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METHODS FOR QUANTIFYING BEHAVIOUR AND CEREBRAL ELECTRICAL ACTIVITY AND THE EFFECT OF DRUGS UNDER CONTROLLED CONDITIONS

BY

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The effects of substances acting on the central nervous system are difficult to analyse with traditional pharmacological methods, particularly when behaviour is studied. The methods to be described were devised to meet the following requirements:

(1) Control known effects of the testing situation in relation to the previous experience of the animal.

(2) Strict control of the environmental conditions, such as noise, light, temperature and partial pressure of oxygen and carbon dioxide.

(3) Ability to observe the animal without the experimenter's presence affecting it.

(4) Quantitative and continuous measurement of behavioural and physiological changes before and after injection of drugs without hampering, disturbing or handling the animal.

(5) Ability to study social interactions between animals for, although study of isolated animals is easier and generally more convenient, the responses observed may be atypical of those customarily found in gregarious species.

The methods have proved simple and successful in practice; they have been demonstrated to the Physiological Society (Dewhurst & Marley, 1963).

METHODS

Experimental box

A box was made, the internal dimensions of which were $15 \times 15 \times 16$ in. The front and one side wall were made of transparent Perspex. The rear wall was made of 0.25-in. translucent Perspex to provide diffuse lighting from external sources; the remaining wall and base were of 0.5-in. plywood. A two-way mirror was fitted inside the front wall. If required, additional illumination was obtained from bulbs mounted on an opaque adjustable partition which could replace the rear wall (Fig. 1). A removable polyethylene floor-tray rested on four crystal microphones (Acos Mic 38; frequency response, ± 3 dB with respect to 1 kcycle from 30 to 12,000 cycles/sec; sensitivity, 62 dB ref 1 V dynes/cm^2; Cosmocord Ltd.). These were surrounded by felt lagging. The lid was made of 0.5-in. plywood and

was flanged and tapered to fit tightly; a crystal microphone (Acos Mic 38) was attached inside. A control box (a mirror image of the experimental box but otherwise identical) was placed so that the transparent side walls of the two abutted. An opaque screen could be interposed between the boxes.

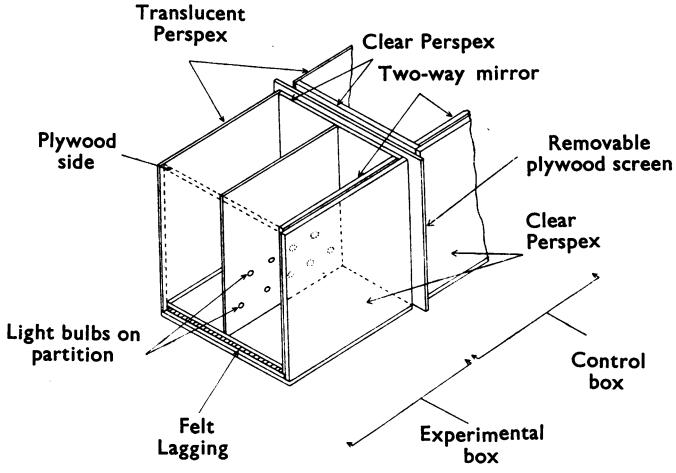

Fig. 1. Details of experimental and control boxes providing controlled environment. The internal dimensions of each box were 15 × 15 × 16 in. The plywood side has been removed to show detail. For further details, see text.

Control of physical environment

Light. As behaviour in the chicken is affected by light intensity, the light bulbs were supplied with stabilized direct current at 24 V.

Temperature. Young chickens have poor temperature control and it was essential to maintain a box temperature optimal for the age of the bird (33° C during the first week falling to 28° C at the third week). Temperature was recorded by a mercury thermometer with the bulb 1 in. above floor level. The light supply usually gave sufficient heat but more was sometimes required. That cheeping increases when birds are cold (Collias, 1952) was confirmed. Other species tested were kept at room temperature (17 to 20° C).

Humidity. Relative humidity was monitored by a hygrometer, and maintained at the optimum (70%). Excess humidity was prevented by silica gel.

