New technique for movement analysis: Application to biological systems[1]

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Summary. A new system for motion analysis is described. Modulation of light intensity by an organism's movement over an opaque and transparent checkerboard grid is monitored by a photocell. The photocell's output is proportional to the organism's amplitude and frequency of movement. This output is analyzed by a continuous interval dot display and spectral analysis. The system was tested by analyzing the activity of 4 Drosophila strains which are known to differ in their activity. General applicability of the system is discussed.

Quantification of animal motor activity has been attempted in the past using methods including direct observation[2], cinematography[3] and more sophisticated electronic devices[4–6]. Some of these methods are suitable for specific organisms such as small insects[7] or small mammals[8]. In determining the activity of an organism, the following 3 basic objectives must be regarded as essential: 1. To define the type of activity occurring at any point in time. 2. To measure the duration of each activity. 3. To measure the frequency of each activity type. Ideally, a method fulfilling these objectives should be applicable to both single organisms and population studies. Although the direct observation method has been employed for activity measurements, it is very time consuming, unobjective and suitable mainly for single animal studies. Other more sophisticated methods have overcome the human error factor and can record either cumulative or ongoing activities automatically. However, they usually do not discriminate between different types of activity. We therefore attempted to develop an activity analysis system which may have the capacity to fulfill the objectives described above. The system described here was tested on 3 Drosophila behavioral mutants. However, it may easily be adapted to studies of motion of various other organism as well.

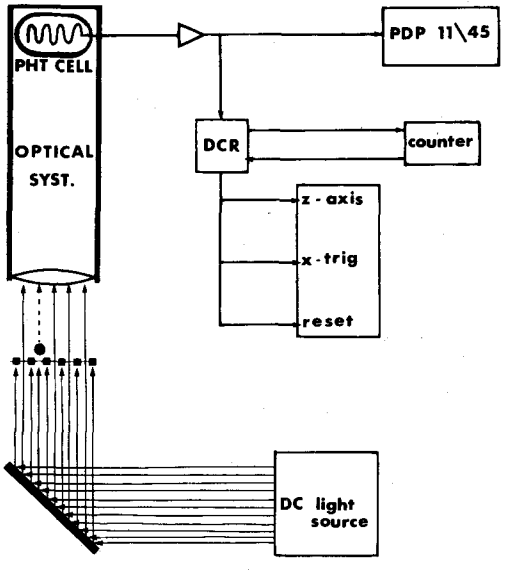

Fig. 1. Schematic representation of the experimental set-up. A DC powered light source is directed by a 45° mirror through an opaque and transparent checkerboard grid on a CdS photocell (PHT CELL). The dot on the checkerboard represents a fly over a transparent square decreasing the amount of light passing through. The photocell output is amplified and fed in parallel to a minicomputer (PDP-11/45) and a discriminator DCR. The discriminator can be switched off by the pulse counter on the right. z-axis, x-trig and reset are inputs to a 5013N Tektronix oscilloscope.

Materials and methods. One or more flies are placed in a shallow (2.5 mm high, 30 mm diameter) petri dish, limitting movement to 2 dimensions only. The experimental system with the recording chamber are shown schematically in figure 1. A light beam from a low ripple DC powered source is transmitted through the petri dish and the image of the fly (flies) is focused on a cadmium sulfide photocell. (The optical system is a trinocular Wild M7 zoom stereoscope enabling simultaneous photometry and visual observation.) A black and transparent checkerboard grid is interposed between the light source and the photocell either just below the petri dish or in juxtaposition to the photocell. The size of the squares in the grid must be adjustered accordingly. When the image of a fly covers a transparent square of the grid less light is transmitted. Thus the total amount of light reaching the photocell at any given point in time is a function of a) number of flies active at any point in time in the sample (in the field of view); b) number of flies or the fraction of their cross-sectional area, covering the transparent (or opaque) region of the photocell; c) relative size of squares to cross-sectional area of fly (a ratio of approximately 1 was found to be optimal).

