STUDIES ON THE BEHAVIOUR AND SENSORY PHYSIOLOGY OF THE RED WOOD ANT (FORMICA RUFA).

A Thesis for the degree: Doctor of Philosophy

by

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Submitted to the University of Southampton

December 1969

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ABSTRACT

FACULTY OF SCIENCE

ZOOLOGY

Doctor of Philosophy

STUDIES ON THE BEHAVIOUR AND SENSORY PHYSIOLOGY OF THE RED WOOD ANT (FORMICA RUFA)

by Anthony Hugh Cosens

Threat behaviour in ants is not normally recognisable. However in the Red Wood Ant (Formica rufa) threat behaviour has developed into a distinctive fixed action pattern. A basis for research into the behaviour pattern was provided by definition of the various postural stages. Subsequent work then revealed the releasers associated with threat behaviour. This ranges from a low intensity response to a high intensity response, the latter taking the form of the "full threat posture".

New, open plan, observation nests were designed. Apparatus, including a behavioural observation arena, was developed in conjunction with the nests, in order to study the responses of foraging ants to a range of sensory stimuli. Methodical presentation of the stimuli permitted assessment of the relative importance of the components of the releaser complex.

As the stimuli involved act directly upon the senses, the threat response could be used as an indicator that the experimental stimuli were being detected by the ants. This meant that the system could be used to

obtain more information about insect sensory receptors.

Substrate-borne vibrations produced negligible responses and acted only to make ants more receptive to other stimuli. Strong threat behaviour was released in response to chemical stimuli, especially ketones with a carbon chain length of seven atoms. The presence of an alarm pheromone was postulated but attempts to isolate one were unsuccessful. Formic acid was not shown to be effective as a releaser of threat behaviour, but is employed in immobilising prey, and defence.

The responses elicited by ketones were of higher intensity than those elicited by visual stimuli, yet visual stimuli were shown to be more fundamental as releasers. The effects of visual stimuli were enhanced by the addition of the chemical stimuli and summation of response intensity occurred.

Ideally, the visual stimuli took the form of a solid figure of large size. Figures subtending an angle of 24^{o} at the insect eye, elicited optimal responses. Movement was the most important component of the visual stimulus. Optimal response was obtained from a stimulus moving at 125 - 130 cms. per second, and with a flicker frequency of 10 flickers per second. The major components of movement were found to be flicker frequency and velocity.

Response intensities elicited by combinations of stimuli far exceeded those expected from the simple summation of responses obtained with individual stimuli.

The origins of, and reasons for, the behaviour pattern are discussed.

CHAPTER ONE

Introduction

1.0 The aculeate Hymenoptera and the Isoptera are the only insects to show true social organisation. With social organisation comes the development of single social units composed of many individual members. Within such units individuals must develop a social bond, and a series of rules and behaviour patterns must be developed for social cohesion to be maintained. The behaviour of the individual should also be orientated towards the well-being of the social unit rather than that of the individual. This is demonstrated by the honey bee returning to the nest and indicating the position of food to other bees, by means of a dance, Von Frisch (1950,53). This behaviour pattern benefits the society rather than the individual. Similarly, very few animals are free from predation and defensive behaviour patterns have been evolved where defence of the society is more important than defence of the individual.

Such behaviour patterns are usually not completely new, but are borrowed from existing insular behaviour patterns and restylised for the new purpose. This is the case with the defensive behaviour of the ant Formica rufa where the behaviour pattern evolved for attacking prey is slightly modified and used as a defensive behaviour pattern. This is for defence of the colony rather than the individual.

Closely allied species of ants do not show such a distinct defence posture (Wallis, 1962a) and the original object of this research project was to work out the parameters of the releasers involved in that part of the aggressive behaviour complex which has become elaborated into a distinct "threat posture".

The Oxford Dictionary defines 'threat' as a 'declaration of intention to punish or hurt' and 'defence' as 'resistance against attack'. Both of these definitions cover the defensive behaviour pattern. It is resistance against attack, but on the other hand it is also a declaration of intention. If the situation developed such that the ant could attack, it would, and the behaviour pattern would contain aggressive rather than defensive components. Hence the behaviour pattern will be termed a "threat posture" rather than a defensive posture, (see also Wallis, 1962b).

1.1 The stereotyped threat behaviour was ideal for classical (typical) ethological, analytical study, and it was hoped that the results, could in the future be used to provide insight into the aggressive behaviour of insects showing very little specialised aggressive behaviour. The fixed action pattern was so distinct that, as the releaser complex included many sensory components, the scope of the research project was extended to cover certain aspects of the sensory physiology of the ant, using the threat posture as a sign that the ants perceived the stimuli to which they were being subjected.

1.2 Although threat in F. rufa had been noted before by Conway (1834), Daniell (1847) and Buchanan White (1871), Donisthorpe (1915)

described the threat posture of F. rufa:

"Formica rufa secretes a large amount of formic acid (HCOOH), the Spiritus Formicarium, and is the most capable of ejecting it into the air in defence of the nest etc.; these ants partly paralyse their prey with acid discharges, and spray the liquid into wounds caused by their mandibles. When alarmed, or enraged, the workers stand upon the tips of their feet, with the gaster bent between their legs, and the acid is ejected to a considerable distance from the anal aperture."

Wallis (1962) mentions the behaviour pattern and the naturalists or zoologists who have shown any interest in F. rufa and noted the behaviour pattern at one time or another are too numerous to mention.

1.3 Threat behaviour can only be elicited from females. Males respond to strong sensory stimulation by fleeing, or are unaffected by stimuli which cause workers to respond strongly. Virgin reproductive females do show threat behaviour but the large thorax and gaster prevent the gaster being easily tucked under the ant and fleeing behaviour is shown more often than threat behaviour. Thus defence of the colony is the prerogative of the sterile worker. Males, when present, are incapable of efficient defence due to their morphology, i.e. small heads and jaws, and large cumbersome abdomens and wings. The reproductive females are too valuable to a colony and do not fight but seek shelter at any sign of danger.

1.4 Thus when alarmed and presented with sensory stimuli suggesting

danger, workers will show threat behaviour with the following features:- (Figs. C1.1 and C1.2)

a) The mandibles are held wide open and the other mouthparts are fully withdrawn.

b) The body of the ant is erect and the ant is alert. The mouthparts are the most anterior, superior part of the body apart from the antennae which are held well forwards and often rigid.

c) The prothoracic legs are held forwards and very often off the ground, being waved synchronously in a position where they could easily be brought into contact with anything moving close to the ant.

d) The gaster is tucked forwards between the legs so that its ventral surface almost touches the ventral surface of the thorax. The apex is held as far forward as is possible, pointing obliquely upwards in the direction in which the ant is facing. The meso-thoracic legs are spread wide and slightly forwards and the meta-thoracic legs are placed well back.

e) Formic acid (+ other volatile compounds?) is sprayed from the apex of the gaster, possibly as far as 15-30 cms.

1.5 This full threat behaviour is elicited only by strong sensory stimuli. If stimuli are weaker then the response is correspondingly weaker. In order that experimental results could be analysed, five stages of threat behaviour response were recognised ranging from a very low intensity response at stage 1, to the full threat posture at stage 5. These stages, although arbitrarily separated for

FIGURE C1.1 Sketch of *Formica rufa* in full threat posture.

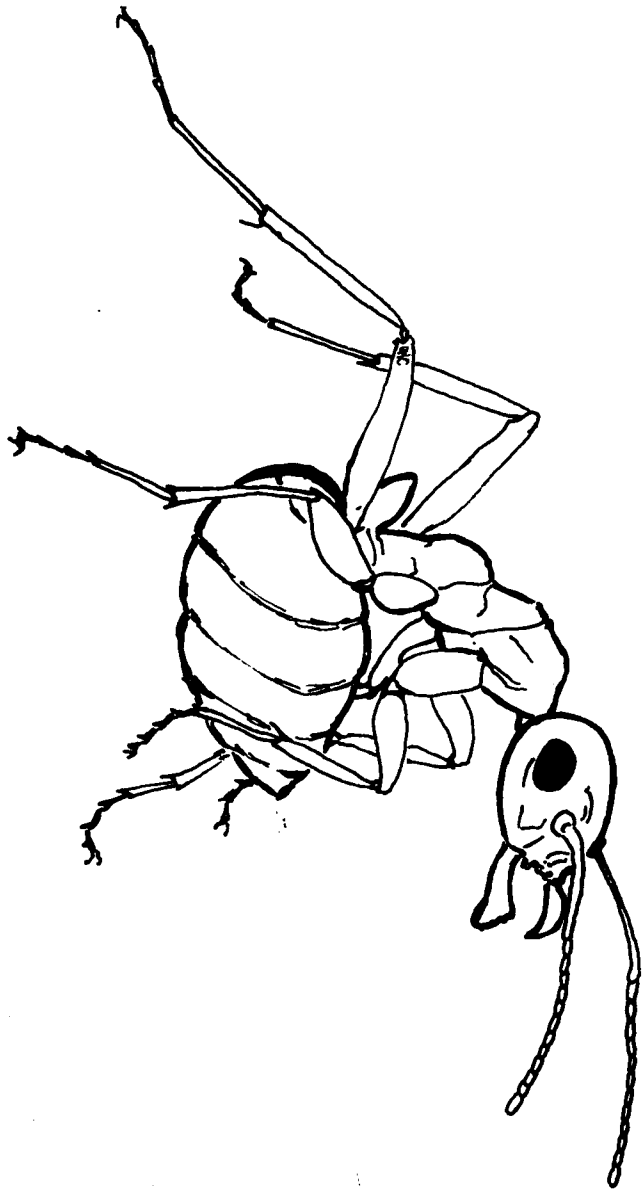
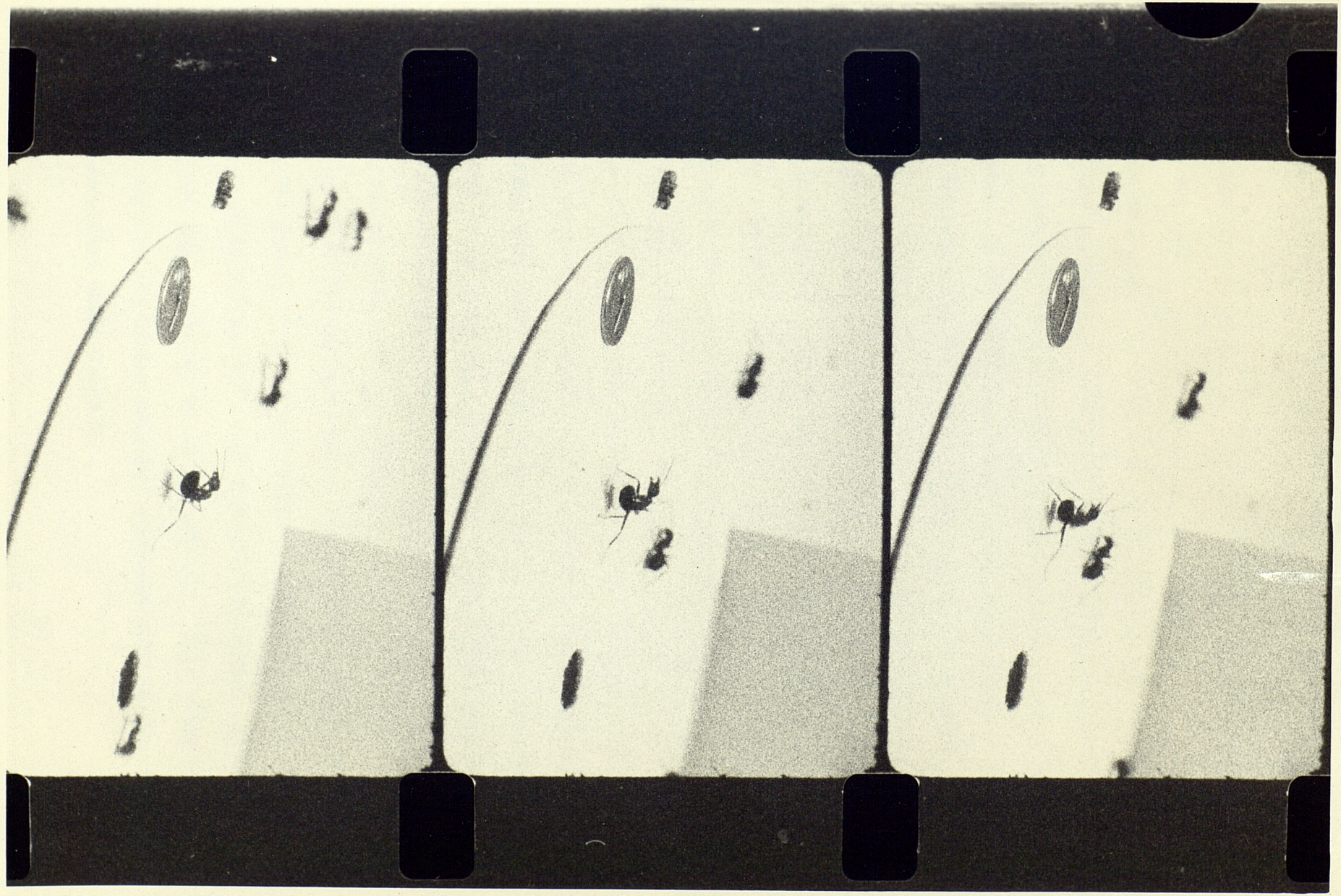


FIGURE C1.2 Full threat posture of *Formica rufa*



convenience, are easily noted by a trained observer and it is usually easy to note the transition from one stage to the next.

Stage 1 This is a very low intensity threat response where the ant has an alert posture, the antennae are held forwards, the mandibles are open and the other mouthparts are withdrawn (Fig. C1.3).

Stage 2 Stage 1 is distinguishable only from stage 2 because in the latter stage the ant stands much more erect, but still with all legs on the ground. The ant may be moving towards the stimulus, in which case the ant runs on all legs either steadily or with frequent stops and possible vertical movement of the thorax and head with respect to the ground. This is brought about by flexing the prothoracic legs. The longitudinal axis of the body may vary from horizontal (Fig. C1.4) to nearer vertical (Fig. C1.5).

Stage 3 (Fig. C1.6) The prothoracic legs cease to be held on the ground and are waved synchronously in the direction of the stimulus. The longitudinal body axis is much nearer vertical than horizontal varying from 45° - 90° from the horizontal. When moving forwards the ant proceeds on the meso- and metathoracic legs either by four leg hopping/jumping or by four leg walking.

Stage 4 (Fig. C1.7) The gaster becomes tucked under the ant, but not folded under enough to touch the ventral surface of the thorax. The body axis varies between 45° - 90° from the horizontal and the prothoracic legs may or may not be on the ground. This stage is usually seen only in stationary ants but sometimes ants do hop forwards as described under stage 3.

FIGURE C1.3 Stage 1 threat posture of *Formica rufa*

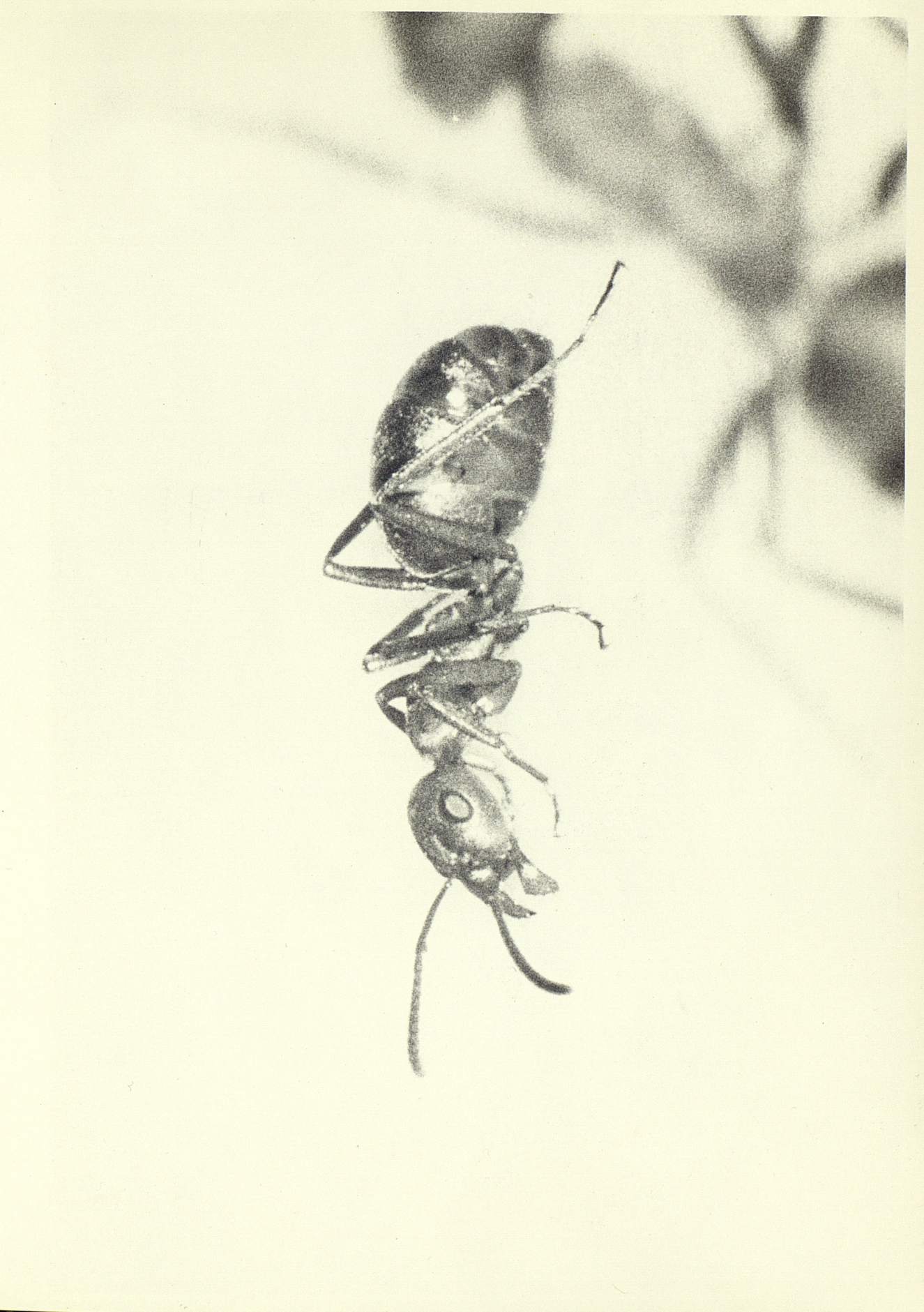


FIGURE C1.4 Stage 2 threat posture of **Formica rufa**

a) Horizontal posture.

FIGURE C1.5 Stage 2 threat posture of Formica rufa

b) Near vertical posture.

FIGURE C1.6 Stage 3 threat posture of Formica rufa

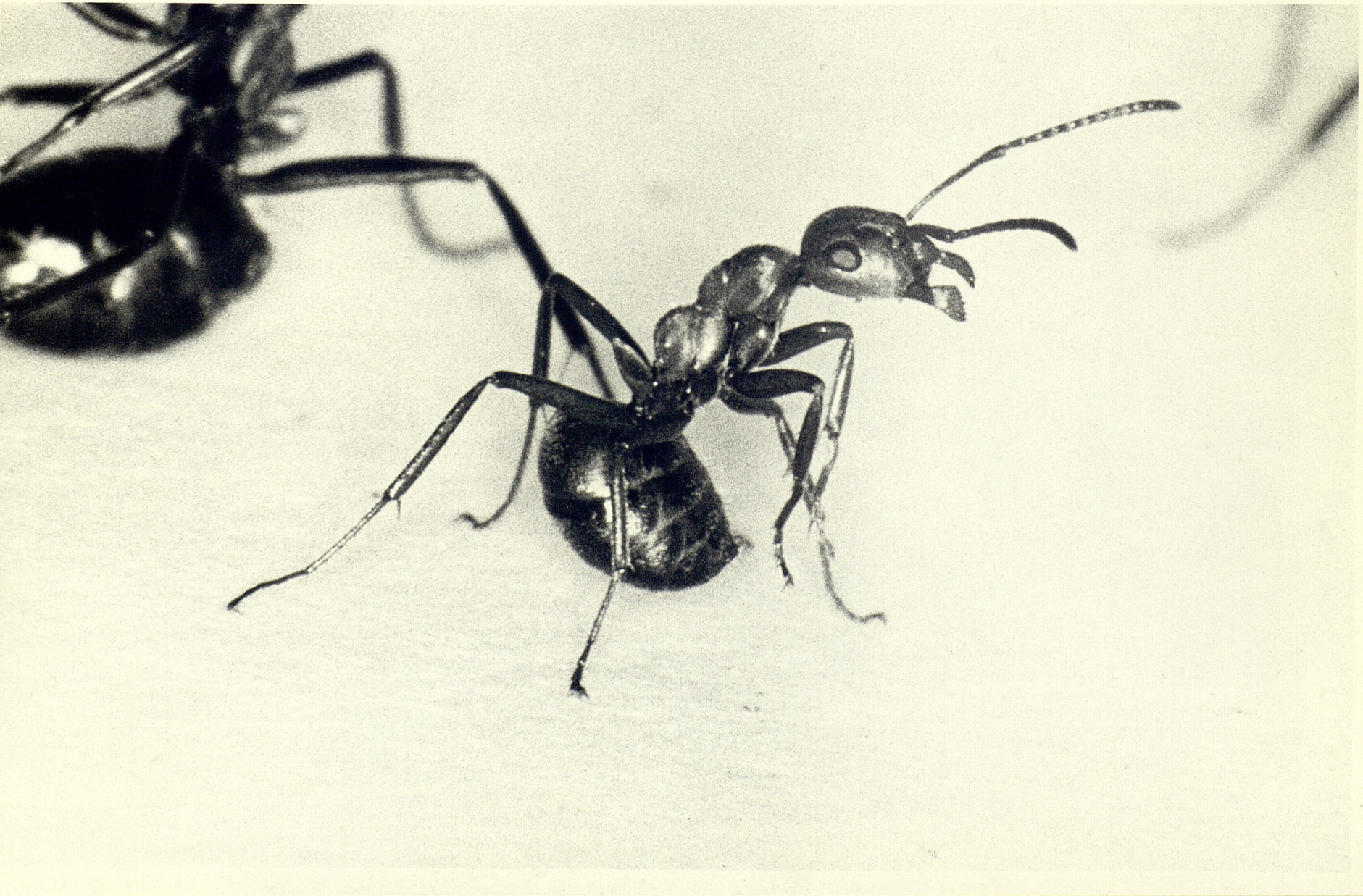


FIGURE C1.7 Stage 4 threat posture of Formica rufa

Stage 5 The full response is as described under Section 1.4 and occurs only when an ant is stationary, although ants do often tend to creep towards the stimulus by shuffling movements.

1.6 Ants were observed for behavioural response in the arena described in Section 2.10. The assessment of the degree of response was necessarily qualitative and subjective, although consistency could be maintained with practice. However, because the results were based on subjective assessments, it proved difficult to compare results from different experiments or tests. Thus for statistical analysis and in order to compare results from any two tests, a statistical ploy was used to reduce the results of each test to a single value which was quantitative and directly comparable with other test values, Krishnan (1967).

In any observation carried out using the behavioural arena described in Section 2.10, a standard of 25 ants per test was used. I.e. for any single stimulus of one or more components, the threat responses of 25 naive ants entering the arena were noted. This unit of 25 ants comprised one test. An experiment either covered one or more tests in which unique stimuli were used, e.g. chemicals, or a set of tests in which a series of discrete stimuli were selected from a constantly variable stimulus, e.g. speed of movement.

1.7 Thus each test could have a number of ants responding at any of six stages (a negative response and the five positive response stages). In Table C1.1A, test 1 shows response at stages 4 and 5 to a hypothetical

A. COMPARISON OF ANY TWO TESTS

	STAGES					
	0	1	2	3	4	5
Test 1	?	?	?	?	5	5
Test 2	?	?	?	?	6	4
Test 3	?	?	?	?	7	3
Test 4	?	?	?	?	8	2
Test 5	?	?	?	?	9	1

? = an unknown quantity

B. RESPONSE STANDARD

	STAGES					
	0	1	2	3	4	5
	0	2	5	$\frac{25}{2}$	$\frac{125}{4}$	$\frac{625}{8}$
OR:	0	0.0155	0.0388	0.0970	0.2425	0.6062
						= 1.0000

C. EXAMPLE

	STAGES					
	0	1	2	3	4	5
Test X	0	5	5	5	5	5

Multiply by the Response Standard

0.0775 + 0.1940 + 0.4850 + 1.2125 + 3.0310 = 5.0000

Thus the Intensity Index for Test X = 5.0 (100% = 15.155)

TABLE C1.1

stimulus, test 2 shows the response to another. In comparison, test 1 shows a better response to the stimulus because more ants respond at a higher stage. The same conclusion is reached when test 3 is compared with test 1. The latter shows a better response. However when test 4 is compared with test 1, the fact that 8 ants are responding at stage 4 and 2 at stage 5 in test 4, means that test 1 with only 5 at stage 4 and 5 at stage 5 is not necessarily a better stimulus, even though more ants respond at the higher stage. Test 4 is therefore taken as the critical point, and Test 5 would then definitely be a better response than test 1. Thus it was decided that there must be a difference of three ants at each stage between the test with the higher number of ants at the lower stage and the test with the lower number of ants at the higher stage before the former could be considered as being the better stimulus.

1.8 Having decided this, a response standard could be established which is based on the relationship 2:5 and which relates to the premise above, Krishnan (1967).

Table C1.1B shows this response standard worked out for each response stage. These response standards are multiplied by the relevant experimental response figures, the totals summed and a single Intensity Index obtained for each test, see Table C1.1C. The Intensity Index enables the results of any series of tests to be compared either together or with those of other series. The higher the Index the better the releasing value of the stimulus. (100% response is when 25 ants respond at stage 5 and provide an

Intensity Index of 15.155). All experiments and tests involving threat response are compared using the Intensity Indices.

1.9 No previous work has been carried out directly on this releaser complex in F. rufa and no records exist of the behaviour pattern being used as a behavioural sign that a stimulus has been perceived. Wallis (1962b) described the aggressive behaviour of the related species F. fusca which shows threat behaviour up to stage 2. He describes the close links between all components in the aggressive behaviour complex, a matter that will be discussed in Chapter 8.

1.10 The intention was to examine the responses to any environmental variables that could act as releasers to threat behaviour. These were to be presented to the ant in such a way that only one variable altered throughout an experiment in order that any changes in response could be attributed to changes in that particular variable. In this way a set of stimuli giving optimum response could be obtained and combined to give a complex stimulus which would, it was hoped, elicit full threat behaviour and be homologous to the natural stimuli, but have the advantage that the stimulus components would be known.

1.11 The physiological condition of the ant could cause endogenous stimulation, which would determine the level of the behavioural threshold but this would not be a direct factor in threat behaviour and is therefore only of indirect importance.

1.12 The external environment can provide visual, tactile, acoustic and chemostimulation. Table C1.2 lists stimulus types investigated together with the experiments carried out under the appropriate headings. Appendix III contains description of a piece of apparatus designed to prove presence or absence of colour vision in F. rufa. Development time was extensive and refinements are still necessary in the optical and mechanical systems. As results depended upon learning and conditioning in the ants and the necessary time was not available, the description of the apparatus is included as an appendix, without any experimental results.

The review of work already carried out in the fields investigated will be included in the relevant chapters.

A. VISUAL STIMULI

Chapter 3 quantitative properties

i) Stationary patterns

a) Variation in shape: Expts. 62,64 and 69

b) Variation in size: Expt. 73

ii) Moving patterns

a) Variation in velocity and flicker: Expts. 26-33,54,58,68 and Expts. EP4-EP14

b) Variation in shape and size: Expts. 35,36,48-53,55-57, 59-61 and 63

c) Variation in pattern regularity: Expts. 34,37-47a,71 and72

Chapter 4 qualitative properties

a) Range of visual sensitivity: Expts CC2-CC14 and Q1-Q3

b) Response to monochromatic light: Expt. 23

c) Descrimination between wavelengths: See Appendix III

B. CHEMICAL STIMULI

Chapter 5 Expt. 8

C. ACOUSTIC STIMULI

Chapter 6

Response to substrate-borne vibrations: Expts. 70,73-75 and 80

D. COMBINED STIMULI

Chapter 7

a) Chemical and visual stimuli: Expts. 8 and 65

b) Chemical and acoustic stimuli: Expts. 83-86

c) Acoustic and visual stimuli: Expts. 78,79,81 and 82

d) Acoustic, chemical and visual stimuli: Expts 88-91

TABLE C1.2 List of stimuli types and experiments.

CHAPTER TWO

Apparatus and Experimental Nests

2.1 Nests

Formica rufa is a mound-building ant. The usual habitat of the insect is in coniferous woodland, although it does occur in mixed woodland. Nests are usually located along the south facing edges of rides and pathways where they receive maximum amount of sunshine. The mounds of F. rufa are thatched mounds, that is to say that the mound consists of plant material, usually pine leaf litter. The needles although apparently laid without design, form a waterproof thatch which is so effective that a heavy shower of rain will run off the nest slope.

Galleries are excavated below ground level, usually round an old tree stump, and in large nests may descend into the substrate to a depth of two to three feet depending upon the height of the watertable. Mounds vary in size from a mound of 10 - 20 cms. high with a nest base diameter of 45 cms. to large mounds 1.5 - 2 metres high and 2 - 3 metres in diameter at the base. The latter nests are only found where the pine woods have been undisturbed for a number of years and the underground tunnels and excavations in such a case extend out beyond the base of the mound and exits are clearly visible up to 60 cms. from the base of the mound. One such nest was discovered in Wentwood, Monmouthshire during September 1966, but it had been destroyed the following summer, probably by the Forestry Commission who had begun a new culling cycle in this wood during 1967.

Even the smallest F. rufa nests have a population of several thousand workers and foraging trails may run for twenty yards or more. In the large nest at Wentwood a foraging trail was followed for a hundred yards and at that point was still continuing.

The problems of keeping such a species in the laboratory are thus much greater than for keeping myrmecines or even the closely related species F. fusca. To keep a thriving colony under laboratory conditions, the typical formicaria of Santschi, Janet and others are obviously inadequate both in size and nature of construction. Even the nests used by present day workers are inadequate and small, Wallis (1963), Vowles (1965). It is not known what social pressures are found in a small colony in a small enclosed laboratory formicarium, but if such conditions can be avoided then such pressures are kept to a minimum. Thus completely new style laboratory formicaria have been designed and used, the main features of which are their large size and open plan nature. The formicaria were designed so that the colonies contained could build as natural a nest mound as possible under laboratory conditions. Construction also allowed the inside of the mound, as well as the outside, to be inspected without disturbing the ants.

2.2 The Church Nest

This was so named because of the similarity between the nest in construction and certain modern church designs (see Figs. C2.1,A1.1d).

The principle of the nest is that the colony live in a sector of

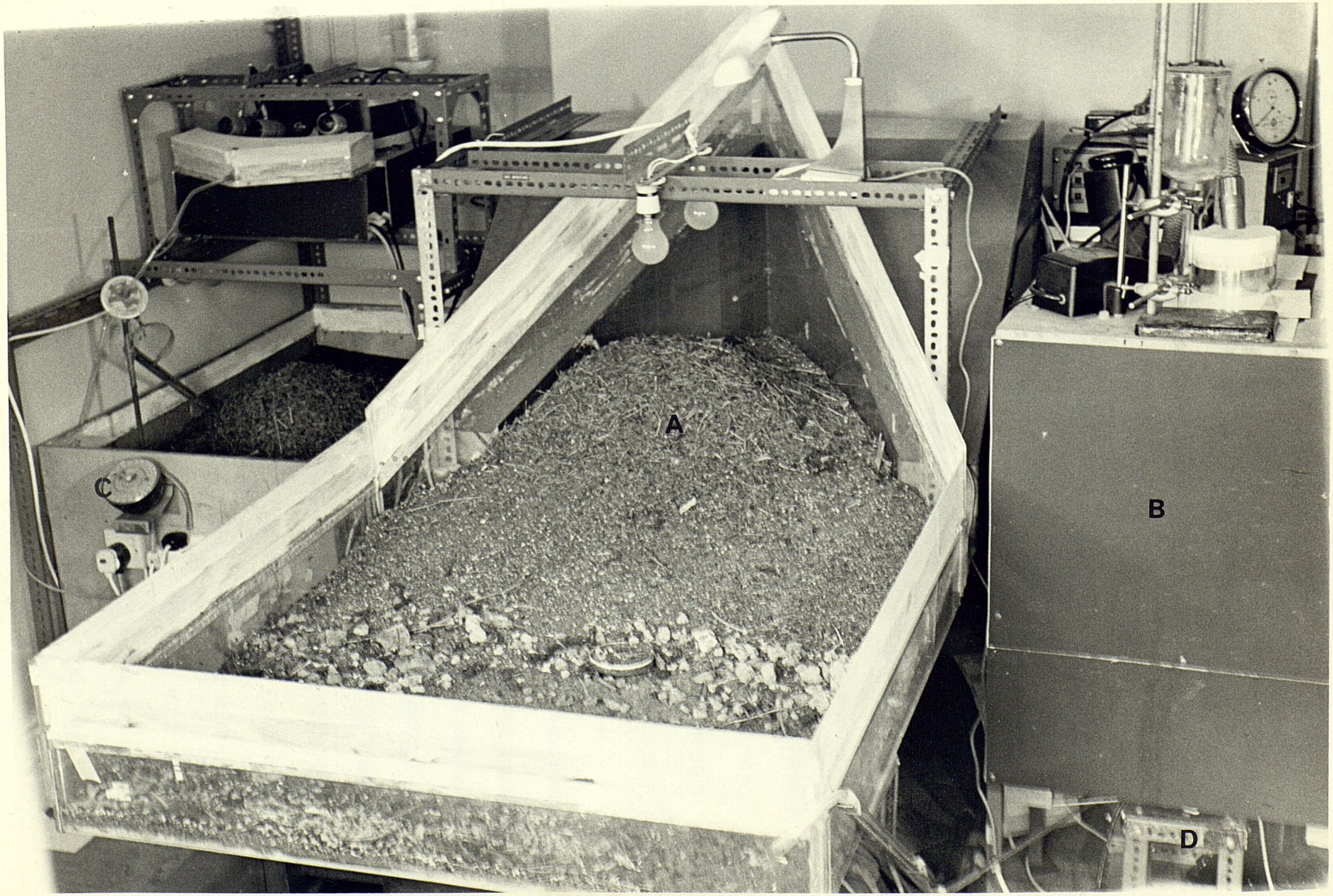
FIGURE C2.1 The Church Nest.

A) The Church Nest

B) The Behavioural Arena

C) Time-switch

D) Gantry for Vibrator (Section 2.23)


A
B
C
D

a mound, rather like a slice of cake. The mound sector is built by the ants into a clear perspex former which constitutes one side of a metre square foraging area. The nest dimensions and plans can be found in Appendix I (Fig. A1.1a-d). The mound slice is surrounded by a light tight chamber with three removable doors. This chamber contains four black lamp heaters and thus ensures that the nest interior is maintained at a satisfactory temperature. By observing ants in The Slice Nest it was found that the optimum interior temperature was in the region of 30°C. This temperature was maintained using a thermostat. The heating chamber is not connected to the nest interior and thus forms an observation chamber for the nest interior. Galleries are easily visible through the perspex walls of the mound slice. Larvae and pupae are often housed in these adjacent galleries.

The nest material and colony were removed complete and unsorted from a suitable location, transported in a plastic dustbin and emptied onto the nest base towards the sector end. Additional nest material was placed in the foraging area. Within a short time the colony attracted by the warmth reconstituted itself and built the nest mound up into the perspex former. The nest slope runs straight into the foraging area and thus activity could be observed as on a field mound.

The foraging area and nest sector are open to the air and there is nothing to prevent animals flying in or out. However exit from the nest up the 25 cm. perspex walls is prevented by a ten cm. wide band of Polytetrafluoroethylene (P.T.F.E. or Fluon). This is

supplied by I.C.I. Plastics Division, Welwyn Garden City in an aqueous dispersion and is simply painted on to the vertical perspex walls. The dispersion, when dry, forms such a smooth surface that the insects are unable to obtain a grip. The P.T.F.E. strip is effective only if painted on to glass, perspex or shiny metal strip; on wood or other porous surface it does not form a non-stick surface. Initially an electric fence was used to retain the ants, but it produced a high mortality rate and was not as efficient as the later P.T.F.E. strip.

Above the nest surface a gantry carries two light bulbs wired into a time switch providing the nest with a twelve hour light cycle throughout the year. A fifteen inch fluorescent tube is also kept alight continuously to suggest summer conditions. The laboratory is heated day and night and the room temperature varies between 20° - 30°C. Colonies are kept active for twelve months every year and thus behavioural experiments are possible at all times.

Exit holes below the P.T.F.E. strip are provided in the end wall of the foraging area to enable experimental apparatus to be coupled into the nest.

2.3 The Slice Nest

The Church Nest was designed primarily for observation on the nest surface and foraging area. The Slice Nest, however, was designed for observation of the nest interior and to obtain a temperature gradient across the nest slice. The nest former consists

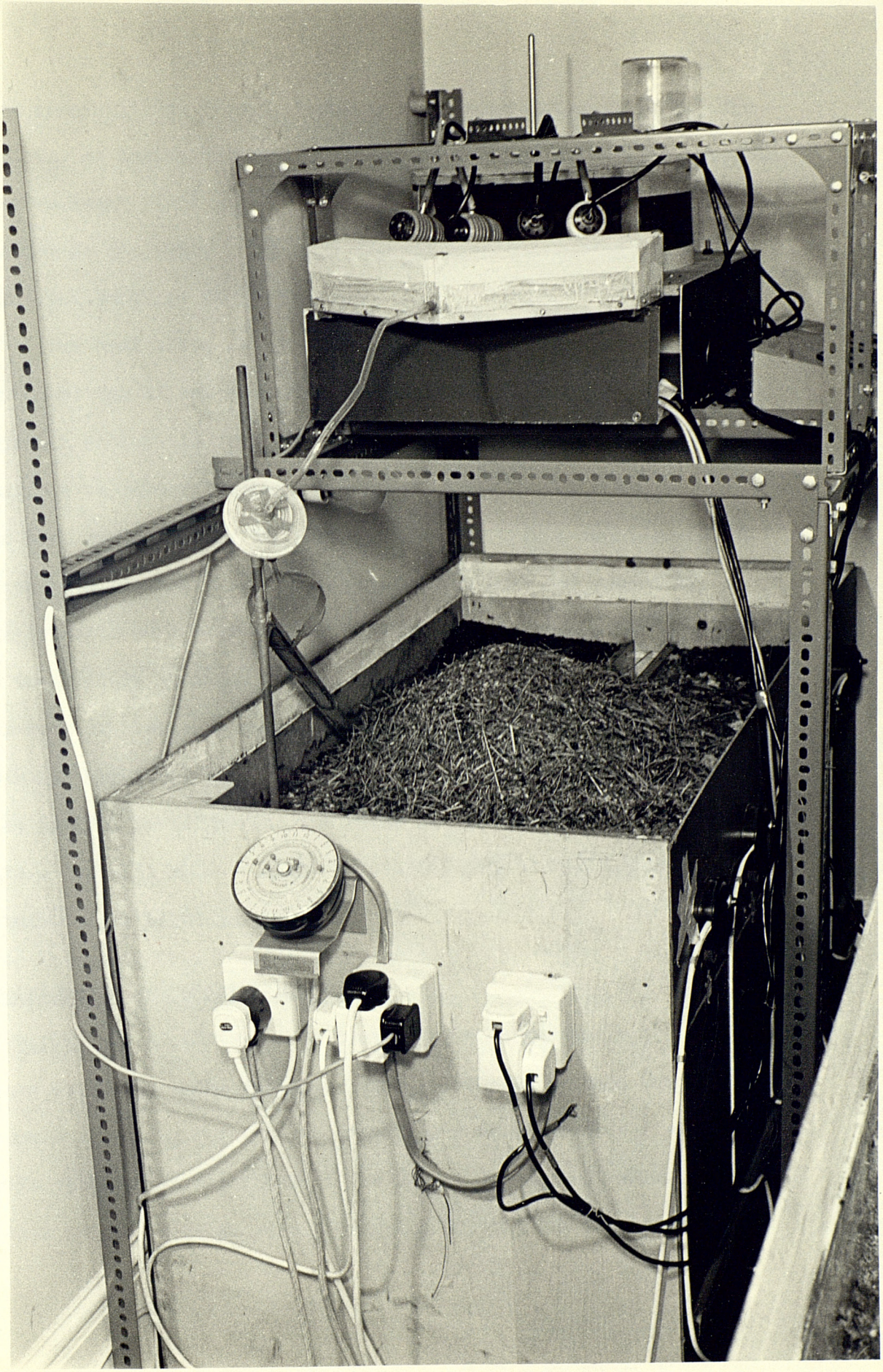
of two clear perspex walls, each 120 x 60 cms., six centimetres apart, sealed at the ends and bottom and forming a vertical slice box. One of the perspex walls forms the fourth wall of a light tight box, 120 x 60 x 30 cms., with a single door opposite the perspex wall. The perspex wall is marked out with a matrix and numbered so that each sector can be referred to again. This box allows observation of the nest interior.

The other perspex wall forms the fourth sides of five small light tight boxes, together equalling the size of the observation box. Each small box is lined with half inch thick expanded polystyrene sheet (except the perspex wall) and has a door opposite the perspex wall. Each box contains a black lamp heater and thermostat, together with two copper/constantan thermo-couples which protrude into the perspex slice box from the top and bottom of each of the five boxes. A reference thermo-couple protrudes from one end of the complete nest box. The roof of the observation box and the five heater chambers forms the floor of a foraging area along the centre of which the perspex slice box opens. The wall of the foraging area is fifteen centimetres high and around the top is a five centimetre aluminium strip painted with P.T.F.E. to retain the ants. This replaces the electric fence used initially to retain the ants. Holes are drilled below this strip to allow the nest to be coupled into experimental apparatus (see Figs. C2.2 and A1.2a-d).

Nest material containing an ant colony is collected as described for the Church Nest and placed in the centre of the foraging area and nest material pushed down into the slice box. Thus when the colony

FIGURE C2.2 The Slice Nest.

Including apparatus described in Appendix III



has reconstituted itself, there is an underground nest slice containing galleries between the perspex walls and above this a normal conventional field mound of perhaps thirty to forty-five centimetres across at the base. The size depends upon the amount of nest material supplied to the colony.

The nest slice is heated from the heater chambers and a heat gradient can be set up across the nest and optimum temperatures for certain activities observed. However in order to keep the nest surface at summer temperature e.g. 20° - 30°C., it would be necessary to cool one or two of the heater chambers. As a cooling unit is expensive and heat preference experiments were not essential, no experiments were conducted on temperature preference in F. rufa. A temperature gradient between room temperature 20° and 40°C. was however achieved for a testperiod during which the ants occupied the section heated to approximately 30°C. This temperature is taken as the optimum preferred and the nests heated accordingly. A gantry above the nest holds a light bulb wired into a time switch giving twelve hours light per day.

Maintenance and general notes

2.4 Watering It is advisable to drench the nest surface and interior well once every two weeks and give light showers in between because with constant heating the nest material has a tendency to dry out quickly. This watering is best carried out with a pressurised spray or a small water pump. A petri dish containing cotton wool is placed in the

foraging areas and kept partially filled with water to provide a constant waterlick for the colonies.

2.5 Feeding The diet consists of carbohydrate in the form of sugar solution (just concentrated enough to give a sweet taste to man) and protein in the form of insects, either cockroaches or blowfly maggots. It is more convenient to use maggots as they can be stored for long periods at low temperature and a quantity fed to the ants once a week. Also they are more easily overcome by the ants and dragged into the nest. Cockroaches, unless their tarsal claws are first removed, climb out of the nest enclosures and escape. They are also harder to catch, kill and drag into the nest. Dead protein matter is not very acceptable and is eaten only if nothing else is available. Maggots have another advantage over cockroaches in that when sucked dry the skins are easily portable and removed to the colony cemeteries. Dried cockroach skeletons are less easily disposed of by the ants.

Sugar solution is not actually supplied in the foraging area unless no experimental apparatus is connected to the nest, in which case an air pressure drip feed vendor is placed in the foraging area (for details see Appendix II). Usually colonies are fed the sugar solution in the experimental apparatus in order to keep a steady stream of ants entering the apparatus.

2.6 Apart from feeding and watering, the nests are left strictly alone, although from time to time a little more leaf litter is placed

in the foraging areas. Thus the ecology of the nests is not disturbed. As the nest material comes straight from the field without cleansing there are numerous myrmecophiles living with the ant colonies and the ecosystem works remarkably well. Due to the fact that I transferred from Cardiff to Southampton a year after beginning this work, I have been unable to keep any colony for longer than eighteen months. The present colony in the church nest was introduced into the enclosure in October 1967 and was still viable in April 1969. It was replaced due to pressure of work and inactivity of the colony. Thus there is no problem about continual replenishment of experimental animals.

2.7 Another indication that the colonies thrive under the conditions available is that during December/January 1966/7 alate reproductives were produced in the slice nest. Only males were observed but during the following December/January alates of both sexes were produced in the church nest. The first two to three batches bred were males but towards the end of the burst of male production, a smaller quantity of females was also produced. During January 1969 a small quantity of males was again produced.

In the wild, F. rufa produces alates during May - July, thus why were they produced during December and January in the laboratory colonies? During the winter months, in order to keep the colonies alive, the room temperature is kept between 20° - 30°C. even at night,

the nests are well watered and food is provided in plenty, especially protein. This would simulate the summer for a normal colony. During the summer months, as outside temperatures are higher and the ants naturally active, the control of laboratory conditions is relaxed and thus the annual reproductive cycle is partially reversed.