Noise. The box was insulated against noise by layers of felt lagging and plastic foam under the floor and by the tight-fitting tapered lid. Adequacy of sound-proofing was judged by the lack of effect of external noise on the chicken's behaviour and by zero counts from the microphone and recording apparatus.

Air. The measures used to exclude noise also restricted atmospheric exchange. The oxygen content of the box was adequate to maintain a 4-week-old chicken for at least 24 hr, as shown by serial testing with a Beckman oxygen analyser. No detectable change of atmospheric oxygen occurred even after respiration had been increased by amphetamine; however, after 24 hr, carbon dioxide reached 5.5%. Thereafter, " indicating soda-lime "

(Carbosorb, B.D.H.) was placed in the box to absorb excess carbon dioxide; this kept the carbon dioxide at or below 0.5% (B.O.C. CO_2-analyser). A hole at floor level allowed gas sampling or replacement without disturbing the animal.

Food and water. These were always available in the box.

Control of social environment

Birds of a feather do indeed flock together and it was essential to compare the behaviour of the solitary animal with that of the animal in a group. The effects of companion birds were tested by placing two birds in the same box. The effects of a visible but inaudible companion on drug action could be assessed by putting a bird in each box and removing the opaque screen; the effects of an audible but invisible companion were studied with an animal in each box, but with the opaque screen interposed, the laboratory quiet, and the lids removed.

Responses and their recording

An animal with electrodes implanted in brain and muscle and a cannula in a jugular vein (Fig. 1 in Dewhurst & Marley, 1965) was placed in the recording box. The lid was closed after the electrode wires and cannula tubing had been led to the exterior through a central hole or a groove in the rim of the lid. The hole was plugged with a rubber stopper which kept the electrodes in place; the bird could move freely and drugs could be given through the cannula without handling the animal.

Observation

Continuous direct observation was made through the " two-way mirror "; the animal saw only its reflection in the mirror.

Electrocortical and electromyographic activity

Bipolar recordings were made with an eight-channel Ediswan electroencephalograph. The balanced power output (impedance 1 to 2 kΩ) was integrated by passing it into the centre-tapped primaries of impedance-matching transformers (WO 1546A, Gilson Ltd.) instead of the pen-coils (Fig. 2). The alternating outputs from the transformer secondaries were fully rectified by a germanium diode bridge (OA81, Mullard Ltd.) and fed into d.c. integrating motors (d.c. resistance 190Ω) with counters. These gave continuous integration of imposed voltages with respect to time. Large potential differences of long duration (for example those associated with sleep) had large integrals, whereas small potential differences of short duration (as in the alert electrocorticogram) had small integrals (Fig. 3). Integrals so obtained were compared with measures derived by direct area planimetry and by weighing paper sections of the same trace; since all measures correlated highly, the cerebral electrical activity could justifiably be expressed as a continuous time integral.

Animal movement, whether spontaneous or drug induced, did not produce artefacts if the preparation was satisfactory. Thus movement increased with alerting whereas the amplitude of electrocortical activity and corresponding integral diminished. However, improperly fixed electrodes could introduce artefacts in the trace which also were integrated. Such animals were excluded from the study.

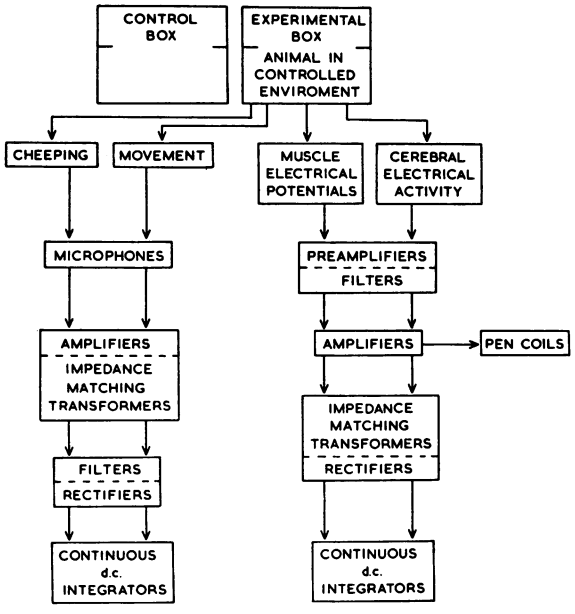

Fig. 2. Block-diagram of apparatus for recording and integrating cerebral and muscle electrical activity, cheeping and movement.