Any movement in or out of the transparent photocell-grid region affects the amount of light hitting the photocell. Thus the voltage output (or resistance change) of the photocell represents the fly's activity, i.e. locomotion and other movements of its appendages. This voltage is amplified (Tektronix 26A2 amplifier), stored on magnetic tape and in parallel fed into a window discriminator (Fraederick Haer & Co.). A standard 5V 1 msec square pulse is obtained for each peak of the voltage input. These pulses are fed into a Tektronix 5013N scope as a) z-axis intensification, b) x-axis trigger pulse, c) sweep termination and fly back. (The latter is achieved by feeding the 5 V pulse externally to the reset switch of a 5B12N dual time base.) Thus a 'significant event' registered as a pulse generates a dot (n) on the osciloscope screen and triggers the sweep; the (n + 1) event is again registered as a dot after a certain interval and terminates the sweep fly back trigger. The (n + 2) event restarts the cycle. The y-deflection plates are supplied with an external slow voltage ramp.

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2 W. D. Kaplan, in: Genetic and Behavioral Studies of Drosophila Neurological Mutants in the Biology of Behavior. Proc. 32nd Ann. Biol. Coll., p. 133. Ed. J. A. Kiger, Jr. Oregon State University Press 1971.

3 R. Williamson, D. K. Kaplan and D. Dagan, Nature *252* (5480), 224 (1974).

4 S. K. de F. Roberts, Science *124*, 172 (1956).

5 R. J. Konopka and S. Benzer, Proc. Nat. Acad. Sci. USA *68* (9), 2112 (1971).

6 K. Connolly, Anim. Behav. *14*, 444 (1966).

7 D. Dagan, W. D. Kaplan and R. Ikeda, Adv. Behav. Biol. *15*, 321 (1975).

8 P. Viand and Y. Le Cain, Behaviour *52*, 313 (1975).

The cumulative dot displays were photographed either from a storage oscilloscope or with long exposure times directly from the oscilloscope screen. The x-axis represents the interval between significant events, while the absolute time span of recording is represented on the y-axis. Thus the higher the frequency of significant events, the closer the cluster of dots generated will appear to the y-axis. Display of a present number of events was obtained by switching off the discriminator by an output pulse from an electronic pulse counter. Information stored on magnetic tape was fed into a PDP-11/45 minicomputer for spectral analysis. The data were plotted 3-dimensionally to show the change in power spectrum with time.

Results and discussion. To test the system, motor activity of wild type and 3 single gene Drosophila mutants was analyzed. Flies were controlled for sex, age and environmental temperature. The strains tested are inactive (iav) [7], wild type – Kiryat Anavim (QA), Hyperkinetic Hk^1 [2] and a new autosomal dominant gene shaker (As).

The activity patterns of the 4 strains can be seen in the dot displays of figure 2. Clusters of dots along the y-axis depict activity composed of significant events occurring at rates higher than 5 Hz. A general impression of the patterns generated over a 60-sec-period is sufficient to classify the records in an increasing order of activity (iav-Hk^1-As), which is in accordance with classification based on visual observation. The number of significant events generated by the 4 strains occurring in 5 different frequency band widths is shown in the histograms of figure 2. In comparing the activity histograms of the 4 strains in all cases the highest number of events occurs in the 1–5 Hz band, while only iav shows activity at frequencies below 0.1 Hz. The most conspicuous differences between the 3 mutants are evident in the 0.5–1 Hz range; however, the entire spectral distribution is more representative of each mutant's activity.

A chi-square test shows significant differences between QA and both As and Hk^1 flies in the 4 arbitrarily chosen band widths from 5 to 0.2 Hz with levels of significance $p = 0.001$, whereas the iav activity recorded is not significantly different from that of QA. Visual observation of iav cultures shows that these flies, although generally inactive, are capable of seemingly normal motor acts once they are stimulated strongly enough. The record in figure 3D is an example of a 60-sec-bout of activity in a normally inactive iav fly. A more intensive analysis of specific activity may show differences between the iav and wild type strains.