2.8 An interesting observation concerning colony behaviour is necrophoric behaviour and the installation of "cemeteries". In the wild, dead ants are transported out of the nest and disposed of some distance away from the nest site. However in an enclosed laboratory nest this is not possible and one of the most prevalent behaviour patterns is the carrying of dead (also noted by Wilson (1958) in Pogonomyrmex badius). A dead ant may be carried round the nest enclosure several times before it is deposited either on a cemetery or elsewhere. It may even be picked up and transported again by another ant for a further period of time. This behaviour suggests that the dead are taken as far from the nest as possible. If this is not possible, as in the enclosed nest, the process is simulated by a long pointless transportation of dead ants until the porter ants fulfill their internal drives.

The dead ants all end up, in time, on a "cemetery". These are sites within the enclosed system where all dead, both ants, myrmec-

ophiles, maggot cases and dead flies are placed in a pile. The cemetery sites are usually at the furthest point possible from the nest proper. This is either a far corner of the foraging area or, if experimental apparatus is coupled to the nest, may turn out to be transferred to this, it being further from the nest.

2.9 The Behavioural Arena and Ancillary Apparatus

For all the behavioural experiments described, except those on colour discrimination, a single obeervation arena was used. This was modified accordingly to suit the sensory stimulus required for any series of experiments. The ancillary apparatus was also changed for different series of experiments and the arena was thus both simple and versatile.

2.10 The Arena

The arena is a much modified and reduced version of the arena used by Jander and Voss (1963) in their experiments on shape discrimination and preference in F. rufa.

It consists of a cylindrical chamber, 55 cms. diameter, inside a square light tight box of 60 cms. side. Both cylinder and box are 75 cms. high. (Figs. A2.1, C2.1, C2.3, C2.4) The cylinder is a tube of thick white cartridge paper held in position by four circular formers, at the top, bottom and equally spaced between. Inside the cylinder on the floor is a peripheral ring of ten equally spaced

FIGURE C2.3 View of the top of the Behavioural Arena

Note:

A) Sugar Solution Container for The Drip Feed Sugar Solution Vendor.
B) Housing for Extractor Fan (see Section 2.22)
C) Motor and Gears
D) Speedometer
E) D.C. Power Supply Unit.
F) Illumination Lamp
G) Stop Clock and Digital Counter
H) Observation Window

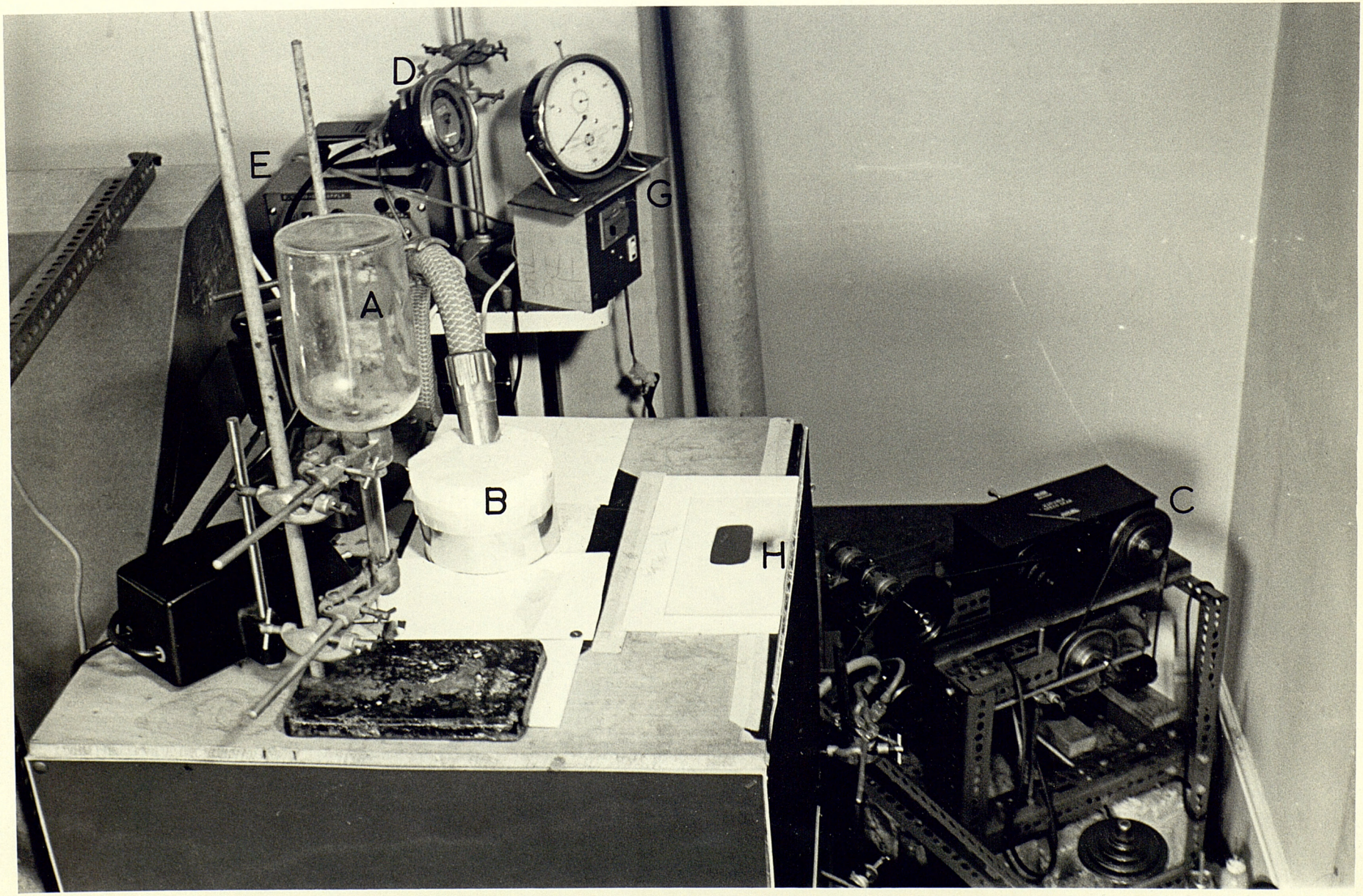

D
E
G
A
B
C
H

FIGURE C2.4

Note:

A) Stimulus Cylinder.

B) Speedometer Drive-cable.

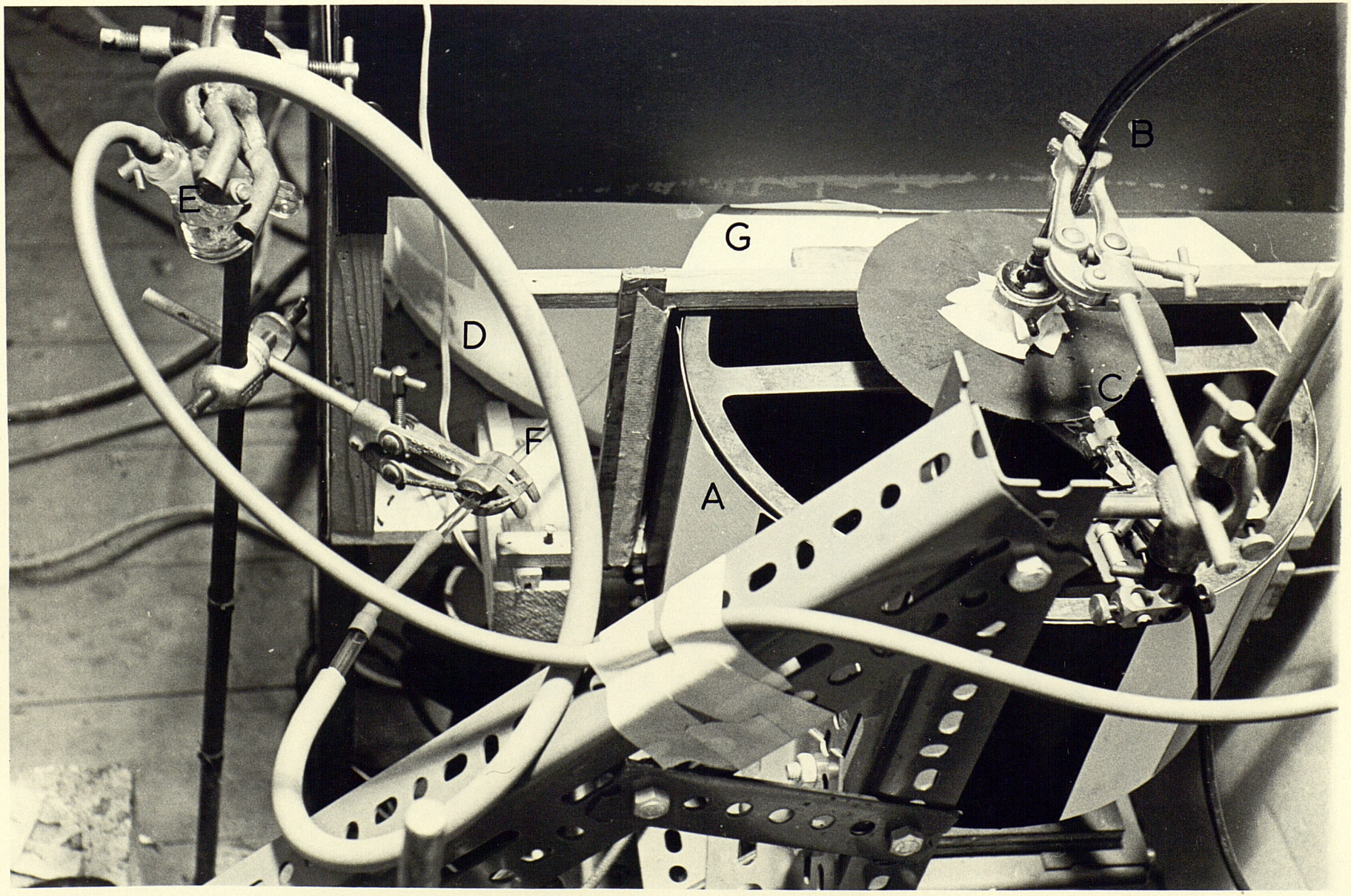
C) Photoelectric Counter.

D) Exterior view of Arena wall.

E) Bubbler.

F) Glass tube, drawn into a jet.

G) Top of Bay-window.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M.E.S. lamp sockets, each holding a 24 volt, 2.8 watt type 993 pilot bulb. The ring of lamps is run in parallel from a 30 volt A.C. supply. Positioned centrally inside the ring of lamps is a circular platform, 45 cms. diameter with a 5 cms. vertical wall of aluminium strip. This wall prevents those ants that are walking on the platform from having direct vision of the lamps. thus providing concealed lighting. On the inner face of the wall is a coating of P.T.F.E. to prevent escape from the platform.

Ants enter onto the platform via a centrally placed hole which is just big enough to take the spout of an inverted 2" glass filter funnel. The spout is cut level with the platform. The funnel thus protrudes down below the floor of the enclosing box, which stands on legs. The funnel is coupled to a tube at right angles to the funnel and the tube leads to one of the nest exits. The funnel is inserted between the tube and the platform in an attempt to provide random direction of entrance on to the platform.

The platform is covered with white paper to provide a good contrast between the floor and ants for purposes of observation. However this soon becomes dirty due to the activity of the ants and spillage of sugar solution. Placed over the central hole is a 12 cm. disc of white card with a 5 mm. central hole and a 5 cm. high white card wall around half of its circumference, the other half being open. This hemi-cylindrical dais is placed with the open side facing that part of the cylinder wall which is used for attachment, projection or

protrusion of the behavioural stimuli. Behind the hemi-wall on the other side of the platform is placed a small petri-dish containing cotton wool. Into this dish is placed an air pressure drip feed sugar solution vendor (for details see Fig. A2.2) so that the dish cannot be approached directly from the central hole and is not visible to ants entering onto the platform.

The top of the box/cylinder is closed off by a 60 x 60 cm. blockboard lid containing three holes; a central slit 13 x 50 cm. and two smaller holes, one for an observation window and the other for an illumination lamp. The underside of the lid and any covers over the holes are black. The central slit provides access to the cylinder for apparatus and in changing lights.

2.11 The white cylinder provides a stimulus free wall and the ring of lamps provides concealed lighting, giving no direction landmarks. The platform and cylinder walls together form an experimental arena which could be used in choice experiments, if required. They will be called 'the Arena'. However as the behavioural experiments observed in the arena are dependant upon fixed action patterns and not orientation responses, the presence of such items in the arena as the dais and the feeding dish, although they provide direction landmarks, is irrelevant once their position is learned. Thus only items which are new to the arena e.g. visual stimuli, are liable to excite a behavioural response from an ant newly entering the arena.

This is, of course, not true for the arena of Jander and Voss (1963) where preference experiments were carried out and the presence of an orientation signpost would have upset the behaviour of an ant entering the arena.

2.12 For all experiments the illumination lamp casts a pool of light over the centre of the arena. The lamp is usually run at setting 8 on the ten point scale of the built in rheostat. The illumination is provided so that an observer can clearly see any behavioural responses in the arena. The aperture is usually opened wide to flood the arena platform. However in the experiments on the behavioural response to colours, because the intensity of the stimuli was low, the arena was blacked out and the central dais was pinspotted in red light using an Ilford Spectrum Filter. This provided enough light under the blacked out conditions to observe behavioural responses.

Another lamp is provided below the arena to illuminate the inverted filter funnel. The funnel is illuminated so that the ants, with eyes adapted to the laboratory lighting intensities, on entering the funnel are partially re light-adapted at a higher intensity for entrance into the arena, which has a high light intensity. Without this adapting link, it was found that many ants needed an initial period, on entering the arena, to adjust to the conditions and spent many seconds or even minutes at self-grooming behaviour and were thus pre-adapted to the stimuli when they finally responded. This meant that they were no longer naive.

2.13 Ancillary Apparatus

In order that responses to visual, chemical and acoustic stimuli could be observed, the arena was gradually modified by addition of ancillary apparatus concerned with the production of the stimuli required. The bulk of the apparatus was positioned outside and adjacent to the arena and consequently to give improved performance could be changed or modified without upset to the ants.

2.14 For the initial experiments using stationary visual stimuli, the arena was unmodified and as described under Section 2.10. The stimuli were geometrical shapes out out of black card (for details see Chapter 3), and were hung from white strings on the inside of the arena wall, the bottom of the stimuli being 6 - 10 cms. from the arena floor.

Further experiments using stationary visual stimuli were carried out after modification of the arena for the moving visual stimuli experiments (Section 2.16). However once the foraging ants had learned the position of the stimulus window, its presence was irrelevant for the reasons stated in Section 2.11, and responses were given only to the stimuli presented. Both the initial and later experiments are thus grouped under Experiments 62, 64, 69 and 73.

2.15 Experiment 23 was concerned with the responses to, and perception of, monochromatic light. A 10 x 10 cm. hole was cut in the arena wall behind the hemi-wall of the central dais. This hole was thus not visible from the central dais. Through this hole a geometrical shape was projected onto the opposite wall of the arena from a

projector outside the arena box. The size of the stimulus was adjustable by moving the projector backwards and forwards in relation to the arena.

Back projection was at first attempted but the translucent screen required, absorbed much of the projected light and the images obtained on the screen were thus indistinct due to the low intensity of illumination. Front projection was substituted as higher intensity stimuli could be obtained.

This is an important consideration when colour filters have low transmission characteristics even before addition of neutral density filters to standardise light intensities.

In order to obtain maximum source intensity, a projector was constructed out of a high source intensity lamp, slide carrier and projector lens (see Fig. A2.3). The slide carrier, designed to take a 2" x 2" spectral filter and several 2" x 2" neutral density filters, and the projector lens were supported on micromanipulators. It was thus easy to align the optics and to position and focus the stimulus on the arena wall. The lamp dimmer rheostat was set at 9 and the colour curve for the source emission is shown in Fig. C4.1. The spectral filters were all corrected to give equal illumination at the ant eye (see sections 4.4 and 4.10 for details).

The camera shutter was set on time release and the lamp was switched on and off by the shutter and cable release to allow the lamp to reach a steady working temperature and consequently constant wavelength emission characteristics. For the first series of tests

in experiment 23, the stimuli were continuously presented during a test and the responses of ants entering the arena observed. For the second series of tests, the stimuli were flashed at 3.25 flashes per second by means of a two bladed rotor driven by a small high speed 250 volt A.C. induction motor geared down by means of a 1 cm. rubber wheel on the motor shaft, friction driving onto the rim of a 20.5 c.m. diameter disc on ball races. The double blade was attached to this disc the length being 35 cms. The motor and rotor were positioned between the projector and the 10 x 10 cm. projection hole into the arena, so that the blades obscured the light source twice every revolution. The power to the motor was supplied via a foot switch which was operated by the observer at the observation window. The motor was left running and the stimulus flashing during the course of any test. Details of the stimuli can be found in Section 4.12.

As the projected stimuli intensities were so low, the ring of pilot lights in the arena was switched off in order to provide semi-dark adapted conditions and increase the sensitivity of the insect eyes to the stimuli. Similarly the laboratory lights were also turned off and the curtains drawn. The lamp illuminating the arena entrance was also turned off. The top illumination lamp remained on but a red filter was placed in front of it (Ilford Spectrum Filter No. 608 - 620nm into the infra red), and the aperture reduced so that it pinspotted the central dais. In the blacked out conditions this

provided sufficient light for behavioural responses to be observed. A red filter was used because insect compound eyes are relatively insensitive to red light.

Stereotyped behaviour patterns should be elicited by the correct stimuli irrespective of the ambient conditions. The drastic reduction in illumination for Experiment 23 should therefore not affect the behavioural responses to the stimuli presented, if in fact the responses do occur under normal lighting conditions. Indeed the blacked out conditions should elicit better responses because there could be no stray reflected white or polarised light to which the ants could react preferentially.

2.16 Experiments with moving stimuli create several problems in apparatus design. For experimental purposes, a moving stimulus must have constant speed, a continuous range of speeds which are easily selected and monitored and provide an adequate area of movement.

As a 15 cm. square was the standard size for stationary visual experiments, the same approximate size of stimulus was used in the movement experiments. To obtain a moving stimulus, e.g. stripes on the wall of the arena, there are three possible methods.

I To position a spinning disc of black/white circle sectors either behind a circular hole in the arena wall, or in front of the wall. In the latter case unless the disc and sectors are precision made

and mounted, irregularities in the disc would be accentuated when spinning and could provide a strong enough stimulus for behavioural response. Also the centre of the disc would need masking with a stationary spot for the same reason. Even a disc behind a circular hole would need positioning in an exact concentric position to the hole. True stripes are also not possible with this method.

II The best method of moving stripes across a section of the arena is to surround the arena with a slightly larger concentric cylinder mounted on ball races and thus revolvable. Stripes could be attached to the inner surface of this and be visible through a window in the arena wall. However this would necessitate expensive precision made bearings and components in order to obtain accurate running and prevent vibrations in the arena.

III For this reason the third method was used for all movement experiments. A cylinder with stripes round the curved surface was made to project through a window into the arena. As the cylinder had a large diameter, the surface seen by the ants in the arena was not too convex and the stripes not unduly distorted at the edges (the advantage of method II is that all stripes are at constant radius from ants in the arena centre). The cylinder 30 cms. diameter and 15 cms. deep, mounted vertically on a $\frac{3}{4}$" shaft running in ball race bearings which were attached to a heavy concrete base. The bearing frame was constructed with Dexion (see Figs.A2.6,02.4).

2.17 A bay window was so constructed that it projected into the arena immediately above the 5 cm. metal wall. This window had a convex contour of 1 cm. larger radius than the cylinder. The aperture of the window was 14 x 16 cms. lengthwise but the bay was deep enough to take the 15 cm. cylinder. The window surround was of white card and suitably masked into the arena wall. The window itself consisted of a celluloid pane curved to fit the bay. In the preliminary experiments using moving stimuli, no actual pane was present, but with the cylinder rotating at speeds of up to 700 r.p.m., winds were created by the rotation causing the ants to react erroneously. The window, although possibly obscuring the stimulus and possibly providing reflections and a partial habituation to objects at that point, is unfortunately necessary and better results were obtained with it than without it.

The cylinder was placed in such a relation to the arena that it filled the bay window although not touching the arena or its supports. In this position the cylindershaft base was screwed and braced to the floor to prevent vibration of the complete unit when the cylinder was rotating at high speed. The cylinder was driven from a pulley system.

2.18 This system was capable of producing infinitely variable constant speeds from 20 r.p.m. up to 700 r.p.m. Any speed in this range could be selected by using the 2:1 variable speed gear box and the correct pulley ratios.

The drive was obtained from a 250 volt, $\frac{1}{2}$ brake horse power induction motor producing 1425 r.p.m. This drove a 2:1 variable speed gear box by leather belt from a $1\frac{1}{2}$" pulley on the motor shaft to a four step cone pulley ($1\frac{1}{2}$" - $4\frac{1}{2}$") on the gearbox input shaft. Thus four input pulley ratios were available. The belt was tensioned by an idler pulley. The output shaft from the gearbox carried a similar four step cone pulley and drove a 5/8" shaft via another reversed cone pulley. This pulley pair provided a further four pulley ratios. The 5/8" shaft carried a $1\frac{1}{4}$" pulley giving the final drive to a final four step cone pulley on the cylinder shaft. Drive from the motor to cylinder was easily disconnected by slipping the belt off the cone pulley pair.

To obtain speeds below 100 r.p.m. it proved necessary to change the pulley ratios between the 5/8" shaft and the cylinder. As this was an inconvenient procedure, for speeds below 100 r.p.m. the drive belt from the cylinder was connected straight to a cone pulley on the shaft of a Palmer "Electric Twelve" Kymograph. This was capable of providing infinitely variable constant speeds up to 100 r.p.m.

2.19 Although the complete gearing system was belt driven, constant cylinder speeds were obtainable because the $\frac{1}{2}$ b.h.p. motor provided more than sufficient power to run the system and was therefore never overloaded, gearing down the drive from 1425 r.p.m. increased the

torque available and the heavy metal cylinder acted as a flywheel. So that once the cylinder was accelerated to a particular speed, the flywheel effect and the ample power/torque maintained it at a constant speed. Also waxing the belts and pulleys reduced belt slip to a minimum. Pulley and belt gearing is simpler and more easily adapted than the equivalent toothed gear system and was thus also better for the purpose required.

The motor and pulley system were mounted on a single Dexion frame which was weighted with a heavy concrete base (see Fig. C2.3). The base stood on a sheet of 1" foam rubber to reduce vibrations transmitted to the arena via the wooden floor. As the cylinder was belt driven, no vibrations were transmitted to the cylinder assembly.

2.20 Speed of cylinder rotation was monitored by two methods. One end of a short length of speedometer cable was attached to the top of the cylinder shaft and the other end to an old speedometer. This only gave readings at speeds above 100 r.p.m. but, once r.p.m. against meter reading had been calculated, the speedometer could be used to set the cylinder speed quickly to the correct r.p.m. Once this was done the cylinder speed could be accurately monitored using a photo-electric counter (for the circuit see Appendix IV) giving one count per shaft revolution. The cylinder speed could then be accurately set to the speed required using the counter, a stopclock and adjusting the variable speed gearbox.

2.21 The apparatus used for the chemical stimulation experiments consisted of a pump, a bubbler and a glass jet, all coupled together. The pump was a simple variable pressure aquarium pump set at constant pressure for all experiments. This was coupled by rubber tubing to a bubbler jar. For the early experiments the jar was a 500 ml. 'Quickfit' bubbler jar and the minimum liquid quantity that could be used was 100 mls. This proved expensive on chemical and a smaller glass stoppered bubbler, which had a working capacity of 10 mls. was used for the later experiments. The outlet from the bubbler was fed into a glass tube with the end drawn out into a fine jet of about 0.5 mm. aperture. The glass tube projected through the arena wall and was set in such a position that the jet sprayed vapour from a distance of approximately 10 cms. at the entrance hole in the centre of the dais. The power supply to the air pump was taken via a foot operated switch (see Figs. A2.8, C2.4).

2.22 The arena was cleared of volatile chemical by means of an extractor fan placed in the top of the chamber which forced the impure air from the arena into a 4 cm. diameter hose leading to the chimney piece of the room. The extractor fan was run after each test involving chemical stimuli for several minutes so that the arena was full of odourless air for the next test (see Fig. C2.3).

2.23 In order to test the responses to substrate born vibrations,

an electro-mechanical vibrator was attached to the floor of the arena. Pure sine waves of single predetermined wavelengths were fed into the $\frac{1}{2}$" blockboard floor, via an aluminium stirrup, from the vibrator which was placed centrally below the arena. The arema floor was mounted on rubber pads to prevent the vibrations spreading to the rest of the arena assembly and to provide damping to the floor (see Fig. A2.9).

As the arena floor was not a perfect diaphragm, as well as the wavelength fed into it from the vibrator, there may also have been harmonics and distortions of the waveform. This was unavoidable without using apparatus designed specifically for vibration experiments.

2.24 The vibrator was fed from a signal generator via a linear power amplifier. Amplitude of vibration at the arena floor varied with wavelength because the floor contained a series of resonant frequencies. These can be seen in Fig. C5.2. The dominant frequency occurs at 840 Hz, the others being partials or harmonics of this fundamental frequency. Consequently constant amplitude of input waveform could not provide constant amplitude at the arena floor and to overcome this the vibration of the arena floor was monitored using a magnetic pickoff transducer. The aluminium stirrup joined the vibrator, via a bolt, directly to the floor. A thin sheet

steel washer captive on the coupling bolt between the stirrup and vibrator, vibrated at a fixed ratio to the floor. The transducer was set to monitor the vertical movement of the washer and the output from the transducer was fed into a variable gain A.C. amplifier (see Fig. A4.15) and then into an oscilloscope where the relative waveform and amplitude could be seen and measured. Thus by varying the input amplitude to the vibrator, a fixed amplitude at the floor could be obtained using the transducer as a monitor. The maximum amplitude obtainable from the wavelength giving the smallest signal amplitude at full amplification (without distortion) was taken as the standard amplitude for all wavelengths.

The vibrator input leads were taken via a push button switch operated by the observer so that the stimulus could be given as an ant entered the arena, or as often as necessary.

2.25 The second channel amplifier of the oscilloscope was used as the power amplifier and consequently visual monitoring of the input waveform to the vibrator was possible. At high frequencies (above 1000 Hz) in order to obtain the power required to vibrate the floor at the standard amplitude, a second amplification stage was inserted between the oscilloscope and the vibrator. This was set at a fixed gain and only relative visual monitoring of the input waveform was possible.

Overloading of any of the amplification stages, causing distorted waveforms, produced distortion in the transducer output and could thus be guarded against. (For the block wiring diagram see Fig. A2.10.)

2.26 The apparatus units described under Sections 2.13 - 2.25 were used in conjunction for the experiments using combined stimuli as no units prevented simultaneous use of any other unit. Visual stimuli were presented continuously but chemical and acoustic stimuli were presented only as ants entered the arena (see Chapter 7).

2.27 In the experiments using a stroboscope to provide a standard flashing stimulus without movement, the cylinder and mountings were removed completely from the arena. The stroboscope was placed in the bay window so that the tube and reflector discharged into the arena. A screen of white paper masked to 14 x 15 cms. from behind was placed behind the window, between the arena and stroboscope so that the rectangle was only seen in the arena during each flash and the stroboscope was not visible from the arena at all (see Fig.A2.9.).

The stroboscope was set to flash at the required pulse/minute and the behaviour of naive ants entering the arena was observed as in previous experiments.

Electrophysiological Apparatus

2.28 To plot a visual sensitivity curve for F. rufa

Simple gross electrophysiological techniques were used for recording generator potentials in the compound eye. An ant with its appendages removed to prevent it struggling was embedded in a trough of plasticine with only the head protruding. The head was so oriented that one compound eye was exposed and looking vertically upwards. The other eye was obscured. The preparation trough was attached to a 3" x 1" microscope slide and placed on the stage of a binocular microscope. A wire mesh cage was placed round the preparation to provide a screen from unwanted signals, particularly of 50 Hz mains supply origin. This cage was just large enough to contain the binocular microscope, a preamplifier, two micromanipulators and the preparation (Fig. A5.1). The lens of an intense microscope lamp protruded into the cage through a hole and the lamp was set in such a position that it could be focussed to a 1 cm. diameter spot of light covering the head of the insect without being obscured by the micromanipulators when these were in position.

The microscope, preparation and micromanipulators were enclosed, within the cage, in a light tight box of black card. This box had a removable lid and front to provide access to the preparation between experiments. The cage itself was fitted with a cover of blackout cloth having a hinged front flap for access to the interior. When

both light tight boxes were sealed the preparation could be efficiently dark adapted. Further to ensure that this was the case, the laboratory curtains were drawn and the lights switched off whilst experiments were in progress.

The intense lamp carried a built in camera shutter and in front of this a filter holder large enough to take several 2" x 2" filters. The camera shutter was operated by cable release from outside the cage and was set on time exposure so that the light was either permanently illuminating the eye or was permanently off.

2.29 Each micromanipulator carried an electrode attached to its movable arm. The electrodes were minute stainless steel headless entomological pins which were etched down to extremely fine points by electrolysis with HC1. Each pin was soldered and clamped firmly to a 6 cm. length of 14/.0076" insulated wire terminating in a miniature plug. The two electrode plugs were plugged into sockets on the end of a length of lightweight screened cable. The indifferent electrode was plugged to the screen. This cable in turn was plugged into the preamplifier. The screened cable was just long enough to reach comfortably from the preparation to the preamplifier. The preamplifier input plug was wired as for a single ended input and the preamplifier was thus not used, as was possible, as a differential amplifier.

The binocular microscope was fitted with a very low power objective and used to monitor the position of the electrodes when they were

being inserted into the insect head. In this way the fine electrodes could be accurately positioned. The stage of the microscope was fitted with a slide manipulator and the preparation could thus be set in the correct position for viewing, illuminating and electrode insertion.

2.30 The size of the generator potential recorded for a simple E.R.G. (Electroretinogram) varied with wavelength and the height of the potentials was thus taken as a measure of response intensity. The recording electrode was inserted into the compound eye in the position shown in Fig. A5.2 until it just broke through the corneal layer. The indifferent electrode was similarly inserted in the head capsule as close to the eye as possible, thus completing the circuit.

The filter holder on the lamp contained a spectral filter (for the type see Section 4.4) and several neutral density filters to correct the light transmission to a standard value for all wavelengths (see Chapter 4 for details).

2.31 The preamplifier output was fed into the upper trace of a double beam oscilloscope. The decay time of the generator potential wave was long enough for its height to be measured on the oscilloscope screen using the calibrations provided. The generator potentials were also filmed with an oscillograph camera to provide a permanent record. The quiescent electrical potential of the eye of an insect is subject to infrequent bursts of increased potential in the form of

spikes. To ensure that such activity did not occur whilst filming was in progress the output from the preamplifier was monitored audibly. The output besides being fed into the oscilloscope was also fed into a simple audio amplifier and a loudspeaker. Any changes in nervous activity were thus easily noted.

The flash synchronisation terminal of the camera shutter was coupled to the lower trace of the oscilloscope with a small voltage supply (Fig. A5.3) so that when the shutter was open there was a permanent displacement of the lower trace until the shutter was closed again. This gave a reference trace of the "on" and "off" positions of the lamp, and could be recorded simultaneously with the response at the eye.

For the layout wiring diagram see Fig. A5.3.

2.32 To record electrical activity at the eye in response to a flashing light

As in section 2.28, simple E.R.G.s were recorded and the height of the generator potentials taken as a measure of response intensity.

An ant minus appendages was placed in a plasticine trough and electrodes inserted as before (Fig. A5.2). The unobscured eye however was positioned so that it faced towards one wall of the wire mesh cage. Behind this wall facing into the cage a stroboscope was set so that it discharged into the arena.

2.33 The preparation trough was placed on the surface of a cork table 4" above the bench top. Micromanipulators were used as before to carry the electrodes which in this case were plugged into twin screened cable and the preamplifier used as a differential amplifier. The preamplifier was more conveniently placed immediately outside the screened cage. The preparation table was only visually screened with black card on two sides. The cage was, as before, covered with blackout cloth and could be made light tight.

The lens of a microscope "intense lamp" projected through the wire mesh into the cage and was used to illuminate the preparation during insertion of electrodes and later as an adapting light (Fig. A5.4).

The electrical output from the eye was fed into the preamplifier differential inputs and the preamplifier output fed into the upper trace of an oscilloscope (Fig. A5.5). An output directly from the stroboscope was fed into the lower trace of the oscilloscope as a reference trace. A small capacitor was placed in series with the stroboscope in order that the on-effect will be seen as a positive spike on the oscilloscope trace.

The binocular microscope used for watching the insertion of electrodes was attached to a swinging arm and could be swung away from the preparation during experiments providing one less object in the field of view of the experimental animal.

2.34 As the frequency of each stimulus was high and the decay time of any response rapid, the responses to different stimulus frequencies

were filmed with an oscillograph camera and the relative heights of the generative potentials measured from the film.

For the layout wiring diagram see Fig. A5.5.

2.35 To record electrical activity at the eye in response to moving stripes

The experimental preparation was set up as in Sections 2.32 and 2.33, however instead of a stroboscope being used as the stimulus a small 3" diameter, 2" deep brass cylinder on ball races was used as the stimulus (Fig. A5.6). Around the circumference of the cylinder were eight equally spaced black stripes on a white ground simulating the same stripe characteristics as the 6 cm. stripes used in the behavioural experiments (see Section 2.16). The brass cylinder was placed inside the screened cage in order that the angle subtended at the eye by the stripes should be the same in both cases. The edges of the cylinder were masked off from the preparation by a small bay window (cf. Section 2.27). The cylinder was revolved by means of a pulley on the cylinder shaft driven by rubber belt from a set of cone-pulley gears giving a series of set speeds. The gears were in turn driven from a stepped-ratio geared motor. With this gearing system speeds throughout the range required were possible.

The microscope "intense lamp" as well as being used to illuminate the eye to facilitate electrode insertion was also used to illuminate the stimulus window during an experiment. A further intense lamp was placed behind the cylinder window screen and was set to project

a pinspot of light onto the cylinder circumference out of sight of the preparation. This pinspot was used with the flicker monitor (Appendix IV) to provide a reference on the lower scope trace. The two traces, from the preparation and the flicker monitor, were filmed with an oscillograph camera and the relative heights of the generative potentials noted at a later date. For the layout wiring diagram see Fig. A5.7.

CHAPTER THREE

Visual Stimuli

Quantitative properties of light stimuli

3.1 Compared with the majority of ant species, wood ants have well-developed eyes. F. rufa workers have about 600 facets in each eye compared with 30-50 facets in Pheidole megacephala, 100-200 facets in Myrmica sp. and Lasius sp. or one facet on each side of the head as in Eciton burchelli (Sudd, 1967). When compared with the honey bee (Apis mellifica), which has more than 4,000 facets in each compound eye, the eye of F. rufa is somewhat less impressive and little research into the vision of ants has therefore been attempted.

3.2 Form vision in insects has been demonstrated in Apis mellifica by Hertz, (1929, 1935) and Wolf and Zerrahn-Wolf, (1937). Form vision can be conveniently subdivided into object recognition (Objektkenntnisse) and locality recognition (Ortskenntnisse) (Jander and Voss, 1963; Voss, 1967). Object recognition is universal in insects having eyes capable of receiving images and telotaxis is common in this group (Kühn, 1919). However locality recognition is not so common and is best known in the Hymenoptera and Odonata, more specifically digger wasps, (Sphecoideae), social wasps (Vespoideae), bees (Apoideae); (review by Carthy, 1958) and ants (Formicinae) (Jander, 1957). Locality recognition can only be acquired by learning. Object recognition is a prerequisite for locality recognition and the majority of visual experiments are designed to gain more information about the mechanisms involved in object and locality recognition.

3.3 Jander and Voss, (1963) and Voss, (1967) on the basis of orientation experiments, showed that F. rufa is capable of recognising objects and localities visually. The present series of experiments using threat behaviour as a response, does not, unlike earlier experiments, rely on orientation of the insect towards a preferred stimulus but depends upon a visual stimulus eliciting a fixed action pattern (Thorpe 1951) from the insects.

3.4 Experiments that rely on directed orientation indicate only that the insect is stimulated to give a qualitative phototactic response (Jander, 1963). It is however possible to obtain a quantitative measure of response if a fixed action pattern is involved (see Section 1.4). This latter method can be used only where an insect has a behaviour pattern which can be elicited by means of visual stimuli. The threat behaviour of F. rufa is therefore ideal for this type of investigation.

3.5 Experiments using the threat behaviour of F. rufa and involving visual stimuli can be sub-divided into experiments using stationary stimuli and those using moving stimuli.

3.6 Stationary Stimuli

Jander and Voss (1963), using a stimulus-free arena, presented F. rufa workers with geometrical shapes placed towards the periphery of the arena. The ants entered the arena, oriented to, and then moved towards the preferred stimulus. Jander and Voss showed that a

solid figure was preferred to a dissected figure and that vertical stripes were preferred to horizontal stripes. They put forward the hypothesis that there were two independent detectors systems in the eyes, one for solid/dissected discrimination and one for vertical/horizontal discrimination.

Voss (1967) extended this work and found that in the vertical/horizontal detector system it was the dark/light border that was the releasing factor and postulated the presence of four visual detectors; for darkness, brightness, vertical edge and disruption.

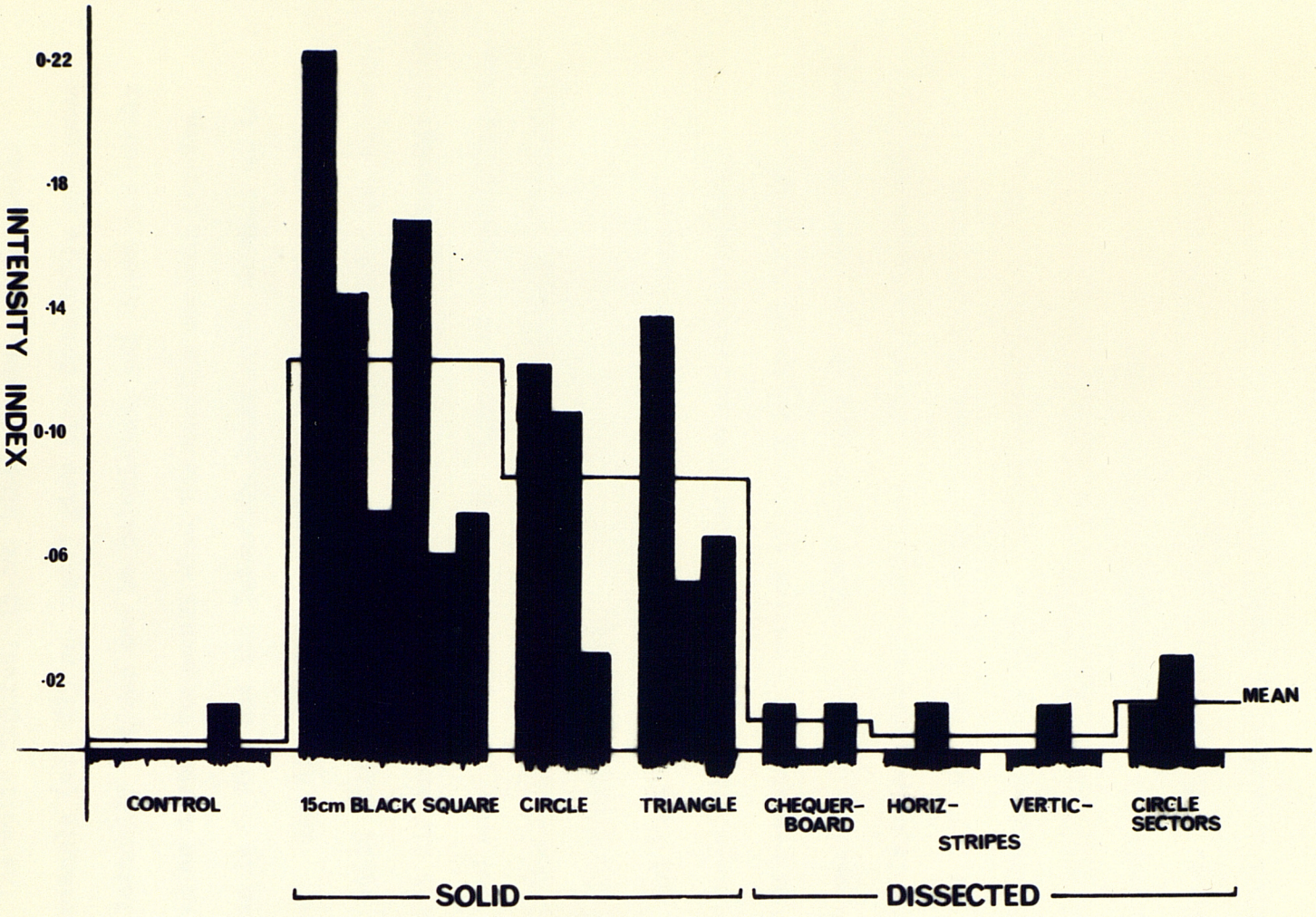
3.7 The main purpose of this research project was to discover the stimuli that elicit threat behaviour in F. rufa and the experimental technique was therefore biased towards discovering the releasers of threat behaviour. Experiments were carried out to ascertain whether stationary stimuli of the type used by Jander and Voss acted as threat releasers. The apparatus as described in Sections 2.10 and 2.14 were used. Black geometrical shapes were cut from fully exposed and developed photographic paper and suspended on white strings against the arena wall 6-10 cms. above the floor in full view of ants entering the arena. Only one stimulus at a time was presented and the responses of 25 naive ants (see Section 1.5) per stimulus were noted as they entered the arena, and the intensity index calculated.

3.8 Table C3.1 lists the Intensity Index per test for each type of stimulus and Fig. C3.1 shows the Intensity Indices plotted as

Stimulus type	Observed Intensity Index					No of Tests	Means
Control	0.0,	0.0,	0.0,			6	0.0025
	0.0155,	0.0,	0.0				
Black Square	0.2250,	0.1474,	0.0775,				
	0.1707,	0.0620,	0.0775			6	0.1266
Circle	0.1241,	0.1086,	0.0310,			3	0.0879
Triangle	0.1396,	0.0543,	0.0698			3	0.0879
Chequerboard	0.0155,	0.0,	0.0155			3	0.0103
Horizontal stripes	0.0,	0.0155,	0.0			3	0.0051
Vertical stripes	0.0,	0.0155,	0.0			3	0.0051
Circle sectors	0.0155,	0.0310,	0.0			3	0.0155

TABLE C3.1 Results of the stationary stimuli tests showing the Intensity Indices for each test together with the mean response for each stimulus

FIGURE C3.1 A histogram to show the relative responses to stationary visual stimuli


INTENSITY INDEX
0·22
·18
·14
0·10
·06
·02
MEAN
CONTROL
15cm BLACK SQUARE
CIRCLE
TRIANGLE
CHEQUER-BOARD
HORIZ-
VERTIC-
STRIPES
CIRCLE SECTORS
SOLID
DISSECTED
NO OF TESTS PER STIMULUS

histograms. The control tests with no stimulus show no threat response. The solid figures, 15 cm. black square, black circle and black triangle (all 225 sq. cms.) give some detectable responses from which Intensity Indices can be calculated, but these can barely be considered as threat responses when the average reading for the solid figures is 0.1072 and the Intensity Index scale extends up to 15.155 units. The dissected figures which have half the surface area of the solid figures give a much lower value. There is, however, no significant difference between threat response in ants of control experiments and ants presented with solid figures or dissected figures.

3.9 Jander and Voss (1963) found that increasing the size of stimulus increased the percentage of positive orientations, Table C3.2 shows the results for a series of tests in which different sized squares were used as stimuli. These were hung in the arena as before and responses from the ants were observed. Three black squares with sides of 15 cm., 10 cm. and 5 cm. were used and no significant difference in responses was found.

3.10 These results do not necessarily conflict with the results of Jander and Voss. Threat behaviour is only rarely elicited by a visual stimulus presented by itself in the visual field of the ant.

Stimulus type	Observed Intensity Index			No of Tests	Means
Black square 15cm sides	0.2250,	0.1474,	0.0775,		
	0.1707,	0.0620,	0.0725	6	0.1266
Black square 10cm sides	0.0155,	0.0310,	0.0465	3	0.0310
Black square 5cm sides	0.0,	0.0310,	0.0155	3	0.0155

Table C3.2 Results of the series of tests in which the size of stimulus was changed, showing Intensity Indices for each size together with the mean responses.

It is immaterial whether the stimulus is solid or dissected, large or small.

3.11 Moving Stimuli

The sense organs of the insect can be thought to act as filters to ensure that stimuli of little importance to the life of the animal do not affect behaviour. Although ants detect and orientate towards stationary stimuli (Jander and Voss, 1963), the stimuli are not sufficiently intense to elicit threat or alarm behaviour.

3.12 The insect eye is very sensitive to movement, Burtt and Catton (1962) obtained electro-physiological responses to displacements of a point source of light through only 0.1^{6} in Locusta migratoria, Calliphora erythrocephala and Phormia terranovae. Using striped patterns they obtained responses to displacements of 0.3° for Locusta and Phormia and 0.28° for Calliphora. If these results are typical for insects, F. rufa will have acute perception of movement, therefore moving stimuli will provide a more intense visual stimulus than stationary stimuli. Vowles (1965) trained workers of F. rufa to find their way through a 'T' maze orienting by means of striped patterns on the maze walls. The patterns did not move but as the ants walked past them, apparent movement was an important factor in their orientation. This orientation to apparent movement has been utilised by researchers in the optomotor reaction of insects. The insect is held stationary and the patterned surroundings moved. Behavioural compensatory movements occur (Kælmus, 1949 and Hassenstein 1961).