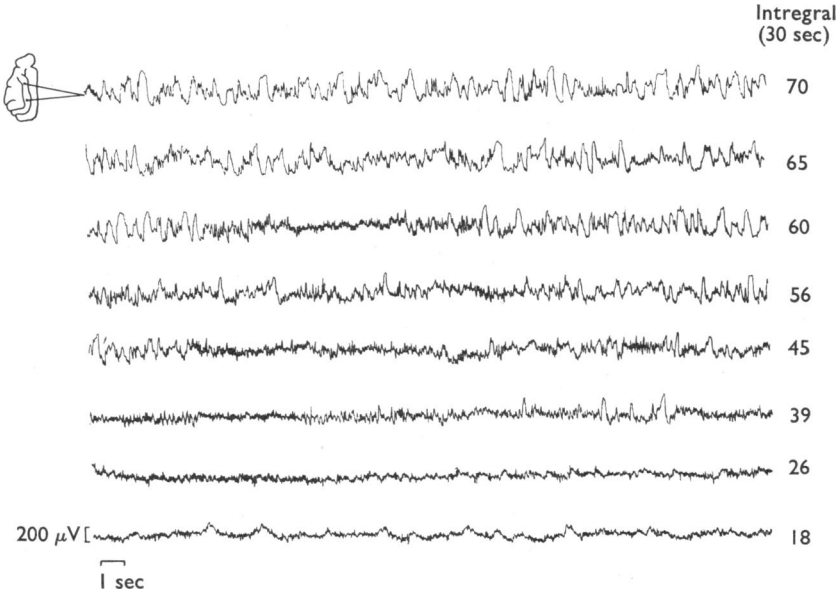

Fig. 3. Correspondence between integral (numbers on right) and amplitude of electrocortical activity. Cat *encéphale isolé*, 2.5 kg. From above downwards, transition from large voltage sleep activity and large integral to small voltage alert electrical activity with associated reduction in integral. Calibrations, 200 μV and 1 sec.

Cheeping

Characteristics. To ensure that the design of the filtering and integrating system was appropriate, cheeping was recorded on a Philips tape-recorder (frequency response ± 3 dB with respect to 1 kcycle from 50 to 15,000 cycles/sec) from the same twenty chickens at ages of 1 to 7 and 21 days (Fig. 4,A, B and C). The recordings were amplified and photographed on a Nagard oscilloscope before and after rectification (Fig. 4,E). The main characteristics were as follows. All variations in shape between a horizontal and vertical ellipse occurred. The overall contour was not modified by filtering; rectification produced half-waves (Fig. 4,E). Frequency content (measured by a frequency analyser) was chiefly 4 to 6 kcycles/sec. Cheep repetition rate varied from zero to a maximal of 4 to 5 per sec recorded in " twittering " produced by amphetamine. Alterations in cheep contour by amphetamine are shown in Fig. 4,D. Mean cheep duration varied from 0.085 sec in the 1-day bird to 0.137 sec at 21 days. Mean rise time was 0.07 sec at 1 day and 0.11 sec at 21 days.