Comparison of specific activities was attempted in one mutant and between mutants. The onset of the dot display was triggered manually when the fly was either walking (figures 3A and 3B) or preening (figure 3C), as determined by visual observation. Locomotion and

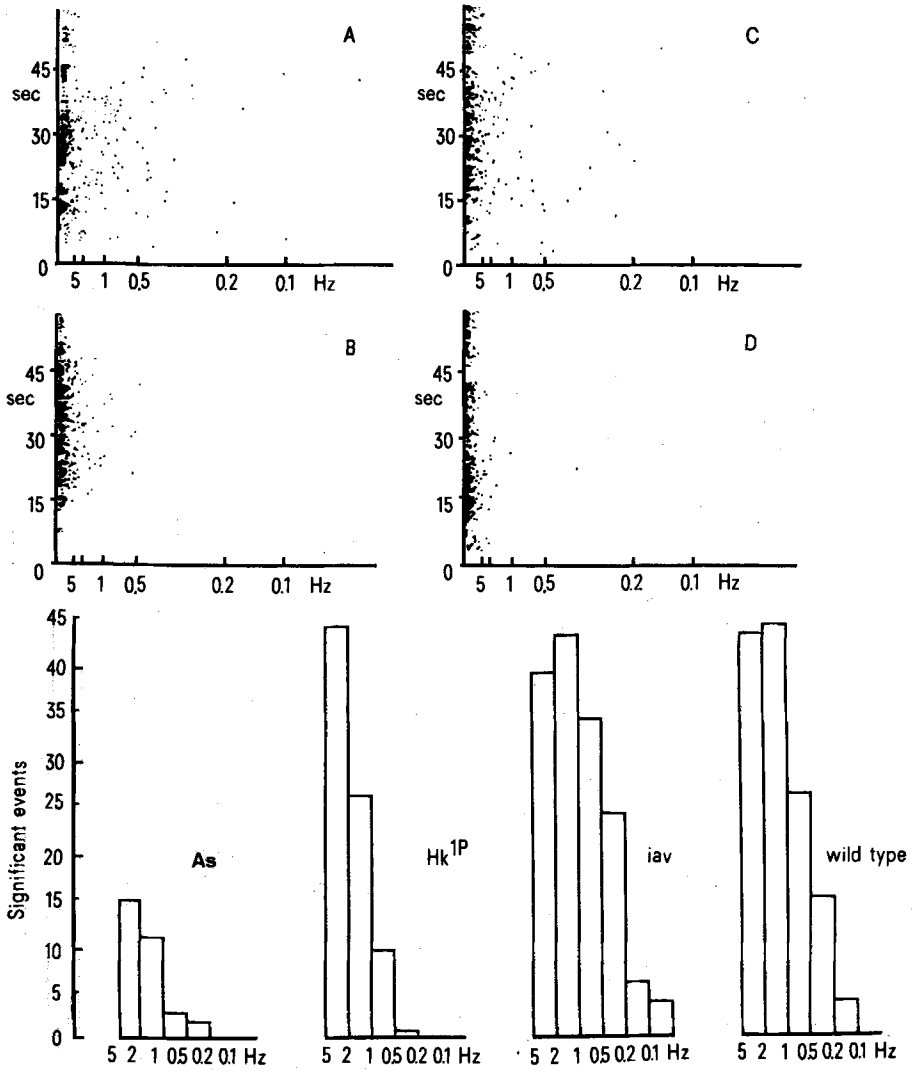

Fig. 2. General activity dot display and histograms of number of significant events occurring at various frequency band widths of 4 Drosophila strains. *A* iav, B Hk^1, *C* QA, *D* As. Note the longer interval activity in *A* and *C* as compared to *B* and *D*.

preening of a 2-day-old female Hk^1 fly are shown in figures 3A and 3C. Walking is characterized by longer interval events, namely in the 5–10 Hz band width, while preening shows higher frequency characteristics 10 Hz. (Note different frequency scales in figures 2 and 3). When preening of an iav fly (figure 3B) is compared with the Hk^1 activity, an intermediate frequency scatter is obtained between Hk^1 walking and cleaning. The general iav cleaning pattern resembles more Hk^1 walking then preening. It is therefore necessary to calibrate the system's output to each type of activity in specific mutants before making comparisons.