3.13 A series of experiments using moving stimuli were designed and carried out in an attempt to find out what size and type of stimulus, and what speed, flicker frequency and regularity of stimulus elicits threat behaviour in F. rufa. The apparatus as described in Sections 2.10 - 2.12 and 2.16 - 2.20 was used to provide the experimental conditions and stimuli.

3.14 In the first series of experiments the responses to size of stripe against speed of movement (velocity) were investigated. The stimulus stripes used were made of black fully exposed and developed photographic paper on a white background and were 1.5 cm., 3 cm., 6 cm., 12 cm. or 16 cm. wide by 15 cm. high. These stripes, when equally spaced, fitted round the stimulus cylinder without overlap forming a continuous moving stimulus when the cylinder was rotated. Each experiment consisted of a series of tests (responses of 25 ants) at a range of velocities from stationary to a speed above the flicker fusion frequency, (the frequency of flicker at which the optic units cease to distinguish the stimulus from each flicker as a separate entity). The flicker fusion frequency was reached when responses dropped to the level recorded for stationary stimulus. Velocity was measured in cylinder revolutions per minute but was then converted to cms./sec. for analysis.

3.15 With the increase in stripe width, the number of stripes on the

cylinder was decreased. To reach the flicker fusion frequency, the velocity of the cylinder was therefore increased to compensate for the decrease in flickers per revolution (see Table C3.3).

3.16 If the rate of flicker at the compound eye is an important factor in threat response, the response peak will show a direct relationship with flicker frequency. Table C3.4 contains the results of the experiments carried out, two experiments per stripe width being shown. As the plotted curves and response peaks for the experiments were ill-defined, it was decided that a mathematical curve fit was necessary in order to provide meaningful comparison between experiments. The best method of handling the data was by using a computer curve fit programme. A suitable programme for multiple regression and trend analysis based on work by Esler, Smith and Davis (1968) had been modified to run on the I.C.L. computer at Southampton University by Fisher and Barrs (1969). This programme was used by J. Barrs, Botany Department, Southampton University, to analyse the experimental results, comparing observed Intensity Indices against flicker frequency and to fit the best curves up to a 5th order polynomial.

3.17 Table C3.4 gives the calculated Intensity Indices as well as the observed Intensity Indices for the experiments. Fig. C3.2a-d shows the observed and calculated Intensity Indices for one of each pair of experiments plotted against flicker frequency. The data for experiment 54 - 1.5 cm. stripes, is almost a straight line and is therefore not plotted. There were no significant differences when the Intensity Indices at each flicker frequency were analysed for variance.

Flickers per sec.	Stripe Width 1.5cm cm/sec	3cm cm/sec	6cm cm/sec	12cm cm/sec	16cm cm/sec
2.66	4.0	7.9	15.9	31.8	42.5
10.66	15.9	31.8	63.6	127.3	163.9
21.33	31.8	63.6	127.3	254.6	361.2
42.66	63.6	127.3	254.6	509.3	682.5
64.0	95.5	191.0	382.0	764.0	1024.0
80.0	119.3	238.7	477.5	955.0	1280.0
100.0	150.0	300.0	600.0	1200.0	1600.0

TABLE C3.3 Table to show the relationship between flicker frequency and speed of movement in cm/sec. for each stripe width.

Flickers per sec.	Responses to Experiments with stripe widths of :- 1.5cm Obs.	1.5cm Calc.	3cm Obs.	3cm Calc.	3cm Obs.	3cm Calc.
0.0	0.0155	0.0150	0.0310	0.0372	0.0310	0.0158
2.66	-	-	0.1183	0.1500	-	-
5.33	-	-	0.1706	0.2857	-	-
8.0	-	-	0.3025	0.3989	-	-
10.66	0.0155	0.0032	0.5470	0.5021	0.2638	0.3939
16.0	-	-	0.8049	0.5930	0.5897	0.4340
16.5	-	-	-	-	-	0.4346
18.5	-	-	-	0.6023	-	-
21.33	0.0155	0.0060	0.5508	0.5855	0.4267	0.4122
26.66	-	-	0.4499	0.4927	0.3297	0.3460
32.0	0.0853	0.0800	0.2791	0.3665	0.1939	0.2677
3500	-	0.0883	-	-	-	-
37.33	-	-	0.2016	0.2205	0.1861	0.1817
42.66	0.0465	0.0353	0.1783	0.1030	0.1163	0.1121
45.0	-	0.0042	-	-	-	-
48.0	-	-	0.0620	0.0429	0.1241	0.0716
51.5	-	-	-	0.0309	-	-
53.33	-	-	0.0620	0.0342	0.0543	0.0536
58.66	-	-	0.0620	0.0660	-	-
61.0	-	-	-	-	-	0.0558
64.0	0.0310	0.0310	0.0155	0.0850	0.0155	-
69.33	-	-	0.0465	0.0212	0.0388	-

continued

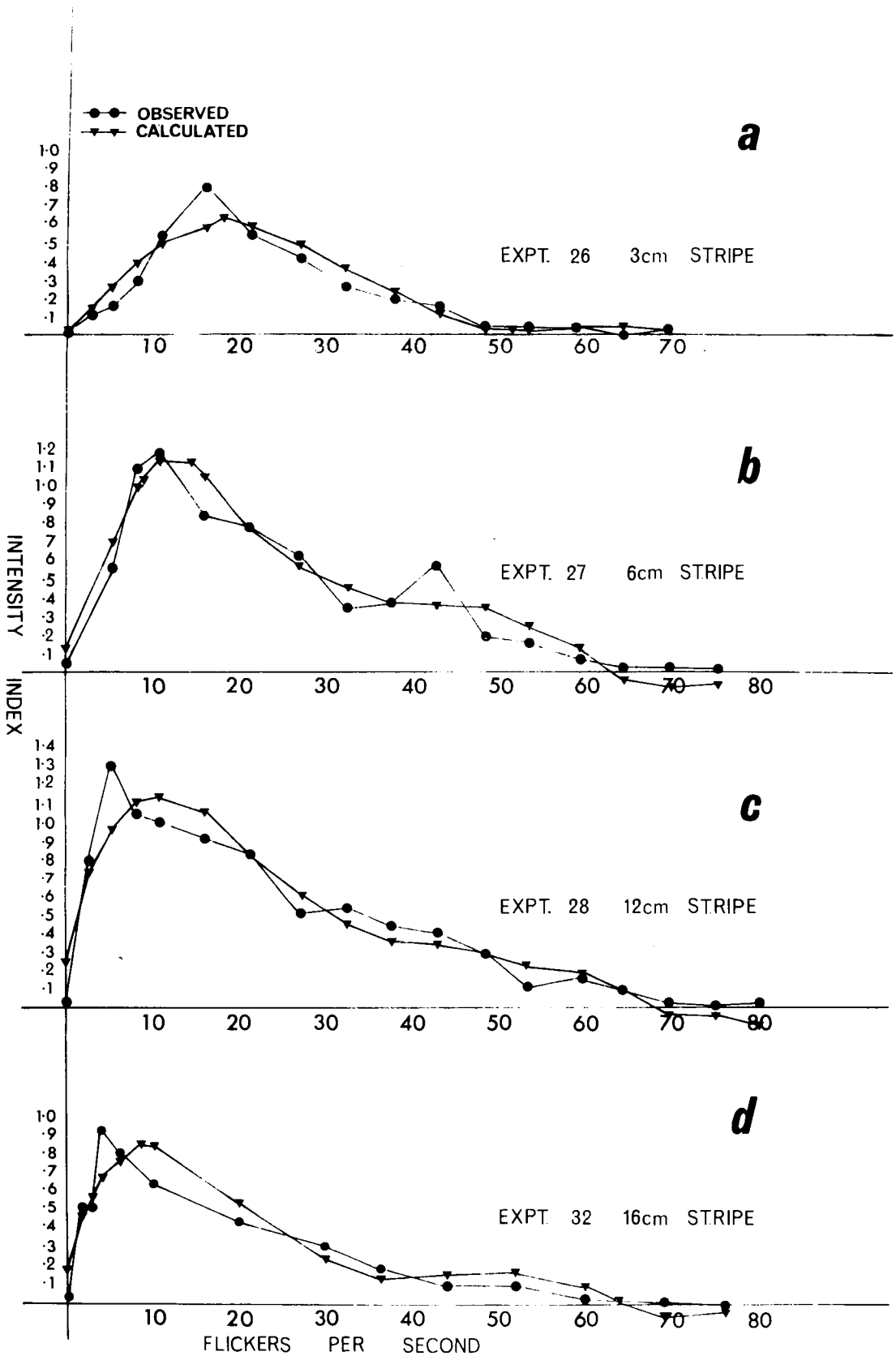
Flickers per sec.	6cm Obs.	6cm Calc.	6cm Obs.	6cm Calc.	12cm Obs.	12cm Calc.
0.0	0.0698	0.1086	0.0155	0.0412	0.0310	0.2337
2.66	0.5780	0.6777	-	-	0.8128	0.7368
5.33	1.0862	0.9527	0.6122	0.6122	1.3172	0.9870
8.0	-	1.0917	0.6905	0.6870	1.0592	1.1193
10.66	1.1775	1.1307	0.6905	0.7301	1.0262	1.1626
11.5	-	-	-	0.7310	-	-
13.0	-	1.1000	-	-	-	-
16.0	0.8496	1.0057	0.7099	0.6877	0.9263	1.0591
21.33	0.7759	0.7705	0.5121	0.5753	0.8574	0.8351
26.66	0.6129	0.5592	-	-	0.5276	0.6118
32.0	0.3646	0.4349	0.4500	0.3982	0.5586	0.4605
37.33	0.3956	0.3723	-	-	0.4577	0.3642
42.66	0.5896	0.3528	-	-	0.4189	0.3267
45.33	-	-	0.3257	0.2965	-	-
48.0	0.2249	0.3310	-	-	0.3103	0.3086
53.33	0.1706	0.2634	0.1396	0.1850	0.1163	0.2777
58.66	0.0853	0.1367	0.0310	0.0644	0.1706	0.2061
61.0	-	-	-	0.0173	-	-
64.0	0.0310	-0.0005	0.0155	-0.0471	0.1008	0.1030
69.33	0.0310	-0.0781	-	-	0.0310	-0.0188
74.66	0.0310	0.0768	0.0388	0.0403	0.0155	-0.0631
80.0	-	-	-	-	0.0388	0.0946

continued

Flickers per sec.	12cm Obs.	12cm Calc.	16cm Obs.	16cm Calc.	16cm Obs.	16cm Calc.
0.0	0.1008	0.3184	0.0155	0.1734	0.0155	0.1981
2.0	-	-	0.5159	0.4583	0.6207	0.5886
2.66	1.0883	0.9330	-	-	-	-
3.0	-	-	0.5120	0.5648	0.7565	0.7310
4.0	-	-	0.9428	0.6504	1.0804	0.8426
5.33	1.4666	1.2341	-	-	-	-
6.0	-	-	0.8125	0.7669	1.0660	0.9863
8.0	1.3473	1.3887	-	-	-	-
8.5	-	-	-	-	-	1.0462
9.5	-	-	-	0.8339	-	-
10.0	-	-	0.6440	0.8325	0.8691	1.0334
10.66	1.1601	1.4334	-	-	-	-
20.0	-	-	0.4422	0.5339	0.3568	0.5277
21.33	1.0145	1.0098	-	-	-	-
30.0	-	-	0.3258	0.2194	0.1939	0.0883
32.0	0.6362	0.5725	-	-	-	-
36.0	-	-	0.2016	0.1462	0.1551	0.0255
42.66	0.5120	0.4648	-	-	-	-
44.0	-	-	0.1096	0.1471	0.0698	0.0989
52.0	-	-	0.1163	0.1539	0.0775	0.1702
53.33	0.4267	0.4599	-	-	-	-
58.66	0.3413	0.3831	-	-	-	-
60.0	-	-	0.0388	0.0762	0.0388	0.0890
64.0	0.2248	0.2507	-	-	-	-0.0045
64.5	-	-	-	0.0001	-	-
69.0	-	-	0.0155	-0.0616	0.0155	-0.1051
69.33	0.0931	0.0732	-	-	-	-
74.66	0.0543	-0.0329	-	-	-	-
76.0	-	-	0.0	0.0310	0.0	0.0471
80.0	0.0465	0.0930	-	-	-	-

TABLE C3.4 Intensity Indices of experiments in which moving stripes were presented as stimuli.

FIGURE C3.2 Threat response plotted against flicker frequency for the experiments in which the stimuli are normal full striped patterns


OBSERVED
CALCULATED
a
EXPT. 26 3cm STRIPE
b
EXPT. 27 6cm STRIPE
c
EXPT. 28 12cm STRIPE
d
EXPT. 32 16cm STRIPE
INTENSITY INDEX
FLICKERS PER SECOND

However when the Intensity Indices of the other experiments were compared for variance, there were very significant variations between flicker frequencies (see Table C3.5).

3.18 Taking as a typical example, experiment 26, with 3 cm. stripes, it can be seen that the threat response increases with increased velocity of stimulation until a peak response is reached at which point response begins to fall off again until the response is again similar to that for a stationary figure. At this velocity it is assumed that the stimulus cylinder is revolving fast enough to provide a flicker frequency at the insect eye such that the individual black/white interfaces cannot be discerned. When this point is reached, the behavioural flicker fusion frequency for the eye of F. rufa has been attained. For each curve the two points of interest are therefore the peak response point and the response cut-off point.

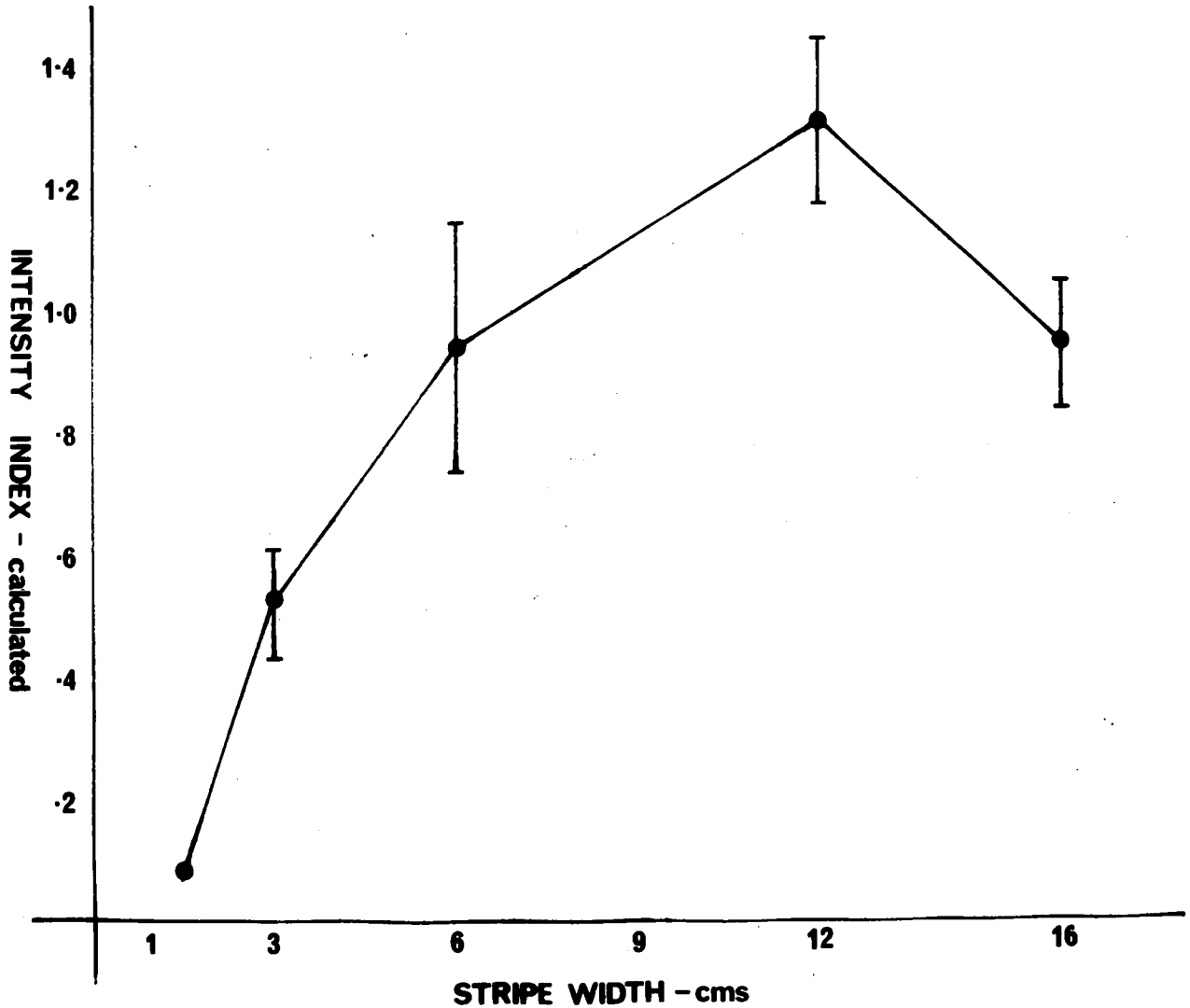
3.19 If the peak response Intensity Index for each experiment is plotted against the size of stimulus (see Fig. C3.3) it can be seen that increase in size of stimulus causes an increase in threat response until the size of stimulus reaches about 12 cm. x 14 cm. when it subtends an angle at the eye of about 24°. The response then begins to fall off again.

3.20 If the flicker frequency and the velocity of movement in cms./sec. of the peak response for each experiment are also plotted against size of stimulus, the curves as shown in Figs. C3.4 and C3.5 are

Stripe Width	Significance level 1%	5%
3cm.	*	
3cm.		*
6cm.	*	
6cm.	*	
12cm.	*	
12cm.	*	
16cm.		*
16cm.	*	

TABLE C3.5 Results of the analysis of variance tests carried out on the normal full-striped patterns. The levels of significance for each experiment are shown.

FIGURE C3.3 Size of stimulus plotted against intensity of response, for normal, full striped patterns.


1·4
1·2
1·0
·8
·6
·4
·2
INTENSITY INDEX - calculated
1
3
6
9
12
16
STRIPE WIDTH - cms

obtained. The data plotted in Figs. C3.4 and C3.5 is tabulated in Table C3.6. Fig. C3.4 shows that with the narrow 1.5 cm. stripes, which subtend an angle of 3^{o} at the eye, the response peak occurs at a flicker frequency of about 35 flickers/sec. With increase in stimulus size the optimal flicker frequency falls until the stimulus subtends an angle of 12^{o} (corresponding to 6 cm. stripes). Then any increase in stimulus size does not alter the flicker frequency at which the optimal response is obtained. This curve indicates a non-linear relationship and in order to analyse the results further, graphs were plotted using transformed variables.

3.21 The only polynomial equation that provided any form of linear relationship was $Y = 1/ao + aiX$, where ao and ai are constants. This indicated that the original curve shown in Fig. C3.4 is probably a hyperbola. However if Y is plotted against 1/X as in Fig. C3.4a, it can be seen that the points do not lie exactly on a straight line but on a slight curve. It is probably safe to assume however that, within the limits of experimental error, there is direct relationship between response peak flicker frequency and stripe width.

When the size of stimulus is plotted against velocity of movement, in cms/sec., the curve obtained is as shown in Fig. C3.5. It can be seen that, within the limits of experimental error, the points lie on a straight line and indicate a linear relationship between velocity of movement and stimulus size.

3.22 It is evident therefore that stimuli involving regular striped

Experiment	Stripe width cms.	Flicker frequency fl/sec.	Speed cm/sec.
54	1.5	35	51.6
30	3	16.5	48.7
26	3	18.5	55.7
27	6	10.5	62.7
31	6	11.5	68.6
28	12	10.5	127.3
29	12	10.5	127.3
32	16	9.5	150.9
33	16	8.5	134.7

TABLE C3.6 Flicker frequency and speed of movement for optimal response with each size of stimulus.

FIGURE C3.4 Optimal response plotted against size of stimulus, for normal, full striped patterns.

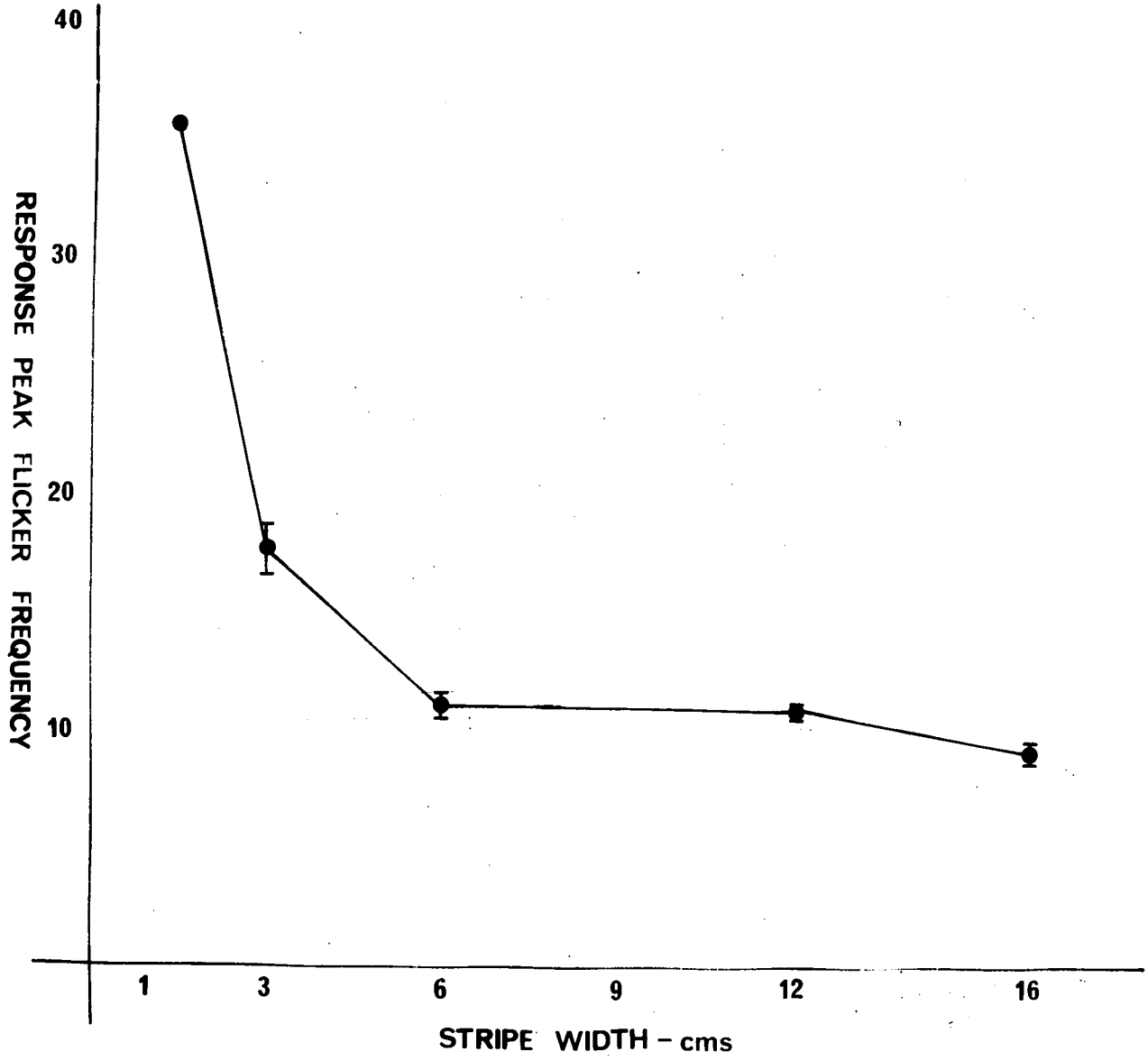

40
30
20
10
RESPONSE PEAK FLICKER FREQUENCY
1
3
6
9
12
16
STRIPE WIDTH – cms

FIGURE C3.4a Data as for Figure C3.4 but with optimal response plotted against the reciprocal of the stimulus size.

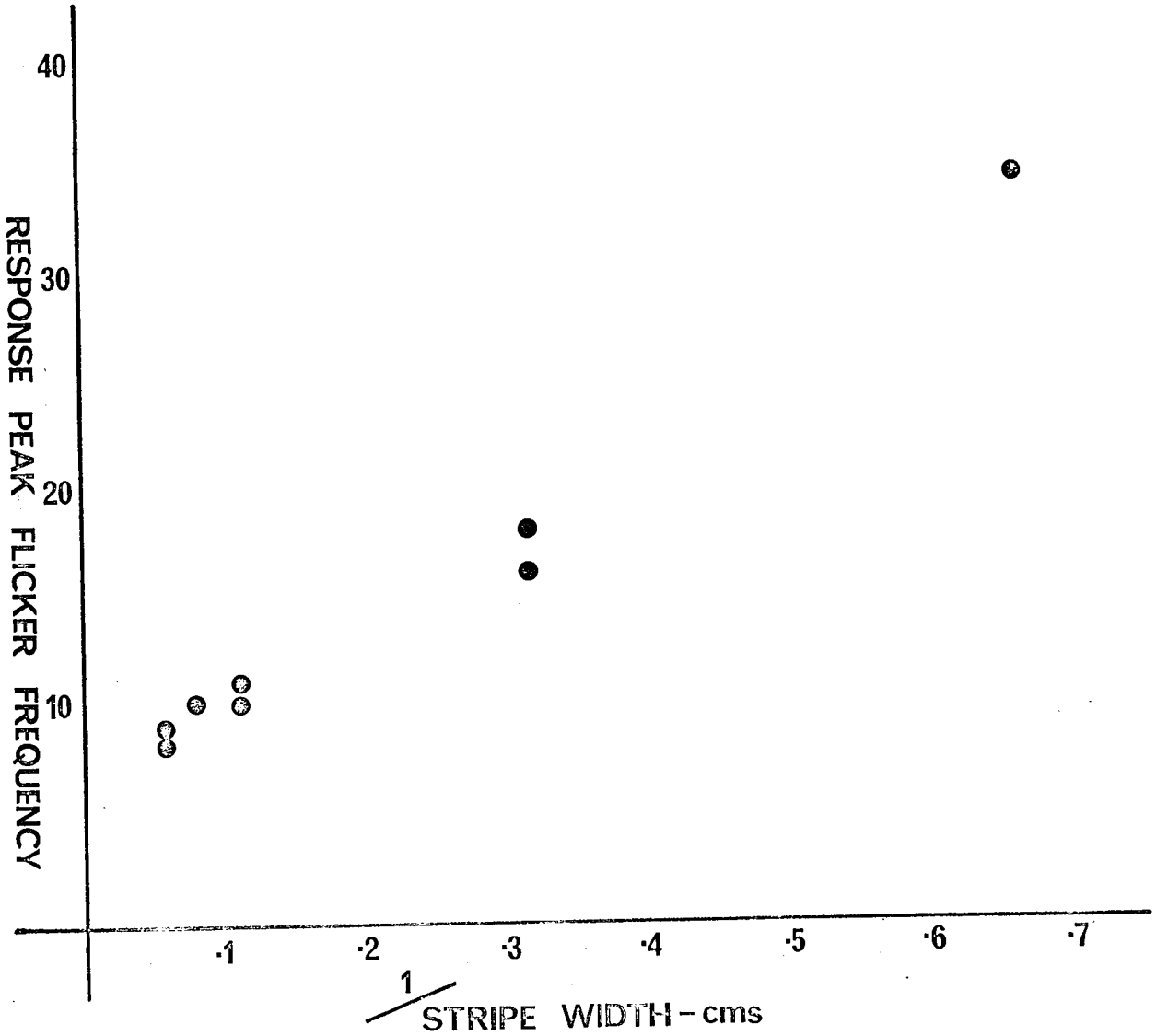
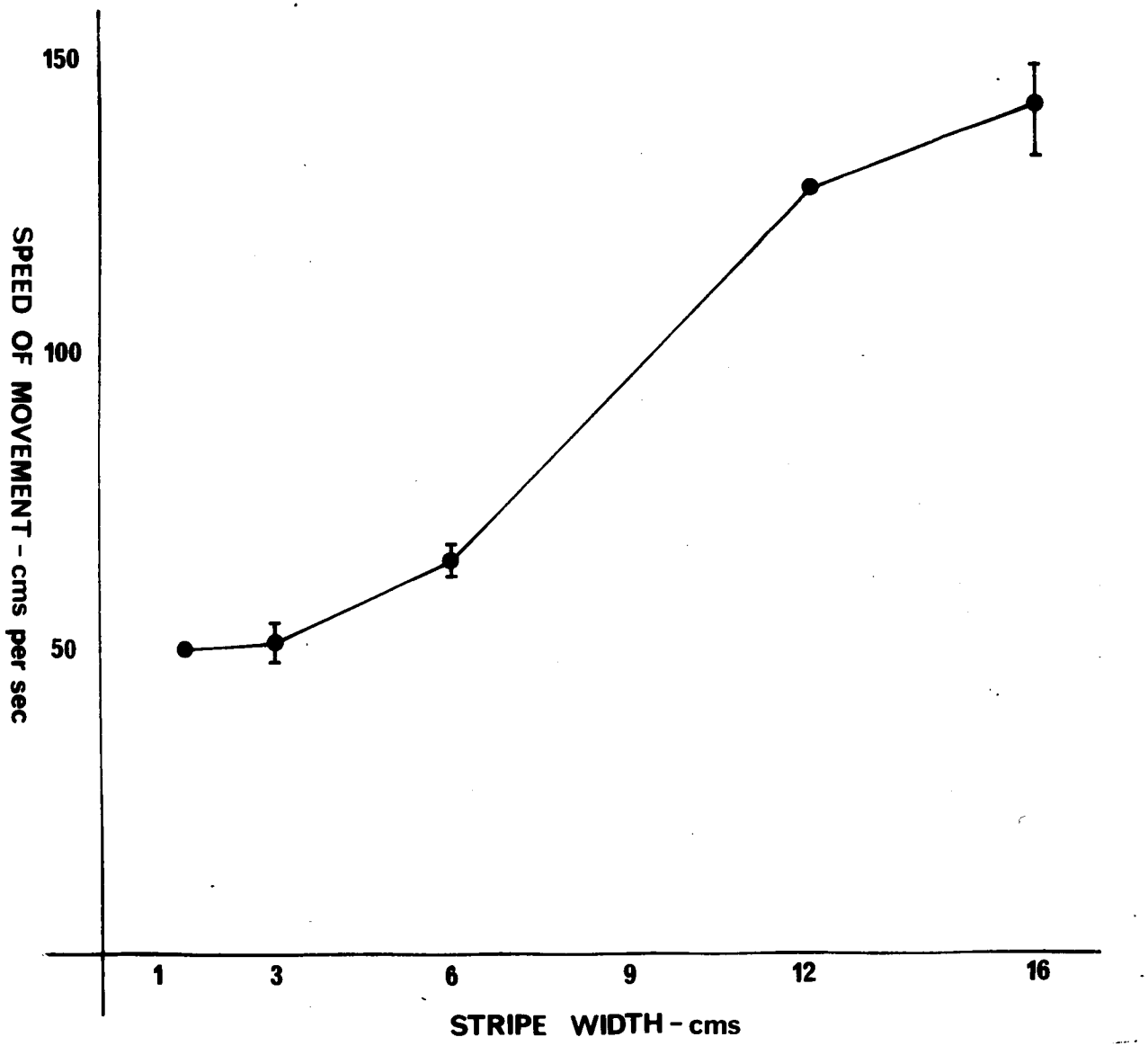

RESPONSE PEAK FLICKER FREQUENCY
40
30
20
10
·1
·2
·3
·4
·5
·6
·7
1
STRIPE WIDTH – cms

FIGURE C3.5 Velocity of optimal response plotted against size of stimulus


150
100
50
SPEED OF MOVEMENT - cms per sec
1
3
6
9
12
16
STRIPE WIDTH - cms

patterns require two components before they can act as efficient releasers of threat behaviour. They require a near optimal flicker frequency and near optimal velocity of movement. That there is definite correlation between velocity of movement and optimum response indicates that the eye of F. rufa may be able to detect velocity of movement independantly of flicker frequency.

3.23 McCann and MacGinitie (1966) mention a velocity sensitive reaction in Musca domestica and Collett and Blest (1966) describe binocular, directionally selective neurones in the eye of Sphinx ligustri that give strong response to the movement of patterns and less response to change in the level of illumination. There is therefore evidence from electrophysiology for velocity detection in insects.

3.24 In an attempt to provide further information on flicker/velocity detection, a series of electrophysiological experiments were carried out. Two sets of experiments were carried out and the apparatus for both is described in Sections 2.32 - 2.35. In the first set, the stimuli were provided by a stroboscope which produced constant repetitive on/off pulses. The experimental animal was subjected in turn to different frequencies of flicker between the range 1 pulse per second and 55 pulses per second. The height of the electro-retinogram was measured at each frequency tested. Table C3.7 shows the results of four such experiments.

3.25 If the height of the electroretinogram is plotted against flicker frequency (Fig. C3.6), it can be seen that the greatest visual response occurs when the flicker frequency is at its lowest. The response falls off rapidly with increase in flicker frequency and becomes insignificant at 50 pulses per second when it is not noticeable above the background noise of the preparation. Fig. C3.7 shows the oscillograph traces of experiment EP 5 for each flicker frequency tested. At all frequencies it can just be seen that the decaytime of the

Stroboscope pulses per second	Experiment EP4 'scope sensitivity m.V.	Experiment EP4 height* of ERG m.m.	Experiment EP5 'scope sensit. m.V.	Experiment EP5 height of ERG m.m.	Experiment EP7 'scope sensit. m.V.	Experiment EP7 height of ERG m.m.	Experiment EP9 'scope sensit. m.V.	Experiment EP9 height of ERG m.m.
1	200	140.0	200	130.0	100	100.0	50	62.5
5	200	120.0	200	120.0	100	85.0	50	42.5
10	100	70.0	100	70.0	100	60.0	50	32.5
Bg. noise**	-	-	100	10.0	100	10.0	-	-
15	50	32.5	50	30.0	50	25.0	20	13.8
20	50	28.75	50	30.0	50	22.5	20	13.2
Bg. noise	-	-	50	5.0	50	5.0	50	7.5
25	50	22.5	50	25.0	20	17.4	20	10.8
30	20	13.6	20	14.7	20	12.2	10	7.7
Bg. noise	-	-	20	2.2	20	3.0	20	4.0
35	20	10.2	10	9.9	10	9.3	10	6.8
40	10	7.3	10	6.5	10	5.8	-	-
Bg. noise	-	-	10	1.9	-	-	10	3.3
45	-	-	10	4.7	5	4.4	10	3.9
50	-	-	10	3.0	5	2.4	10	3.0
55	-	-	10	2.1	5	2.2	10	3.0
Bg. noise	-	-	-	-	5	1.8	-	-

TABLE C3.7 Results of the electrophysiological experiments in which a stroboscope was used to provide the visual stimulus.

* The height of the ERG is the height after correction due to the differences in oscilloscope sensitivity

** The Background noise is a measure of electrical output from the preparation when dark-adapted

FIGURE C3.6 Height of the ERG. as measured for each experiment plotted against flicker frequency.

Measurements taken from oscillograph paper traces (see Fig. C3.7) and corrected for oscilloscope sensitivity

Note: The dotted trace is the background noise level

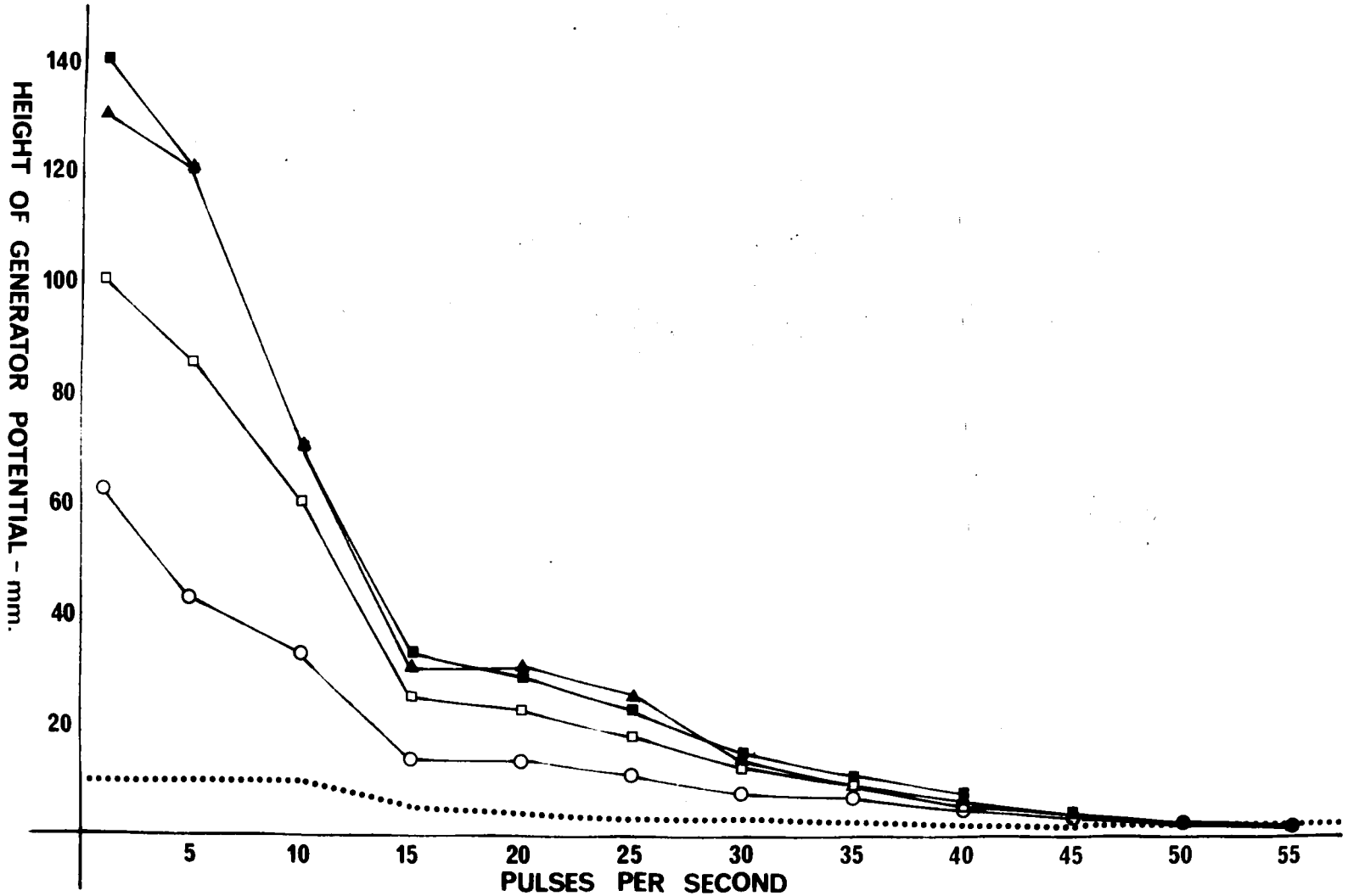
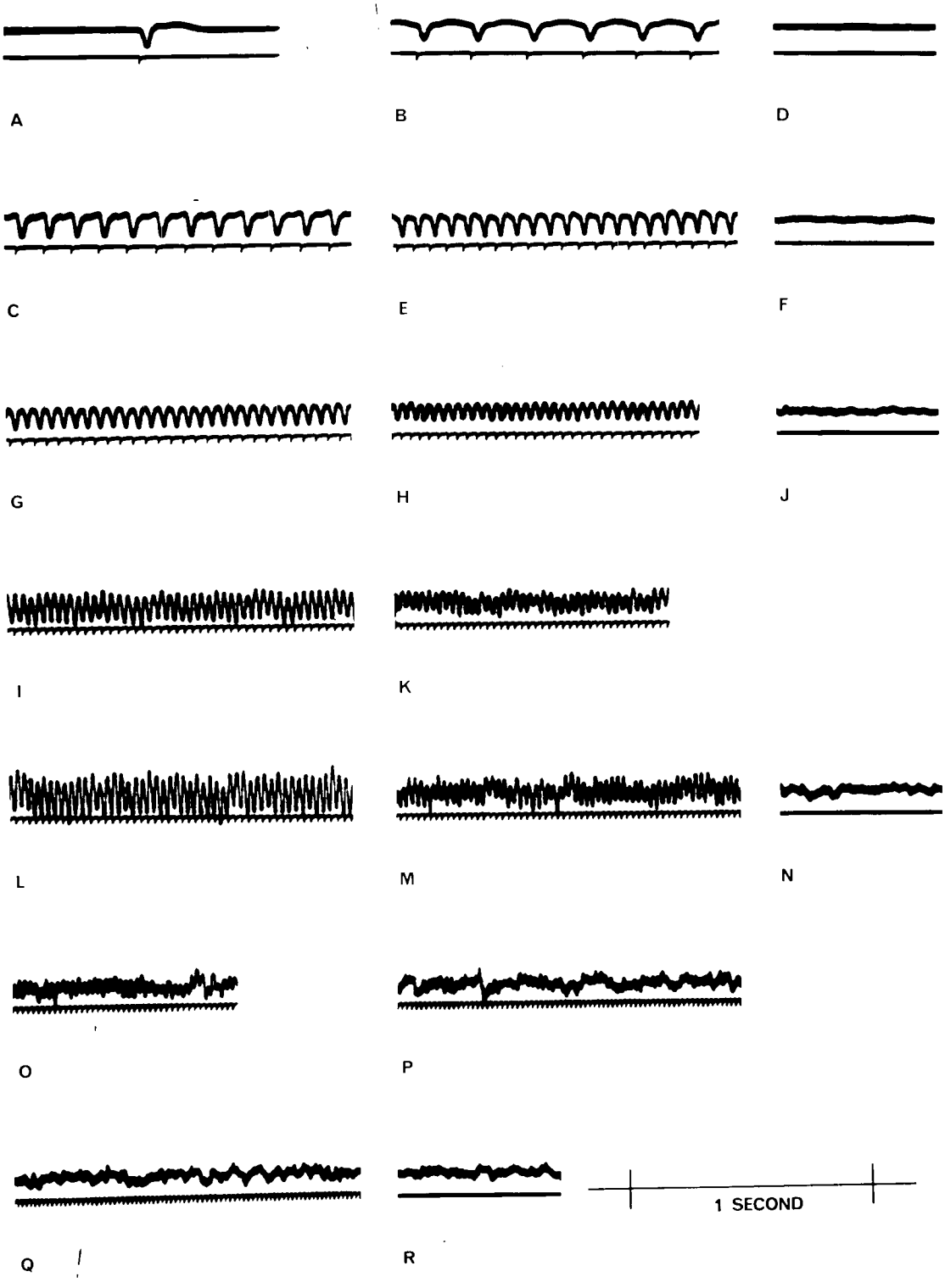

HEIGHT OF GENERATOR POTENTIAL - mm.
140
120
100
80
60
40
20
5
10
15
20
25
30
35
40
45
50
55
PULSES PER SECOND

FIGURE C3.7 Results of Experiment EP5 recorded on oscillograph paper

Trace A.	1 pulse/sec.,	200mV	oscilloscope sensitivity
B.	5 pulses/sec.,	200mV	" "
C.	10 pulses/sec.,	100mV	" "
D.	Background noise,	200mV	" "
E.	15 pulses/sec.,	50mV	" "
F.	Background noise,	100mV	" "
G.	20 pulses/sec.,	50mV	" "
H.	25 pulses/sec.,	50mV	" "
I.	30 pulses/sec.,	20mV	" "
J.	Background noise,	50mV	" "
K.	35 pulses/sec.,	20mV	" "
L.	35 pulses/sec.,	10mV	" "
M.	40 pulses/sec.,	10mV	" "
N.	Background noise,	20mV	" "
O.	45 pulses/sec.,	10mV	" "
P.	50 pulses/sec.,	10mV	" "
Q.	55 pulses/sec.,	10mV	" "
R.	Background noise,	10mV	" "


A
B
D
C
E
F
G
H
J
I
K
L
M
N
O
P
Q
R
1 SECOND

stroboscope discharge is constant at approximately 12.5 msec., which is fast enough to provide full power for each discharge even at 55 pulses per second. Possible loss of power output in the discharge tube at higher frequencies of discharge is therefore not the cause of the drop in response from the preparation. Trace A shows the response in the visual units to one pulse per second. It can be seen that the E.R.G. consists of a negative "on" wave followed by a positive overshoot. This overshoot has a decay time of 200 msec. in returning to the resting potential. As the frequency of stimulation increases, the time period between each discharge becomes less than 200 msec. (see Trace C) and the decay of the positive overshoot is not completed. This causes reduction in the subsequent negative on wave. Therefore as the permitted decay times of the positive overshoot decrease, the heights of the negative potential decrease providing the results observed.

3.26 In the second set of electrophysiological experiments, the recording technique was the same as that used in the previous experiments but the stimulus was provided by a striped cylinder similar to that used in the behavioural experiments, but smaller. The cylinder was made to revolve at constant speeds and was visible to the experimental preparation through a bay window of similar proportions to the window used in the behavioural arena. The black and white stripes on the cylinder presented the same flicker frequency

per revolution as the 6 cm. stripes of the behavioural experiments, and the angle subtended at the eye of the preparation was also 12°.