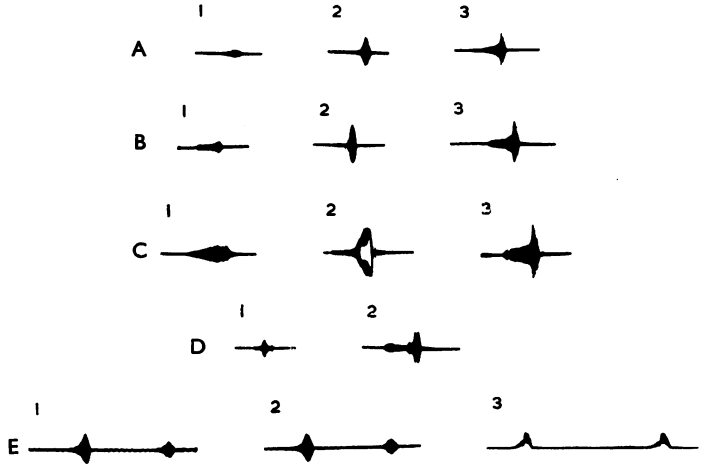

Fig. 4. Single-cheep contours recorded on an oscilloscope from a group of twenty Rhode Island Red chickens. Cheeps of various intensities from same birds: A, aged 1 day ; B, at 7 days; C, at 21 days. D, Cheep contour in 1-day old chicken before (1) and after (2) dexamphetamine (1.0 μmole/100 g, intraperitoneally) showing prolonged duration and increased intensity. E, Cheeps (1) before filtering; (2) after filtering; (3) after rectification.

Measurement. The method was modified from that devised by Parkes & Lessin (1960) to measure movement. The essential difference was the inclusion of filters (Fig. 2). Voltages induced in the roof microphone were amplified by an A.F. amplifier (Linear Ltd.). Contaminating low frequencies were removed by a simple high-pass T-type filter (inductance 410 mH, capacitances 4,000 pF in each arm) and the output then rectified (diode bridge) and integrated. The integrating motor had a time constant (10 msec) which ensured faithful registration. The amplifier output impedance (3 to 16 Ω) was matched by transformers to the motor. A standing voltage could be applied to the motor from an L.T. stabilized supply. Calibration with a 25-mV, 4,000-cycles/sec sine-wave signal gave counts that varied by only 2% over several months.

Activity

Characteristics. Sounds made by animals moving on the tray covered with food crumbs or silica gel granules were tape-recorded and photographed. Both pulse shape and repetition rate were irregular; frequency content varied from 100 cycles/sec to several kcycles/sec; the duration from 0.01 to 0.02 sec; and rise times were random and quite frequently less than that of the time constant of the integrator. Counts were therefore statistically but not absolutely accurate. Intensity of activity noise of some animals was slight (for example chick walking) so greater amplification and a four-microphone input were required.

Measurement. This was similar to that for cheeping. To make cheeping and activity counts comparable, either an integrating motor responding to a smaller imposed voltage or a more powerful amplifier (30 W, Linear Ltd.) was used. Low-pass filtering (T-type filters, capacitance 0.05 μF and inductance 420 mH in each arm) was essential to eliminate contamination by integration of the cheeping. Calibration at 400 cycles/sec was similar to that used for cheeping.

Posture

Of a number of measures tried, two were selected as most useful. These were the position in space of body or body-part relative to fixed external axes, and the relation of different parts to each other. Measurements were made by a grid superimposed on the outer surface of the two-way mirror and calibrated in cm and degrees; the two-way mirror gave a double image of the grid and it was possible to sight any portion of the chicken and measure its position accurately by parallax. Similar grids on the rear and interior sides of the box provided additional checks. To ensure further accurate recording, mirrors were placed in the base of the box so that joints could be seen in profile.

Only exceptionally was the alert bird still; mostly it moved continuously through a succession of postures. The average of these during a given minute would not provide the information required, which was the degree to which normal attitude was impaired. Hence control observations were made of the maximum degree which the measures attained in normal upright standing. The degree of postural change induced by drugs was recorded as the nearest the bird approached during any minute to its normal standing position.

The measures relating position to external axes were the vertical distances between the floor and points on the vertebral column, ventral trunk, head and wings. Angular measures employed comprised the included angle between the tarsus and floor (α), and between the trunk and a horizontal plane passing through the hip joint. Measures of the spatial relation between different parts of the body were expressed as degrees of flexion and extension of various joints, for example rotation of the trunk about the hip (θ). The most useful indices of postural change were α and θ as they combined sensitivity with reliable end-points. Overall postural changes due to certain drugs were also graded in intensity. Descriptions of these are given in the subsequent paper (Dewhurst & Marley, 1965).

