as the ganglion. Ganglia in Na^+-Ringer give rise to reciprocal plots of similar shape.

Table 1

Number of experiments	K^+-Ringer induced an increase in motion	K^+-response enhanced by CN^-	K^+-response reduced by CN^-	K^+-response unaffected by CN^-
10	10	7	2	1

Effects of cyanide on the potassium-response

To test the metabolic dependence of the potassium-response, cyanide (1–2 mM KCN) was added to the K^+-Ringer when the elevated steady level of motion had been achieved. Cyanide did not abolish the potassium-response in any of ten experiments. Typically it increased the potassium-response by about 20% and prevented its reversal in sodium (Table 1). Control experiments with various potassium chloride concentrations in the Ringer confirmed that the enhanced response was due to the presence of cyanide ions rather than potassium ions. Thus the motion induced by potassium was not dependent on metabolic energy.

Effects of calcium on the potassium-response

The effects of calcium on the potassium-response were studied in the following ways: by varying the calcium ion concentration; by the addition of a chelating agent (EGTA); by substitution of calcium ions by magnesium ions; by the addition of low concentrations of cobaltous ions. Only the cobalt treatment produced a positive result. Adding cobaltous ions to normal Ringer at concentrations of 2–10 mM in each of six experiments reduced the amplitude and, in most cases, slowed the onset of potassium-response. Amplitude reductions of 10–60% were noted and changes in the $t_{0\cdot5}$ (onset) varied from no change to a $\times 2$ increase. The result of an experiment in which the presence of cobaltous ions (5 mM) reduced the potassium-response is shown in Fig. 11.

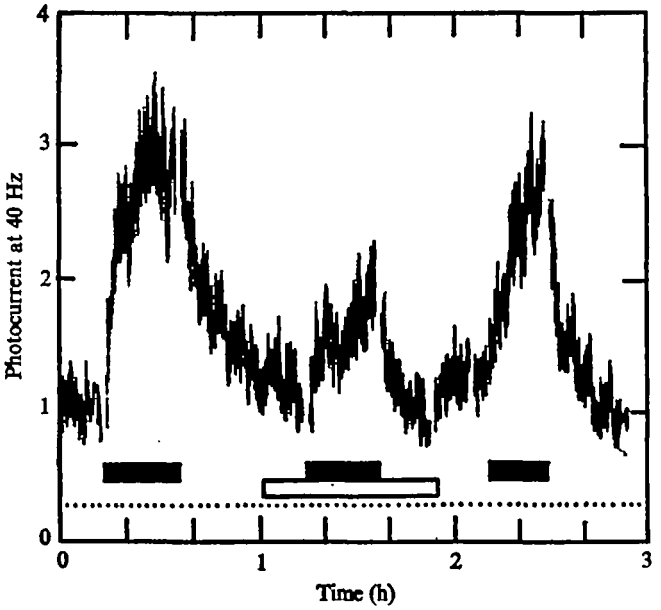

Fig. 11. Inhibitory effect of cobalt on the potassium-response. Changes in photocurrent (arbitrary units) at 40 Hz during successive exposures to K^+-Ringer (black bars). For the rest of the experiment the preparation is bathed in Na^+-Ringer. During the period denoted by the white bar, cobaltous ions (5 mM) are added to the bathing media. (Time constant of random-noise voltmeter = 30 sec.)

Omitting calcium ions from normal Ringer (2 experiments) did not affect either the amplitude or the time-course of the potassium-response. The response was similarly unaffected when 1 mM EGTA was added to calcium-free Ringer (2 experiments). Elevating the calcium ion concentration of normal Ringer from 2 mM to 10 mM was without effect in two experiments. The substitution of calcium ions in normal Ringer by magnesium ions also failed to modify either the time-course or the magnitude of the motion increase produced by potassium ions (2 experiments).

Analysis of scattered light at various scattering angles (Θ)

(*a*) *Light-beating experiments*

Spectral data were obtained from ganglia at two well-separated scattering angles (Θ) of 90° and 18°. Results were plotted as $1/S_f$ against F^2. Using spectral data in the range 6·3–40 Hz, for a ganglion in K^+-Ringer $\Gamma_{90°}$ was 16·3 Hz and $\Gamma_{18°}$ was found to be 3·2 Hz. Although problems of multiple scattering complicate the interpretation of data obtained from a complex tissue such as a ganglion this five-fold reduction in the half-bandwidth accompanying a reduction of Θ from 90° to 18° is in the direction that would be predicted for a diffusive process (Cummins, 1974).