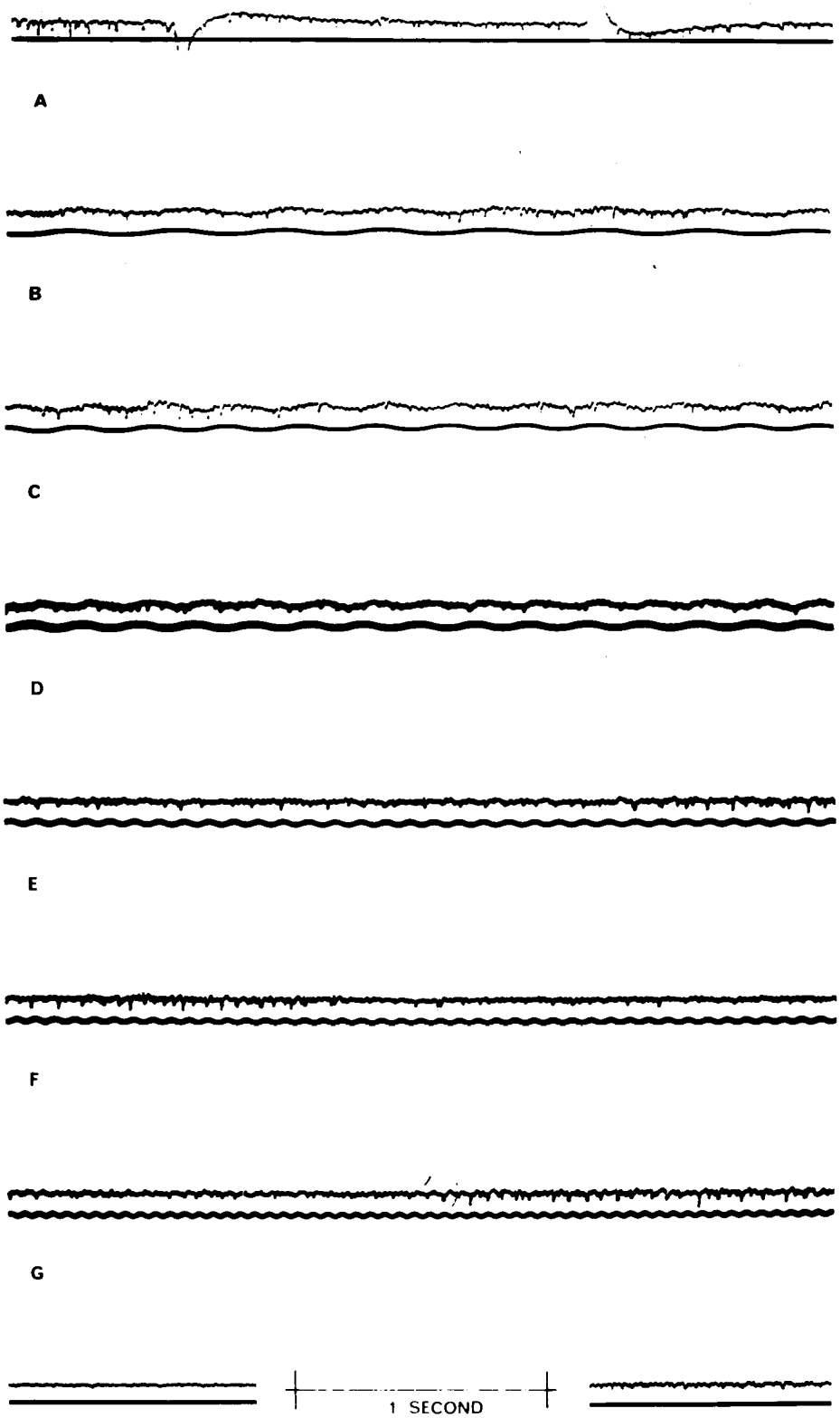
3.27 Attempts were made to record the E.R.G. in response to a range of flicker frequencies, but it proved almost impossible to obtain any measureable responses to the movement of the stripes (see Fig. C3.8). Trace A shows the responses to switching the light on and off to provide a comparison with Fig. C3.7. It was found impossible to obtain any clearly defined responses even with the cylinder window illuminated with the maximum light intensity that was practically possible.

3.28 Abortive attempts were also made to obtain responses to visual stimuli by recording from the ventral nerve cord (Burtt and Catton, 1954) but although spikes were readily recordable, there was no correlation with the visual stimuli.

3.29 The ant detects movement and flicker and reacts behaviourally but has insufficient nervous activity in the eye to provide a measurable E.R.G. If however a bright lamp (discharge tube) is switched on and off in the visual field, strong nervous activity occurs, providing well defined E.R.G.s. Bullock and Horridge (1965) state that in the light of present knowledge, the insect eye, as judged behaviourally, is sensitive to illumination which is too weak to produce an E.R.G. I would amend this statement to read that the insect eye, as judged behaviourally, is sensitive to changes of illumination from light to dark which are too weak to produce an E.R.G.

FIGURE C3.8 Results of Experiment EP12 recorded on oscillograph paper

Trace A.	Illumination light switched off and then on again to show the response given to flashes of light,	1.0V/cm oscilloscope sensitivity
B.	5.0 flickers/sec.,	500mV 'scope sensit.
C.	7.2 flickers/sec.,	500mV " "
D.	10.0 flickers/sec.,	500mV " "
E.	21.3 flickers/sec.,	500mV " "
F.	26.6 flickers/sec.,	500mV " "
G.	36.0 flickers/sec.,	500mV " "
H.	Background noise,	1.0volt " "
I.	Background noise,	500mV " "


A
B
C
D
E
F
G
1 SECOND
H
I

3.30 If F. rufa is subjected to pulsed stimulation in the behavioural arena, using the apparatus described in Section C2.27 it can be seen from the results in Table C3.9 that there is negligible threat response.

3.31 There is therefore a situation where visual flicker from moving stripes causes strong behavioural response but does not provide a measureable E.R.G. and where pulsed flicker from a stroboscope provides an easily measureable E.R.G. but no behavioural response. It can only be assumed that at least two active mechanisms are present in the visual system of the ant. A movement/flicker detector (presence of which has been indicated in previous sections), which connects directly to motor pathways via the C.N.S. producing the behavioural reflexes of threat behaviour, and the flicker/pulse detector which detects on/off stimuli with no immediate behavioural reaction. The information can be passed to the central nervous system for analysis and thus can be monitored electrophysiologically. The latter mechanism may make use of the darkness and brightness detectors (Jander and Voss, 1963 and Voss, 1967).

3.32 Velocity and flicker frequency are not the only parameters associated with moving stimuli and experiments were carried out to analyse the effect of change in shape, change in size (surface area) and change in pattern regularity.

3.33 Shape and surface area of a stimulus are linked because as the shape changes, the surface area will also alter unless the size is rigorously controlled. Table C3.10 shows the results of experiments

Stroboscope pulses per second	Blacked-out Arena Intensity Index	Illuminated Arena Intensity Index
1	0.0543	0.0
5	0.0155	0.0155
10	0.0155	0.0
20	0.0310	0.0
40	0.0	0.0
60	0.0155	0.0

TABLE C3.9 Results of the behavioural experiments using a stroboscope to provide flicker without velocity of movement.

Flickers per sec.	CHEQUERBOARDS Experiment 35 observ.	calc.	Experiment 36 observ.	calc.	STRIPES Experiment 27 observ.	calc.
0.0	0.0155	0.0215	0.0310	0.0903	0.0	0.1086
5.33	0.3103	0.3430	0.4267	0.3558	1.0862	0.9527
8.0	0.4499	0.4296	0.4422	0.4289	-	1.0917
10.66	0.5198	0.4856	0.5431	0.4773	1.1775	1.1307
15.5	-	0.5240	-	0.5130	-	-
16.0	0.5353	0.5234	0.4189	0.5129	0.8496	1.0057
26.66	0.3258	0.3949	0.3647	0.4059	0.6129	0.5592
37.33	0.2094	0.1780	0.2327	0.2061	0.3956	0.3723
48.0	0.0465	0.0352	0.0775	0.0552	0.2249	0.3310
55.5	-	0.0015	-	0.0070	-	-
58.66	0.0310	0.0025	0.0310	0.0033	0.0853	0.1367
64.0	0.0	0.0177	0.0155	0.0177	0.0310	-0.0005
69.33	0.0155	0.0361	0.0	0.0532	0.0310	-0.0781
80.0	0.0	0.0091	0.0155	0.1362	-	-

TABLE C3.10 Observed and calculated Intensity Indices from the 6cm chequerboard experiments for each flicker frequency tested. The results of a 6cm stripe experiment are also shown to provide a comparison

35 and 36 in which the stimulus was a regular 6 cm. chequerboard presented to ants in the arena as described in Section 2.16. The chequerboard maintained the same areas of black and white as the striped patterns.

The vertical black/white and white/black interfaces were shorter than in the striped stimuli and the vertical edge detector (Voss 1967) may well have had less stimulation. From the data shown in Table C3.10 it can be seen that the responses for a range of stimulus speeds are similar to the responses obtained with 6 cm. stripes. The optimal response is lower using chequerboards, showing that a chequerboard is not such an efficient releaser of threat behaviour as are the equivalent stripes, and the optimal response speed is higher with chequerboards than with stripes. The response cut-off point (flicker fusion frequency) is also lower for a 6 cm. chequerboard (55.5 flickers per second) than for 6 cm. stripes (64.0 flickers per second). The smaller surface area of each stimulus unit (i.e. each black square) in the chequerboard when compared with the surface area of each black stripe in the striped pattern may account for these discrepancies.

Analysis of the calculated Intensity Indices at each flicker frequency in experiments 35 and 36 shows a 1% significant variation between tests

3.34 In order to obtain further information concerning the effect on threat behaviour of changes in shape and surface area, experiments were carried out in which the width of each stimulus was held constant but the height was changed. The stimuli were presented as in Section 2.16 and consisted of black rectangles, 3cm., 6cm. and 12cm. wide The rectangles, like the 15cm. high stripes, were equally spaced round the stimulus cylinder. Each rectangle was either 3cm., 6cm. or 12cm. high, and all combinations of stimulus were used. Each stimulus size was tested at a limited number of discrete speeds. The results together with those obtained for 15cm. high stripes (Table C3.4) provide enough information for the relationship of stimulus width to stimulus height to be ascertained.

3.35 Table C3.11 shows the experimental results for all experiments together with the relevant results for 15cm. high stripes, and in Fig. C3.9 the Intensity Indices are plotted against flicker frequency for each stimulus. It can be seen that as the area of stimulus increases, the intensity of response also increases. If the mean optimal Intensity Index for each width of stimulus is plotted against height of stimulus (Table C3.12), it can be seen that as the height of stimulus increases, the response intensity also increases.

3.36 In order to find out the effect of stripe width and stripe height on threat behaviour, the Intensity Indices were analysed for variance using a two way classification table and a randomised block design.

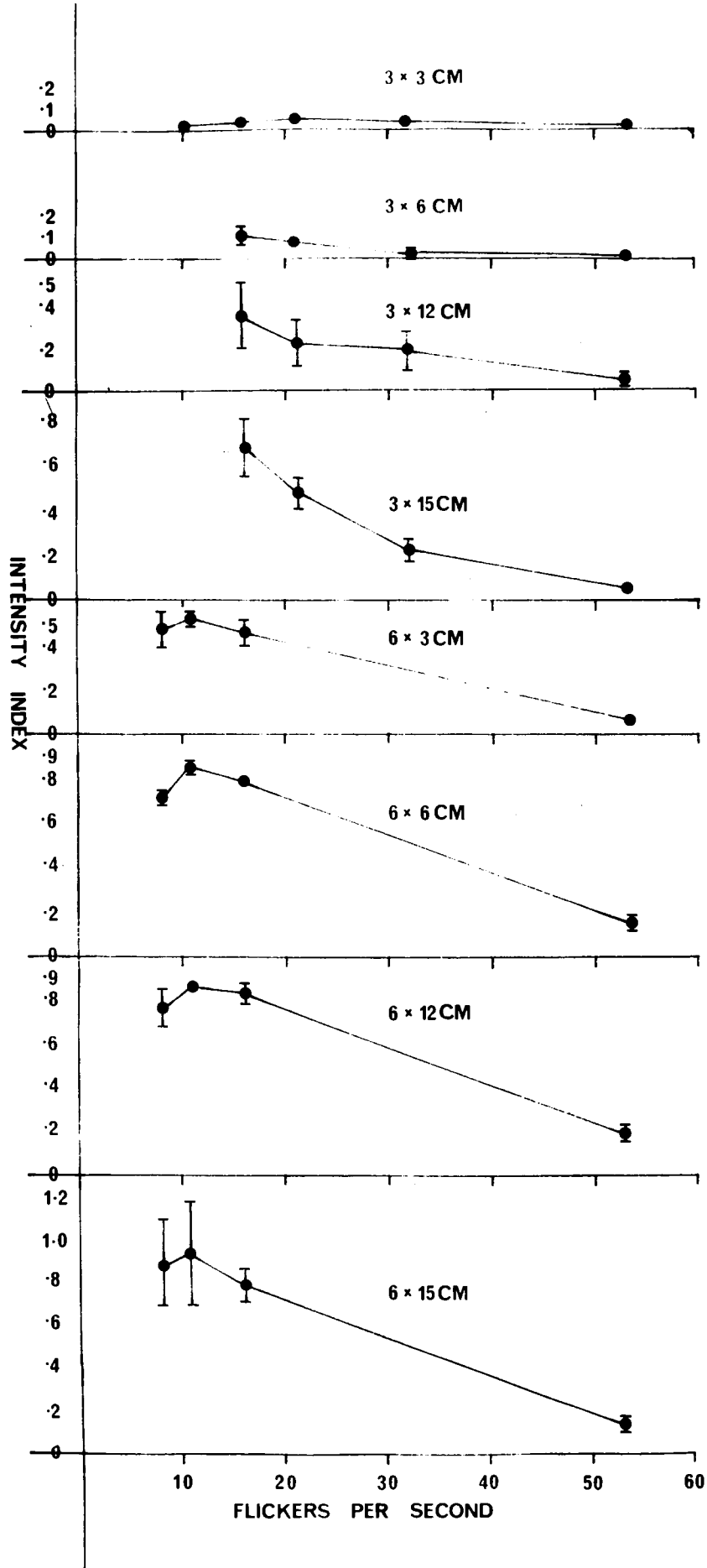
Stimulus size in cms	Flickers per sec.	Expt.	observed Intensity Index	Expt.	observed Intensity Index
3 x 3	10.66	52	0.0155	52a	-
	16.0		0.0310		0.0310
	21.33		0.0543		0.0310
	32.0		-		0.0310
	53.33		0.0		0.0155
3 x 6	16.0	53	0.1319	53a	0.0853
	21.33		0.0698		0.0853
	32.0		0.0310		0.0
	53.33		0.0		0.0155
3 x 12	16.0	55	0.2250	55a	0.4966
	21.33		0.1319		0.3103
	32.0		0.1008		0.2638
	53.33		0.0310		0.0698
3 x 15	16.0	26	0.8049	30	0.5897
	21.33		0.5508		0.4267
	32.0		0.2791		0.1939
	53.33		0.0620		0.0543
6 x 3	8.0	56	0.5509	56a	0.4112
	10.66		0.5044		0.5587
	16.0		0.5121		0.4190
	53.33		0.0776		0.0776
6 x 6	8.0	57	0.6751	57a	0.7371
	10.66		0.8138		0.8720
	16.0		0.7992		0.7905
	53.33		0.1241		0.1939
6 x 12	8.0	59	0.6751	59a	0.8342
	10.66		0.8342		0.8652
	16.0		0.8603		0.7905
	53.33		0.1784		0.2017
6 x 15	5.33	27	1.0862	31	-
	8.0		-		0.6905
	10.66		1.1775		0.6905
	16.0		0.8496		0.7099
	53.33		0.1706		0.1396

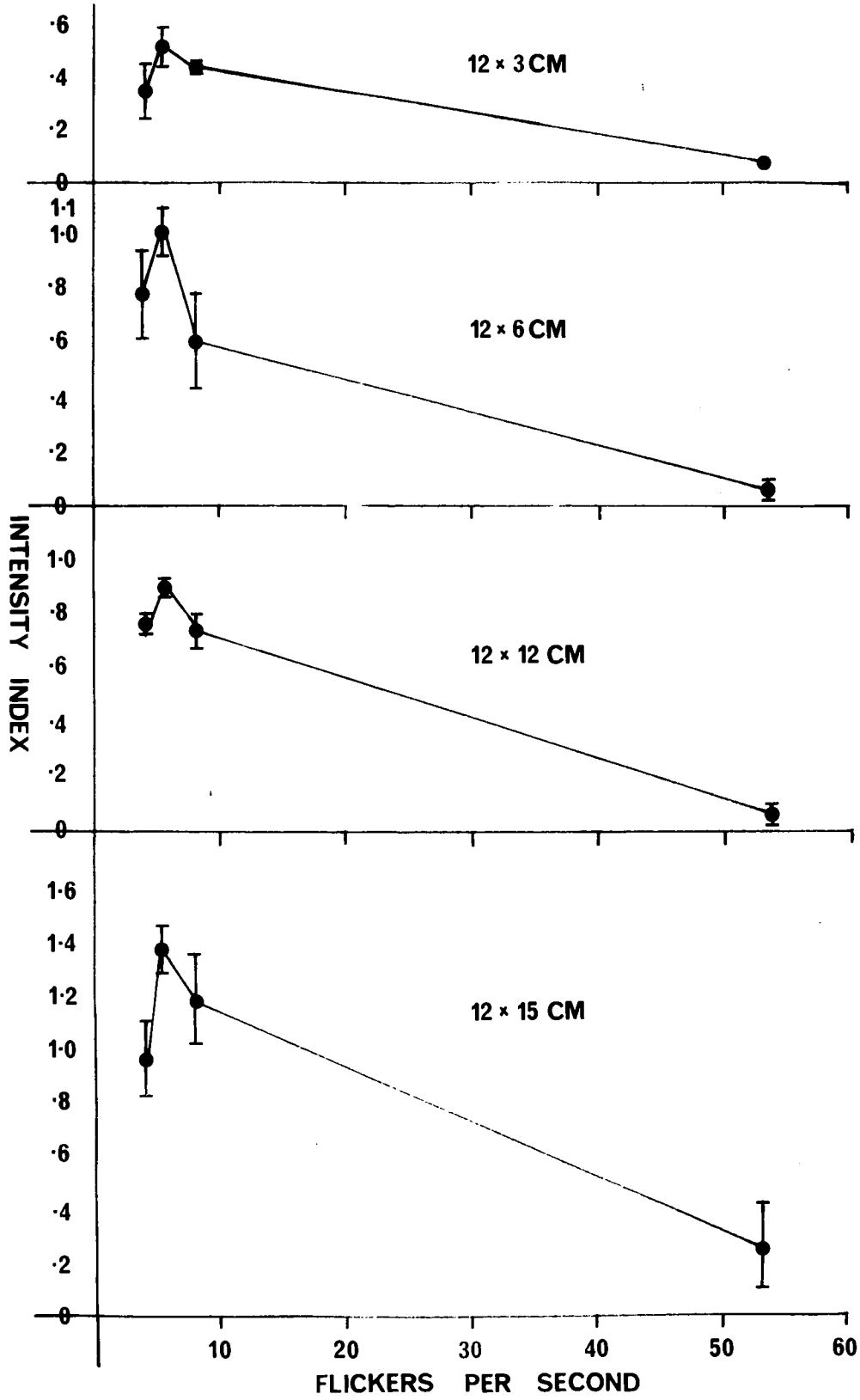
continued

Stimulus size in cms	Flickers per sec.	Expt.	Observed Intensity Index	Expt.	Observed Intensity Index
12 x 3	4.0	60	0.4423	60a	0.2405
	5.33		0.5820		0.4423
	8.0		0.4035		0.4423
	53.33		0.0543		0.0931
12 x 6	4.0	61	0.6208	61a	0.9263
	5.33		0.9312		1.0951
	8.0		0.4423		0.7682
	53.33		0.0931		0.0310
12 x 12	4.0	63	0.7876	63a	0.7139
	5.33		0.8681		0.9108
	8.0		0.6751		0.7954
	53.33		0.0853		0.0155
12 x 15	2.66	28	0.8128	29	0.0883
	5.33		1.3172		1.4666
	8.0		1.0592		1.3473
	53.33		0.1163		0.4276

TABLE C3.11 Intensity Indices for the "increase in size" experiments shown for each flicker frequency and each stimulus size.

FIGURE C3.9 Intensity Indices for each stimulus size (3cmx3cm - 12cmx15cm) plotted against flicker frequency


3 × 3 CM
3 × 6 CM
3 × 12 CM
3 × 15CM
6 × 3CM
6 × 6 CM
6 × 12CM
6 × 15CM
INTENSITY INDEX
FLICKERS PER SECOND
10
20
30
40
50
60


12 × 3 CM
12 × 6 CM
12 × 12 CM
12 × 15 CM
INTENSITY INDEX
FLICKERS PER SECOND
·6
·4
·2
0
1·1
1·0
·8
·6
·4
·2
0
1·0
·8
·6
·4
·2
0
1·6
1·4
1·2
1·0
·8
·6
·4
·2
0
10
20
30
40
50
60

Size of stimulus cms	Flickers per sec.	Speed cm/sec.	Mean optimal Intensity Index
3 x 3	21.33	63.6	0.0426
3 x 6	16.0	47.7	0.1086
3 x 12	16.0	47.7	0.3608
3 x 15	16.0	47.7	0.6973
6 x 3	10.66	63.6	0.5315
6 x 6	10 66	63.6	0.8429
6 x 12	10.66	63.6	0.8497
6 x 15	10.66	63.6	0.9340
12 x 3	5.33	63.6	0.5121
12 x 6	5.33	63.6	1.0131
12 x 12	5.33	63.6	0.8894
12 x 15	5.33	63.6	1.3919

TABLE C3.12 Flicker frequency and speed of movement at which optimal response occurs for each stimulus size

The variance ratios obtained showed that there is no significant variation in threat behaviour due to differences in height or width of a stimulus. There is however slight indication that the larger the stimulus the better a releaser of threat behaviour it is.

3.37 Experiments were also carried out with a stimulus that increased rapidly in size throughout one cylinder revolution and then returned instantaneously to negligible height at the beginning of the next revolution. If there is no well developed distance detector in the compound eye of F. rufa such a stimulus could create the illusion of movement towards; i.e. increase in relative size, but with a lateral movement component.

The stimulus consisted of a solid black isosceles triangle with a 15cm. base and 72cm. height. The triangle was wrapped round the surface of the stimulus cylinder (Section 2.16) with the base vertical. The apex formed a stimulus of negligible height and as the cylinder was revolved, the stimulus size increased until the base of the triangle formed a stimulus of 15cm. height. In order to prolong the 15cm. high stimulus so that it would fill the

stimulus windows a black square of 15 cm. sides was butted onto the triangle base. This left a 9 cm. space between the free side of the square and the triangle apex.

As the cylinder was revolved the stimulus increased in size and then disappeared to reappear again after an interval of one cycle per revolution. The reverse was also possible where the stimulus diminished once every cylinder revolution.

3.38 Table C3.13 shows the results for an experiment where the size of stimulus was increased and another where the size was decreased. From the calculated Intensity Indices it can be seen that there is optimal response at 320 cm. per sec. or 6.66 flickers per sec.

The optimal results of these two experiments are compared in Table C3.14 with the optimal results obtained for striped stimuli. It can be seen that if the results for striped stimuli are projected beyond the 16 cm stripe to a 48 cm stripe, the increasing size stimuli provide results that could be expected for such a stripe width. As the Intensity Indices obtained for the change in size stimuli are no better than those for striped stimuli, there can be no importance attached to the increase/decrease in size. Sudden change in size is therefore not important as a component of threat behaviour. The results do not rule out the possibility that F. rufa has a distance detector. There are no significant variations between tests in the calculated results.

Flickers per sec.	Speed of movement cm/sec.	Increasing size		Decreasing size	
		obs.	calc.	obs.	calc.
0.33	16.0	0.0853	0.1861	-	-
1.0	48.0	0.2560	0.2172	0.2482	-
1.33	64.0	0.4035	0.2596	0.4423	0.3594
2.0	96.0	0.3957	0.3091	0.4423	0.4610
3.33	160.0	0.3259	0.4653	0.6751	0.5599
4.23	203.2	0.3802	0.5484	-	-
5.0	240.0	-	-	0.6130	0.6664
5.33	256.0	0.7372	0.5973	-	-
6.66	320.0	0.6518	0.6049	0.7750	0.6952
8.33	408.0	0.4811	0.5036	0.5043	0.6109
10.0	480.0	-	-	0.5354	0.4960
10.5	504.0	0.2871	0.2954	-	-
13.33	640.0	0.4035	0.3954	0.4966	0.5014

TABLE C3.13 Observed and calculated Intensity Indices for increasing and decreasing size stimuli shown for each speed and flicker frequency

Stimulus stripe width cms.	Optimal calculated Intensity Index	Optimal response speed cm/sec.	Optimal response flicker frequency flickers/sec.
1.5	0.0883	51.6	35.0
3.0	0.6023	55.7	18.5
3.0	0.4346	48.7	16.5
6.0	1.1307	62.7	10.5
6.0	0.7310	68.6	11.5
12.0	1.1626	127.3	10.5
12.0	1.4334	127.3	10.5
16.0	0.8339	150.9	9.5
16.0	1.0462	134.7	8.5
increasing size stimulus	0.6049	320.0	6.66
decreasing size stimulus	0.6952	320.0	6.66

TABLE C3.14 Results of the experiments in which striped stimuli were used together with the results of experiments in which size of stimulus changed, to show correlation between the speed and flicker frequencies of optimal response

3.39 Regular stripes stimulate the eye in a regular temporal sequence and the threshold level for each successive stimulus is the same. However, when irregular stripes stimulate the eye, the temporal sequence is irregular and response thresholds are different for each stimulus. To accommodate this variation a lower total response threshold will occur and an irregular stimulus should therefore elicit a greater response from the ants than will an equivalent regular stimulus (for an extension of this theory see Schneirla, 1967).

To test this basic premise, experiments were carried out using irregular visual stimuli. These were of three types:-

1) A pattern of black stripes 1.5, 3, 6 and 12 cms. wide spaced round the stimulus cylinder as described in Section 3.14, but with only half the number of stripes used in those experiments. This provided periods between stimulation (i.e. white background) that were three times the length of the alternate periods between stimulation (black stripe).

2) Striped patterns where the number of stripes was not reduced where the width was constant but where the intervals between the stripes were irregular.

3) A pattern consisting of stripes of different widths, irregularly spaced.

All stimuli were presented to the ants as in Section 2.16.

3.40 Table C3.15 shows the experimental results together with the calculated Intensity Indices for type 1 stimuli. Fig. C3.10 shows the mean of the observed and of the calculated Intensity Indices plotted against flicker frequency for comparison with Fig. C3.2.

As the number of stimuli is reduced by half, the flicker frequency for each revolution is halved and to obtain the flicker frequencies used in earlier experiments the speed of movement must be doubled. It can be seen that the response curves are similar to those obtained for experiments in which the normal full striped patterns were used. Analysis of the calculated optimal response flicker frequencies for each test (see Table C3.16) shows no significant variations in the flicker frequencies.

3.41 However if the calculated optimal response speeds for each test are compared (see Table C3.16) there is a 1% significant difference between speeds. These results reinforce the conclusions obtained from the normal full striped patterns examined earlier.

Flickers per sec.	1.5cm STRIPE* Expt. 71 obs.	Expt. 72 obs.	3cm STRIPE Experiment 42 obs.	calc.	Experiment 43 obs.	calc.
0.0	0.0388	0.0388	0.0155	0.0053	0.0310	0.0683
5.33	-	-	0.6208	0.6923	0.6401	0.5780
8.0	-	-	0.8807	0.8199	0.6712	0.6736
10.66	0.0931	0.1629	0.8322	0.8631	0.7294	0.7054
16.0	0.2017	0.1241	0.8244	0.7543	0.5314	0.6247
18.66	-	-	0.6401	0.6491	0.4267	0.5470
21.33	0.0930	0.0931	-	-	-	-
24.0	-	-	0.3258	0.3795	-	-
26.66	-	-	0.2172	0.2626	0.3025	0.2628
37.33	-	0.0388	0.0465	-0.0281	0.1008	0.0492
40.0	0.0543	-	-	-0.0326	-	0.0447
48.33	-	-	0.0155	0.0589	0.0465	0.0970
53.33	0.0310	0.0155	-	-	-	-
58.66	-	-	0.0	0.0005	0.0	0.0056

continued

* No calculated values available

6cm STRIPE

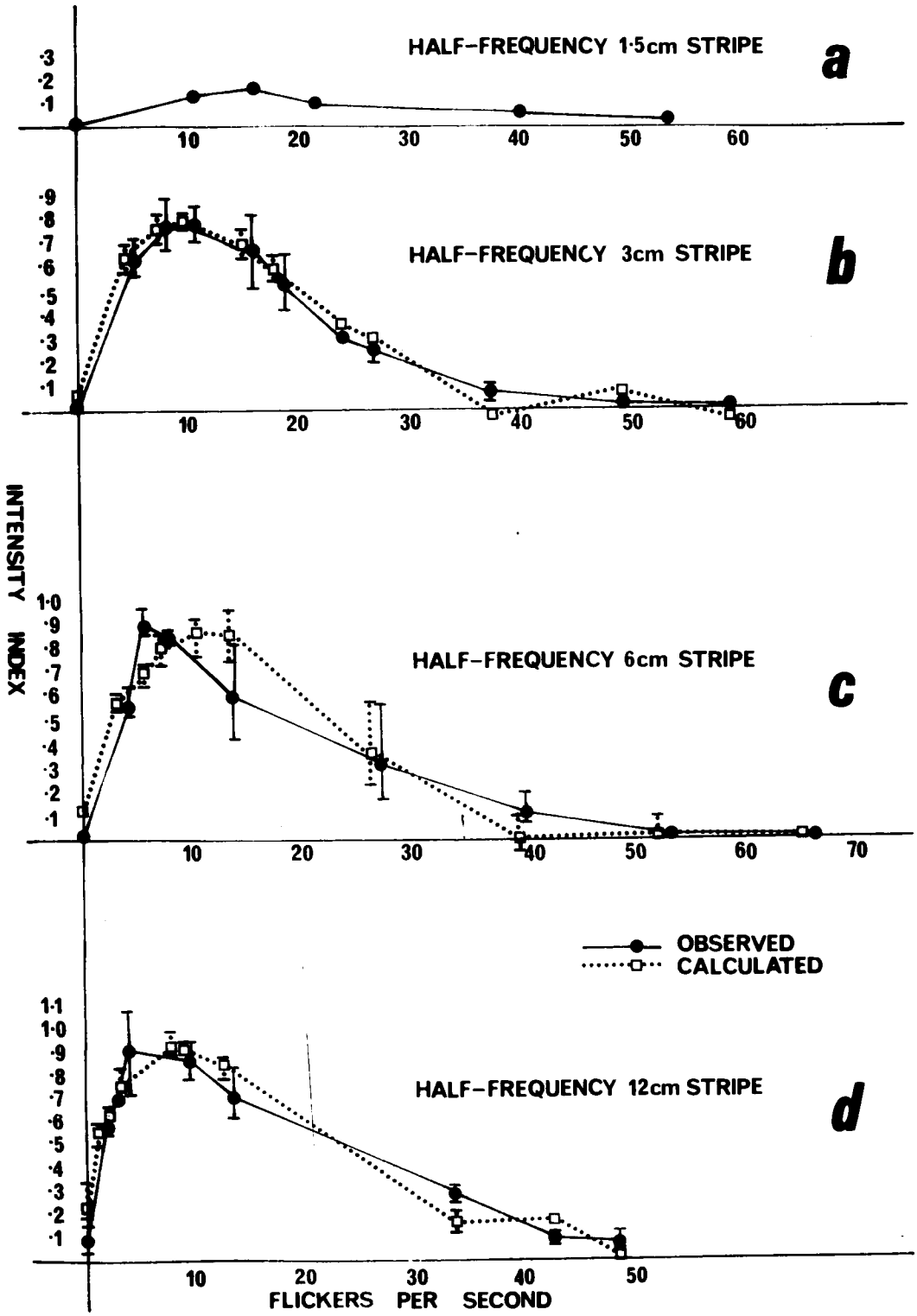
Flickers per sec.	Experiment 44 obs.	Experiment 44 calc.	Experiment 45 obs.	Experiment 45 calc.	Experiment 45a obs.	Experiment 45a calc.
0.0	0.0310	0.1140	0.0	0.1341	0.0	0.1385
4.0	0.5509	0.5947	0.6285	0.5831	0.5354	0.5398
5.33	0.9690	0.7157	0.8652	0.6830	0.8924	0.6308
8.0	0.8264	0.7818	0.8681	0.7818	0.8293	0.7235
10.66	-	0.9334	-	0.8107	-	0.7550
13.33	0.8186	0.9561	0.5664	0.7746	0.4422	0.7305
26.66	0.5742	0.5799	0.1707	0.2372	0.2793	0.2759
40.0	0.1939	0.1293	0.0775	-0.0852	0.0853	-0.0251
49.5	-	0.0384	-	0.0358	-	0.0515
53.33	0.0	0.0476	0.0155	0.1230	0.0310	-
66.66	0.0155	0.0082	0.0155	0.0023	0.0	-

continued

Flickers per sec.	12cm STRIPES Experiment 46 obs.	calc.	Experiment 47 obs.	calc.	Experiment 47a obs.	calc.
0.0	0.0930	0.2105	0.0310	0.1944	0.1551	0.3432
2.0	0.5431	0.5837	0.6052	0.4970	0.6052	0.5689
2.66	0.6983	0.6542	0.6984	0.5572	0.7139	0.6147
4.0	1.0786	0.8176	0.7449	0.7047	0.9467	0.7293
8.0	-	0.9706	-	0.8963	-	0.8911
9.33	0.9418	0.9495	0.7876	0.9091	0.8448	0.9079
13.33	0.6052	0.7702	0.8293	0.8385	0.7294	0.8682
29.0	-	0.7620	-	0.2367	-	0.3114
33.33	0.2948	0.1265	0.2560	0.1698	0.3181	0.2029
37.33	-	0.2271	-	0.1575	-	0.1570
42.33	0.1163	0.2941	0.0620	0.1543	-	-
44.0	-	-	-	-	0.1085	0.1752
48.33	0.0465	0.0163	0.0698	0.0477	-	-
61.33	-	-	-	-	0.1241	0.1217

TABLE C3.15 Results of experiments using irregular temporal visual stimuli. Observed and calculated Intensity Indices are shown for each flicker frequency tested

FIGURE C3.10 Threat response plotted against flicker frequency for the experiments in which the stimuli were half-frequency stripes


HALF-FREQUENCY 1·5cm STRIPE
a
HALF-FREQUENCY 3cm STRIPE
b
HALF-FREQUENCY 6cm STRIPE
c
HALF-FREQUENCY 12cm STRIPE
d
INTENSITY INDEX
OBSERVED
CALCULATED
FLICKERS PER SECOND

Page 85 was not
included in
the bound thesis.

Stimulus	3cm STRIPE Mean Intensity Index	cm/sec.	fl/sec.	6cm STRIPE Mean Intensity Index	cm/sec.	fl/sec.	12cm STRIPE Mean Intensity Index	cm/sec.	fl/sec.
Full-flicker frequency Expt.	0.5184	50.9	17.0	0.9304	62.7	10.5	1.2972	127.3	10.5
Half-frequency flicker Expt.	0.7842	62.7	10.5	0.8330	127.3	10.5	0.9219	222.8	9.5

TABLE C3.16 Mean Intensity Indices for each stripe width shown against speed and flicker frequency for comparison between experiments in which the maximum number of stripes are used, i.e. normal stripe stimulus and experiments in which only half the number of stripes are used

Irregular temporal stimuli show no increase in threat response above that for normal regular striped patterns. Analysis of the Intensity Indices for each test shows a 5% significant variation between flicker frequencies for the 3cm. stripes but no significant variation for other stripe widths.

3.42 Striped patterns providing irregular temporal stimulation, but with maximum flicker frequencies per revolution (3cm. = 32 flickers per rev.; 6cm. = 16 flickers per rev.) were used to provide an irregular stimulus of type 2 (Section 3.39). The patterns consisted of stripes 3cm. or 6cm. wide presented as in Section 3.14 but irregularly spaced round the stimulus cylinder.

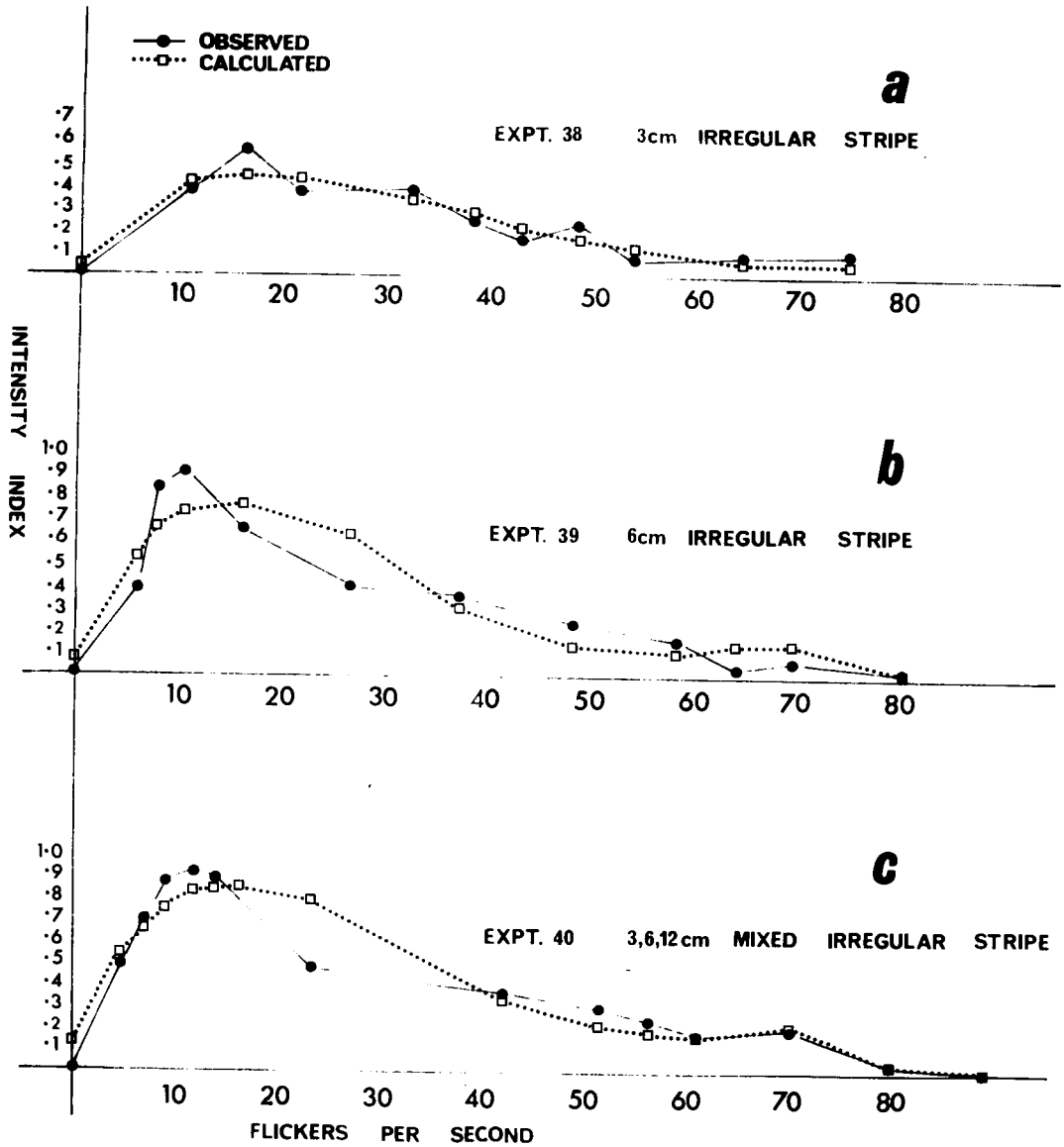
Table C3.17 shows the experimental and calculated results for experiments in which these stimuli were used and it can be seen that the calculated optimal response is lower than that for full regular flicker frequency experiments (Table C3.4). Fig. C3.11 is provided for comparison with Fig. C3.2.

A visual stimulus with temporal irregularity does not form a better stimulus for threat behaviour than does a regular temporal visual stimulus. The Intensity Indices for each test within each

Flickers per sec.	3cm STRIPES Experiment 37 obs.	calc.	Experiment 38 obs.	calc.	6cm STRIPES Experiment 39 obs.	calc.	Experiment 34 obs.	calc.
0.0	0.0310	0.0126	0.0	0.0018	0.0	0.0846	0.0	0.1324
5.33	-	-	-	-	0.4073	0.5359	0.4888	0.4808
8.0	-	-	-	-	0.8575	0.6568	0.6673	0.5800
10.66	0.2016	0.3123	0.3801	0.4038	0.9234	0.7459	0.7837	0.6483
16.0	0.5121	0.3807	0.5664	0.4694	0.6595	0.7872	0.6828	0.7092
19.0	-	0.3987	-	0.4765	-	-	-	-
21.33	0.3801	0.4052	0.3879	0.4705	-	-	-	-
22.5	-	0.4059	-	0.4655	-	-	-	-
26.66	-	-	-	-	0.4112	0.5990	0.4654	0.6000
32.0	0.3568	0.3707	0.3956	0.3711	-	-	-	-
37.33	0.3413	0.3278	0.2482	0.2987	0.3878	0.3243	0.2637	0.3602
42.66	0.3025	0.2825	0.1629	0.2350	-	-	-	-
48.0	0.1939	0.2314	0.2405	0.1752	0.2560	0.1466	0.2094	0.1935
53.33	0.1551	0.1835	0.0775	0.1312	-	-	0.1553	0.1015
58.66	-	-	-	-	0.1784	0.1178	0.1706	0.0745
64.0	0.1707	0.1118	0.0941	0.0937	0.0543	0.1366	0.0776	0.0751
68.5	-	-	-	0.0916	-	-	-	-
69.33	-	-	-	-	0.0853	0.1496	0.1008	0.1004
74.66	0.0465	0.0717	0.0941	0.0916	-	-	-	-
80.0	-	-	-	-	0.0310	0.0121	0.0620	0.1684
90.66	0.0465	0.0448	-	-	-	-	-	-
96.0	-	-	-	-	-	-	0.0465	0.0143

TABLE C3.17 Observed and calculated Intensity Indices for the experiments in which irregular stripes were used as stimuli

FIGURE C3.11 Threat response plotted against flicker frequency for the experiments in which :-
a) and b), the stimuli were irregular standard width stripes
c) the stimuli were irregular mixed width stripes


OBSERVED
CALCULATED
a
EXPT. 38 3cm IRREGULAR STRIPE
b
EXPT. 39 6cm IRREGULAR STRIPE
c
EXPT. 40 3,6,12cm MIXED IRREGULAR STRIPE
INTENSITY INDEX
FLICKERS PER SECOND
10 20 30 40 50 60 70 80

experiment were compared for significant variations. Variations between the flicker frequencies of experiments 37 and 38 were obtained at the 5% level and for experiments 34 and 39 at the 1% level.

3.43 In the last set of irregular stimulus experiments, a stimulus pattern was used which had stripes of different widths, irregularly spaced round the stimulus cylinder. The seven stripes provided a flicker frequency of 14 flickers per revolution and Table C3.18 and Fig. C3.11 show both the experimental and calculated results from two experiments in which this stimulus was used. The calculated optimal response is in the region 15 - 16.5 flickers per second or 102.3 - 112.5 cms. per second, which is lower than for regular temporal stimuli (see Table C3.4).

Comparison of the Intensity Indices for each test in experiments 40 and 41 showed 1% significant variation in both cases.

3.44 None of the irregular stimuli tested therefore act as better releasers of threat behaviour than does a regular stimulus. The speed of movement and flicker frequencies at which optimal responses occur

in each experiment, are usually significant when compared with the response levels at other speeds and flicker frequencies. A few puzzling exceptions occur e.g. results shown in Table C3.15A which would be expected to show significant variations.

3.45 Using the results from all relevant movement experiments, the frequency range over which the flicker fusion frequency of F. rufa occurs can be established. In Section 3.14 the flicker fusion frequency was assumed to have been reached when the intensity of threat response dropped to the level recorded for stationary stimuli. Table C3.1 lists the mean response for each stationary stimulus (see Section 3.7) and from these figures the overall response mean is 0.0426. This figure will be used as the standard value for the Intensity Index at flicker fusion frequency. Table C3.19 shows the flicker fusion frequencies for the moving stimuli experiments, from which a relationship between fusion frequency and stimulus size can be obtained. From the values for the regular stripe experiments it can be seen that the fusion frequency varies with the size of stimulus. A small stimulus, e.g. 1.5 cm. stripes, provides a fusion frequency at only 42.5 flickers per second, but a larger stimulus, e.g. 12. cm. stripes, provides a fusion frequency at a higher flicker frequency, 66.5 - 70.5 flickers per second. Above this stimulus size the fusion frequency begins to fall again.