Validation of methods

For recording of electrocortical and electromyographic activity

The frequency response of the electroencephalograph integrators was substantially flat from 1.5 to 30 cycles/sec, the range of relevant frequencies in the electrocorticogram,

using an Ediswan low-frequency oscillator as a sine-wave source (Fig. 5,A); there was a linear relation between a balanced input voltage so delivered and the integral (Fig. 5,B); phase shift was less than 5° over the appropriate frequency range as measured with a Nagard double-beam oscilloscope.

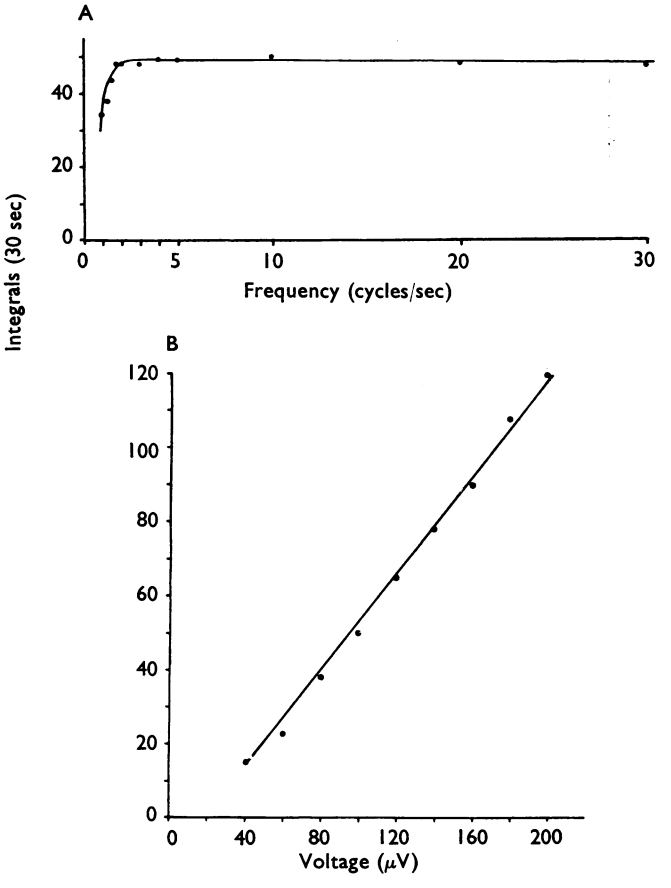

Fig. 5. Calibration of impedance-matching transformer and d.c. integrating motor. A: Integral independent of signal frequency; substantially flat response from 1.5 to 30 cycles/sec. Input: balanced 1 mV sine-wave signal for 30 sec. B: Integral dependent on signal voltage; substantially linear response for 40 to 200 μV balanced sine-wave signals at 15 cycles/sec for 30 sec (electroencephalograph setting: time constant 1.0 sec; no H.F. attenuation).

A linear relation between input voltage and the power output of the Ediswan electro-encephalograph depends on satisfactory discrimination by the balanced amplifier against in-phase signals, and accurate setting of the electrical zero. To check this when pen-coils were by-passed, the d.c. voltages between the centre tap and anode connections of the transformers were measured and the electroencephalograph was adjusted until they were equal. Maximal discrimination was achieved by applying in-phase signals and adjusting the balance potentiometer until the integrator gave minimal readings. In later experiments, a

Kaiser electroencephalograph was used (TR60); since the output was single-sided and of low impedance, no matching transformer was needed. Further, the integrating motor could be used simultaneously with the pen-writer on the same channel.