(*b*) *Photon-correlation experiments*

Examples of the autocorrelation functions obtained from a ganglion in K^+-Ringer are shown in Fig. 12. Similar curves were recorded from four ganglia at 90°. The effect of increasing the scattering angle (Θ) to 120° was investigated. The autocorrelation function at 120° was more dished (faster) as illustrated in the insert of Fig. 12. This

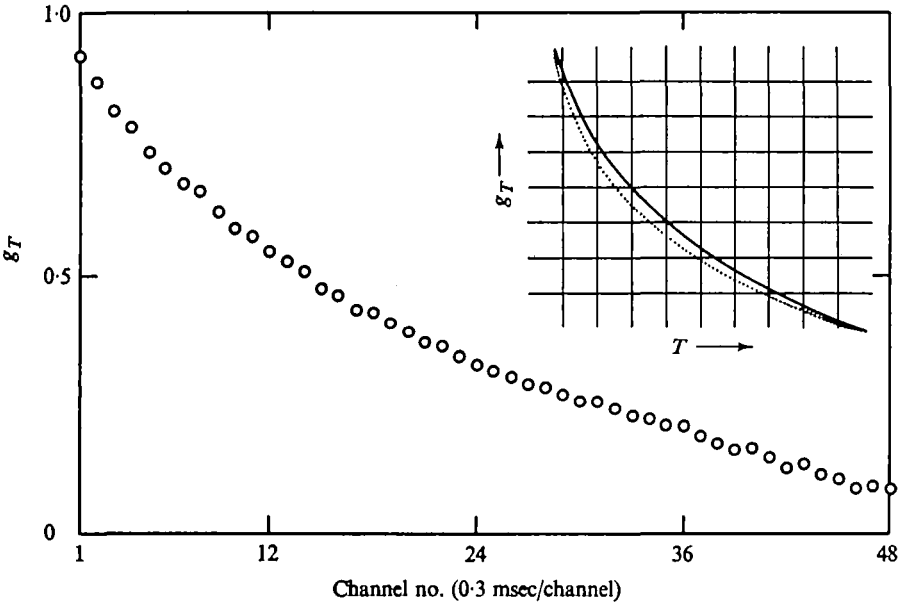

Fig. 12. Autocorrelation function recorded from a ganglion in K+-Ringer, plotted as correlation (g_T) against channel number which is proportional to time. Insert is tracing of oscilloscope display of autocorrelation functions from a ganglion in K+-Ringer at 90° (solid line) and 120° (dotted line). The increased 'dish' at 120° is in the direction expected for particles undergoing Brownian motion (see Cummins & Pike, 1974).

shift in the autocorrelation function was in the direction that would be predicted for a diffusive process (Jakeman, 1974). (The shape of the autocorrelation functions also indicates a distribution of particle sizes as would be expected from the light-beating spectra. Our earlier comments on multiple scattering also apply.)

DISCUSSION

The techniques of light-beating spectroscopy and photon-correlation spectroscopy employed in this study are of particular value in studying the diffusive motion of particles of uniform size (monodisperse) in solution (Pusey, 1974). Various kinds of directed motion or flow can also readily be analysed when scatterers are introduced into an otherwise homogeneous fluid (Crosignani & Di Porto, 1974). However, as the polydispersity of the scattering system and the heterogeneity of the medium (fluid) increase, the interpretation of light-scattering data becomes less quantitative. Considerable caution is therefore required in some aspects of the interpretation of results obtained from a preparation as complex as nervous tissue. It is nevertheless clear that locust ganglia in normal (Na^+) Ringer show a level of motion which is easily detectable by means of light-beating spectroscopy and photon correlation spectroscopy. This movement derives from less than 10% of the tissue, the remainder acting as a fixed solid scatterer (e.g. sheaths, tracheae, etc.). Substituting the sodium ions in normal Ringer by potassium ions leads to an approximately four-fold increase in the modulated level of the scattered light and hence the level of motion in the ganglion. Desheathing

the ganglia decreases the half-time for both onset and recovery of the potassium response by a factor of 3-4. It is relevant to note that the half-time for the change in the concentration of potassium ions in the fluid immediately bathing the surfaces of a giant axon in a *desheathed* central nervous connective of *Periplaneta americana* is about half a minute (Treherne *et al.* 1970). Bearing in mind that the extracellular diffusion pathway is more extensive in the ganglion than in the connective, it is reasonable to conclude that the potassium-induced motion increase is linked closely to the depolarization of the tissue. Gross electrical stimulation can produce a K^+-like response (Piddington & Sattelle, 1975*a*).

Cyanide (1–2 mM KCN) failed to abolish the potassium-response and in seven out of ten experiments enhanced it. Metabolic energy does not, therefore, appear necessary for the motion increase. A comparison of spectra obtained from a ganglion in K^+-Ringer reveals a five-fold reduction in the half-bandwidth (Γ) accompanying a reduction of the scattering angle (Θ) from 90° to 18° ($\Gamma_{90°} = 16{\cdot}3$ Hz; $\Gamma_{18°} = 3{\cdot}2$ Hz). This is in the direction that would be predicted for a diffusive process (Cummins, 1974). Similarly, increasing the scattering angle (Θ) from 90° to 120° decreases the apparent time-constant of the autocorrelation function and this is also in the direction that would be predicted for a diffusive process. In both cases problems of multiple scattering from a complex tissue preclude a more detailed analysis but the evidence presented here strongly suggests that the potassium-response is best explained by a passive physical mechanism.