A third approach used in processing the output of the photocell-grid system was continuous spectral analysis. For this analysis, the analog voltage of the photocell was amplified and fed directly into a PDP-11/45 minicomputer. A low pass filter was interposed for display of low frequency activity. The data was sampled, digitized and a continuous power spectrum was computed. Figure 3 is an example of a 3-dimensional display of a continuous spectral analysis. The 3 axes in the display are frequency, time and amplitude. Whereas in the previous displays (figures 2 and 3) the amplitude of each movement was not incorporated due to the standardized discriminator output, here in the power spectral analysis it is evident that slow, frequency events have a higher amplitude than higher frequency activity.

An 8-min-span of activity of a 2-day-old female wild type fly is given in figure 3. Recording was started immediately upon the introduction of the fly into the recording chamber and continuous activity can be seen occurring for the first 400 sec with a gradual decrease. The decrease in activity is evident first in the 1–4 Hz band width with the concomitant decline in amplitude of these movements.

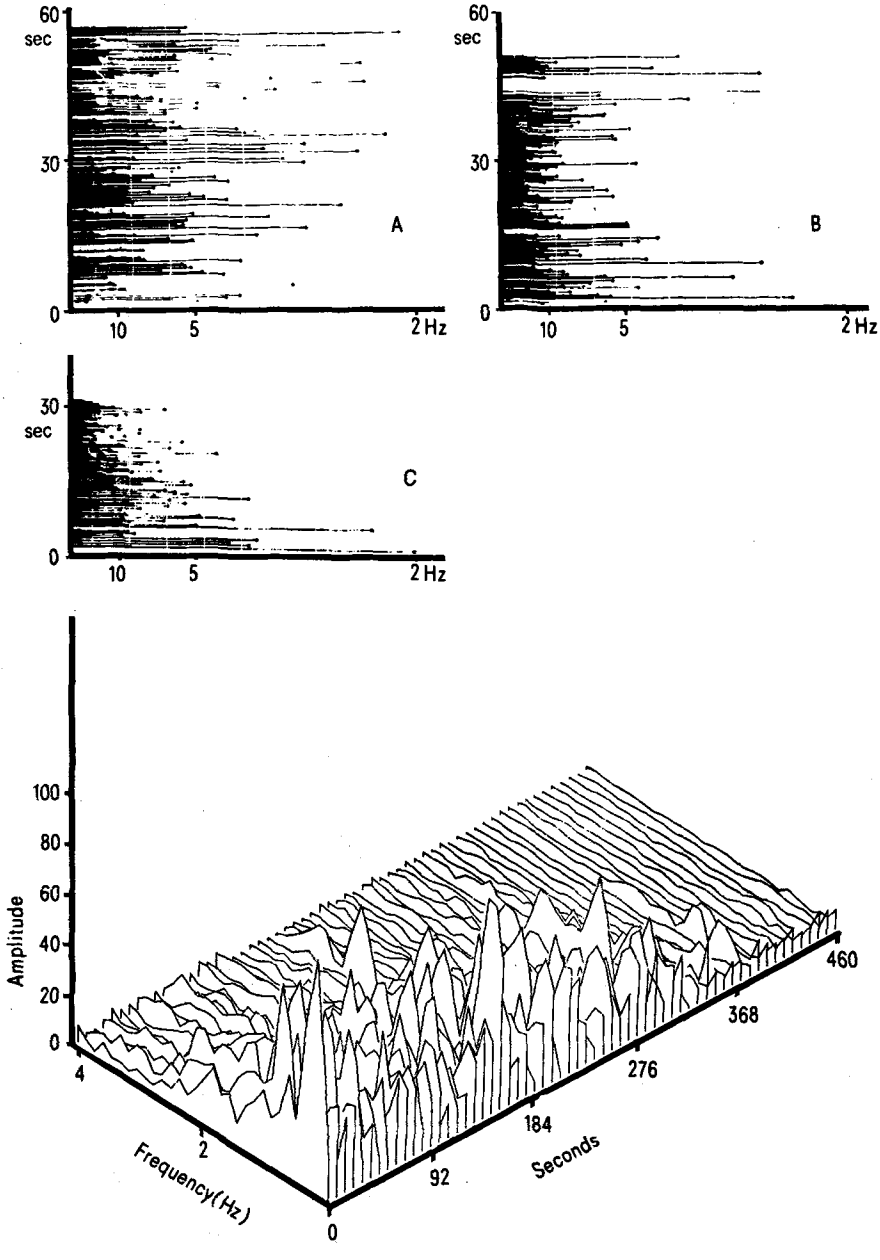

Fig. 3. Recordings of specific activity from 2 Drosophila strains. *A* Walking of Hk^1, *B* iav preening, *C* Hk^1 preening. Example of spectral analysis of a QA fly displayed over 460 sec. Amplitude units are arbitrary. Note change in amplitude and frequencies over the 460-sec-span.

Following this, the activity diminishes progressively both in frequency and amplitude until very low amplitude movements only can be seen from 400 to 464 sec.

Qualitatively, all the results presented here can be obtained by visual observation. Thus preening movement appears 'faster' than walking and Hk^1 flies seem 'more active' than an iav fly. Thus the validity of the method is more in quantification and objective comparison of various types of activities. Once the system is calibrated for specific activities, these can be characterized by spectral analysis. It should then be possible to detect specific activities from continuous spectral display recordings, thus comparisons of the duration of each type of activity, or how much time a certain mutant spends on preening, walking, etc., can be made. Further experimentation along these lines is in progress. Silent periods between bursts of activity may be used as well to characterize behaviour of different mutants. This is evident in the more even scatter of black patches on the y-axis in figure 2C representing more or less equally interspersed rest and activity periods in the wild type flies, as compared with longer spans of activity in figure 2A, representing the long bursts of tumbling as observed visually exhibited by the As mutant.