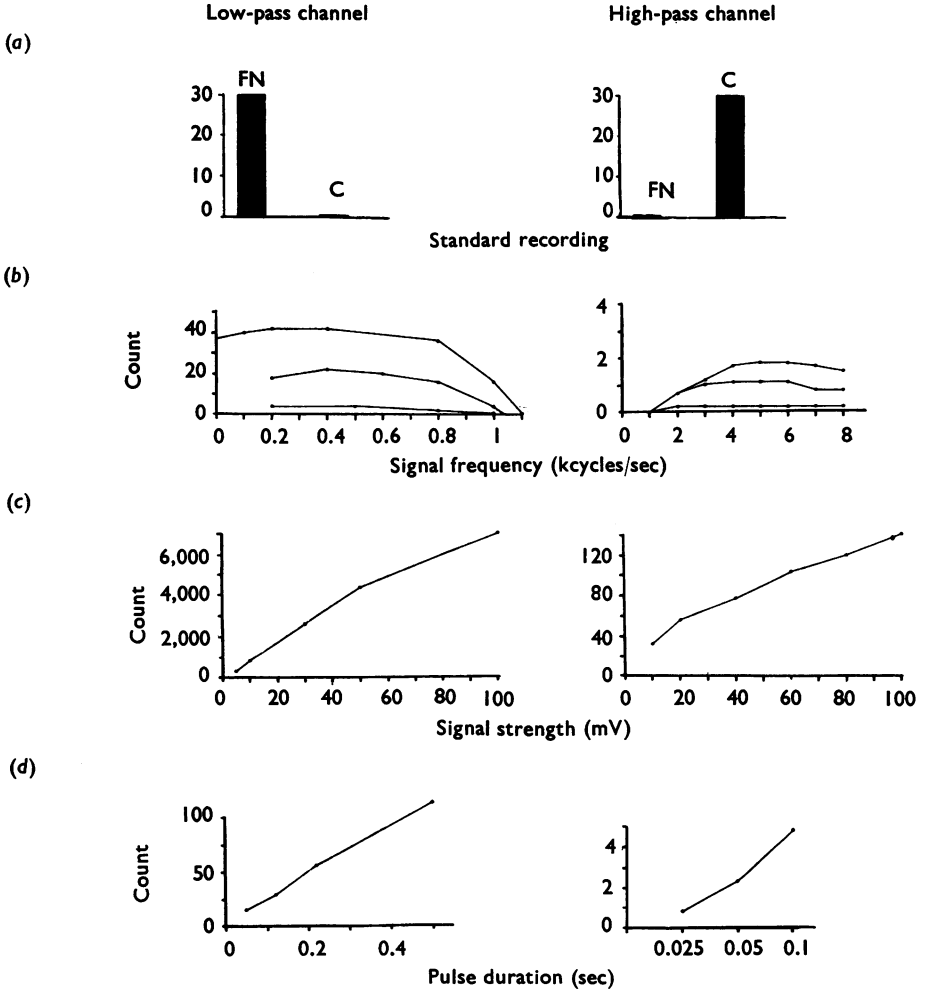

Fig. 6. Performance of low-pass (left) and high-pass (right) channels (for activity and cheeping).

Low-pass channel. (*a*) Good response to floor noise (FN) but not to cheeping (C). (*b*) Flat frequency response with 2.5 mV signal at various pulse durations (from above downwards, 0.25, 0.125 and 0.06 sec) with cut off at 1.1 kcycles/sec. (*c*) Linear increase in count with increase in signal voltage (frequency 400 cycles sec; pulse width 0.1 sec). (*d*) Linear increase of count with increase of pulse width (frequency 400 cycles/sec at 20 mV).

High-pass channel. (*a*) Good response to cheeping but not to floor noise. (*b*) Frequency response with constant frequency and pulse widths (from above downwards, 20 mV, 0.05 sec; 10 mV, 0.1 sec; and 5 mV, 0.1 sec) with cut-off at 1 kcycle/sec. (*c*) Linear increase in count with increase in signal voltage (frequency 4 kcycles/sec; pulse width 0.1 sec). (*d*) Linear increase of count with increase of pulse width (frequency 4 kcycles/sec at 20 mV).

For recording cheeping and activity (high- and low-pass systems)

Pulses of sine waves, varied in duration and frequency and counted by a digital frequency-meter, were applied to the inputs of the two systems. Frequencies above 1.1 kcycles/sec were not registered by the low-pass channel. The frequency response was substantially flat from 100 to 800 cycles/sec for signals of magnitude similar to those produced by movement (Fig. 6,*b*). Response to varying pulse width was satisfactory (Fig. 6,*d*). The frequency response of the high-pass channel and the effects of varying signal voltage and pulse width were similarly tested (Fig. 6,*a* to *d*).