An attempt has been made to determine the size range for the bulk of the moving scatterers. A particle-size range of 0·3–3·0 μm was estimated assuming a viscosity equal to that of water. If the viscosity of cytoplasm is taken to be 0·06 poises, which is the average value obtained for giant fibres in the lobster ventral nerve cord (Rieser, 1949) the size range is reduced to 0·05–0·5 μm. (The viscosity figures for the axoplasm of lobster giant fibres were estimated by following the movements of oil droplets injected into axons. This method may well be insensitive to local changes in viscosity within the fibre and the relevant viscosity may be closer to that of water. See also Piddington & Sattelle, 1975*a*).

It is known from work on squid axon and crab nerve that calcium entry into these cells accompanies depolarization (Fluckiger & Keynes, 1955; Keynes & Lewis, 1956; Baker Meves & Ridgway, 1973*b*). Shaw & Newby (1972) report a reduced potassium-response in the presence of EGTA (1 mM). However, varying the calcium ion concentration (0–10 mM), the addition of a chelating agent (EGTA 1 mM) and the substitution of calcium ions by magnesium ions are all without effect in our experiments. Nevertheless, cobaltous ions (2–10 mM) consistently reduce the amplitude of the potassium induced motion increase by between 10% and 60%. This cation is known to block inward calcium movements in invertebrate nerves (Geduldig & Junge, 1968; Baker, Meves & Ridgway, 1973*a*). The potentiating effect of cyanide on the potassium-response is also relevant in this context. It might be expected that the presence of this inhibitor would increase the intracellular calcium concentration first by reducing the active extrusion of sodium from the cells thus increasing the likelihood of calcium entry and, secondly, by releasing calcium from intracellular stores (Baker, Hodgkin & Ridgway, 1971). Thus, although more experiments on calcium are required, preferably with simpler preparations, the results to date suggest that blockage of

calcium entry lowers the amplitude of the potassium-response and that elevation of intracellular calcium levels enhances it.

Osmotic phenomena such as a potassium-induced increase in the swelling of the tissue, thereby allowing greater displacements of cellular components, could conceivably account for the observed changes in motion. It is relevant to note here that potassium-induced swelling of guinea-pig cerebral cortical slices (Lipton, 1973) and nerve ending particles from rat brain cortex (Kamino, Inouye & Inouye, 1973) have been detected using light-scattering techniques. However it seems unlikely that the results presented here can be interpreted on this basis for two main reasons. First, the work on the rat cortex reveals that the potassium-induced swelling is reduced by increasing the calcium ion concentration, an effect in the opposite direction to the interaction of potassium and calcium in the locust ganglion. Also, deliberately varying the osmolarity of the Ringer from 70 to 110% of its normal osmolarity produces no change in the level of motion detected from the ganglion (see Piddington & Sattelle, 1975*a*). It is relevant to note here that B. J. Newby (unpublished observations) detected no weight changes when the Na^+-Ringer bathing the ganglion was replaced by K^+-Ringer.

The most likely hypothesis to account for these results is that a potassium-induced calcium entry into the cells leads to a gel-sol transition in at least part of the cytoplasm. It has been known for some time that axoplasm normally exists as a gel (Bear *et al.* 1937; Hodgkin & Katz, 1949; Chambers & Kao, 1952) and more recently it has been demonstrated that this gel-like structure penetrates the finer branches of nerve cells (Gilbert, 1972). It has also been shown that extruded axoplasm can be liquified by calcium ions (Hodgkin & Katz, 1949; Chambers & Kao, 1952). Furthermore, in preliminary experiments with axoplasmic gels of *Myxicola infundibulum*, an increase in the level of motion is produced by the application of low concentrations of dinitrophenol (DNP), which would be expected to release calcium from storage sites within the preparation (see Piddington & Sattelle, 1975*b*).

Our results demonstrate increases of particle motion within nervous tissue which appear to be related to the entry of calcium into the cell on depolarization; these changes are reversible and share some of the properties of the liquefaction process noted in samples of isolated axoplasm. These findings may have implications for a number of physiological processes. For example a depolarization-induced, calcium linked coupling mechanism has been postulated for the release of hormones (see Matthews, 1970), for neurotransmitter release (see Hubbard, 1970) and for neurosecretion (see Douglas, 1968). It may be that a reversible gel-sol transition of part of the cytoplasm is a common feature of the stimulus-secretion coupling mechanism of all these processes. Alternatively, the motion increase could represent the release of previously bound structures which would then be free to participate in active processes involving motion such as growth and axoplasmic transport.

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