We are also using the same grid-photocell-spectral analysis method to characterize sperm motility. Thus the method may have general applicability to motion analysis if a suitable optical system (macro, micro or telescopic) is used.

CONGRESSUS

DDR – Czechoslovakia

Federation of European Biochemical Societies, 12th FEBS-Meeting

in Dresden (DDR), 2–8 July 1978

A post-congress FEBS-symposium on 'Antimetabolites in Biochemistry, Biology and Medicine' will be held in Prague (CSSR), 10–12 July 1978

Preliminary registration form (mailed together with the preliminary registration form for FEBS-Meeting, before 1 September 1977) to the following address: FEBS-Meeting, P.O. Box 313, DDR–806 Dresden.

Italy

EUCHEM Conference. Phase-transfer catalysis and related topics

in Gargnano (Lake Garda), 5–10 June 1978

Plenary lectures will include: A. Brändström, Sweden; J. A. Fendler, USA; H. H. Freedman, USA; G. N. Gokel, USA; J. M. Lehn, France; M. Makosza, Poland; F. Montanari, Italy; J. P. Sauvage, France; C. M. Starks, USA; R. Ugo, Italy.

Enquiries to Prof. Mauro Cinquini, Istituto de Chimica Industriale, Università di Milano, via C. Golgi 19, I–20133 Milano, Italy.

Protons and ions involved in fast dynamic phenomena

30th international meeting of the Société de Chimie physique, Paris, 28 November–2 December 1977

Contributions and requests for information should be addressed to the general secretary of this 30th meeting: Dr. C. Troyanowsky, 10, rue Vauquelin, F–75231 Paris Cédex 05 (France).

CONSTRUCTIONES

European Pineal Study Group

An association of European scientists working on, or interested in, the vertebrate pineal organ has been formed. The aims of the European Pineal Study Group are to promote the development of pineal research in Europe, and to facilitate the contacts between the different European teams. It will do so especially by organizing small colloquia on pineal research.

Application forms and further information can be obtained from: Dr P. Pevet, Secretary of the E.P.S.G., The Netherlands Institute for Brain Research, Ijdijk 28, Amsterdam-O., The Netherlands.