Practical assessment was made by reproducing standard recordings of cheeping and activity from a loud-speaker in the box to ensure there was no registration from cheeping on the low-pass system nor of activity on the high-pass system (Fig. 6,*a*).

Plotting of integrals

These were plotted either cumulatively (Fig. 7) or as 30-sec or 1-min integrals (Fig. 8). When plotted cumulatively for an activity at a steady basal level the integrals were linearly related to time (Fig. 7, left); saline injections produced transient changes. Drug effects changed the slope to give sigmoid or exponential curves. The total integral is thus a measure of both duration and amplitude of drug response; the cumulative type of curve is useful in assessing changes of rate, for example onset and offset of action. The steeper slope for cumulative cheeping integrals after dexamphetamine (1.0 μmole, intravenously) is shown in Fig. 7 (right); antagonism by various doses of (±)-α-methylnoradrenaline produces a

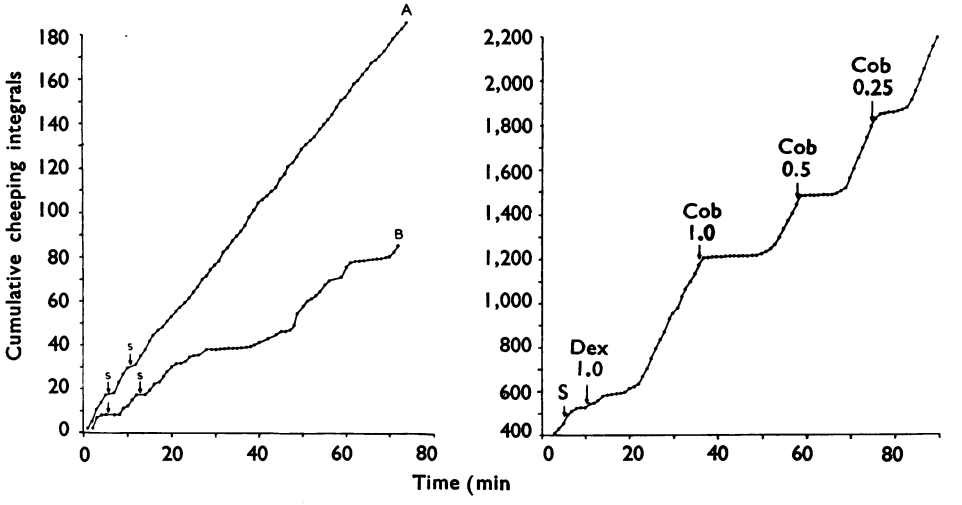

Fig. 7. Cheeping integrals plotted cumulatively. Left-hand graph: A, Linear plot of minute integrals against time for first session of chicken in experimental box. B, Second session of chicken in box; bird adapts with diminished cheeping (contrast with Fig. 8). Right-hand graph. Same chicken as in (*a*) and (*b*), and adapted to experimental box. Increased amount of cheeping produced by dexamphetamine (Dex, 1.0 μmole); note steeper slope. Antagonistic effect of (±)-α-methylnoradrenaline (Cob, 1.0, 0.5 and 0.25 μmole). On recovery, slopes are parallel to that produced by dexamphetamine. In both graphs, S= 0.1 ml. of saline, intravenously.