Such a change of fusion frequency with stimulus size does not appear to have been reported before and if the flicker fusion

Flickers per sec.	Experiment 40 obs.	calc.	Experiment 41 obs.	calc.
0.0	0.0	0.1393	0.0	0.0331
4.66	0.5897	0.5248	-	-
7.0	0.7138	0.6686	0.4733	0.5146
9.33	0.8865	0.7698	0.6905	0.5973
12.5	0.9389	0.8432	0.6983	0.6492
14.0	0.9078	0.8629	0.6906	0.6590
15.0	-	-	-	0.6603
16.5	-	0.8745	-	-
23.33	0.4810	0.8009	0.3878	0.5542
32.66	-	-	0.3801	0.3447
42.0	0.3724	0.3561	-	-
51.33	0.3025	0.2120	0.1551	0.1070
56.0	0.2404	0.1835	0.0698	0.1180
60.66	0.1861	0.1776	0.1551	0.1435
70.0	0.2016	0.2044	0.0543	0.1760
79.33	0.0465	0.1801	-	-
80.16	-	-	0.0155	-0.0158
88.66	0.0	0.0602	-	-

TABLE C3.18 Observed and calculated Intensity Indices for the mixed stripe width, irregular temporal stimuli experiments

Stimulus	Flickers per sec.	Intensity Index
Regular stripes		
1.5cm	42.5	0.0421
3cm	55.0	0.0526
	48.0	0.0429
6cm	62.0	0.0530
	59.5	0.0526
12cm	70.5	0.0442
	66.5	0.0451
16cm	62.0	0.0443
	62.0	0.0434
Half-frequency stripes		
3cm	57.0	0.0519
	39.5	0.0444
	32.5	0.0498
6cm	34.0	0.0474
	31.5	0.0493
	47.0	0.0440
12cm	39.5	0.1517
	37.5	0.1575
	29.0	0.0762
Irregular stripes		
3cm	68.5	0.0916
	90.5	0.0448
6cm	61.0	0.0711
	55.5	0.1124
Chequerboard		
6cm	49.0	0.0455
	47.0	0.0441
Mixed irregular stripes		
	51.5	0.1070
	59.5	0.1773

TABLE C3.19 Flicker frequencies of the calculated Intensity Indices which fall nearest the standard flicker fusion frequency Intensity Index of 0.0426

frequency values obtained with all movement experiments are considered the overall range at which flicker fusion in F. rufa occurs is from 29 flickers per second to 90.5 flickers per second. Such a wide range may be caused by other visual factors, in the stimuli involved, masking or altering the true fusion frequency or could be explained by experimental error. If the extreme values are ignored, the majority of values lie within the range 40 - 70 flickers per second. Further experiments, specifically designed to test for the flicker fusion frequency, would be necessary before more accurate results could be obtained.

CHAPTER FOUR

Visual Stimuli

Qualitative properties of light stimuli

4.1 Observations of colour preference in insects are numerous, dating back to the 1880s, e.g. Plateau (1888). There is therefore little doubt that insects can perceive monochromatic light. Insect compound eyes are sensitive to wavelengths from about 253 nm. (the ultra-violet) to about 730 nm. (the near infra-red), Dethier (1963). Lubbock (1885,86) and Forel (1886) showed that ants were sensitive to ultra-violet light and Buck (1937) observed responses in the firefly, Photinus pyralis to red flashes (560 - 690 nm.) emitted by other individuals. Autrum and Stumpf (1953) obtained responses in Calliphora to 730 nm. (the near infra-red). Many experiments have been carried out to find out whether or not the insect eye is equally sensitive at all wavelengths and whether insects possess colour vision, i.e. the ability to discriminate between different monochromatic sources of equal luminosity.

4.2 Experiments fall into two groups, behavioural and electro-physiological. Many behavioural experiments have been based on phototactic responses but mainly for studying spectral sensitivity, as other behavioural techniques have also been employed in providing evidence for colour vision, e.g. optomotor reaction.

4.3 It was intended to carry out behavioural experiments using monochromatic stimuli to ascertain whether threat behaviour in

F. rufa was elicited more strongly by some wavelengths of light than others. However before any behavioural experiments could be attempted, the spectral filters used had to be corrected to transmit equal energy intensity throughout the spectrum when fed from a single light source. Then because of the difference in sensitivity of the eye at different wavelengths, the transmission characteristics of the filters had to be changed to provide as near as possible equal illumination energy at the ants eye throughout the spectrum.

In any example, the transmission of a filter will be:-

$$\frac{E\ T\ V}{E\ T}$$

(Data supplied by Ilford Ltd.)

where E, T and V are the source emission, filter transmission and receptor sensitivity respectively, all at common wavelengths. Thus the spectral sensitivity curve of the eye must be known in order to obtain equal illumination at the eye.

4.4 In all experiments, both behavioural and electrophysiological, the same stimulation light source was used. This was a 24 volt, 150 watt Quartz Iodine intense lamp with a source emission curve as shown in Fig. C4.1. The curve was obtained by shining the lamp into a monochrometer and plotting the output reading against wavelength in nanometers. The absorption curves and percent transmission of the Ilford Spectrum Filters used are shown in Fig. C4.2. The filter transmissions were standardised using the monochrometer. The reading

FIGURE C4.1 Intense lamp energy emmission curve

Monochrometer galvamometer reading (full scale deflection is 14.0) plotted against wavelength (peak wavelength of filter).

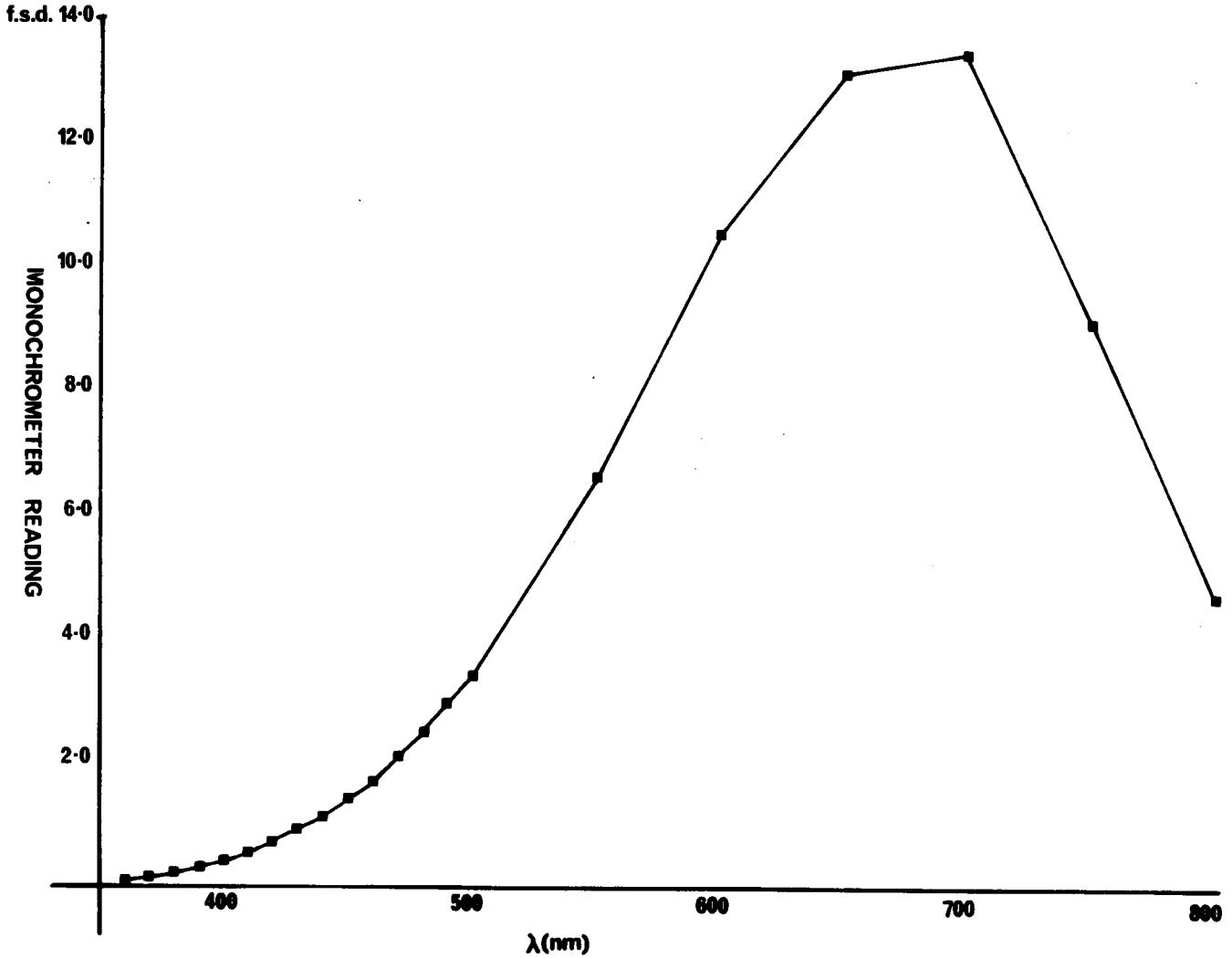
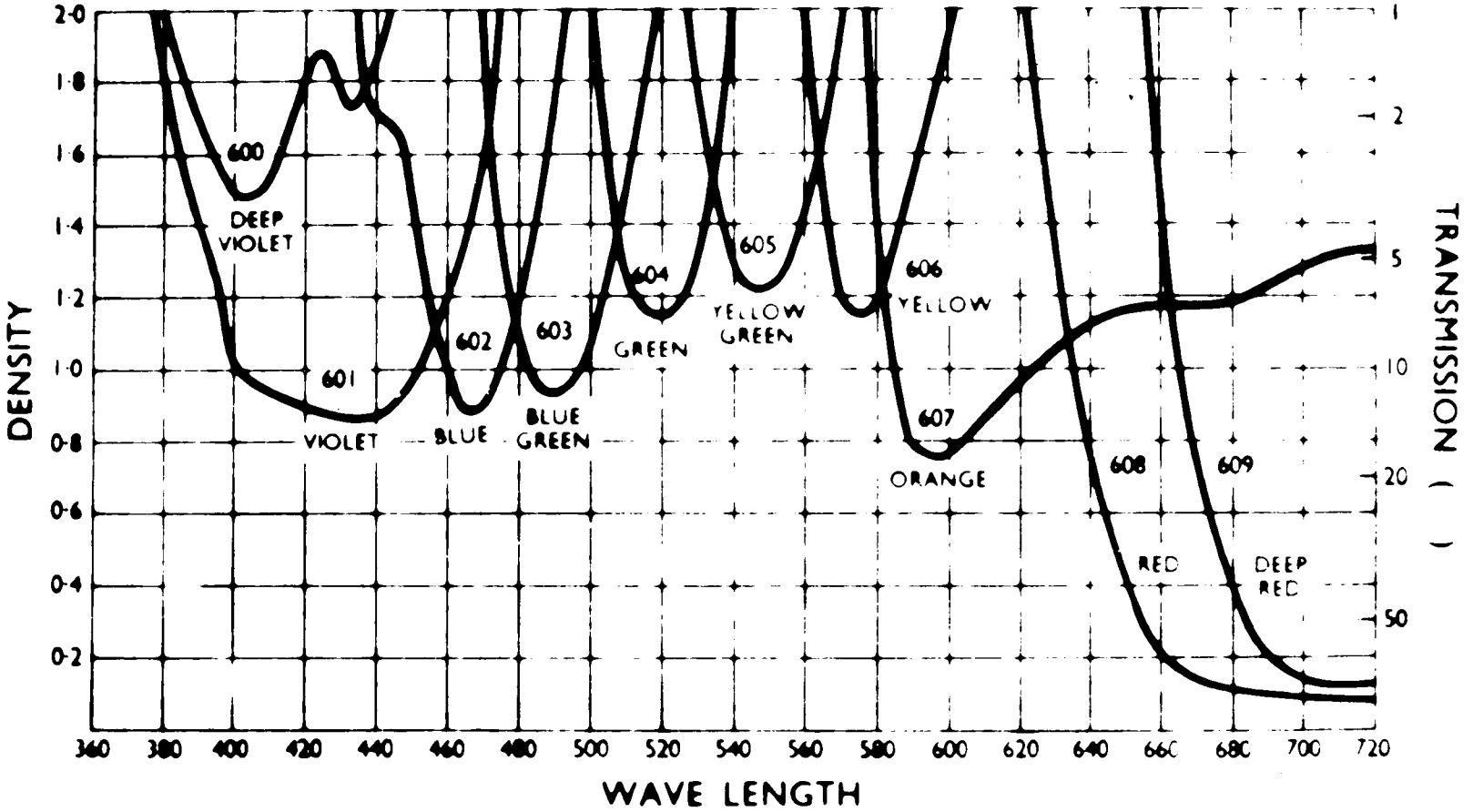

f.s.d. 14·0
12·0
10·0
8·0
6·0
4·0
2·0
MONOCHROMETER READING
400
500
600
700
800
λ(nm)

FIGURE C4.2 Filter absorption curves

ABSORPTION CURVES

Ilford Spectrum Filters, Standard and Supplementary, Nos. 600 to 609



given when the intense lamp with violet filter (the densest filter, Table C4.1) was shone into the monochrometer was taken as a standard and the transmission intensities of all the other filters were adjusted with neutral density filters until they gave the same reading. This did not provide constant energy output at each wavelength (see Table C4.1) because units of neutral density less than 0.1 were not available to match the intensities more accurately.

4.5 Phototactic responses have been used to measure the relative efficiency of different wavelengths for many species of insect. Bertholf (1931a, 31b, 32) showed peak sensitivity at 365 nm. (Ultra-violet) for Drosophila and Apis, with a second peak at 487 nm. (blue-green) for Drosophila and at 553 nm. (yellow-green) for Apis. Saunder (1933) found peaks at 570 nm. (yellow-green) and 470 nm. (blue) in Apis but none in the ultra-violet. Goldsmith (1960) found a peak at 365 nm. (ultra-violet) in Apis. It seems certain that Apis and Drosophila have maximum sensitivity in the near ultra-violet and to a lesser extent in the blue-green and yellow-green. Weiss et Al. (1941, 42, 43a, 43b and 44) experimented with 14,000 insects of forty species and obtained composite group peaks at 492 nm. (blue-green) and at 365 nm.(ultra-violet). They tested so many species with the same results that the results are probably true for all insects.

4.6 Behavioural experiments to indicate spectral sensitivity are

Wavelength nm.	Colour	Monochrometer Scale Reading	Neutral Density added	Light Energy $Ergs\ cm^{-2}\ sec^{-1}$
430	Violet	0.60	0.0	250.9
470	Blue	0.70	0.1	196.5
490	Blue-green	0.75	0.3	206.3
520	Green	0.60	0.0	157.2
550	Yellow-green	0.70	0.1	205.0
580	Yellow	0.80	0.4	186.6
600	Orange	0.85	0.8	211.2
700	Red	1.20	1.3	240.7

TABLE C4.1 Correction of spectral filters to give standard stimulus intensity by addition of Neutral Density Filters.

The light energy striking the eye was measured using a thermopile and was therefore the energy received by the eye, not the energy emitted from source.

dependent upon the physiological state of the insect. Ilse (1928) observed that cabbage butterflies, Pieris brassicae normally land on blue or yellow flowers, while gravid females ready to oviposit show preference for green or blue-green. Thus behavioural responses are not always easy to interpret. Electrophysiological techniques are more reliable and have confirmed the more general behavioural results.

4.7 Crescitelli and Jahn (1939), Jahn and Crescitelli (1939) using electrophyisiological techniques, found a spectral sensitivity peak at 460 - 530 nm. in the moth Samia cecropia and the grasshopper Melanoplus differentialis. Jahn and Wulff (1948) found a peak at 520 - 575 nm.(y'low-green) in Dytiscus fasciventris. Sensitivity peaks in the green and near ultra-violet were found in four species of Diptera by Donner and Kriszat (1950) and Autrum and Stumpf (1953) found two peaks in Calliphora erythrocephala at 540 nm. (yellow-green) and 630 nm. (red). Walther and Dodt (1959) obtained a maximum sensitivity peak at 341 nm. (ultra-violet) and Burkhardt (1962) showed sensitivity peaks in Calliphora at 490 nm. (blue-green) and 350 nm. (ultra-violet). Calliphora therefore has sensitivity peaks at 630 nm. (red), 490 - 540 nm.(y'low-green) and 341 - 350 nm. (ultra-violet). Periplaneta shows the same sensitivity but lacks the peak at 360 nm. (Walther and Dodt, 1957, 1959).

The drone honey bee shows a maximum sensitivity at 400 nm. (violet) for the compound eye (Goldsmith, 1958a, 58b), at 447 nm. (blue) and

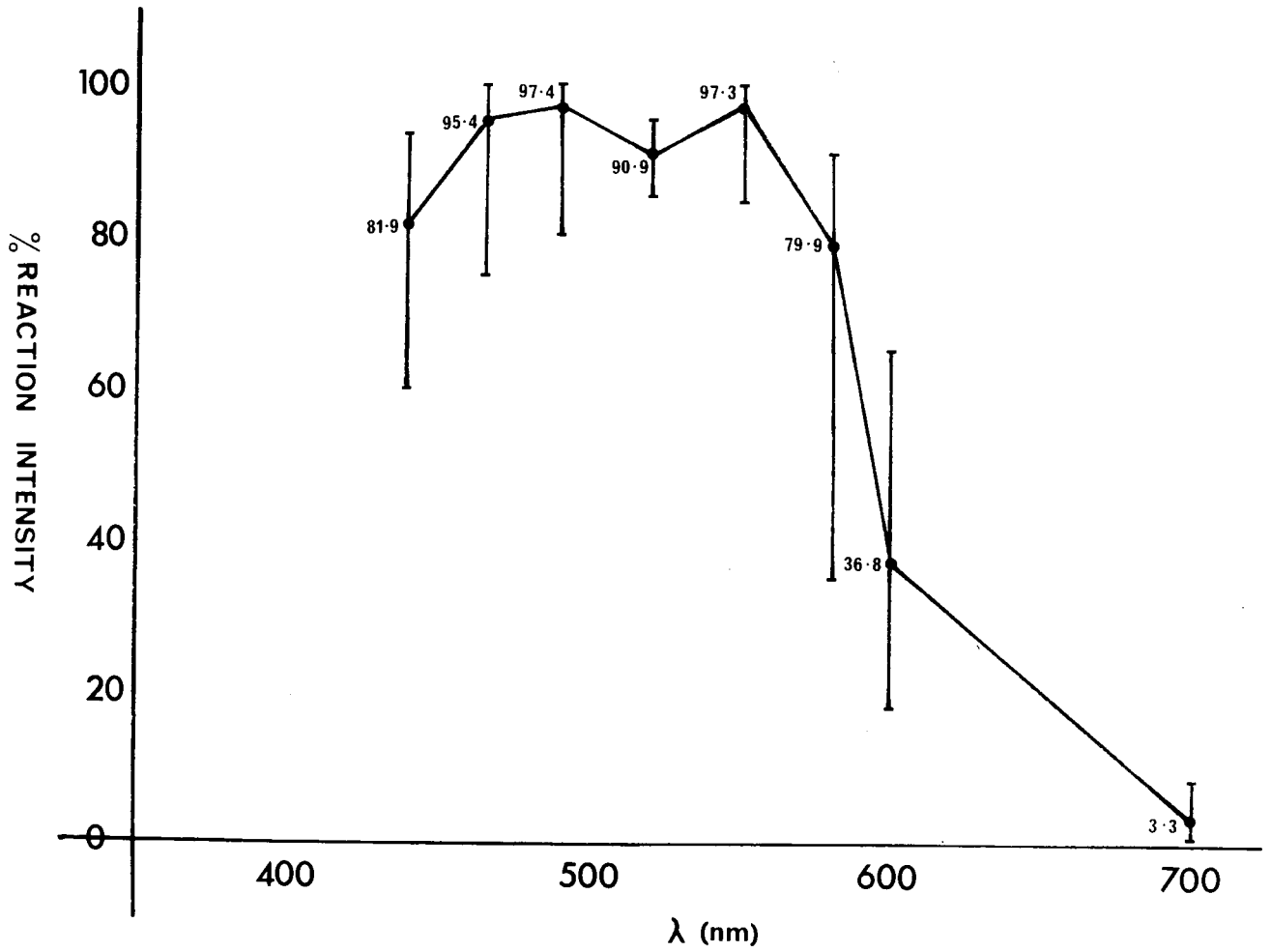
530 nm. (yellow-green) (Autrum and Zwehl, 1962) and at 340 nm. (ultra-violet), 450 nm. (blue) and 530 nm. (blue-green) (Autrum and Zwehl, 1964). Hasselmann (1962) obtained sensitivity peaks in Carabus, Phalera and Macroglossa at 430 nm. (violet), 500 nm. (blue-green) and 620 nm.(red). Bennet, Tunstall and Horridge (1967) found two sensitivity peaks at 430 nm. (violet) and 515 nm. (blue-green) in Locusta migratoria. It would seem that most insects have three sensitivity peaks.

4.8 Sensitivity to monochromatic light in F. rufa was measured throughout the spectrum using the apparatus described in Section 2.28. The height (percent of maximum height per experiment) of the E.R.G. generator potential was plotted against wavelength to obtain a spectral sensitivity curve. The results of all experiments were combined as in Fig. C4.3. It can be seen that two peaks at 490 nm. (blue-green) and 550 nm. (yellow-green) are evident. No measurements were taken in the ultra-violet and there is obviously no sensitivity peak in the red as in Calliphora Walther and Dodt (1957), Carabus, Phalera and Macroglossa Hasselmann (1962).

4.9 Whether the two peaks obtained in F. rufa are indicative of more than one receptor type as claimed for Calliphora; Burkhardt (1962) and Apis; Autrum and Zwehl (1962, 64) or Locusta; Bennet, Tunstall and Horridge (1967) is debatable although experiment CC3 showed only one

FIGURE C4.3 Visual sensitivity curve for *Formica rufa*

Percent E.R.G. generator potential plotted against wavelength in nanometres.


100
80
60
40
20
0
% REACTION INTENSITY
81·9
95·4
97·4
90·9
97·3
79·9
36·8
3·3
400
500
600
700
λ (nm)

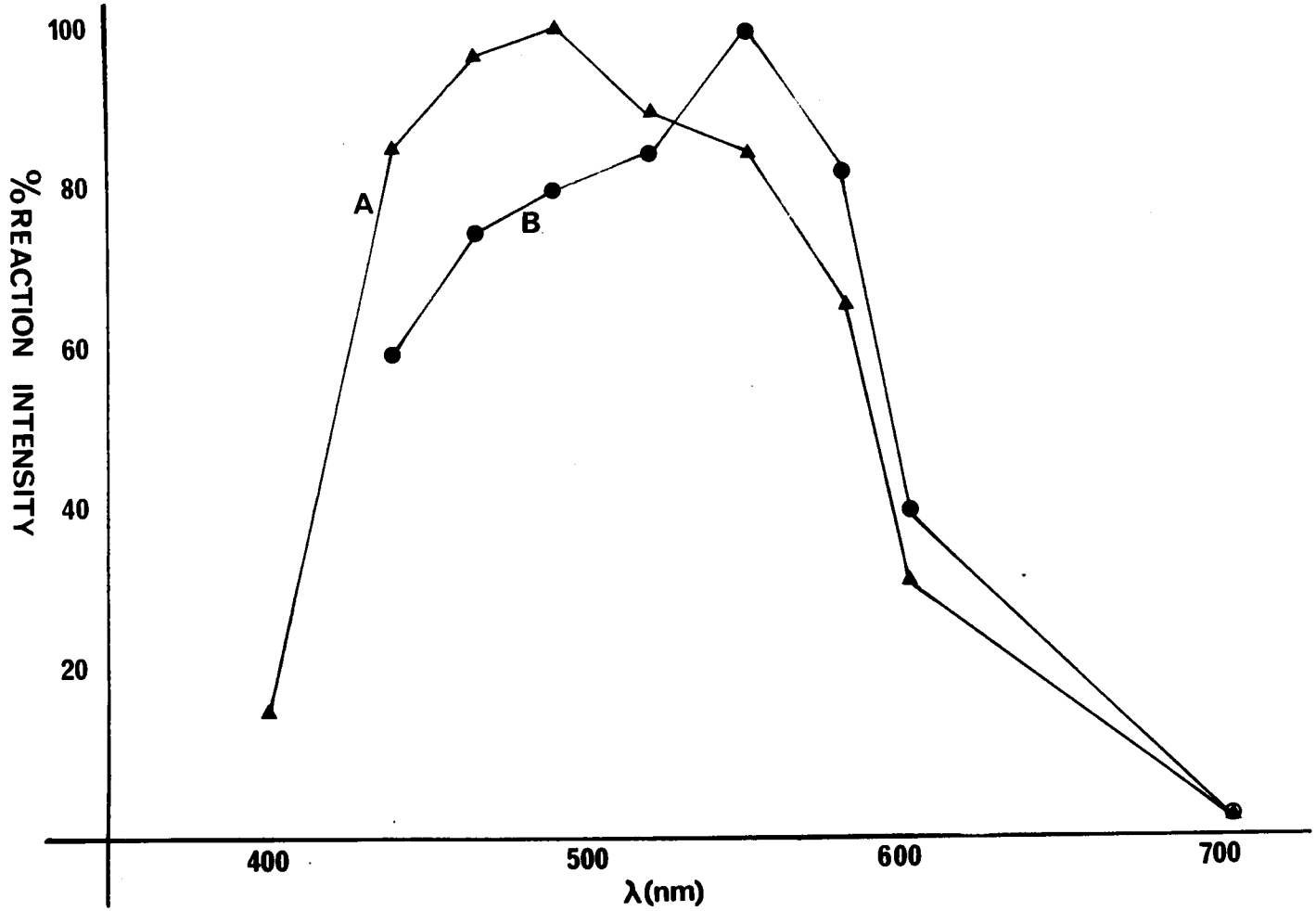
response peak at 550 nm. and experiment CC4 showed a single response peak at 490 nm. (Fig. C4.4). It is improbable but possible that in these two experiments the recording electrode only just entered the eye and made contact with one visual unit. In this case the curves would be explained if they were from single visual units of blue-green types, in which case discrimination between blue-green and yellow-green should be possible. An experimental arena was designed to test for colour discrimination in F. rufa (Appendix III) but due to development difficulties was not completed in time to obtain any results.

Kiepenheuer (1968) carried out experiments to show that F.polyctena is able to discriminate between 325 nm. (ultra-violet) and 570 nm. (yellow-green) of equal intensity. This does appear to be the case. Further experiments would be necessary to extend this work to indicate support for the tentative suggestion above.

4.10 Having obtained the visual sensitivity curve for F. rufa, the spectral filters were recalibrated to provide equal illumination at the eye taking into account the differences in spectral sensitivity by removal of neutral density until all filters provided the same intensity of stimulus at the eye as the blue-green filter (table C4.2). The values were obtained by calculation from the data obtained on source emission, filter transmission and receptor sensitivity and also from further electrophysiological experiments in which filter densities were matched to give equal E.R.G. generator potentials.

FIGURE C4.4

Visual Sensitivity Curves plotted from the results of experiments CC3 (trace B) and CC4 (trace A)


100
80
60
40
20
% REACTION INTENSITY
A
B
400
500
600
700
λ(nm)

Wavelength nm.	Colour	Recalibrated Neutral Density	Light Energy for a 15cm sq. Ergs cm^{-2} sec^{-1}
430	Violet	0.0	0.4218
470	Blue	0.4	0.1776
490	Blue-green	0.8	0.0888
520	Green	0.4	0.1332
550	Yellow-green	0.6	0.0888
580	Yellow	0.3	0.1776
600	Orange	0.7	1.1100
700	Red	0.0	4.3068

TABLE C4.2 Correction of spectral filters to give standard illumination intensity at the eye by recalibrating the Neutral Density Filters.

The illumination energy plotted against wavelength approximately follows the visual sensitivity curve.

4.11 In order to ascertain whether any wavelength of monochromatic light is involved in the releaser complex of threat behaviour, experiment 23 was carried out using the apparatus as described in Sections 2.10 and 2.15. Naive ants entering the low-illuminated arena were observed for response to the monochromatic stimuli projected onto the arena wall. The light energy from a 15 cm. square reaching an ant as it entered the arena is shown in Table C4.2. As ants were kept under observation until they either responded or walked off the 12 cm. central dais, the position of the ant could vary by + or - 6 cm. with relation to the screen and light source. This therefore caused variation in the light energy reaching the eye.

4.12 Tests were carried out using seven geometrical shapes (see Table C4.3) with both continuous and flickered stimulation. The fourteen test stimuli were projected at each wavelength using the corrected filters and the responses observed as before. Light energy varied slightly with each stimulus but caused no important errors.

The results of experiment 23 are shown in Table C4.3 where it can be seen that threat response to monochromatic light is negligible throughout the spectrum. 375 ants were observed per filter and the average threat response was only 1.6% with a maximum at yellow of 3.5%. It is clear that monochromatic light does not enter into the threat releaser complex.

TABLE C4.3

See Section 4.12

								FLASHED							
COLOUR	●	■	▲	III	≡	⁘	⊛	■	▲	III	≡	⁘	⊛	●	TOTAL
VIOLET			1		1	1	1	2			1			1	8
BLUE							1	1	2					2	6
BLUEGREEN					2	1	2		1	1	1	1	1		10
GREEN											1				1
YELLOW GREEN						1			2	1	1				5
YELLOW			1			1	1	1	2	3	3	1			13
ORANGE	1			1					1		1		1		5
RED															0

BEHAVIOURAL RESPONSE TO COLOUR

CHAPTER FIVE

Chemical Stimuli

5.1 Research over the past thirteen years has shown that among insects, communication of alarm is predominantly by chemical means.

Alarm signalling by means of an odour substance was discovered by Huber in 1814 in worker honey-bees. Goetsch in 1934 noted chemical alarm in ants but did not follow up his observation. Sudd (1957), Wilson (1958) and Butenandt, Linzen and Lindaver (1959), were the first to study this problem in social insects.

5.2 Alarm substances are both pheromones and releasers and can be defined as glandular substances which release specific alarm reactions in conspecifics (Maschwitz, 1966). The object of the present research was to find out whether chemical stimulation acted as part of the releaser complex of threat behaviour in F. rufa, which although not strictly alarm behaviour, is closely related and stimuli eliciting threat behaviour may also elicit the typical alarm behaviour of erratic rapid running.

5.3 Formicine ants possess no sting but their production of formic acid far exceeds that of any other animals or plants. On an individual weight basis the formic acid content can amount to 20% in F. rufa (Beard, 1963); or 1.8 - 2.1 mg. of acid per ant (O'Rourke, 1950). Acid concentration in the secretion has been reported to be as high as 72% (O'Rourke, 1950) although a more reliable

estimate is 50% (Stumper, 1960a and b). In addition to formic acid, small amounts of volatile and non-volatile substances including polypeptide-like compounds are present in Formicine venom (Kaiser and Michl, 1958).

In lower animals formic acid can cause severe injury or even death. The venom acts both as an acid and an aldehyde exerting its corrosive effects through the active H ion of the OH radical. Workers of F. rufa when struggling with a strongly resistant predatory animal or prey, spray it with venom which makes resistance weaker and also attracts other ants in the vicinity.

5.4 It was therefore decided to test the reaction of F. rufa to formic acid in an attempt to discover whether the acid released threat behaviour. The experimental apparatus was set up as described under Sections 2.10, 2.21 and 2.22. Stimuli were given as a haive ant entered the arena. Each stimulus took the form of a short blast of air, (1 - 2 secs.) through the bubbler, which drove the vapour from the volatile chemical into the arena in a fine jet directed at the ant under observation. One stimulus was usually sufficient to elicit a response but sometimes several were necessary. Stimulation was therefore at the highest intensity possible at normal temperatures and pressures. Responses were noted and the Intensity Index worked out for each test.

5.5 Tests were carried out using different concentrations of both formic acid and acetic acid and also a series of concentrated

carboxylic acids from formic (C1) to Octanoic acid (C8) to find out whether increase of carbon chain length affects stimulus efficiency. Fig. C5.1 shows the Intensity Index plotted against stimulus for different concentrations of formic acid (a.,Trace a.) acetic acid (b.,Trace c.) compared against a control stimulus of air without vapour. In Control A tests (Trace c.), the air from the pump was bubbled through distilled water only. Table c5.1 shows the experimental results. The Mean of the Intensity Indices is substituted for the actual Intensity Indices of any set of tests.

An analysis of variance was carried out on all chemical data and the mean error thus calculated was used to carry out t-tests on significance between individual test means. The results of the analysis of variance showed that the responses to chemicals varied significantly at the 1% level

5.6 Fig. C5.2a shows the Intensity Index plotted against stimulus for the carboxylic acid series and Table C5.2 gives the experimental results. It can be seen that carboxylic acids and formic acid in particular do not elicit threat behaviour and neither is there any significant difference in any acid tested when compared with Control A. (Trace e.).

5.7 In addition to threat responses, the occurance of distinct alarm behaviour was also noted. Tables C5.1, 5.2 and 5.3 show the

FIGURE C5.1

a) Intensity Index plotted against concentration of formic acid.

Trace a. Chemical only - see Section 5.5
Trace b. Chemical and visual stimulus - see Chapter 7.

b) Intensity Index plotted against concentration of acetic acid.

Trace c. Chemical only - see Section 5.6
Trace d. Chemical and visual stimulus - see Chapter 7.

Trace e. Pure air only - see Section 5.5
Trace f. Pure air and visual stimulus - see Chapter 7.

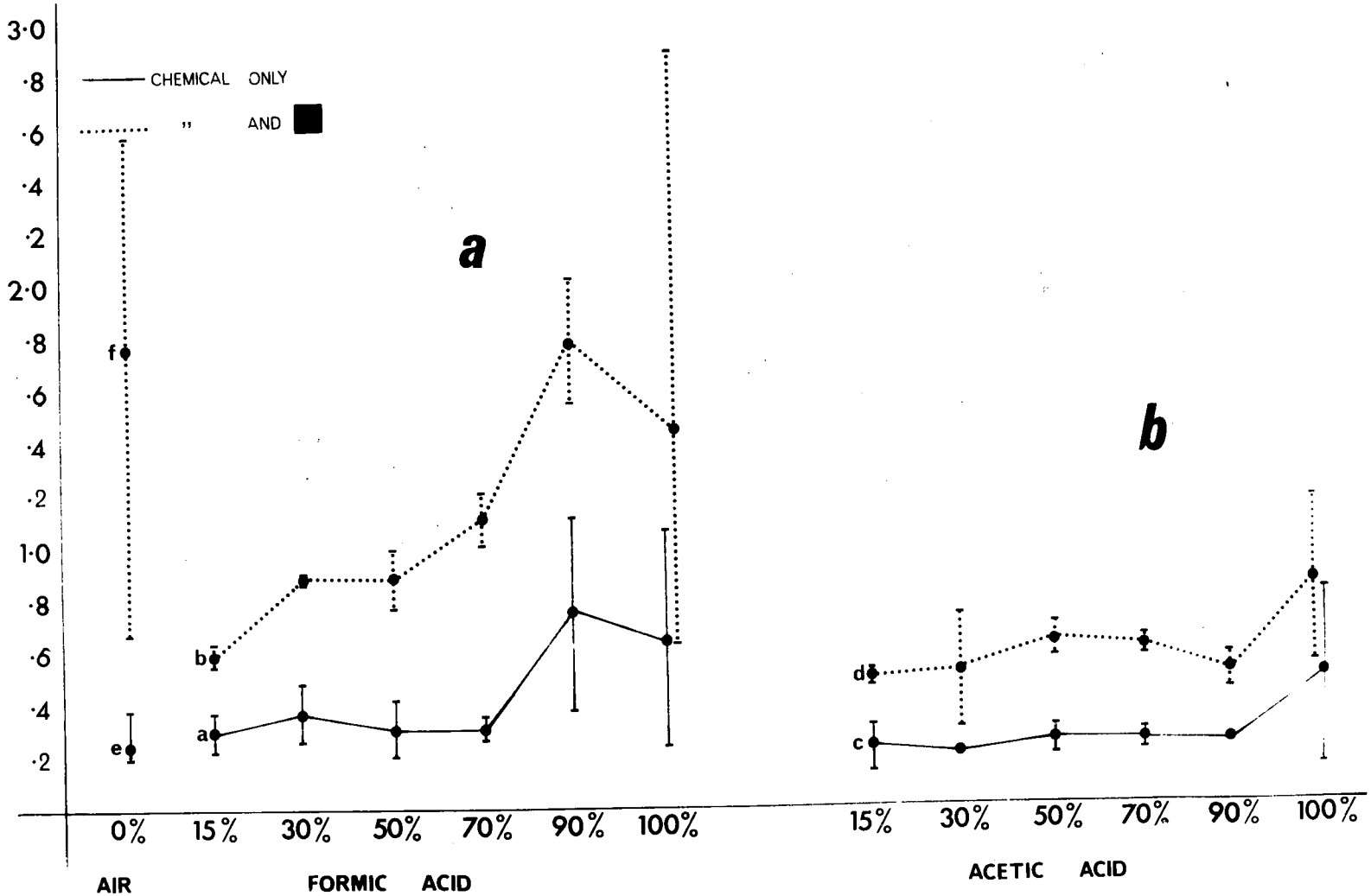

3·0
·8
·6
·4
·2
2·0
·8
·6
·4
·2
1·0
·8
·6
·4
·2
CHEMICAL ONLY
" AND
a
b
f
e
a
b
c
d
0%
15%
30%
50%
70%
90%
100%
AIR
FORMIC ACID
15%
30%
50%
70%
90%
100%
ACETIC ACID

FIGURE C5.2

a) Intensity Index plotted against the stimulus of a black square, air movement only and carboxylic acids of carbon chain length of C1 - C8

b) Intensity Index plotted against the stimulus of straight-chain ketones with a carbon chain length of C3 - C13

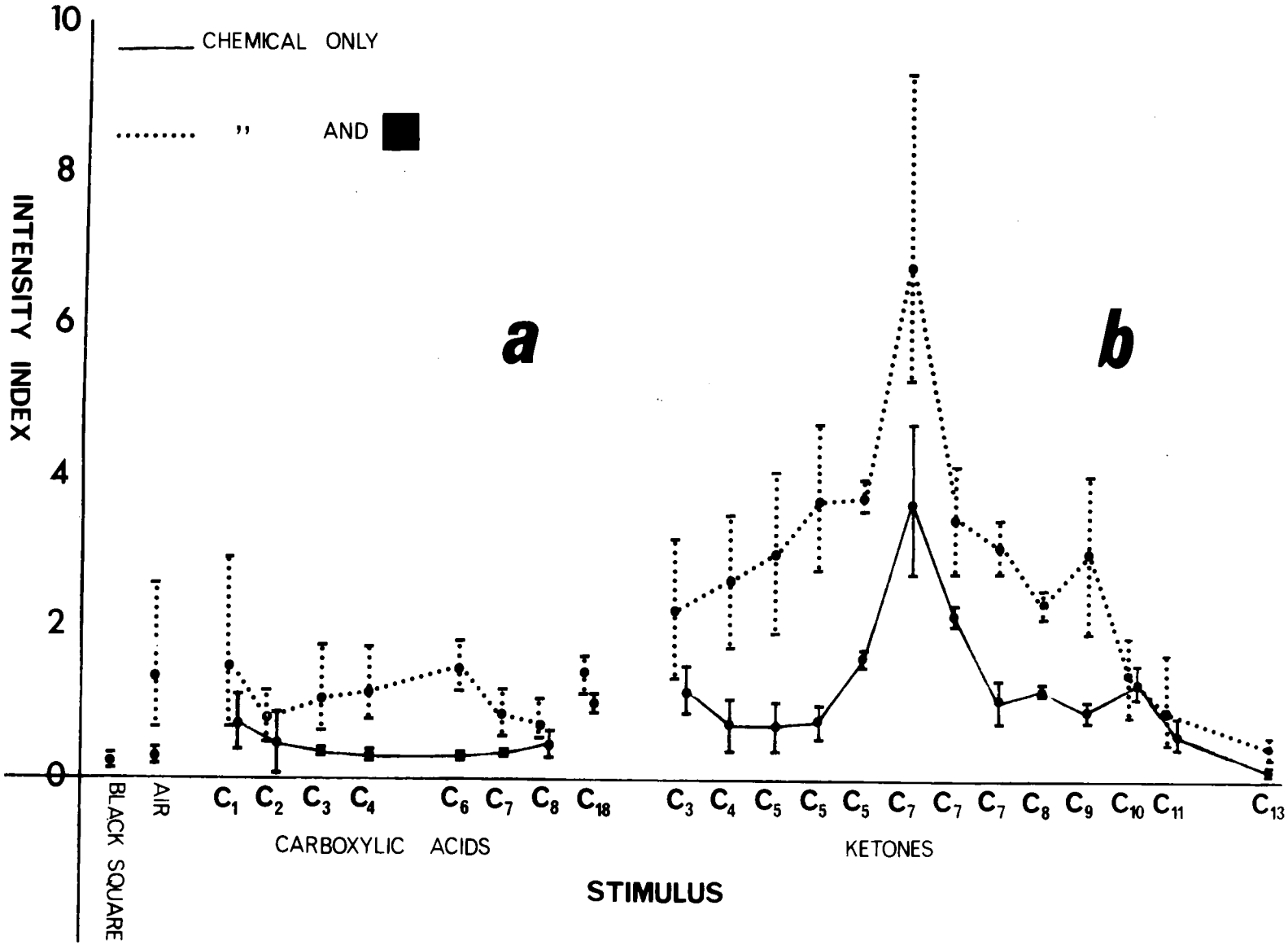
c) Intensity Index plotted against the stimulus of acetates with a carbon chain length of C4 - C8

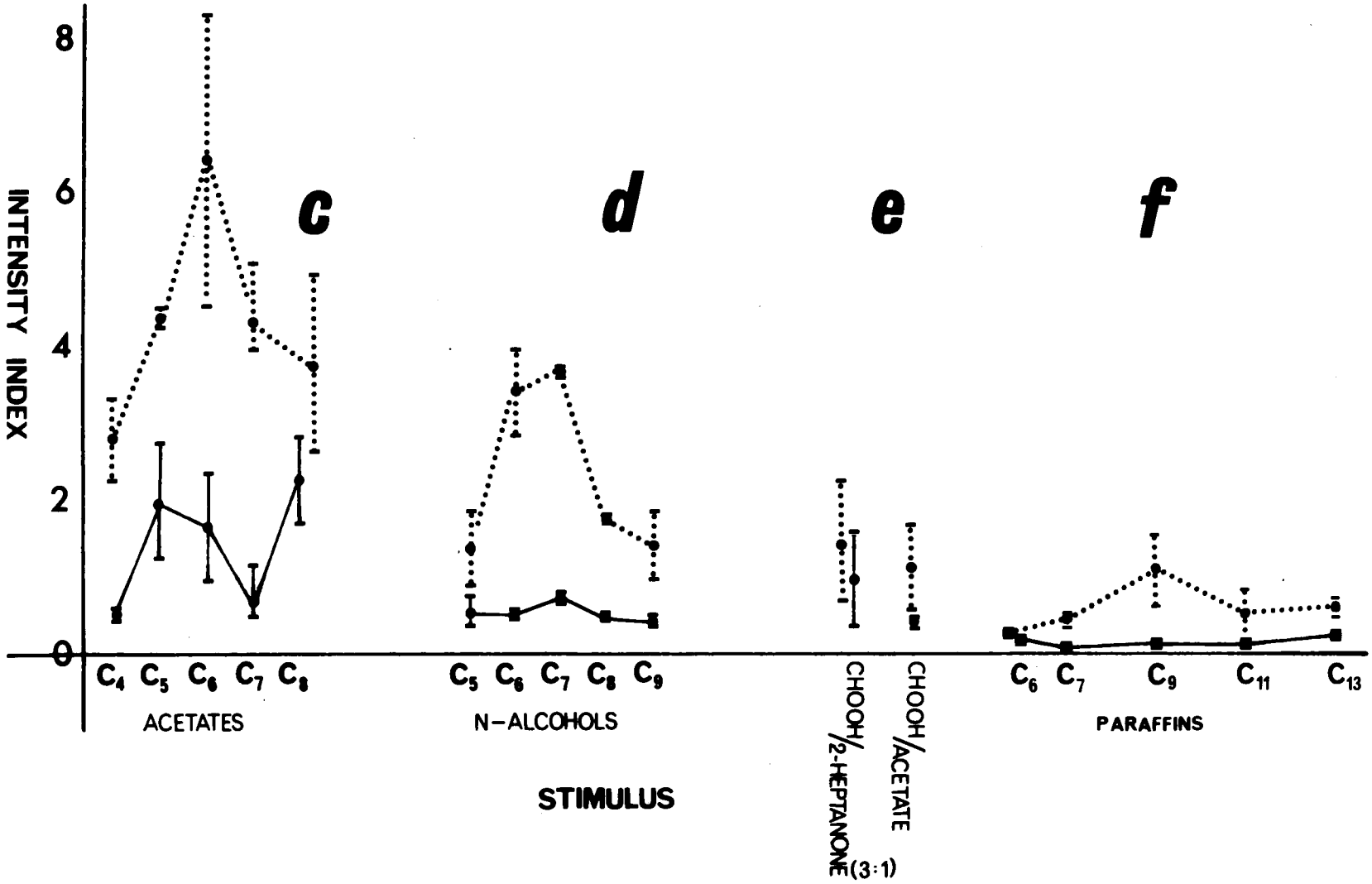
d) Intensity Index plotted against the stimulus of N-alcohols with a carbon chain length of C5 - C9

e) Intensity Index plotted against the stimulus of combined chemicals (Section 5.9)

f) Intensity Index plotted against the stimulus of paraffins with a carbon chain length of C6 - C13

Solid trace, chemicals only - see Section 5.6
Dotted trace, chemicals and visual stimulus - see Section 7.3.


10
8
6
4
2
0
INTENSITY INDEX
CHEMICAL ONLY
" AND
a
b
BLACK SQUARE
AIR
C1 C2 C3 C4 C6 C7 C8 C18
CARBOXYLIC ACIDS
C3 C4 C5 C5 C5 C7 C7 C7 C8 C9 C10 C11 C13
KETONES
STIMULUS


c
d
e
f
INTENSITY INDEX
8
6
4
2
0
C4 C5 C6 C7 C8
ACETATES
C5 C6 C7 C8 C9
N-ALCOHOLS
CHOOH/2-HEPTANONE (3:1)
CHOOH/ACETATE
C6 C7 C9 C11 C13
PARAFFINS
STIMULUS

TEST CHEMICAL	INTENSITY INDEX MEAN	NO. OF TESTS PER CHEMICAL	TEST REF. NO.	5% SIGNIFICANT VARIATION FROM CONTROL A.	AVERAGE ALARM RESPONSE (MAX. 25)
Control A Pure air only	0.2540	8	1	-	1.8
98% Formic Acid	0.6271	5	3	-	2.4
90%	0.7501	4	2	-	7.2
70%	0.3155	2	4	-	5.0
50%	0.3141	2	5	-	2.0
30%	0.3810	2	6	-	2.5
15%	0.2954	2	7	-	5.0
Glacial Acetic Acid	0.4974	3	8	-	0.0
90%	0.2326	2	9	-	1.0
70%	0.2481	2	10	-	2.0
50%	0.2500	2	11	-	1.5
30%	0.2054	2	12	-	1.5
15%	0.2229	2	13	-	2.0

TABLE C5.1 Response to increasing concentrations of Formic and Acetic Acids.

TEST CHEMICAL		INTENSITY INDEX MEAN	NO. OF TESTS PER CHEMICAL	TEST REF. NO.	5% SIGNIFICANT VARIATION FROM CONTROL A.	AVERAGE ALARM RESPONSE (MAX. 25)
Formic	C1	0.6271	5	3	-	2.4
Acetic	C2	0.4974	3	8	-	0.0
Propriomic	C3	0.3645	2	14	-	3.0
Butyric	C4	0.3101	2	15	-	0.5
Hexamoic	C6	0.2790	2	16	-	1.5
Heptamoic	C7	0.3024	2	19	-	1.0
Octanoic	C8	0.4712	2	17	-	4.5

TABLE C5.2 Response to Carboxylic Acids.

TEST CHEMICAL	INTENSITY INDEX MEAN	NO. OF TESTS PER CHEMICAL	TEST REF. NO.	5% SIGNIFICANT VARIATION FROM CONTROL A.	AVERAGE ALARM RESPONSE (MAX. 25)
Ketones					
Acetone C3	1.1617	2	20	-	3.0
Butanone C4	0.6752	2	21	-	2.5
2-Pentanone C5	0.6907	2	22	-	0.0
3-Pentanone C5	0.7209	2	23	-	6.0
2-Hexanone C6	1.5877	2	24	yes	3.0
2-Heptanone C7	3.6514	4	25	yes	6.25
3-Heptanone C7	2.1613	2	26	yes	5.5
4-Heptanone C7	1.0027	2	27	-	1.0
2-Octanone C8	1.2045	2	28	-	2.0
2-Nonanone C9	0.9576	2	29	-	2.0
2-Decanone C10	1.3462	2	30	yes	7.0
2-Undecanone C11	0.6789	3	31	-	9.0
2-Tridecanone C13	0.2017	2	32	-	2.0
Cyclohexanone C6	1.0536	2	33	-	1.5

Aldehydes					
Citral	0.3781	2	34	-	0.0
Acetates					
Ethyl Acetate C4	0.5700	2	35	-	0.5
Propyl Acetate C5	1.9929	2	36	yes	0.0
Butyl Acetate C6	1.6507	2	37	yes	1.5
Pentyl Acetate C7	0.7214	3	38	-	2.6
Hexyl Acetate C8	2.1927	2	39	yes	1.0
Alcohols					
Pentanol C5	0.5319	2	40	-	2.0
Hexanol C6	0.4730	2	41	-	2.0
Heptanol C7	0.6854	2	42	-	2.0
Octanol C8	0.4331	2	43	-	2.0
Nonanol C9	0.4021	2	44	-	0.5
Paraffins					
Hexane C6	0.1710	2	47	-	8.0
Heptane C7	0.0775	2	48	-	2.5

Nonane C9	0.1279	2	49	-	2.0
Undecane C11	0.1590	2	50	-	6.5
tridecane C13	0.2249	2	51	-	6.5
Combined Chemicals					
Formic Acid + 2-Heptanone 3:1	0.9462	2	45	-	0.0
Formic Acid + Butyl Acetate 3:1	0.3818	2	46	-	0.0

TABLE C5.3 Response to Organic Compounds

average alarm response for a test of 25 ants per chemical. Maschwitz (1964) reported that formic acid released strong alarm behaviour in three species of Formica tested. This does not appear to be true for F. rufa. It seems probable therefore that formic acid, which constitutes the major part of the venom, is present solely as a toxic agent to overcome prey (see Section 5.3).

5.8 Schall in 1892 noted the presence of Undecane in the volatile secretions of F. rufa. Regnier and Wilson (1968,69) identified many of the volatile compounds from the ants Acanthomyops claviger and Lasius alienus and showed that Undecane was an efficient spreading agent for formic acid in both ants. All compounds isolated with a carbon chain length of C10 - C13 were alarm substances for A. claviger and similarly compounds isolated from L.alienus with chain lengths of C9 - C10 were alarm substances.

Blum et Al (1963, 65b) isolated 2-Heptanone from Iridomyrmex pruinosus and Conomyrma pyramica, and showed that it acted as an alarm substance. Blum et Al (1965a) after testing I.pruinosus with 49 ketones, decided that C6 - C9 ketones were most effective as alarm substances and that straight chain compounds were better releasers than branched compounds.

Other compounds isolated as alarm substances include:-

Citral and Citronellal, Chadha et Al (1962)

4-Methyl-hexan-2-one, Blum et Al (1963)

4-Methyl-3-heptanone, McGurk et Al (1966)

Moser et Al (1968)

2-Tridecanone, N-Hendecane, N-Tridecane, Blum (1968)

Citronellal, Tridecane, Citronellol, 2-Tridecanone, 2-Pentadecanone,

Regnier and Wilson (1969)

It is therefore evident that the Formicoidea and indeed Hymenoptera in general, make use of many organic compounds for alarm substances and these include paraffins, alcohols, aldehydes, ketones and esters.

It was therefore decided to see what responses could be elicited from F. rufa with a range of straight chain ketones with a carbon chain length of C3 - C13; acetates, from C4 - C8; N-Alcohols, C5 - C9 and paraffins, C6 - C13. See Fig. C5.2b,c,d,e and f, and Table C5.3.

5.9 Fig. C5.2b shows the response to ketones, with a definite peak at 2-Heptanone which is significant at the 5% level. 2-Decanone also shows a significant response. Fig. C5.2c shows response to acetates where once again there is significance at the 5% level, but not with alcohols, Fig. C5.2d, or paraffins, Fig. C5.2f. Combined chemicals, Citral (aldehyde) and a ring ketone (Cyclohexanone) also stimulate little in the way of threat behaviour. Oleic acid, a reputed necrophoric behaviour releaser, Wilson (1958) also elicits no significant responses. It is interesting to note that combinations of formic acid and 2-Heptanone do not elicit a stronger response than separate presentation, but elicit much weaker responses.

5.10 Alarm behaviour is elicited by most of the chemicals tested, but not to a significant degree. Even so, ketones of chain length C6 - 7, and paraffins elicit more than the general background level of alarm response and the results indicate correlation with the work of Regnier and Wilson, and Blum et Al.

Threat behaviour in F. rufa is probably best elicited by ketones with a chain length of 6 or 7 carbon atoms. To test further chemicals in order to obtain more revealing results would be a very expensive occupation and a more rewarding approach would be in attempting to isolate and identify volatile compounds from the ant.

5.11 The obvious response to chemical stimuli from F.rufa workers must be an adaptive response to an environmental stimulus. Predators will not intentionally advertise their presence by chemical signals, but their specific body odour may act as an alarm releaser. Conversely alarm substances secreted by the ants themselves after visual or acoustic perception of predator or prey may be released to attract other ants either to assist in overpowering the prey or in attacking the predator, in defence of the nest.

Occasionally when an ant reacted vigorously to a stimulus at Stage 5 the entire population of ants in the experimental arena, (which may total over a hundred) erupted into frenzied alarm behaviour for up to 10 - 15 seconds. Such sudden changes in behaviour cannot be traced to external stimuli. The same collective

behaviour pattern can often be elicited when a worker is picked up in a pair of clean forceps, partially squashed and dropped amongst the foraging workers in the arena, suggesting that the injured ant is secreting a chemical alarm releaser. The alarm behaviour is not elicited if an unharmed ant is dropped into the arena and is therefore not elicited by a visual stimulus. Wilson (1958) observed the same effect in Pogonomyrmex badius.

5.12 Blum (1968) found the Dufour's Gland of a Neoformica species to be rich in low boiling point volatiles and also found that Lasius species produce 2-Tridecanone, N-Hendecane and N-Tridecane which all act as alarm pheromones. Regnier and Wilson (1968,69) also isolated alarm substances produced by Acanthomyops claviger and Lasius alienus. It is therefore clear that ants in the subfamily Formicinae do secrete pheromones.

Several attempts were made to isolate and analyse low boiling point organic compounds from the complete bodies of F. rufa workers, all unfortunately unsuccessful. Extraction of 50 ants in Di-ethyl Ether by refluxing produced on traces and successive attempts to extract up to 15 gms. of ants by steam distillation and re-extract into Ether or Petroleum Ether also met with no success. The final extracts were concentrated by fractional distillation and analysed by vapour phase chromatography in the Chemistry Department of this University. Unfortunately the chromatography apparatus was destroyed

by fire before other methods of extraction could be tried. The only proof that F. rufa secretes an alarm substance is therefore behavioural.

Little is known concerning perception of predator body odour although mammal body odours may contain aldehydes e.g. civet (the smell of cat) or ketones (e.g. producing a musky smell). There is therefore a possibility that predator odour may be perceived by workers of F. rufa and stimulate alarm or the defensive threat behaviour.

CHAPTER SIX

Auditory Stimuli

6.1 Sound production and reception in insects has been extensively studied. Dethier, (1963) has reviewed the relevant papers up to 1962.

Sound production in ants was noted long before any serious study was undertaken. In 1874 Landois reported that Ponera quadridentata and Lasius fuliginosus both possessed true stridulatory organs. Sharp (1893) denied that they existsd in L.fuliginosus and stated that there were no stridulatory organs in the several species of Camponotidae that he studied. Janet (1893) described a possible stridulatory organ in Tetramorium caespitum L. In a review, Sudd (1967) stated that it is exceptional for an ant to make a noise that can be heard by a human observer. However, Messor spp. and Aphaenogaster testaceus stridulate when picked up. Santschi (1923) observed that Megaponera foetans produced a shrill whistling noise when disturbed during foraging. This signal alarmed other ants over a distance of several metres.

Autumn (1936) found that substrate borne vibrations were produced by Myrmica sp., but no sounds were produced by Formica spp. or Lasius spp. Markl (1965, 1967) described stridulation in the leaf-cutting ant, Atta cephalotes. He found the vibrations were detected by other workers and soldiers and acted as a "distress alarm". Airborne vibrations (sound) were ineffective.

6.2 Fielde and Parker (1904) described experiments in which ants were subjected to vibrations. No responses were obtained to sounds in the range 27-60,000 Hz but responses to vibrations were obtained. The response threshold varied with the species tested and it was decided that stimulation was detected through the legs. Donisthorpe (1915) mentions experiments using a Galton-Edelmann Whistle, but he obtained no positive responses to sounds in any of the ants tested.

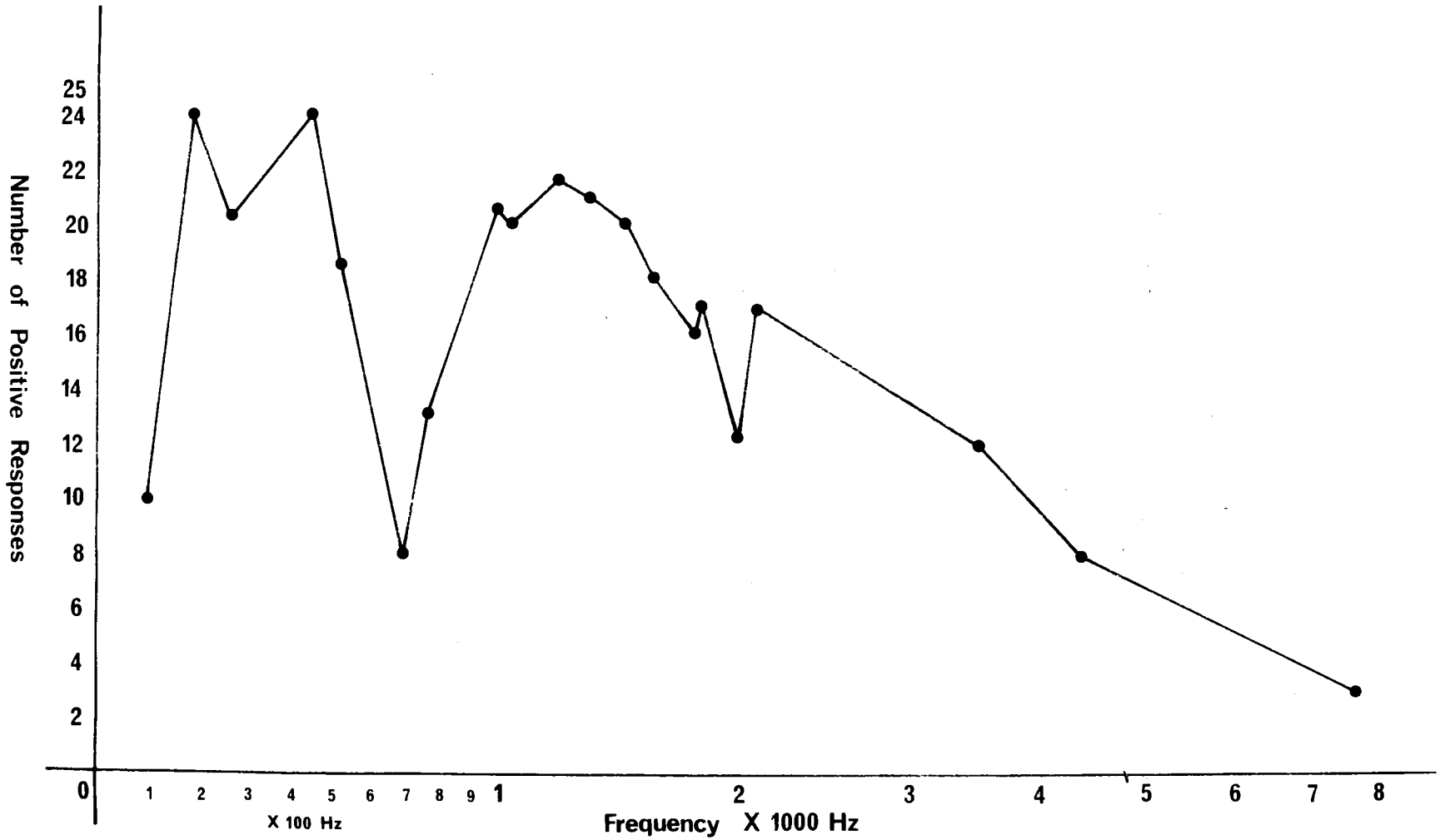
6.3 Autrum (1936) carried out experiments to test whether ants were more sensitive to pressure changes or to displacement of air. He decided that they were more sensitive to displacement. Using electrophysiological techniques, Autrum and Schneider (1948) showed that Camponotus sp. had an optimal response frequency to vibrations of 2,000 Hz and that non-formicine Hymenoptera tested had optimal response frequencies in the range 1,000-2,500 Hz. Markl (1966) using similar techniques demonstrated a response range in Atta cephalotes between 50 Hz and 1500 Hz.

F. rufa is not known to produce sounds and although it has vibration-sensitive sense organs it is relatively insensitive to sounds (Fielde and Parker, 1904; Donisthorpe, 1915 and Autrum, 1936) but when subjected to vibrations will respond behaviourally in the region 80-8,000 Hz (Fig. C6.1).

6.4 Experiments were carried out using the experimental apparatus described in Sections 2.10 and 2.23 - 2.25. As with previous

FIGURE C6.1

Total number of ants showing a simple behavioural response in each test, plotted against frequency of stimulus vibration.


Number of Positive Responses
25
24
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9
X 100 Hz
1 2 3 4 5 6 7 8
Frequency X 1000 Hz

experiments, 25 ants were observed in each test. As an ant entered the arena, a vibrational stimulus was initiated by the observer using the press button switch which controlled the vibrator (Fig. A2.10). The stimulus took the form of a "figured pulse train", (Broughton, 1963) (irregular pulses) of a minimum of three pulses and a maximum of eight, but always the minimum number necessary to obtain an observable response.

The amplitude of the pulses was maintained at a constant level above the response threshold throughout all experiments even with change of frequency. The amplitude was checked by monitoring vibration of the arena floor with a magnetic pick-off transducer (Fig. A2.8) and if necessary corrected by altering the signal generator or amplifier gain. This cancelled out possible errors caused by resonances of the floor (Fig. C6.2). At frequencies above 3,500 Hz, the power available to vibrate the floor could not supply the amplitude maintained at lower frequencies and the stimulus amplitude fell with the increase in frequency. This may be one reason for the fall off in the behavioural response shown in Figs. C6.1 and C6.3.

The main object of experiments 70-75 was to ascertain whether vibration forms part of the threat behaviour releaser complex. It was noted that although most of the ants did not respond to vibration with threat behaviour, they did respond to the stimuli either by ceasing movement with every stimulus, by showing alarm

FIGURE C6.2

Amplitude of vibration at the arena floor plotted against frequency, all at constant input amplitude to show the resonant frequencies of the floor.

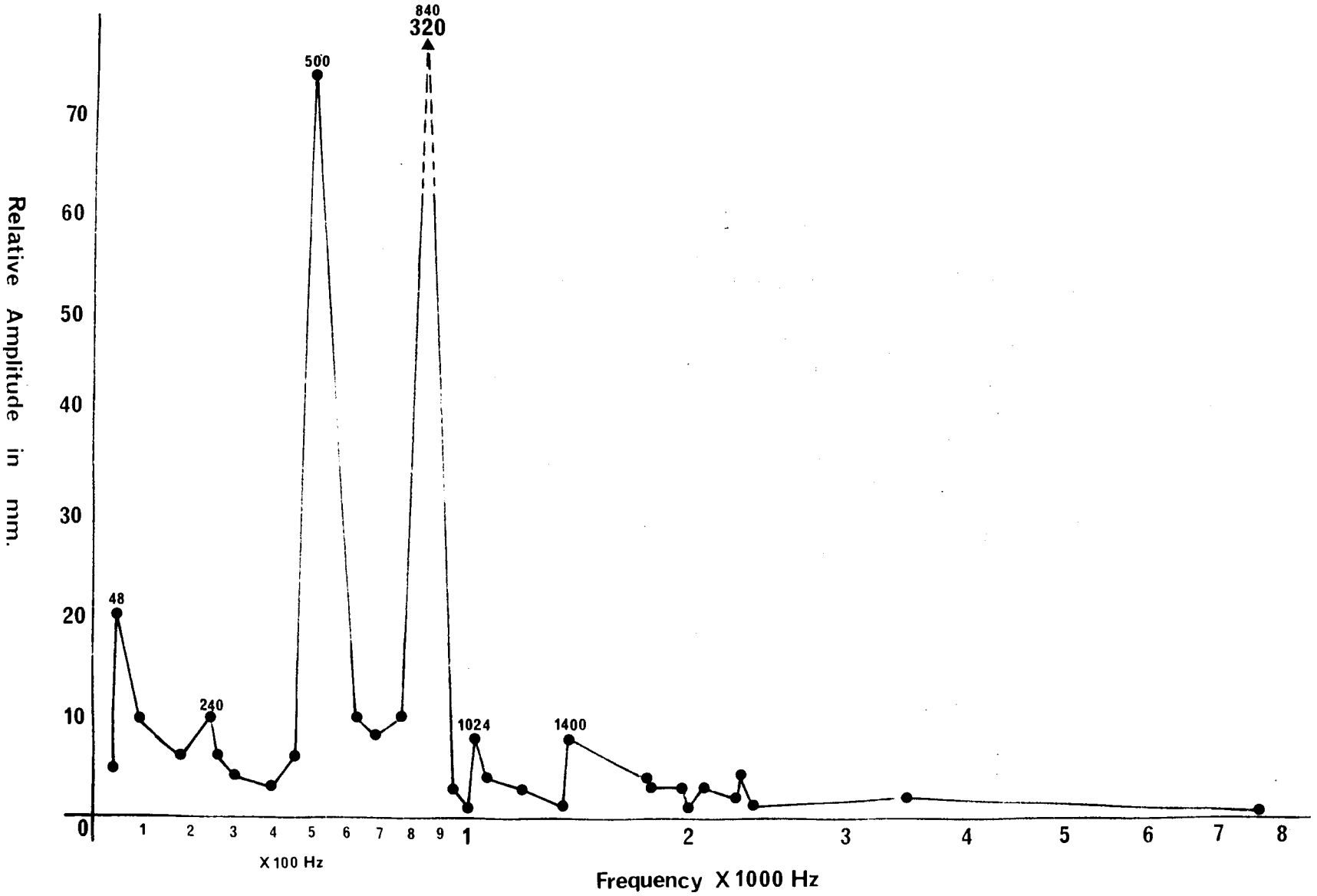
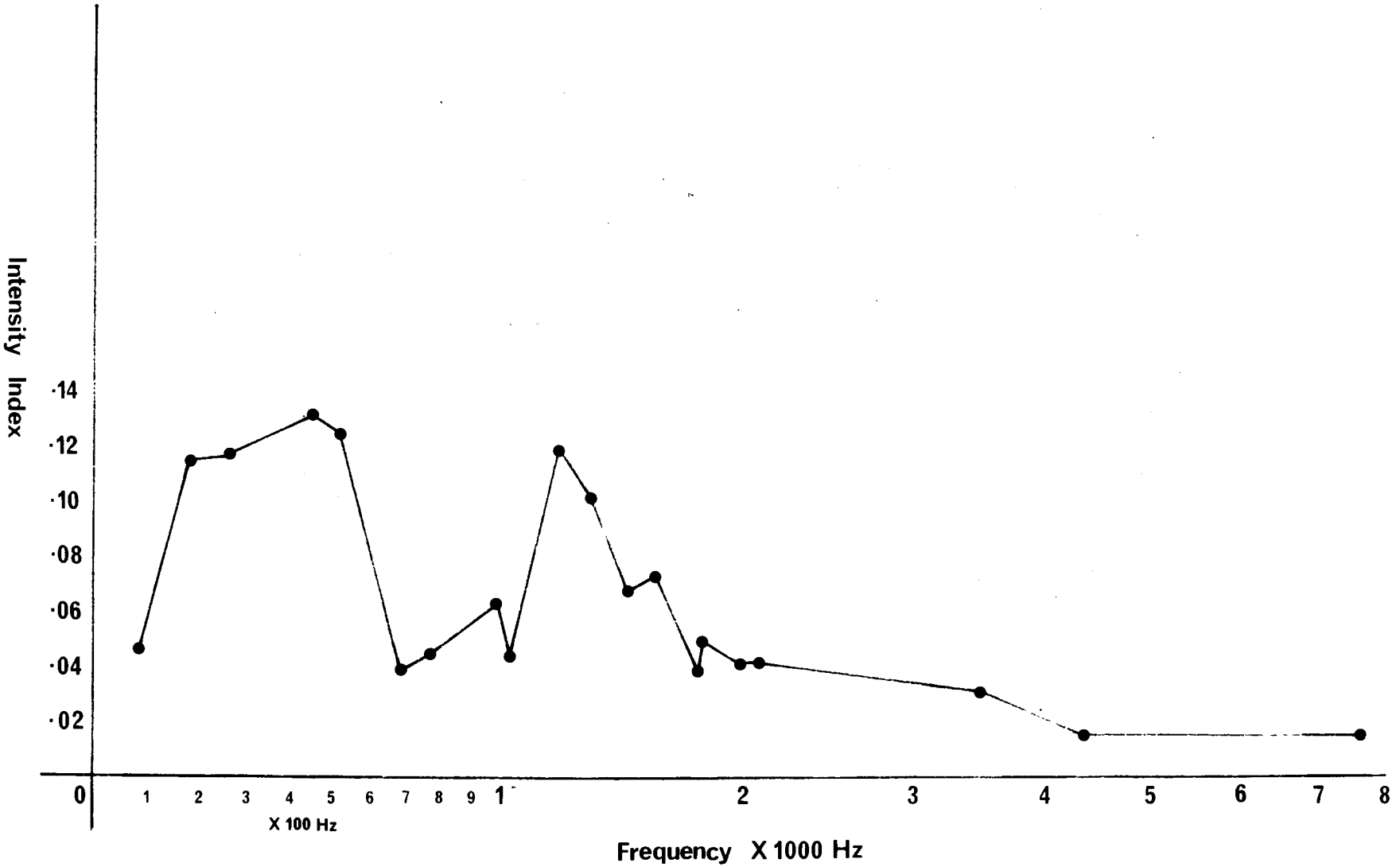

840
320
500
48
240
1024
1400
Relative Amplitude in mm.
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9
X 100 Hz
1 2 3 4 5 6 7 8
Frequency X 1000 Hz

FIGURE C6.3

Mean Intensity Index for experiments 70 - 75 plotted against frequency of stimulus vibration.


Intensity Index
·14
·12
·10
·08
·06
·04
·02
0
1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8
X 100 Hz
Frequency X 1000 Hz

behaviour, i.e. rapid erratic running, or by jerking movements at every stimulus. Thus two sets of response data were noted:-

1) Threat behaviour response.

2) Behavioural response other than threat, presence or absence.

6.5 The results for experiments 70-75 are shown in Table C6.1 and Fig. C6.1 shows the number of ants out of 25 giving a behavioural response, plotted against the log of the stimulation frequency. Peaks are evident at 170-450 Hz and at 950-1600 Hz.

Fig. C6.3 shows the Intensity Index plotted against the log of the stimulation frequency. It should be remembered that although the abscissa is expanded, it represents only a very small section of the scale maximum (15.155) and that there is very little threat response. However there is indication of two peaks, one in the 170-500 Hz range and the other at 1,200 Hz.

There was comparatively little alarm behaviour and this occurred mainly at the optimum response peaks.

6.6 Autrum (1963) on reviewing sound receptors in invertebrates stated that there are two main groups of vibration receptors in insects which can be distinguished by electrophysiological methods due to their difference in sensitivity to vibrations. There is a sensitive group including all Orthoptera, Hymenoptera and Lepidoptera and an insensitive group including Hemiptera, Diptera and Coleoptera. The range of optimal response frequencies by the Hymenoptera is 1,000-3,000 Hz. The threshold amplitude is very low and the vibration-sensitive organs are the sub-genual organs situated in the

Wavelength in Hertz	Number of ants responding positively per test --Experiments--						
	70	71	72	73	74	75	Mean
100	8	16	13	11	6	7	10
200	-	-	23	24	25	-	24
300	20	20	-	-	-	22	20.3
500	-	-	23	25	24	-	24
600	16	21	-	-	-	19	18.6
800	-	-	6	10	8	-	8
900	16	15	-	-	-	8	13
1100	-	-	21	21	20	-	20.6
1200	18	23	-	-	-	19	20
1400	-	-	22	25	18	-	21.6
1500	-	21	-	-	-	21	21
1700	-	-	20	23	17	-	20
1800	15	18	-	-	-	21	18
2000	-	-	13	18	15	-	16
2100	15	14	-	-	-	22	17
2300	-	-	12	16	9	-	12.3
2400	21	13	-	-	-	18	17.3
4000	-	-	11	15	10	-	12
5000	4	16	-	-	-	4	8
9000	0	0	6	2	5	6	3.1

TABLE C6.1a

Wavelength in Hertz	The Intensity Index - measure of Threat Behaviour --Experiments--						
	71	72	73	74	75	76	Mean
100	0.0310	0.0930	0.0310	0.0155	0.0465	0.0620	0.0465
200	-	-	0.1473	0.1086	0.0930	-	0.0163
300	0.1629	0.0930	-	-	-	0.1008	0.1189
500	-	-	0.1396	0.1396	0.1163	-	0.1316
600	0.0465	0.0310	-	-	-	0.0465	0.1240
800	-	-	0.0465	0.0310	0.0310	-	0.0361
900	0.0853	0.0310	-	-	-	0.0155	0.0439
1100	-	-	0.0698	0.0310	0.1008	-	0.0630
1200	0.0620	0.0155	-	-	-	0.0543	0.0439
1400	-	-	0.0465	0.2249	0.0853	-	0.1189
1500	-	0.0775	-	-	-	0.1241	0.1008
1700	-	-	0.1086	0.0310	0.0620	-	0.0672
1800	0.1241	0.0465	-	-	-	0.0465	0.0723
2000	-	-	0.0310	0.0465	0.0310	-	0.0395
2100	0.0853	0.0155	-	-	-	0.0465	0.0491
2300	-	-	0.0310	0.0465	0.0465	-	0.0413
2400	0.0620	0.0310	-	-	-	0.0310	0.0413
4000	-	-	0.0310	0.0310	0.0310	-	0.0310
5000	0.0310	0.0	-	-	-	0.0155	0.0155
9000	0.0	0.0	0.0155	0.0	0.0155	0.0155	0.0026

TABLE C6.1b

tibia. Weber (1933) described the subgenual organ of Formica sanguinea and the structure of sub-genual organs has been described by Eggars (1928), Debarsieux (1935 and 1938) and Howse (1965 and 1968). These are known to be vibration receptors.

6.7 The second optimal response peaks shown in Figs. C6.1 and C6.3 show close correlation with the optimal response range for aculeate hymenoptera but there is no optimum response range to which the first optimal response peak can be matched and its presence is therefore not accounted for.

Autrum (1963) described the characteristics of the insensitive group of insects where threshold amplitudes are high and where the optimal frequencies are between 200-400 Hz with no action potentials occurring at higher frequencies. The vibration receptors are chordotonal organs located between the tibia and tarsus and hair sensilla on the articular membranes. Insects which belong to this insensitive group have no subgenual organs. Hymenoptera however, do possess chordotonal organs between the tibia and tarsus and hair sensilla on the articular membranes. The optimal response peak observed between 170-500 Hz in F. rufa could therefore be due to detection of vibrations by receptors of this second type.

6.8 F. rufa response to vibrations between 80-8,000 Hz with two optimal frequency peaks at 170-500 Hz and 12,000 Hz. The receptors are probably chordotonal organs/hair sensilla and sub-genual organs respectively.

Vibrations rarely elicit threat behaviour which is negligible throughout the frequency range of behavioural responses and vibrations do not therefore supply an important direct component to threat behaviour. However, vibrations are detected by, and do alert ants and alarm behaviour is elicited more often than with visual stimuli. Vibrations within the range 80-8,000 Hz probably alert ants and make them more receptive to other stimuli.

CHAPTER SEVEN

Combined Stimuli

7.1 Experiments using a single sensory stimulus have indicated that threat behaviour in F. rufa can be released by visual, chemical or acoustic stimuli. In all experiments in which the response to one type of stimulus was tested, a single stimulus variable was altered in each test in order that any changes in behavioural response could be attributed directly to that variable.

This situation is not found in nature where the sense organs can be stimulated simultaneously from independent sources. It was therefore necessary to test the releasing value of combined stimuli in order that any interactions or inhibitions could be observed and noted.

7.2 Five series of experiments were carried out to test the releasing efficiency of combined stimuli. In all tests only one variable was allowed and the remaining stimuli not under test were kept constant. These constant stimuli were set throughout each experiment at the optimal levels. In this way the biggest responses possible could be obtained and any reduction in response due to the combination of two or more stimuli could be noted as well as any possible increase in response.

The apparatus used in the experiments to test responses to single stimuli was used again without modification as this still allowed visual, chemical and vibratory stimuli to be applied concurrently.

Test Chemical	Mean Intensity Index	No of tests per Chemical	Test Ref. No:	5% Significant difference from Control		
				A.	B.	C.
98% Formic Acid	1.4582	5	4	+	+	+
90% Formic Acid	1.7662	3	3	+	+	+
70% " "	1.1092	2	5	+	+	+
50% " "	0.8884	2	6	-	-	-
30% " "	0.8874	2	7	-	-	-
15% " "	0.3934	2	8	-	-	-
Glacial Acetic Acid	0.8598	2	9	-	-	-
90% Acetic Acid	0.5042	2	10	-	-	-
70% " "	0.6148	2	11	-	-	-
50% " "	0.6323	2	12	-	-	-
30% " "	0.5191	2	13	-	-	-
15% " "	0.4945	2	14	-	-	-
Proprionic Acid	1.1221	3	15	-	-	-
Butyric Acid	1.1538	3	16	+	-	-
Hexanoic Acid	1.4138	3	17	+	+	+
Heptanoic Acid	0.8693	2	20	-	-	-
Octanoic Acid	0.6691	3	18	-	-	-
Oleic Acid	1.3843	3	19	+	+	+
Acetone	2.2355	2	21	+	+	+
Butanone	2.5716	2	22	+	+	+
2-Pentanone	3.0010	2	23	+	+	+
3-Pentanone	3.6876	2	24	+	+	+
2-Hexanone	3.7375	2	25	+	+	+

2-Heptanone	6.7730	4	26	+	+	+
3-Heptanone	3.4067	2	27	+	+	+
4-Heptanone	3.0649	2	28	+	+	+
2-Octanone	2.3278	2	29	+	+	+
2-Nonanone	2.9908	2	30	+	+	+
2-Decanone	1.4466	2	31	+	+	+
2-Undecanone	0.9357	3	32	+	+	+
2-Tridecanone	0.4927	2	33	+	+	+
Cyclohexanone	2.1018	2	34	+	+	+
Citral	1.3784	3	35	+	+	+
Ethyl Acetate	2.7657	2	36	+	+	+
Propyl Acetate	4.3249	2	37	+	+	+
Butyl Acetate	6.4184	2	38	+	+	+
Pentyl Acetate	4.3192	3	39	+	+	+
Hexyl Acetate	3.7693	2	40	+	+	+
Pentanol	1.3432	2	41	+	-	-
Hexanol	5.3559	2	42	+	+	+
Heptanol	3.6100	2	43	+	+	+
Octanol	1.7506	2	44	+	+	+
Nonanol	1.3592	2	45	+	+	-

Hexane	0.2211	2	48	-	-	-
Heptane	0.4660	2	49	-	-	-
Nonane	1.1241	2	50	-	-	-
Undecane	0.5489	2	51	-	-	-
Tridecane	0.6032	2	52	-	-	-

TABLE C7.1

The Mean Intensity Index for each combined chemical and stationary visual stimulus test is shown together with indication of significance at the 5% level from the three Controls A., B. and C.

Speed of visual stimulus cm/sec.	Flickers per sec.	Expt. 65a 6cm stripes + 2-Heptanone	Expt. 65b 6cm stripes + 2-Heptanone	Expt. 65p 6cm stripes + Butyl Acetate	Expt. 65q 6cm stripes + Butyleacetate	Expt. 27 6cm stripes only
0.0	0.0	1.0484	1.4045	1.0184	0.9806	0.0
31.8	5.33	-	-	-	-	1.0862
47.7	8.0	3.7499	4.1321	3.6908	3.5743	-
63.6	10.66	9.1804	9.1320	5.0730	5.9895	1.1775
95.5	16.0	7.2843	6.5035	4.1563	5.5966	0.8496
318.3	53.33	1.3191	1.3101	1.1271	1.2745	0.1706
366.0	61.33	1.1251	0.5897	1.0079	1.0301	-
382.0	64.0	-	-	-	-	0.0310
445.6	74.66	-	-	-	-	0.0310
477.5	80.0	0.7895	0.5276	0.5819	0.7138	-

TABLE C7.2 The Intensity Indexes for each experiment incorporating moving stimuli and chemical stimuli, where the moving stimulus is the variable, are shown for each stimulus speed and flicker frequency.

7.3 In the first series of experiments, chemical and stationary visual stimuli were presented together. The visual stimulus consisted of a single black square with 15 cm. sides which was presented to the ants as described in Section 2.12. The square was fixed in position in the arena and the chemical under test was changed for each test, thus forming the variable stimulus. As in earlier experiments, the chemical stimulus was presented to ants as they entered the arena. Thus as naive ants entered the arena the strength of the threat response to the combined stimuli could be assessed and the Intensity Index calculated. Table C7.1 shows the results obtained for each test and also the results of the 'T' tests carried out to test for any significant differences from the three control stimuli; Control A, air movement only; Control B, black square only; and Control C, black square with air movement. If these results are compared with those for single visual or chemical stimuli, (See Figs. C5.1, C5.2 and Fig. C3.1) it can be seen that stronger threat response is elicited by the combined stimuli than by the single stimuli (for significance levels see Table C7.1).

7.4 Visual and chemical stimuli when combined and presented to ants, cause a greater response than would be expected if simple summation occurred.

These responses to combined stimuli are optimal at 2-Heptanone

in the ketone series, (Fig. C5.2b); at Hexyl Acetate (Fig. C5.2c) in the acetate series; and at Heptanol (Fig. C5.2d) in the alcohol series. The acid and paraffin series provide no response peaks although concentrated Formic Acid acts as a better releaser of threat behaviour than any other carboxylic acid.

7.5 **Analysis of the responses to combined visual and chemical stimuli to find any significant differences in threat behaviour elicited by the different chemicals, showed significant variation between the chemicals at the 1% level.**

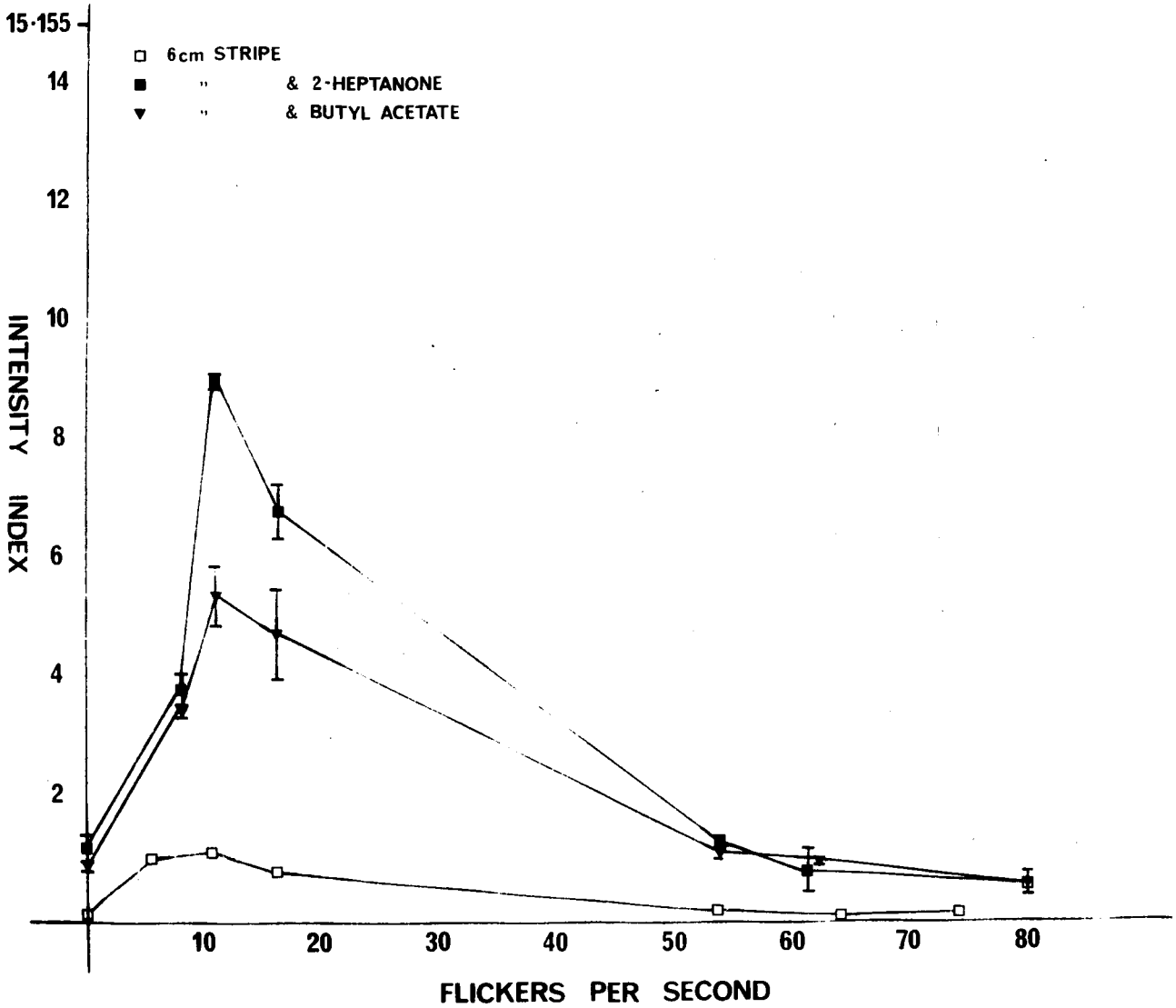
7.6 A much stronger threat response is obtained if both visual and chemical stimuli are presented to the ant than if either stimulus is presented alone. It was therefore expected that if a moving visual stimulus was substituted for the stationary black square, then the threat response would again increase. In a series of experiments chemical stimuli were presented together with a moving visual stimulus to naive ants entering the experimental arena.

7.7 The apparatus described in Section 2.16, incorporating a 6 cm. striped pattern (see Sections 3.20 and 3.21) was used with the apparatus described in Section 2.21. Experiments were carried out with one chemical and changes in visual stimulus or with a constant visual stimulus and a sequence of chemicals. The visual stimulus was presented continuously in the arena, but the chemical stimulus, as before, was presented only as naive ants entered the arena.

7.8 Table C7.2 shows the results of experiments in which the visual stimulus was changed and which were designed to compare the combined responses with the reponses to movement only. 2-Heptanone, the ketone releasing the optimal response, was presented to the ants together with the 6 cm. moving strip pattern. Tests were carried out at seven speeds from a stationary stimulus to a speed above the flicker fusion frequency (477.5 cm/sec.). The same technique was repeated using Hexyl Acetate, the acetate which caused optimal threat response. These particular chemicals were used because they initiated optimal threat responses when presented alone to the ants. Figure C7.1 compares the results of the 6 cm. moving stimulus experiments with the results of the experiments using both the 6 cm. moving visual stimulus and chemical stimuli.

7.9 It can be seen that the threat response obtained with a chemical present is many times better than for the visual stimulus alone. It is interesting to note that although 2-Heptanone by itself elicits a response with a mean Intensity Index of over 6.0000 (see Fig. C5.2), the Intensity Index when the visual stimulus is added drops below this value except at the optimal response for the visual stimulus alone peak. A similar anomaly occurs in the response to Hexyl Acetate when this is combined with a visual stimulus. This could be explained if the visual stimuli are the major stimuli of threat behaviour and mask the responses to lesser stimuli except when both stimuli are near the optimum releasing value, at which point summation occurs.

FIGURE C7.1 Threat response plotted against flicker frequency for visual and visual with chemical stimuli


15·155
14
12
10
8
6
4
2
INTENSITY INDEX
6cm STRIPE
" & 2-HEPTANONE
" & BUTYL ACETATE
10
20
30
40
50
60
70
80
FLICKERS PER SECOND

7.10 Table C7.3 shows the results of the experiments in which the visual stimulus was maintained at a constant speed and the chemicals were changed. These tests provided comparison with the tests in which chemicals only were used. Fig. C7.2 shows a comparison between the results of the experiments in which only chemical stimuli; chemical and stationary visual stimuli; and chemical and moving visual stimuli were used. In the latter experiments the visual stimulus was maintained at 63.6 cm. per second (near the optimal response) in order to obtain maximum response to the visual stimulus. Thus any increase or decrease in the intensity of the threat response could be attributed to the presence of the chemical stimuli alone.

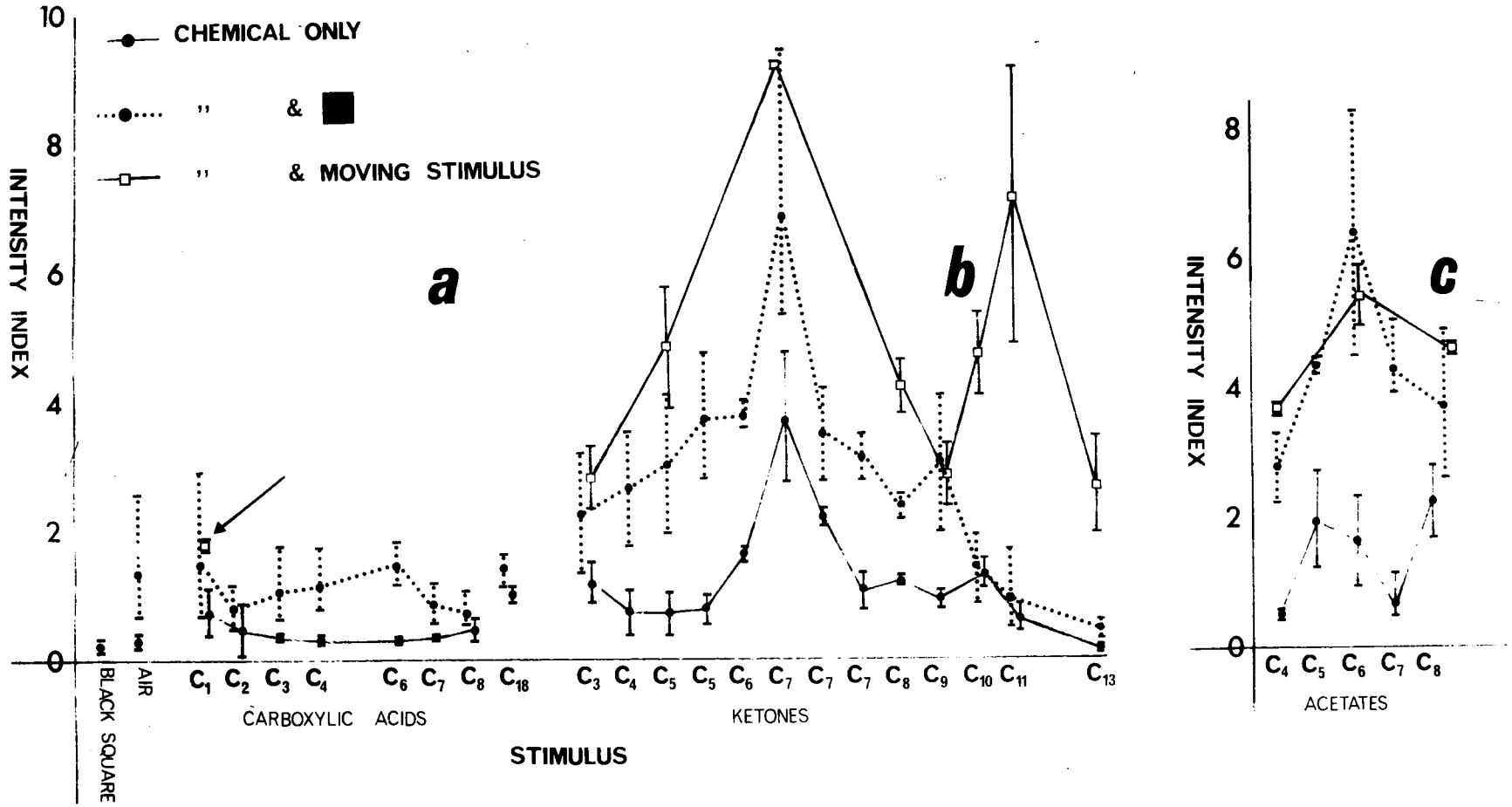
7.11 It can be seen from Table C7.3 that a moving visual stimulus is made neither more nor less effective than a stationary stimulus by the addition of Formic Acid. Similarly the responses to stationary or moving stimuli are not noticeably affected by acetates. However, when ketones are present in addition to the visual stimulus the threat response is greater if the visual stimulus is moving. A ketone combined with a moving visual stimulus is therefore a better releaser of threat behaviour than a stationary visual stimulus with chemical which in turn is a better releaser than either stationary visual stimulus, moving stimulus or chemical stimulus presented to the ants alone. In other words, summation of responses occur.