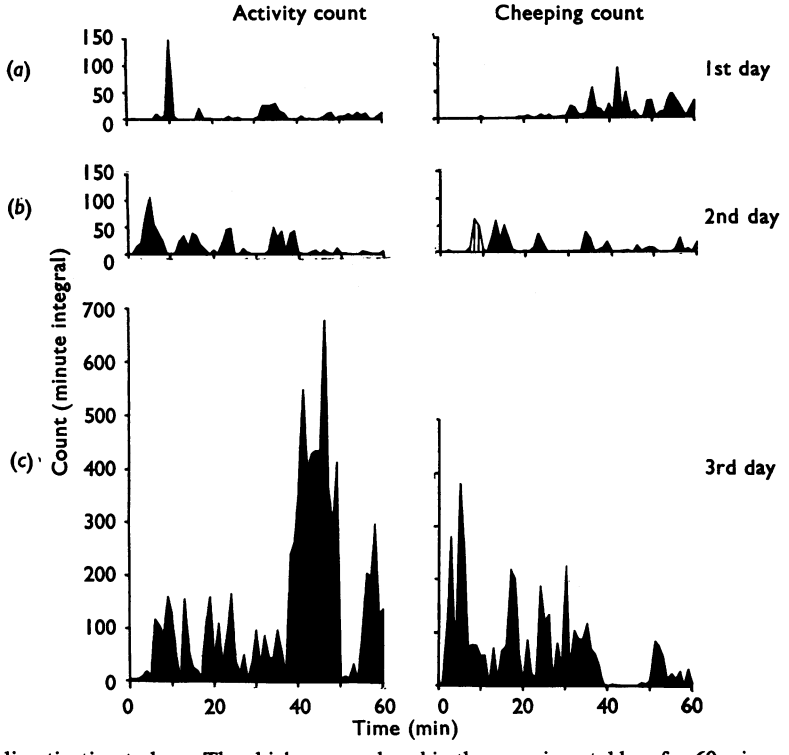

Fig. 8. Acclimatization to box. The chicken was placed in the experimental box for 60 min on three successive days. The chicken adapts to the box with increased activity and cheeping with a more rapid onset of cheeping. Results are plotted as minute integrals (ordinate); individual integrals are shown in the unshaded portion for the 2nd day cheeping counts. (*a*), (*b*) and (*c*): 1st, 2nd and 3rd days.

break in the curve. When integral per minute is plotted against time the graph becomes essentially the differential of the previous type, and amplitude of response is seen more clearly (Fig. 8).

Non-drug factors affecting drug response

Social factors

When first isolated in the experimental box, animals may respond either with excessive motor activity and/or cheeping, or with greatly diminished activity. Such reactions to strange environments are not indicative of the animal's usual behaviour. Not until the bird had been in the box for some time could representative behavioural measures be obtained. Another bird in the experimental or the control box and visible to the test bird could lead to reduced activity if the bird was previously overactive or to increased activity if the bird was tranquil. Qualitative and quantitative responses to certain sympathomimetic amines were found to vary with these factors.

Previous experience

Previous exposure to the experimental situation modified behaviour. As shown in Fig. 7 (left), the chicken adapted to the experimental box by diminished cheeping (compare slope A

with slope B). Another chicken (Fig. 8) adapted to the box by increased cheeping and activity. Thus, when placed alone in the box on the first day, the chicken stayed immobile for about 10 min and showed minimal activity for the ensuing 50 min (Fig. 8). On testing the next day, the period of " freezing " was much reduced; on the third day, full activity and cheeping were recorded for most of the hour. In this last chicken, the effect of a central inhibitory amine, for example (±)-α-methylnoradrenaline, would show least clearly on the first day, but be easily demonstrable on the third.

Intercorrelation of activity, cheeping, electrocorticogram, electromyogram and posture

Maximum activity was usually associated with little cheeping, whereas cheeping was maximal when the bird was immobile (Fig. 8; compare activity and cheeping on third day at 40 to 50 min). Activity and large electromyographic potentials were invariably associated with an alert electrocorticogram, but alert electrocortical activity often occurred with the bird sitting quietly. Cheeping was less consistently associated with an alert electrocorticogram and occurred with the bird's eyes closed and drowsy electrocortical activity. Of the variables, the electrocorticogram was the best index of alertness or drowsiness. Cheeping tended to be more sensitive than electrocortical activity to depressant drugs.

SUMMARY

1. Apparatus and methods are described for the quantitative recording of cheeping, movement, electrocortical and electromyographic activity in small animals under strict control and for testing the effects on these of drugs and environmental and social stimuli. Drug injections could be made without handling or disturbing the creature.

2. The methods are suitable for recording behaviour and the physiological variables of small animals and have been used for young chickens, rats, guinea-pigs and kittens.

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