7.12 Another interesting observation concerning the tests with ketones is that as well as the expected peak at 2-Heptanone, there

Chemical	Speed of visual stimulus	Intensity Index	Mean Intensity Index for Chem. + black square
Conc Formic Acid	63.6cm/s.	1.8819	1.4582
"	"	1.7906	
Acetone	"	2.3435	2.2355
"	"	3.2689	
2-Pentanone	"	3.8023	3.0010
"	"	5.6898	
2-Heptanone	"	9.1804	6.7730
"	"	9.1320	
2-Octanone	"	4.5636	2.3278
"	"	3.7489	
2-Nonanone	"	2.3749	2.9908
"	"	3.2107	
2-Decanone	"	5.2639	1.4466
"	"	4.0194	
2-Undecanone	"	9.0930	0.9357
"	"	4.7140	
2-Tridecanone	"	3.3610	0.4927
"	"	1.9206	
Ethyl Acetate	"	3.5859	2.7657
"	"	3.7877	
Butyl Acetate	"	5.0730	6.4184
"	"	5.9895	
Hexyl Acetate	"	4.7022	3.7693
"	"	4.5442	

TABLE C7.3

FIGURE C7.2 Intensity Index plotted against chemical stimulus to show a comparison between chemical and chemical with visual stimuli


CHEMICAL ONLY
" &
" & MOVING STIMULUS
INTENSITY INDEX
10
8
6
4
2
0
a
b
c
BLACK SQUARE
AIR
C1 C2 C3 C4 C6 C7 C8 C18
C3 C4 C5 C5 C6 C7 C7 C7 C8 C9 C10 C11 C13
C4 C5 C6 C7 C8
CARBOXYLIC ACIDS
KETONES
ACETATES
STIMULUS

is also an optimal response at 2-Undecanone, which is not found with the combined stationary visual and chemical stimuli or with chemical only. Blum, Warter and Traynham (1966) although they obtained optimal alarm responses from Iridomyrmex pruinosus with 2-Heptanone (alone) obtained no response with 2-Undecanone, and although Undecane has been isolated as an alarm substance in certain species (Regnier and Wilson, 1968, 1969) no importance has been attached to the substance. Whether Undecanone is an important part of the chemical alarm releaser complex or not, is unknown.

7.13 Ketones are used as alarm substances throughout the Hymenoptera and have been isolated from many species of ants (see Section 5.8). The fact that ketones release stronger threat behaviour in F. rufa than do other organic chemical groups is not unexpected.

7.14 The three remaining series of combined stimuli experiments involve testing the responses to vibrations, i.e.to vibration and chemical, vibration and visual, and vibration, chemical and visual stimuli combined.

7.15 The apparatus described in Sections 2.21 and 2.23 was used to test the responses to vibrations and chemicals combined. Both stimuli were presented only as naive ants entered the arena in order that prehabituation or presentization to the vibration or chemical odour could not occur. As 2-Heptanone had released the strongest threat behaviour of any chemical tested, it was used as a constant chemical stimulus in all subsequent experiments involving chemicals. If no response could be released with 2-Heptanone then

none of the other chemicals tested would elicit a response. The experimental variable in these experiments was the frequency of vibration in the arena floor. The amplitude of vibrations at all frequencies was maintained at a constant level (see Section 6.4); only the frequency was varied.

7.16 Table C7.4 shows the results of four experiments in which the responses to combined acoustic and chemical stimuli were observed. In Fig. C7.3 the mean of the Intensity Indices is plotted against frequency of substrate-borne vibrations. It can be seen that there are two peaks at 450 Hz and 1460 Hz. These peaks correspond to the two peaks at 500 Hz and 1400 Hz obtained in Chapter 6 when only vibrations were used as stimuli. The addition of the chemical stimulus to the vibration however has caused summation of response and increased the threat response almost fourfold.

Peak Response Intensity Index to Vibration at 500 Hz = 0.1316

at 1400 Hz = 0.1189

Peak Response Intensity Index to Vibration at 450 Hz = 0.4771

and chemical stimulus at 1460 Hz = 0.4542

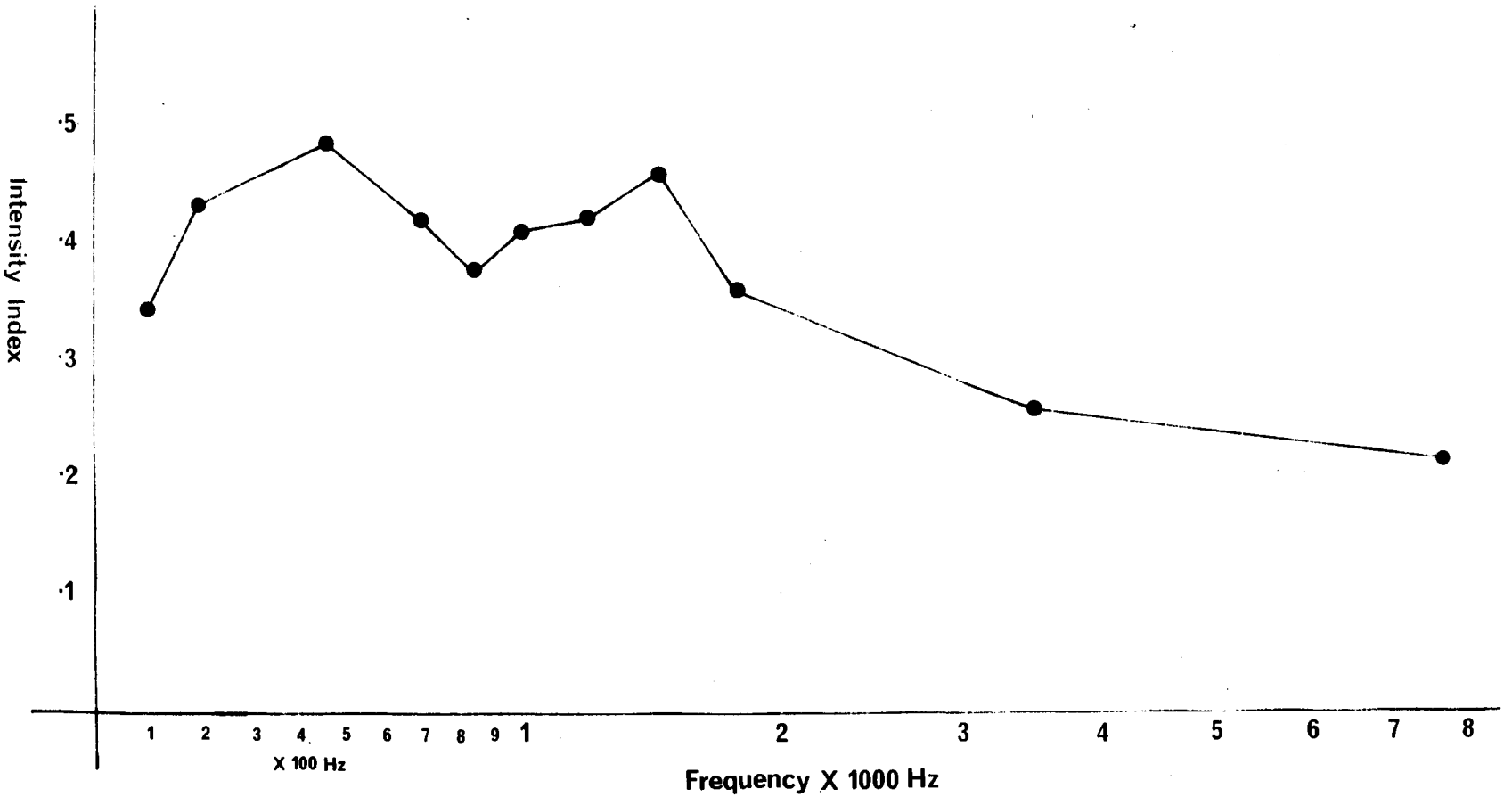
It is again interesting to note that the maximum response intensity obtained with 2-Heptanone alone is not attained when the chemical stimulus is combined with the vibration stimulus. In fact, the threat response to the combined stimulus is still negligible when compared to the full Intensity Index possible threat response which is 15.155.

Frequency of S.B.V. Hz	Expt. 83	Expt. 84	Expt. 85	Expt. 86	Mean
	Intensity Index				
86	0.3567	0.3622	0.2846	0.3568	0.3401
176	0.3645	0.5508	0.3801	0.4266	0.4305
450	0.6090	0.3103	0.5159	0.4731	0.4771
690	0.4654	0.4926	0.3801	0.3335	0.4179
840	0.4034	0.4382	0.3723	0.2714	0.3714
980	0.5198	0.4422	0.2947	0.3723	0.4073
1200	0.5314	0.3723	0.3723	0.4033	0.4198
1460	0.3956	0.5981	0.4732	0.4398	0.4542
1780	0.4189	0.2869	0.3801	0.3257	0.3542
3480	0.2171	0.3179	0.2171	0.2714	0.2559
7600	0.2094	0.2637	0.0930	0.3101	0.2191

TABLE C7.4 Chemical Constant = 2-Heptanone

Intensity Indexes for experiments using combined vibration and chemical stimuli.

FIGURE C7.3 Intensity Index plotted against frequency of substrate-borne vibrations


Intensity Index
·5
·4
·3
·2
·1
1 2 3 4 5 6 7 8 9 1
X 100 Hz
2 3 4 5 6 7 8
Frequency X 1000 Hz

7.17 The intensity of the threat response to combined visual and vibration stimuli is shown in Table C7.5. The constant visual stimulus was provided by the apparatus described in Section 2.16 using the standard 6 cm. stripe moving at 63.6 cms. per second. It was decided that experiments using the stationary 15 cm. square would provide no additional useful information to that obtained using the moving stimulus and consequently such experiments were omitted in order to save time. The vibration stimuli were presented as described in the previous sections and constituted the variable stimulus. Fig. C7.4 shows the mean Intensity Index of the combined visual and vibration experiments plotted against vibration frequency. The double peak of previous experiments can be seen. The threat response intensity is greater than the response intensity to vibrations alone.

Peak Response Intensity Index to Vibrations at 500 Hz = 0.1316
at 1400 Hz = 0.1189

Peak Response Intensity Index to Vibration and Visual Stimulus at 450 Hz = 1.0407
at 1460 Hz = 1.2233

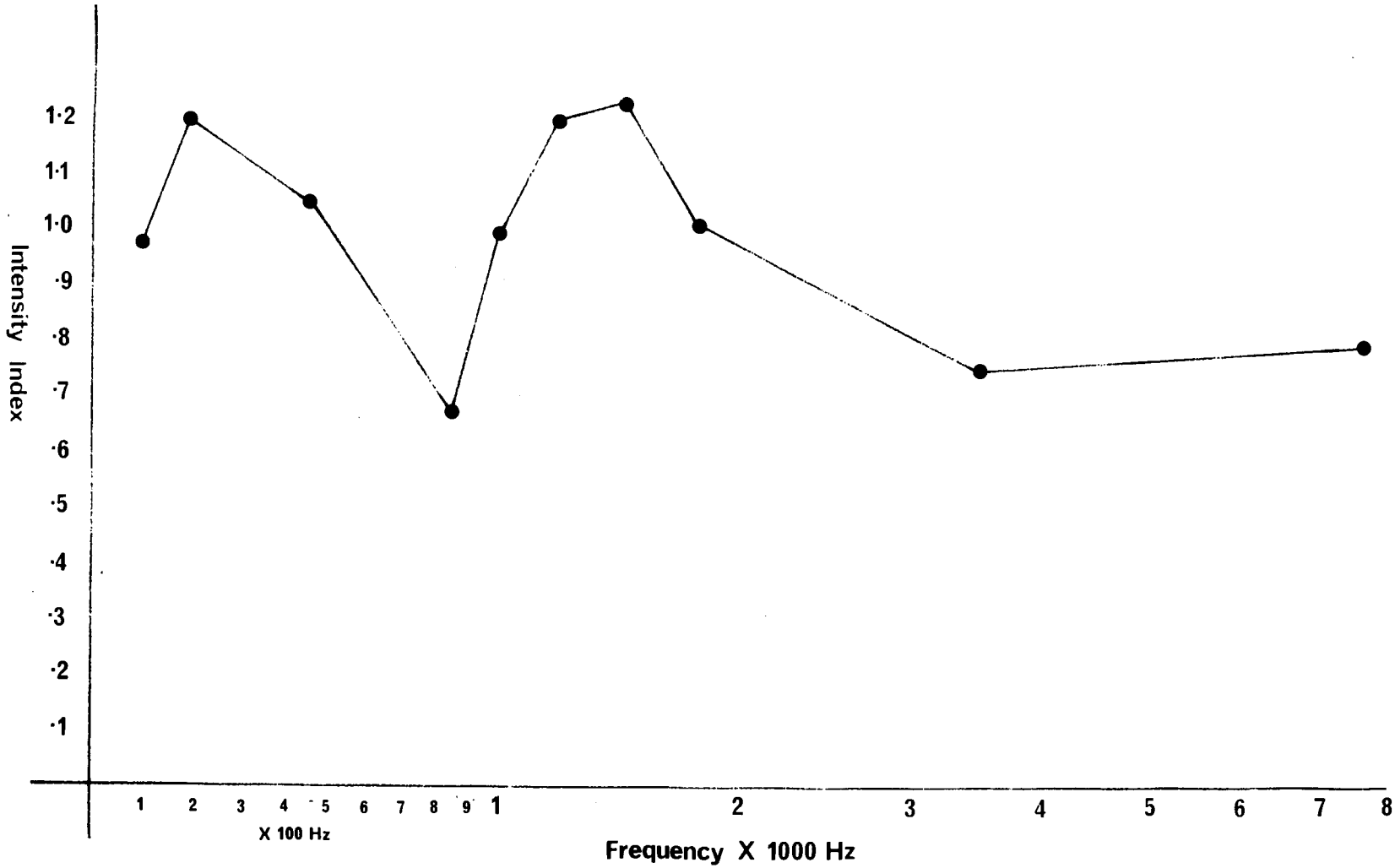
The mean Intensity Index for the combined vibration and visual stimuli throughout the vibration range tested is 0.9664, when compared with the mean Intensity Index for a 6 cm. strip moving at 63.6 cm. per second, which is 0.9297 (100% response is 15.155), it can be seen that there is a negligible difference in response. This suggests that vibrations do not have an active role in the threat releaser complex.

Frequency of S.B.V. Hz	Expt. 78	Expt. 79	Expt. 81	Expt. 82	Meam
	Intensity Index				
86	1.1212	0.9641	0.7837	1.0388	0.9770
176	1.2590	1.1077	1.1019	1.3249	1.1984
450	1.5636	0.9156	0.8846	0.7992	1.0407
840	0.7992	0.4344	0.6518	0.7837	0.6673
980	0.8807	0.7643	0.9253	1.3666	0.9842
1200	1.5171	1.0204	0.8225	1.4355	1.1989
1460	1.2803	0.8380	1.5451	1.2299	1.2233
1780	0.7216	0.7837	1.2696	1.3860	1.0402
3480	0.8215	0.6828	0.6246	0.8574	0.7466
7600	0.8109	0.8215	0.6906	0.8081	0.7978

TABLE C7.5 Visual Constant = 6cm stripe moving at 63.6cm/sec.

Intensity Indexes for experiments using combined vibration and moving visual stimuli.

FIGURE C7.4 Mean Intensity Index of the combined visual and vibration experiments plotted against vibration frequency


Intensity Index
1·2
1·1
1·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8
X 100 Hz
Frequency X 1000 Hz

7.18 In the last series of combined stimuli experiments, all three sensory stimuli were presented at the same time to naive ants entering the experimental arena. The visual stimulus was the standard 6 cm. stripe moving at 63.6 cms. per second, the chemical stimulus was 2-Heptanone presented as before. Changes in the vibration frequency, presented as before, provided the variable stimulus.

7.19 Table C7.6 shows the results of the experiments using all three stimuli combined and Fig. C7.5 shows the mean Intensity Indices plotted against vibration frequency. Unlike the previous experiments there is no double response peak, although if the curve from 86 Hz - 450 Hz is disregarded the double peak is present. Instead the response intensity is at its maximum at 86 Hz and as the frequency of vibration is increased, the intensity of response falls, with a hump at 1200 Hz until it reaches a resting level just below 2000 Hz. Above this frequency further decline in response intensity is negligible.

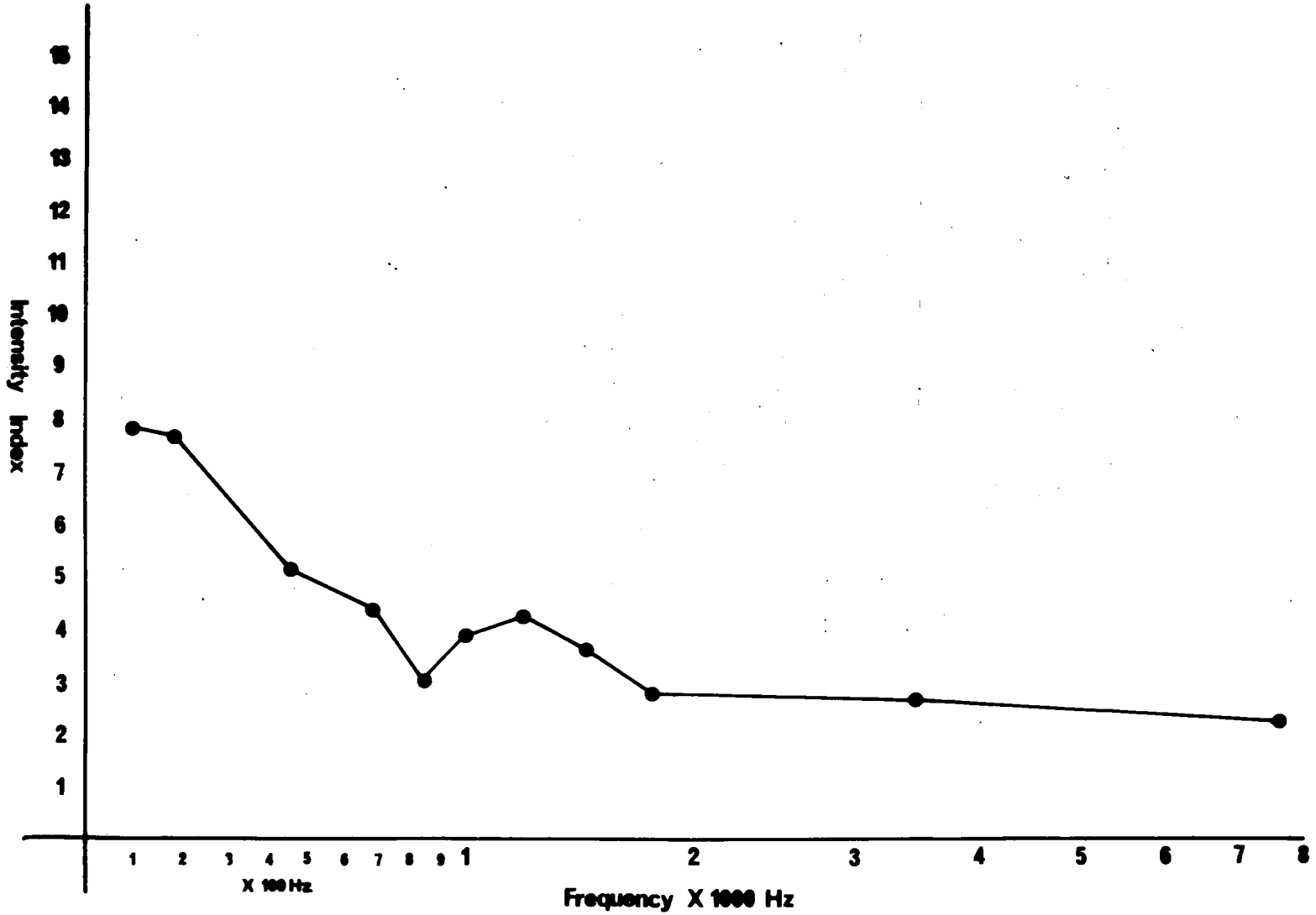
7.20 Why does the response decline like this? Ants are more receptive to vibrations at 450 Hz and 1400 Hz than at 86 Hz and peak responses would be expected at these points if, as is the case, other stimuli remained constant. This is not so. Perhaps a parallel situation to that in Section 3.25 occurs where the decay time of the visual units in the eye is longer than the period between each successive stimulus. A similar situation does not occur when only two of the senses are optimally stimulated. However, if all three senses are optimally stimulated at any one time, the time period

Frequency of S.B.V. Hz	Expt. 88	Expt. 90	Expt. 91	Expt. 89	Mean
	Intensity Index				
86	5.9070	7.9098	8.6955	9.0522	7.8911
176	6.2222	7.9535	8.9185	7.5355	7.6577
450	4.4142	6.0379	5.9166	4.3696	5.1846
690	3.8071	4.6121	4.5781	4.4812	4.3696
840	3.9284	2.8769	2.3075	3.0127	3.0314
980	2.5316	2.5704	2.8332	3.5995	3.8837
1200	5.9118	4.5694	3.1727	3.1815	4.2089
1460	4.0156	3.7983	2.7353	3.8817	3.6077
1780	4.0311	3.0360	2.0990	1.9788	2.7862
3480	2.4615	4.1853	2.1620	1.6936	2.6256
7600	1.4802	1.6780	2.9196	3.0612	2.2598

TABLE C7.6 Constants = 2-Heptanone and 6cm stripes moving at 63.6cm/sec.

Intensity Indexes for experiments in which chemical, visual and vibration stimuli were used.

FIGURE C7.5 Mean Intensity Index for all three stimuli combined plotted against vibration frequency


Intensity Index
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9
X 100 Hz
1 2 3 4 5 6 7 8
Frequency X 1000 Hz

required to analyse the total sensory input and initiate motor responses may be longer than the period between each successive stimulus peak, in this case the time period of each vibration cycle. Thus as the frequency of vibration is increased the response falls.

7.21 When visual and chemical stimuli are presented together in the experimental arena (Sections 7.8 and 7.10) the intensity of the threat responses obtained from naive ants are greater than for visual stimuli by themselves, or for chemical stimuli by themselves. The stimuli act on the sense organs of the ants in such a way that the combined threat response intensities summate and increase as long as the visual stimulus is near maximum releasing intensity. When the visual stimulus does not release maximum response intensity, the effect of the chemical stimulus is masked and the overall response is lower than expected.

7.22 Stimulation of threat response by substrate born vibrations is low. Only the vibration frequencies at which F. rufa is maximally sensitive, i.e. 450 Hz and 1400 Hz, release more threat behaviour than a low threshold level, and even then the response is still negligible (see Section 6.4 , Fig. C6.3). Even when the stimulus of 2-Heptanone is combined with the vibrations, the response intensity is not as great as could be expected from 2-Heptanone alone. Presence of vibrations may partially inhibit response to chemicals (Fig. C7.3). Vibration and visual stimuli combined elicit no

greater response intensity than for the same visual stimulus by itself (Section 7.17). These observations suggest that vibrations do not in fact act as threat releasers, and may even inhibit response to more important releasers of threat behaviour. Certain chemicals e.g. ketones, are efficient releasers of threat behaviour and increase the responses obtained to moving stimuli at optimum visual stimulation levels.

7.23 Threat response in F. rufa is elicited by visual and chemical stimuli, but acoustic stimuli seem to play no direct role in releasing threat behaviour. Certain chemicals by themselves elicit greater threat responses than visual stimuli, but the responses can be inhibited by the presence of sub optimal visual stimuli. This being the case, these chemicals cannot constitute the major threat stimuli or their effect could not be masked. Visual stimuli are therefore the major releasers of threat behaviour. The responses to visual stimuli are only increased by the addition of other stimuli. Chemical stimuli act in support of visual stimuli and when present better threat responses are obtained, especially at optimal stimulation levels for both releasers when summation of response occurs over and above the value expected for simple addition of responses. Vibrations act on threat behaviour indirectly by alerting ants whichthen become more responsive to releasers of threat behaviour. The presence of further chemicals also causes ants to become alert and some of these chemicals may be used as alerting pheromones.

CHAPTER EIGHT

Conclusions

Certain types of behaviour lack any regular rhythm, or appetitive or searching phases. These behaviour patterns are triggered largely or entirely by external stimuli and the behavioural sequence is normally terminated by eliminating the eliciting stimulus in some way. Aggression belongs to this group of behaviour patterns.

A simple type of aggression occurs in the maintenance of a free space around the individual. In the case of ants, the free space is maintained around each colony and not each sister ant. Indeed, within each colony contact between individuals is a necessity. Maintenance of the free space around the colony involves limiting the approach of non-sibling ants and predators (violation of the free space by recognised prey is however welcome), or by driving away alien ants and predators. The other alternative of withdrawing from an occupied area is not feasible with an established ant colony, unless the unwelcome "guest" is likely to be present over a long period.

The aggressive behaviour of F. rufa when directed towards alien ants either of the same species or different species takes the form of seizing and dragging (Wallis, 1962 b). Prolonged

seizure can result in dismemberment of the attacked ant or even death following puncture of the exoskeleton. Wallis found that, in F. fusca, threat behaviour was a component part of aggressive behaviour and was included in the chain of behaviour patterns elicited when one or more conspecifics encountered an alien ant.

Wallis, (1962 a) described the threat behaviour pattern in F. fusca: "The head is raised, both head and antennae being directed towards the other ant. The mandibles are held wide open with the labial mouthparts tightly withdrawn. The position of the mouthparts is diagnostic of threat. Threat may be directed towards ants from other colonies, other species or inanimate objects."

This posture can also be observed in F. rufa where it is classified as a Stage 1 threat response (see Section 1.5). Workers of an F. rufa colony do open their mandibles wide when they encounter an alien ant and do have their labial mouthparts withdrawn, but this is usually only preparatory to seizing. Only if the distance between attacker and attacked remains such that the attacker is only just unable to seize the attacked ant, will the mandibles be held open in this way for a period of more than a second or two. Thus the threat behaviour described by Wallis is simply an arrested preparatory step for attack behaviour. F. rufa will use this same behaviour pattern when attacking prey. When prey is encountered, an immediate attempt is made to seize. If this is effective and the prey is active, the ant either attempts

to grapple with the prey using the forelegs and to anchor itself to the substrate by means of the remaining legs until the prey becomes sluggish, at which time it can be overpowered; or it clings to the prey with all legs and curls the gaster under its own thorax and between the legs until the apex of the gaster is facing forwards. Formic acid is then sprayed over the skin of the prey and into any incisions made with the mouthparts.

This post-seizure behaviour pattern may also be used if an alien ant is attacked, although normally dragging occurs (Wallis, 1962 b). The attacking ant attempts to drag the attacked ant over the substrate.

Thus the behavioural sequences used by F. rufa when attacking both alien ants and prey are:-

1) The preparatory seizure stance - Stage 1 threat posture
2) Seizure
3) Either biting and overpowering with formic acid, or dragging/anchoring (which stops the possibility of the ant being dragged away by the prey).

Threat behaviour has been observed to be directed towards prey only when the prey was large, e.g. cockroach and only when it was moving rapidly past the ant. This was a very infrequent occurrance. Threat behaviour towards alien ants has not been observed by the author.

The complex threat behaviour pattern of F. rufa does not act

as a warning of intention to alien ants or prey.

The experiments described in Chapters 3 - 7 were designed to find out what types of sensory stimuli were involved in the releaser complex of threat behaviour. It was found (Chapter 3) that the visual stimulus acting as the best releaser of threat behaviour was a moving solid dark figure subtending an angle at the eye of the ant of about 24°. The optimum velocity varied with the size of the stimulus but with a stimulus subtending 24° at the eye, the optimal velocity was between 125 - 130 cms./sec. The stimulus also presented a flicker frequency of about 10 flickers per second, a value that did not vary with change in stimulus size. Velocity of movement and flicker frequency are both important components of any efficient visual releaser of threat behaviour.

Regular visual stimulation was found to be a better releaser of threat behaviour than irregular visual stimulation (Chapter 3). No particular wavelengths of monochromatic light within the visual range of the ants were better releasers of threat than were others (Chapter 4) and it was concluded that stimulus colour is unimportant as a threat releaser.

Formic acid (Chapter 5) was not an effective releaser of alarm or threat behaviour and is secreted only to overpower prey. A ketone, probably secreted with the formic acid, and with a carbon chain length of seven carbon atoms (Chapter 5) is probably an alarm

pheromone, and acts to alert ants making them receptive to visual stimuli (Chapter 7). Vibrations (Chapters 6 and 7) are of importance only in so far as they alert ants, which are then more responsive to the releasers of threat behaviour.

Thus the main component releasers of threat behaviour are visual and chemical. In all probability the chemicals are secreted as pheromones by other ants in the vicinity of an ant that has itself been stimulated to threaten an "intruder". Vibrations if present are probably only initiated by the "intruder" and provide negligible releasing value for threat behaviour.

One of the original aims of the research project was to find a combined stimulus that, when presented to the ants, would release full threat posture "nine times out of ten". This was not however found, probably because the laboratory colonies became habituated to the constant movements and changes in smell, together with the vibrations set up by people in the laboratory. Thus stimuli that initially released strong behaviour did not do so after the colonies had been kept in the laboratory for several months and stimuli that would release strong threat behaviour in "Wild" ants produced only weak threat behaviour in the laboratory ants. Sufficient results were, however, obtained to indicate the main releasers, although this problem should be borne in mind when deciding the overall effects that the stimuli had in releasing threat behaviour.

If the threat behaviour of F. rufa has not been evolved as a warning of intention to alien ants and prey, what is the reason for its occurance and how did it develop?

Threat behaviour in Pogonomyrmex badius (Wilson 1958); Formica fusca (Wallis 1963b) and Lasius spp and Myrmica spp (personal observations) take the form of a Stage 1 threat behaviour (Section 1.5). Such threat behaviour is arrested seizing behaviour. If the behavioural components involved in the complex threat postures of F. rufa (Section 1.5) are analysed it can be seen that they correspond to the components involved when F. rufa attacks prey:-

The labial mouthparts are withdrawn, the mandibles are held wide open, the forelegs are used to grapple with the prey and the remaining legs to anchor the attacking ant either to the prey or to the substrate. The gaster is tucked under the body with the apex pointing forwards and formic acid is squirted from the anal orifice. It would seem probable therefore that the threat posture of F. rufa is simply arrested attack behaviour and that by loosing the tactile stimuli that are necessary for actual attack, the behaviour patterns used for seizing and overpowering prey have been adapted and modified as threat.

Why was the fixed action pattern of threat evolved? Threat behaviour is not used when other insects are encountered; these will be either prey or alien ants. The Stage 1 threat posture is

an initial behavioural stance for different types of aggressive behaviour and is therefore not true threat behaviour. Threat behaviour must therefore have evolved as a means of defending the colony against attack by predators. It is known from personal communication with foresters and naturalists that the Greenwood-pecker, (Picus viridis) and badgers (Meles meles) will take the adult ants and grubs for food. In the past when much more of the country was afforested and more species of insectivores present, these may not have been the only predators of wood ants. Thus an effective method to deter large predators from digging into the nest-mound would be an advantage.

The pungent formic acid vapour released by wood ants in the vicinity of a disturbed nest is an irritant to the human nose and eyes and the liquid is very caustic if allowed to enter a cut in the skin, and will even cause blisters (acid secreted on to my finger and thumb by 50 ants picked out of the observation nest by hand, for use in an experiment, caused large, painful blisters). It is also stated by foresters that when horses were used to haul felled timber from the woods that they would not enter the vicinity of a wood ants' nest because of the smell of formic acid. Thus formic acid could be an effective deterrent to predators.

By a process of natural selection, the well established attack behaviour patterns were modified and reformed into the ritual threat behaviour pattern which is a well adapted method of releasing formic

acid vapour into the air. The stereotyped posture of threat, in itself, is not important as a signal because if seen at all by the predator, it would be insignificant against the background of the nest material. The threat stance is therefore only a vehicle for releasing formic acid into the air in the most efficient way. The stance, although unimportant to the predator, is important because if the predator approached too close, the threatening ant could then develope the threat behaviour into attack and seizing could occur followed by an attempt to overpower. This could cause discomfort to the predator due to the presence of formic acid on the skin and also in possible lesions made by the mandibles of the attacking ants.

The threat behaviour of F. rufa is probably just another example of arrested attack behaviour which occurs in a different environmental context (lack of tactile stimuli) from the original behaviour pattern and is modified to become the end product rather than just a stage in a behavioural chain.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor Dr P.E. Howse for his advice and encouragement, to Mr G. Callow for invaluable advice on electronics, Mr J. Barrs for running my data on the University Computer, Dr T. Krishnam for advice and help with statistics, Dr R. Baker for analysing chemical extracts by gas chromatography, Mr R. Bailey for help with optical measurements and Mr J. Baynham for invaluable help with photography.

I would also like to thank Professor J. Brough for allowing me to use the facilities of the Zoology Department, University College, Cardiff and also Professor L. Brent for extending the same facilities at the Zoology Department, Southampton University.

I am also grateful to the Science Research Council for their financial support of a research studentship.

APPENDIX I

Diagrams of the Experimental Nests

A) The Church Nest, Figure A1.1

B) The Slice Nest, Figure A1.2

FIGURE A1.1 Diagrams of the Church Nest.

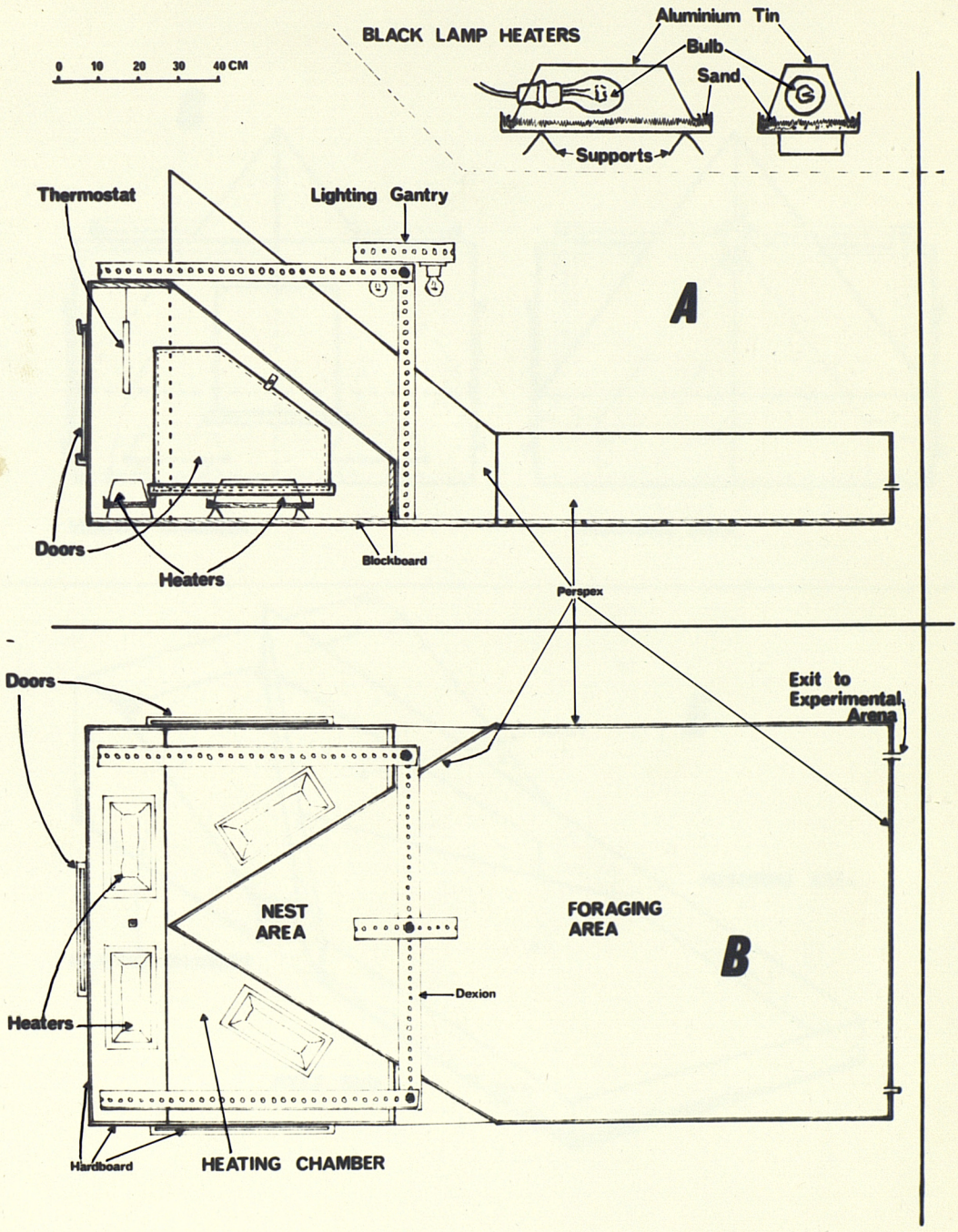
A) Side view, with inset to show construction of the Black Lamp Heaters

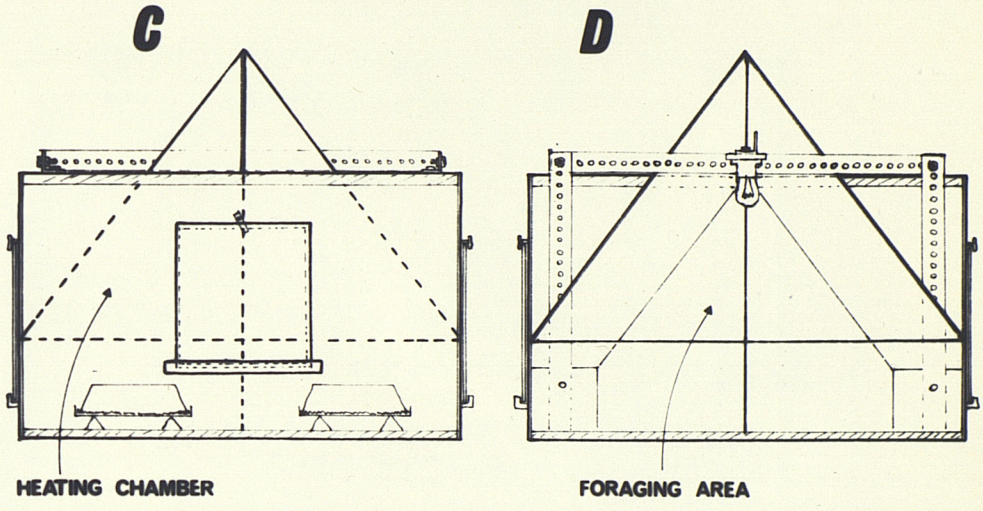
B) Plan view

C) Rear view

D) Front view

E) Oblique sketch to indecate three-dimentional structure, see also Figure C2.1


BLACK LAMP HEATERS
Aluminium Tin
Bulb
Sand
Supports
0 10 20 30 40 CM
Thermostat
Lighting Gantry
A
Doors
Heaters
Blockboard
Perspex
Doors
Exit to Experimental Arena
NEST AREA
FORAGING AREA
B
Dexion
Heaters
Hardboard
HEATING CHAMBER


C
D
HEATING CHAMBER
FORAGING AREA

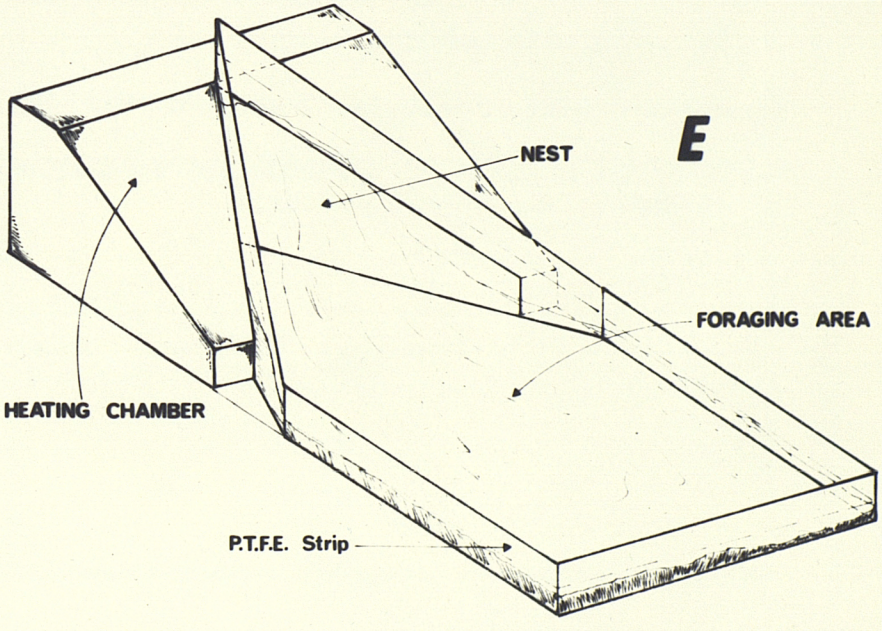

E
NEST
FORAGING AREA
HEATING CHAMBER
P.T.F.E. Strip

FIGURE A1.2 Diagrams of the Slice Nest.

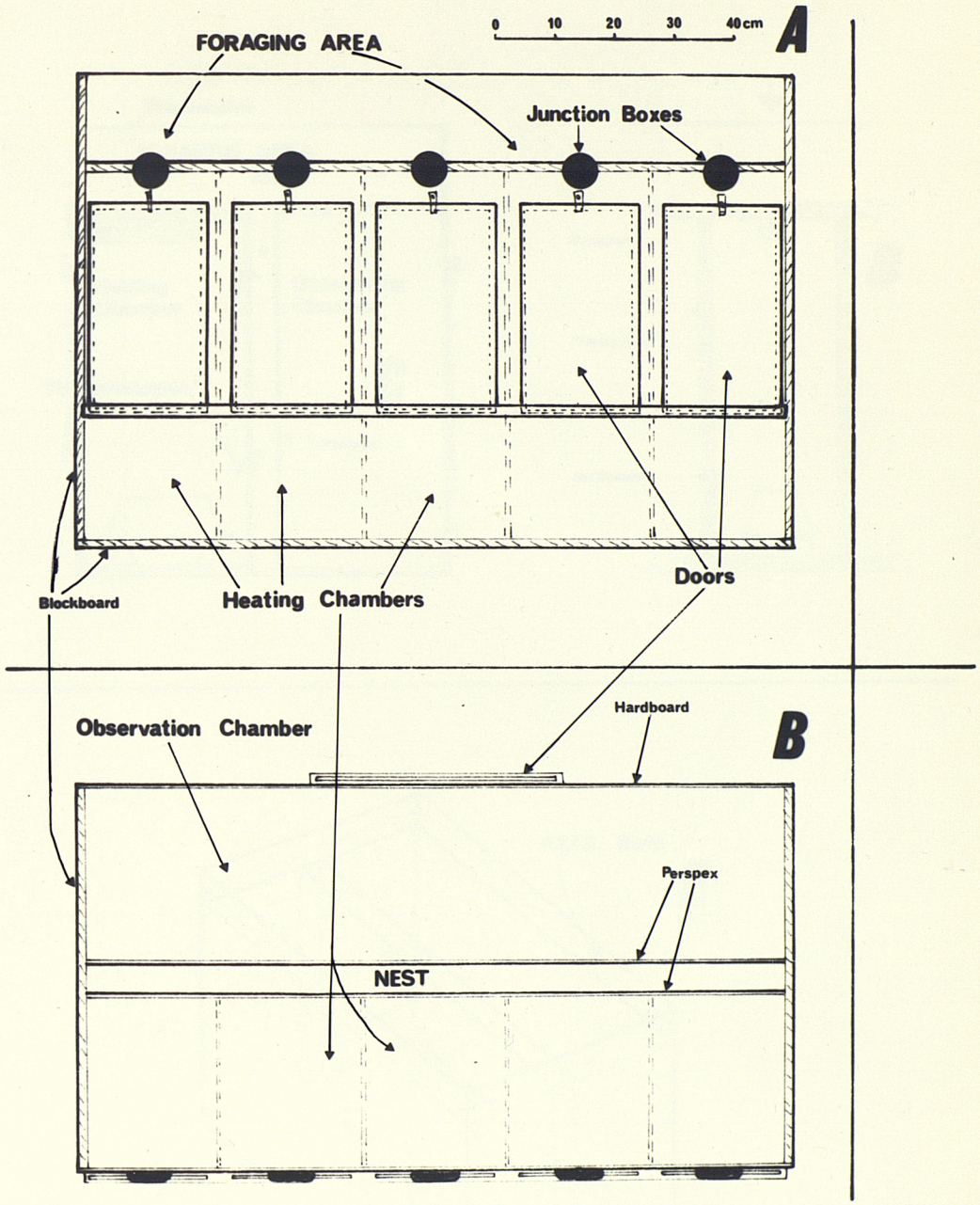
A) Side view, from the side containing the Heating Chambers and showing no interior detail

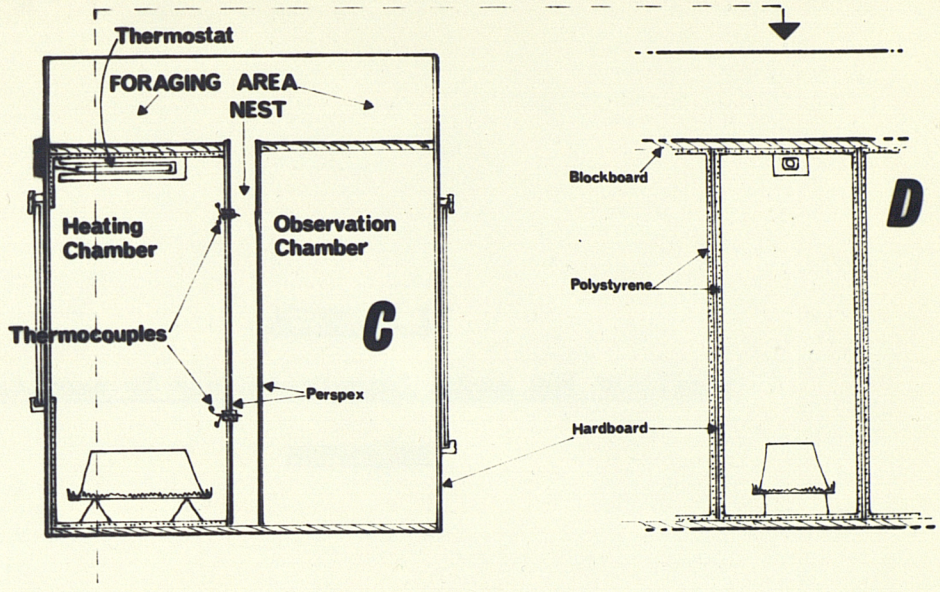
B) Plan view, showing no interior detail

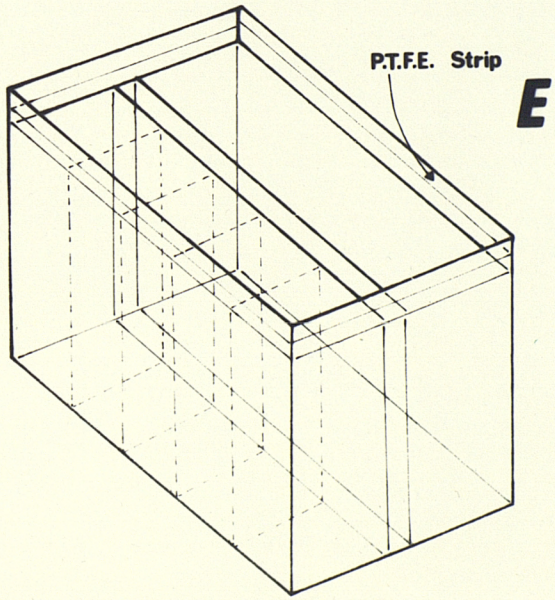
C) End view, with interior detail

D) Section across one Heater Chamber to show internal detail

E) Transparent sketch to indecate the three-dimentional structure, see also Figure C2.2


0 10 20 30 40 cm
A
FORAGING AREA
Junction Boxes
Blockboard
Heating Chambers
Doors
B
Observation Chamber
Hardboard
Perspex
NEST


Thermostat
FORAGING AREA
NEST
Heating Chamber
Observation Chamber
C
Thermocouples
Perspex
Blockboard
Polystyrene
Hardboard
D


P.T.F.E. Strip
E

APPENDIX II

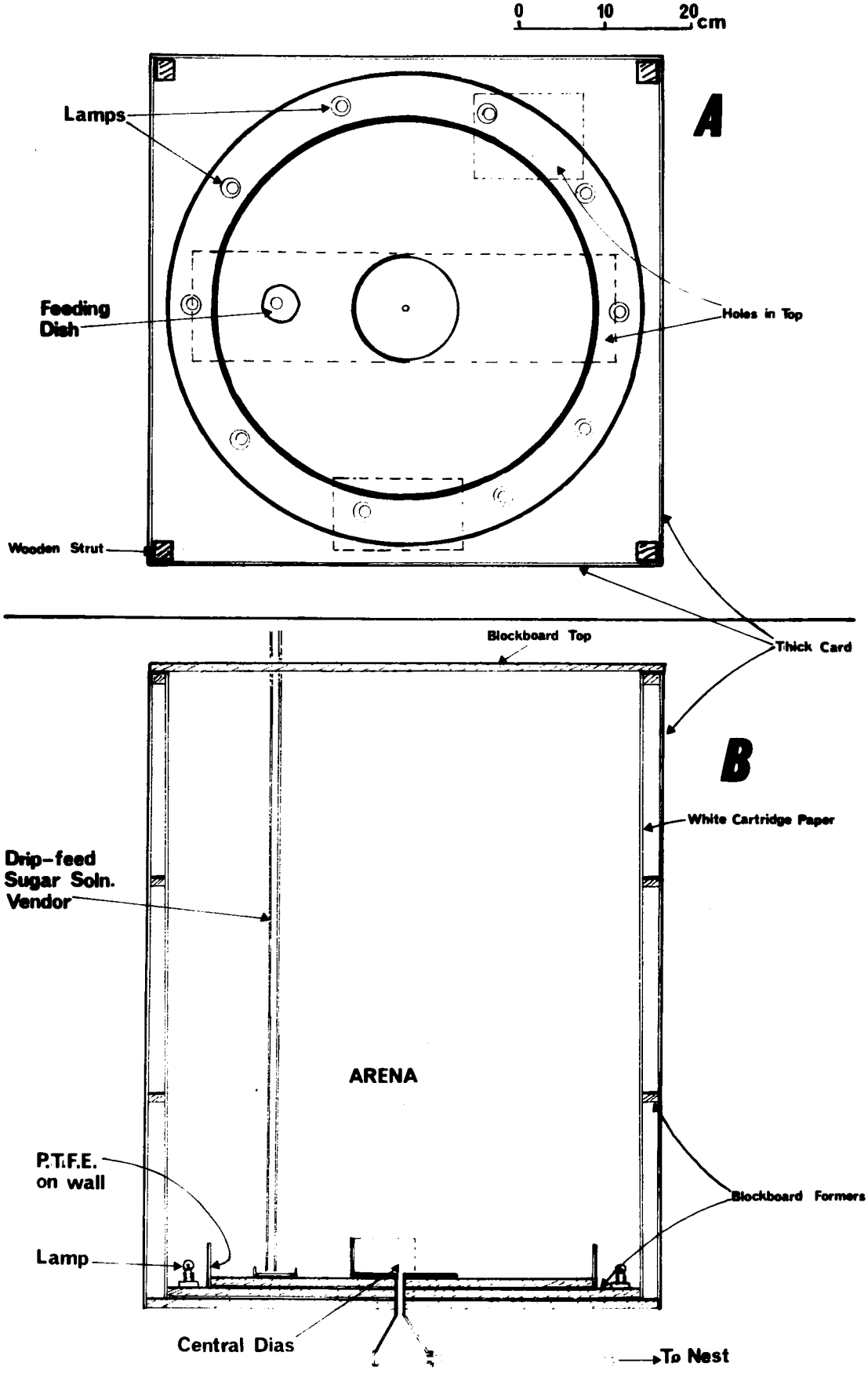
Diagrams of the Behavioural Arena and ancillary apparatus

FIGURE A2.1 The Behavioural Arena

A) Plan view

B) Side view

C) Oblique cut-away view


0
10
20 cm
A
Lamps
Feeding Dish
Holes in Top
Wooden Strut
Thick Card
Blockboard Top
B
White Cartridge Paper
Drip-feed Sugar Soln. Vendor
ARENA
P.T.F.E. on wall
Blockboard Formers
Lamp
Central Dias
To Nest

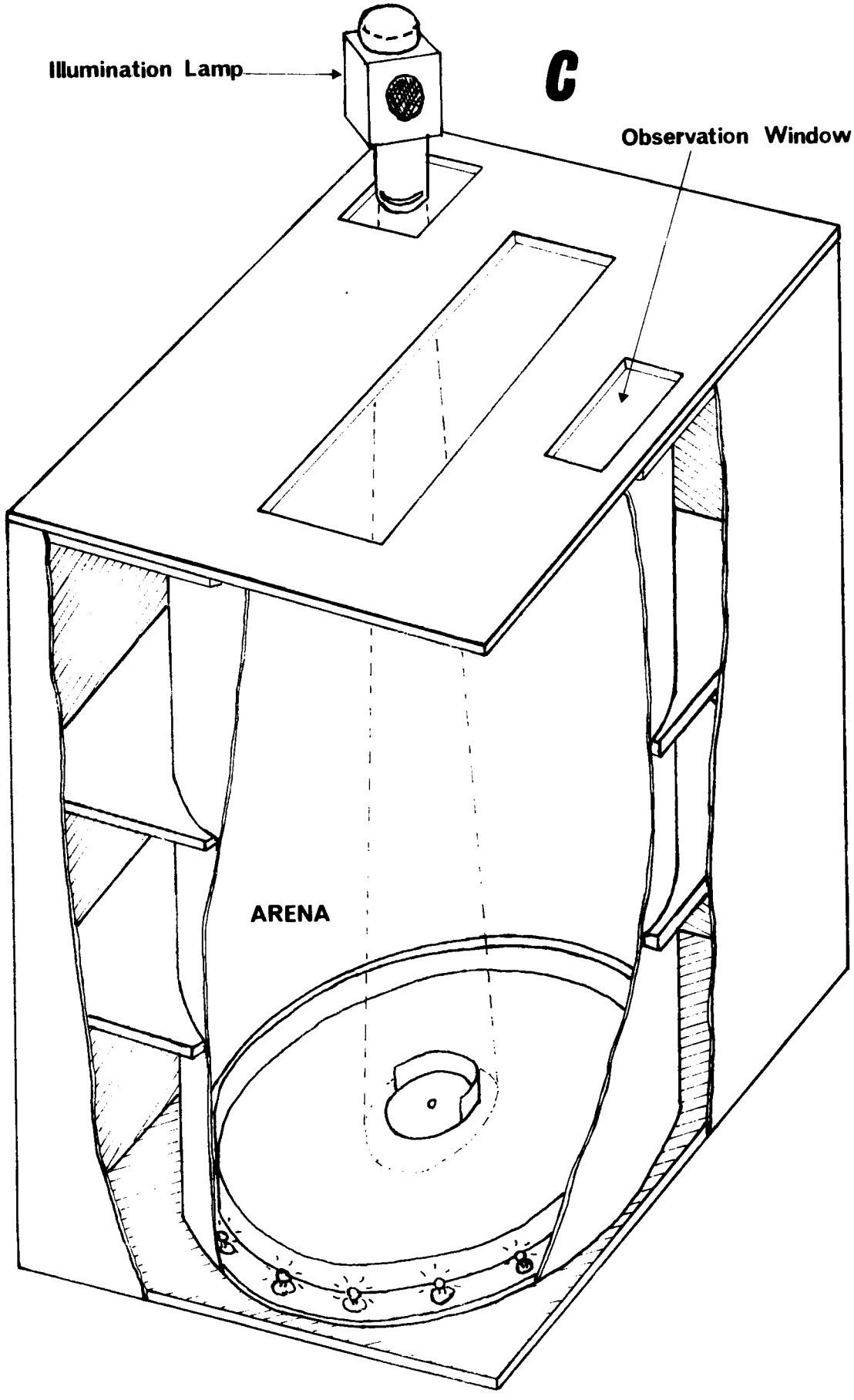
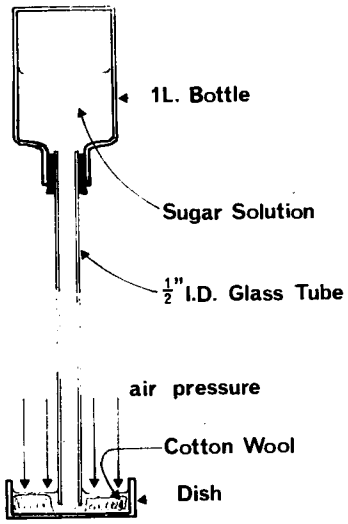

Illumination Lamp
C
Observation Window
ARENA

FIGURE A2.2 Air Pressure drip feed Sugar Solution Vendor.

FIGURE A2.3 Colour Perception Projector.

A) Projectina, Q.I. Intense Microscope Lamp
B) Slide Carrier
C) Micromanipulator
D) Hanimar 85mm, f2.8 Projector Lens
E) Aperture in Arena wall for projection purposes
F) Image projected onto Arena wall
G) Variable speed Iris Camera Shutter and Cable Release


1L. Bottle
Sugar Solution
½" I.D. Glass Tube
air pressure
Cotton Wool
Dish

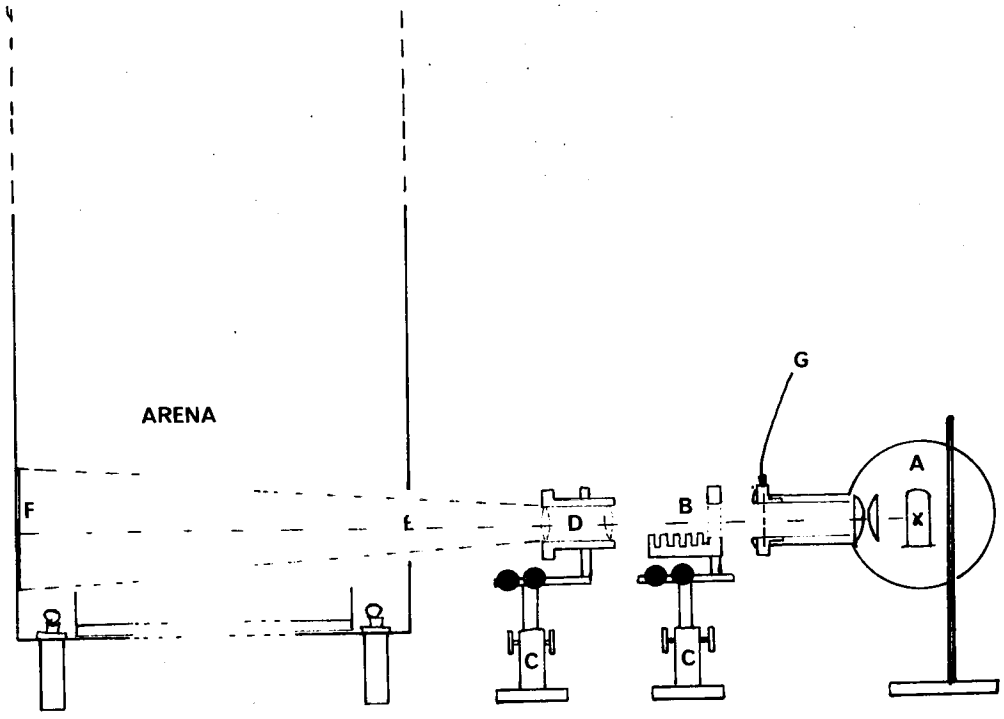

ARENA
F
E
D
C
B
C
G
A
K

FIGURE A2.4 Section through Cylimder Assembly, Bay Window and Arena.

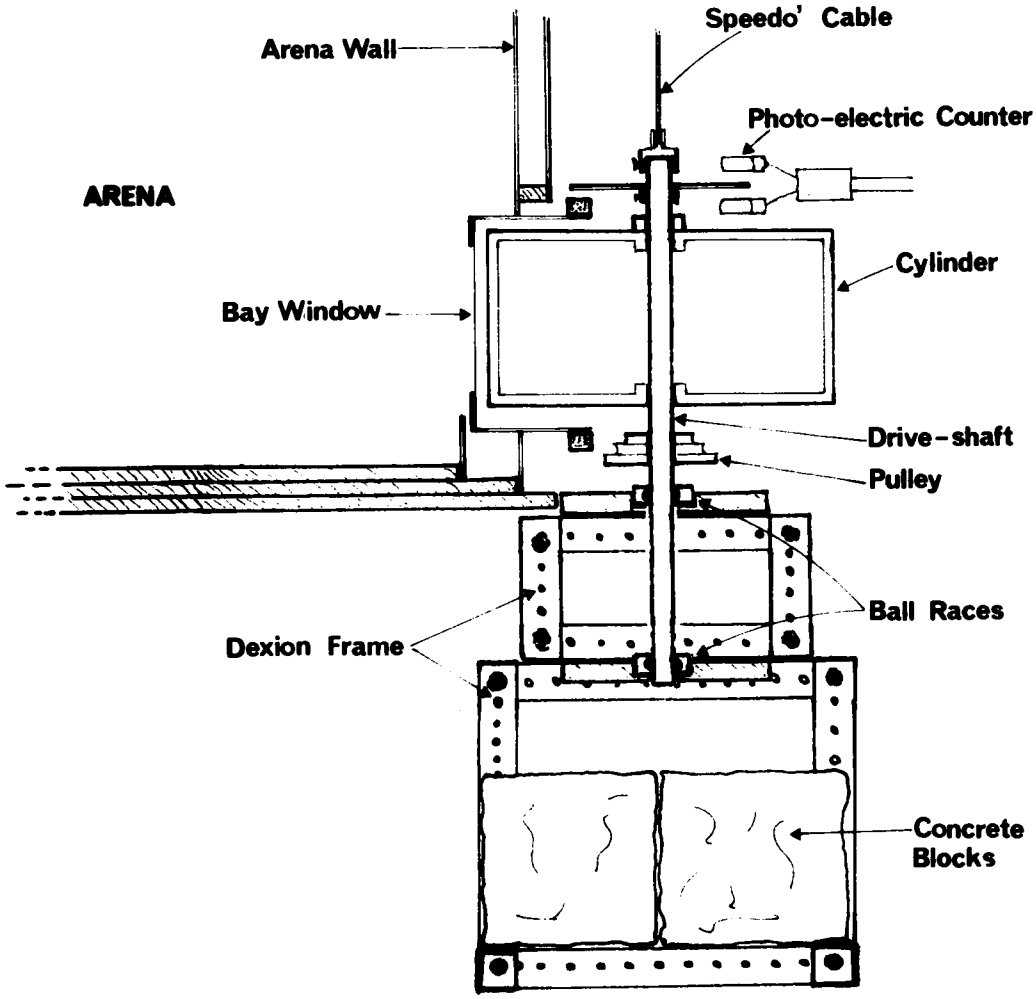

Arena Wall
Speedo' Cable
Photo-electric Counter
ARENA
Cylinder
Bay Window
Drive-shaft
Pulley
Ball Races
Dexion Frame
Concrete Blocks

FIGURE A2.5 Block diagram of Arena and Apparatus used in Movement Experiments to show relationships.

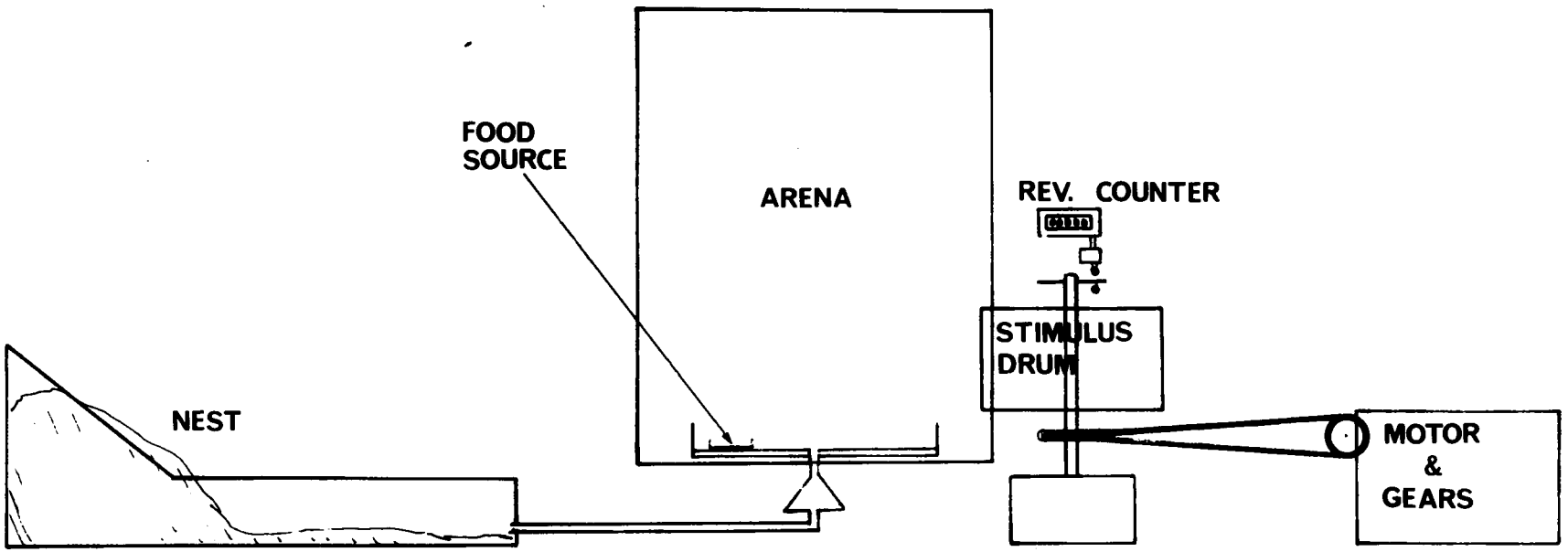
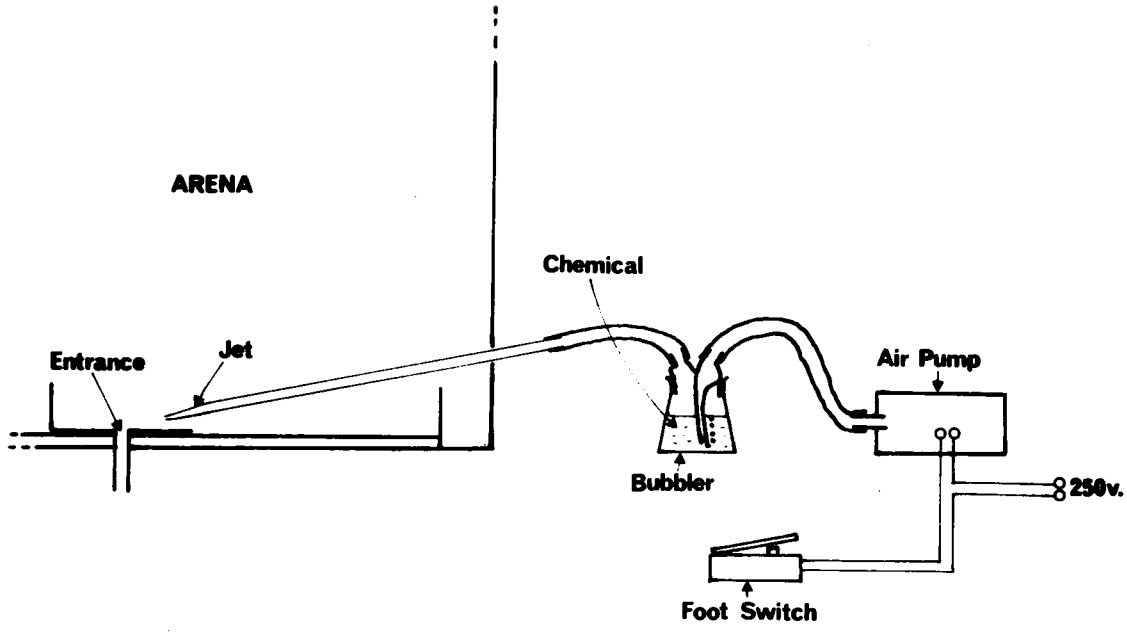

FOOD
SOURCE
ARENA
REV. COUNTER
STIMULUS
DRUM
NEST
MOTOR
&
GEARS

FIGURE A2.6 Diagram of Apparatus used im Chemical Experiments to show relationships.

FIGURE A2.7 Diagram of Vibrator Assembly and attachment to the floor of the Arena


ARENA
Chemical
Entrance
Jet
Air Pump
Bubbler
250v.
Foot Switch

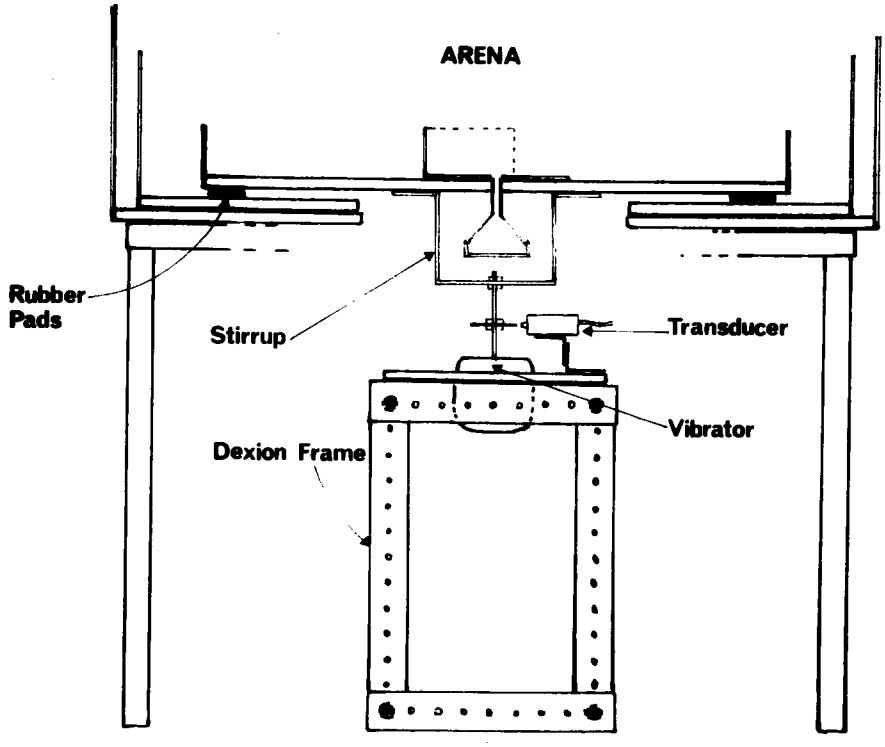
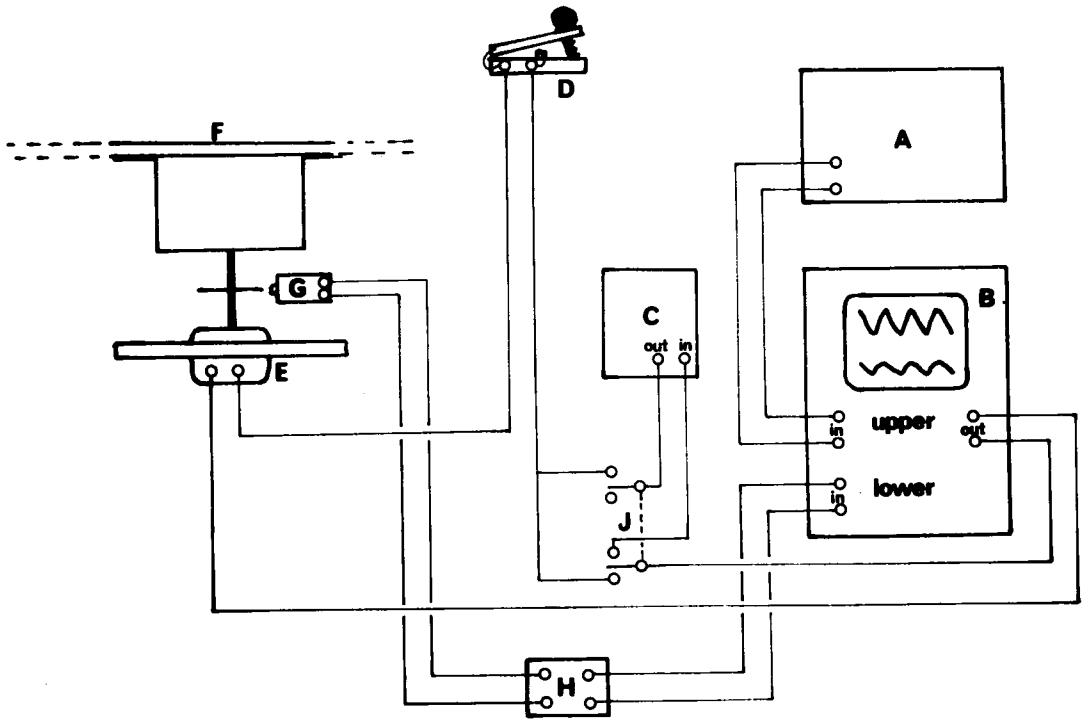
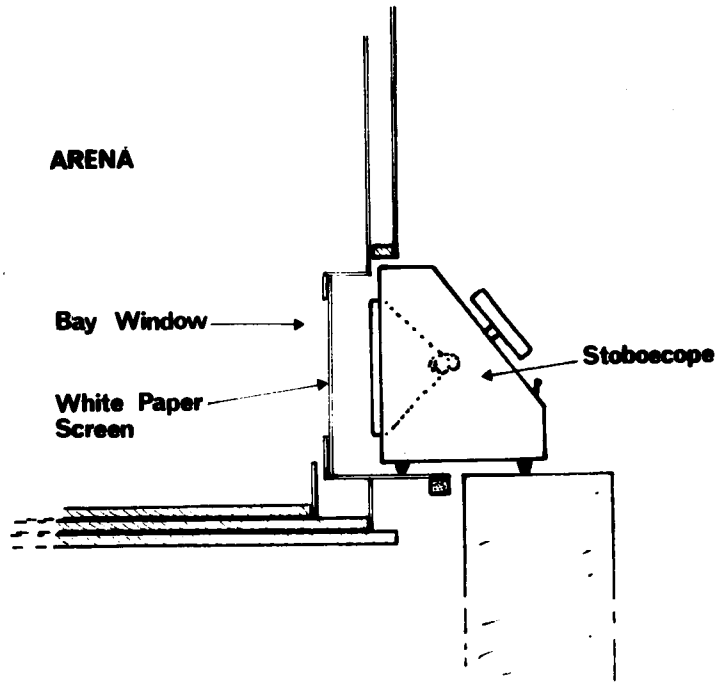

ARENA
Rubber Pads
Stirrup
Transducer
Vibrator
Dexion Frame

FIGURE A2.8 Block diagram of wiring layout used in all vibration experiments

A) Cossor Signal Generator
B) Tektronix Type 502 dual beam Oscilloscope
C) One valve Audio Amplifier
D) Manually operated push button switch
E) Pye-Ling Electro-mechanical Vibrator
F) Vibrating Arena floor
G) Orbit Controls non-contacting, self-powered electro-magnetic transducer
H) Simple A.C. Amplifier
J) Two way, two pole Switch

FIGURE A2.9 Diagram of Stroboscope position


D
F
A
G
C
out in
B
E
upper
in
out
lower
in
J
H


ARENA
Bay Window
White Paper Screen
Stoboecope

APPENDIX III

A Colour Discrimination Arena

Colour discrimination in the winged aculeate Hymenoptera has been known since 1914. Von Frisch (1914) first trained Apis mellifica workers to collect food from dishes placed on coloured papers. These experiments were later repeated and verified using spectral colours projected on to a white background (Kuhn and Pohl, 1921; Kuhn, 1927). Apis can distinguish four regions of the spectrum, 510 - 650nm (yellow-green to orange), 480 - 500nm (blue-green), 400 - 480nm (blue and violet) and 300 - 400nm (the near ultra-violet).

Very little work had been carried out on colour discrimination in ants (Lubbock, 1882; Forel, 1910; Turner, 1907 and Abbott, 1927) until Tsuneki (1950) attempted to find out whether Leptothorax congruus spinosior Forel responded to the colour of lights. He used an arena designed to allow the ants to orientate using coloured light bulbs. Tsuneki used domestic coloured light bulbs which produce light over a wide range of wavelengths. Any conclusions are therefore invalid beeause the light was not monochromatic. Later, Tsuneki (1953) repeated his experiments using spectral light of approximately the same "physiological intensity" to the ants but he varied the intensities with a rheostat and thuschanged the colour curve of the light sources. He tested both Leptothorax congruus and Camponotus herculeanus obscuripes, Mayr. No colour discrimination

was found in Camponotus but indication of orientation by colour discrimination was found in Leptothorax. Kiepenheuer (1968) using learning experiments found that F. polyctena could distinguish between ultra-violet and yellow-green. There is thus evidence of colour discrimination in ants.

During 1967, in conjunction with preliminary experiments to test threat response to monochromatic light, a colour discrimination arena was designed and finally built. It was thought that the homing instinct of ants would be stronger than the foraging instinct as exploited in experiments with bees (Von Frisch, 1914; Kühn and Pohl, 1921; Kühn, 1927; Hertz, 1939; and Daumer, 1956). The experimental arena was therefore designed so that the foraging ants, using visual cues, could easily learn their way to a fixed food source but on returning to the nest were required to recross a stimulus free arena where they were presented with a choice of four exits to the nest, each with or without a monochromatic "signpost" directly over the pathway. Experiments carried out earlier with two pathways from the nest to a single food source indicated preference in all foragers for only one pathway at a time. It was expected that this preference would hold true under the experimental conditions in the arena and that the ants could be taught to associate one colour with the homeward pathway. Then by rearranging the signposts it was expected that, if F. rufa does possess colour vision, there would be a significant change in the numbers using each pathway related to the repositioning of each signpost. In this way tests using different wavelength combinations

should indicate whether the ants could discriminate between certain wavelengths. The monochromatic signposts were spectral filters standardised to give equal illumination at the eye of the ant as described under Sections 4.4, 4.8 and 4.10 although the energy intensities experienced would only be relative to those quoted.

The number of ants using each pathway were to be monitored using photo-electric counters. The original idea was to use simple counters as in Fig. A4.1 and to monitor the total number of ants using each pathway. However when the signposts were rearranged the ants would continue to enter the arena from the nest via the original learned pathway, but assuming orientation using a particular colour the foraging ants would be expected to leave the arena via the pathway labelled with the exit signpost. The change in count per pathway may not have been clear and it was decided that directional counters were necessary so that the ants leaving the arena could be monitored separately from those entering the arena and a true picture of the numbers of ants exiting via each pathway could be seen.

Such a circuit was not available, but finally the circuit shown in Appendix IV was developed, tested, modified and accepted. Four of these circuits were built.

The apparatus (Fig. A3.1) consists of a chamber A with one exit to the nest and four exits to the next chamber B. Chamber B is a shallow cylinder of 33.5 cms. diameter and 15 cms. height. The cylindrical perspex wall is covered with white card and the whole cylinder surrounded with a light tight cover. Chamber B forms an experimental arena. The floor of the arena (a) is also perspsx

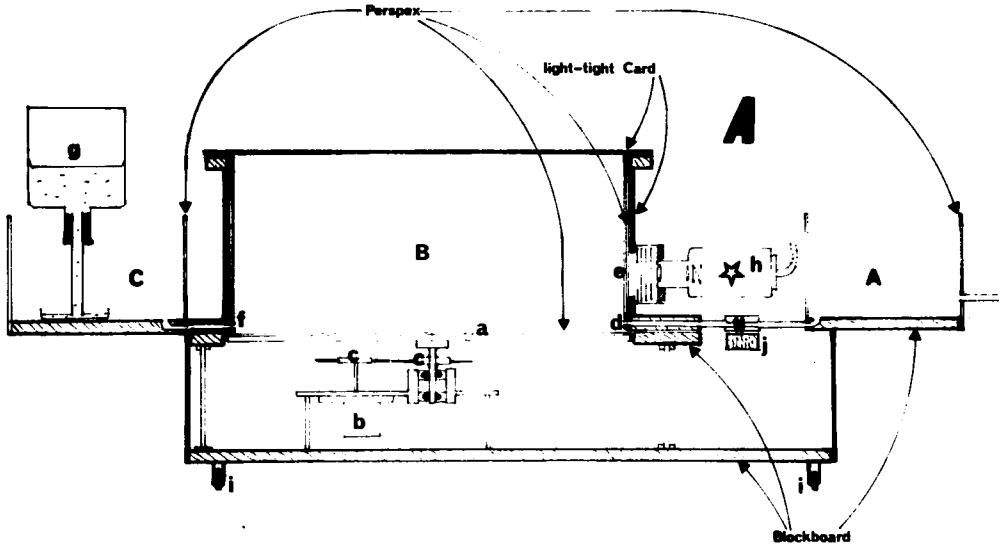
FIGURE A3.1 A Colour Discrimination Arena

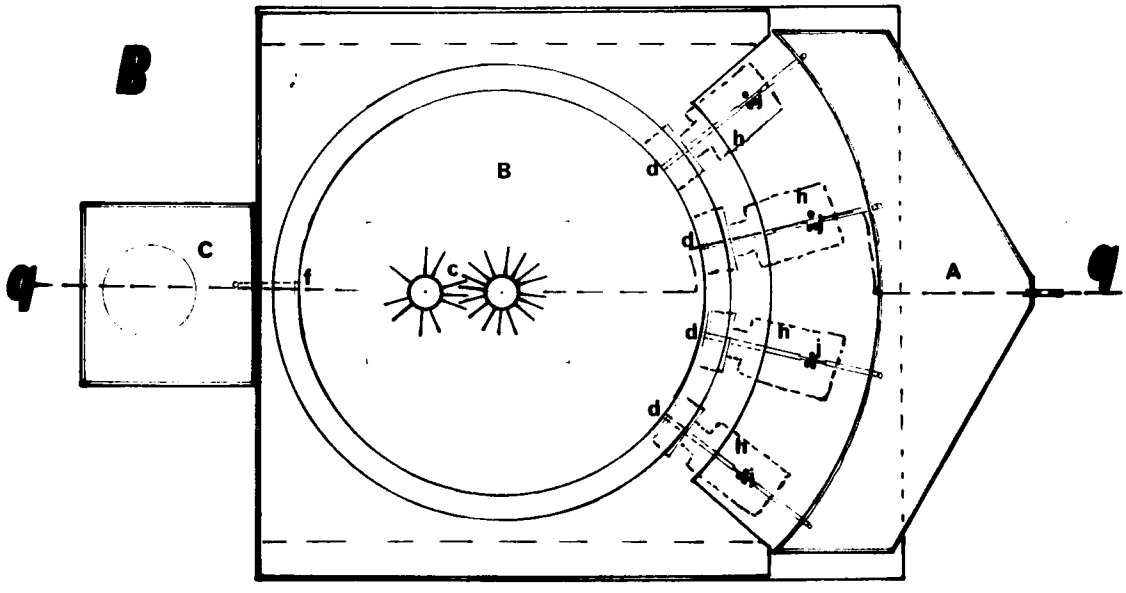
A) Section through q - Side View

B) Plan View

A - Chamber A, see text
B - The experimental arena, chamber B, see text
C - Chamber C, see text

a) Arena floor
b) 240volt, 6rph., syncronous motor made by Smiths Industries Ltd.
c) Drive cogs
d) Exits from arena to the nest
e) White screen
f) Exit from arena to food supply
g) Air pressure drip feed sugar solution vendor
h) Watson System 70 microscope intense lamp
i) Adjustable legs for setting the arena floor horizontal
j) Photocell head


Perspex
light-tight Card
A
B
C
A
g
h
e
f
a
c
d
j
b
i
i
Blockboard


B
B
C
A
f
c
d
h
j
i

covered in card but is mounted on a central spindle on ball races which allows the floor to revolve. The floor is driven round by a 6 rph. synchronous motor (b) at irregular intervals for irregular periods of time by means of two large cogs (c). The cog on the motor-shaft has a number of teeth missing and does not always mesh with the cog on the floor support shaft. The floor assembly is screened from daylight and no visual cues are present from below. The roof of the arena is black and also light tight.

The four entrances from chamber A (d) enter the arena just above floor level and are equally spaced at 25° from each other around the arena circumference. Above each entrance a 3.75 cm. sq. window is cut in the blackout cover providing four white screens (e). Back-projection through each screen is possible using four small microscope intense lamps (h). Each lamp lens is equipped with a slide carrier containing a blackout frame to ensure that only light via the filters enters the arena through the windows. When not in use each window can be sealed with a shutter.

Directly opposite the four exits to the nest (d) there is another exit (f) which goes directly to chamber C. This chamber contains only an air pressure drip feed sugar solution vendor (g) (see Appendix II Fig. A2.2).

The four photocell counter heads are situated between chamber A and chamber B and the pathway is modified as in Fig. A4.12. The counter circuits and power supply are placed at a distance from the

apparatus.

The floor is designed to revolve so that no trail pheromone, if present, could act as a direction signal. The floor of the arena is set horizontal by means of the adjustable legs (i) so that gravity and slope can not act as cues. Apart from a maximum of four 2 cm. diameter visual signposts (each lamp is shuttered to project a 2 cm. diameter spot of light on the screen), no direction indicators are present in the arena. Ants are prevented from leaving chambers A and C by a strip of P.T.F.E. painted on the vertical walls.

The apparatus was run for a test period, but it was found that the optical system (Fig. A4.12) and the back projection system (h) were inadequate and as research time was getting short and the learning experiments involved in this experiment would be time consuming, it was decided that no experimental results would be possible and that the apparatus concept and design should be added to the thesis as an appendix.

APPENDIX IV

Electronic Circuits

A) A Simple Photo-electric Counter

a) A Rotation Monitor

b) A Flicker Monitor

B) A Directional Photo-electric Counter

C) A simple A.C. Amplifier

A) A simple Photo-electric counter

a) A Rotation Monitor (see Section 2.20).

A circular disc with a segment missing is attached to the cylinder driveshaft and the photo-electric counter positioned so that the disc interrupts the light between the pilot bulb and the phototransistor. This arrangement provides a flash of light once every revolution of the cylinder.

The modulated output obtained from the phototransistor T11 is fed into transistor T12 and amplified. The output from T12 drives a small D.C. relay. T12 is protected by resistor R34 which limits the maximum collector current and diode D3 provides protection against large back E.M.F. spikes when the relay latches. The relay triggers a 6 digit electromagnetic counter capable of 2,400 counts per minute. Used with a stop clock, the counter provides accurate monitoring of the stimulus speeds. (For circuit and layout diagrams see Fig.A4.1.)

b) A Flicker Monitor (see Section 2.35).

The same circuit as used in the rotation monitor but the sensitive face of the phototransistor is pointed towards a spot of light shining onto the striped, rotating cylinder (out of sight of the experimental animal) and the relayis replaced with an output attenuator. The modulated output from the phototransistor T11, provided by the reflective differences between the black and the white stripes on the cylinder, is amplified by transistor T12 and the output fed into an oscilloscope to provide a test trace against which a trace from the experimental preparation can be compared. As the counter gives

FIGURE A4.1 A Rotation Monitor

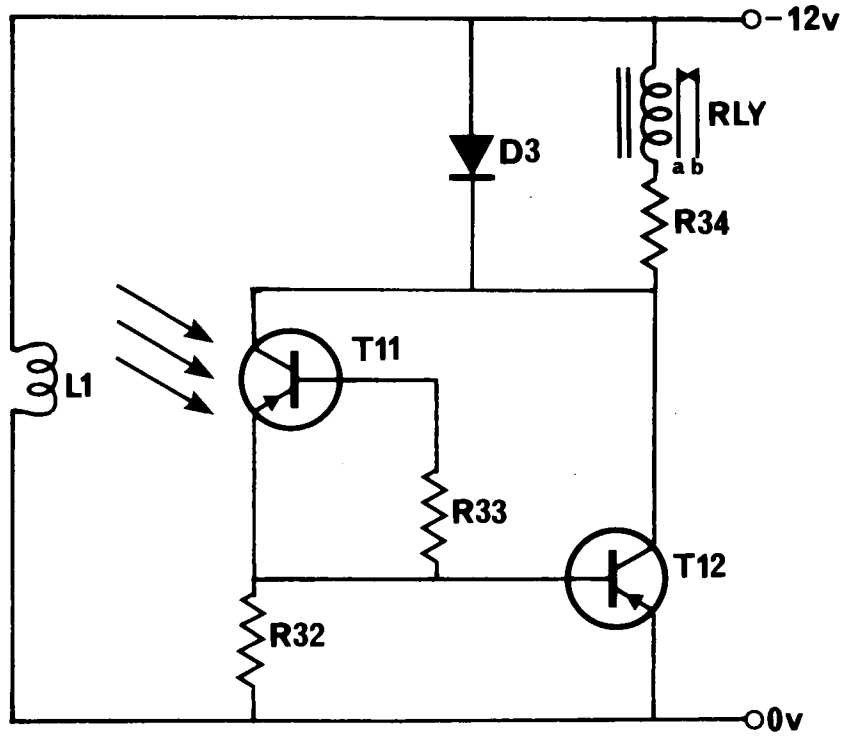
A) Circuit Diagram

Components	
R32	330ohms
R33	4K7
R34	390ohms
R35	500ohms variable
D3	OA81
T11	OCP71
T12	OC83
Rly	6volt, 335ohms, D.C. relay (Radiospares) normally open
L1	L.E.S. pilot bulb, 12-14volt 0.75watts

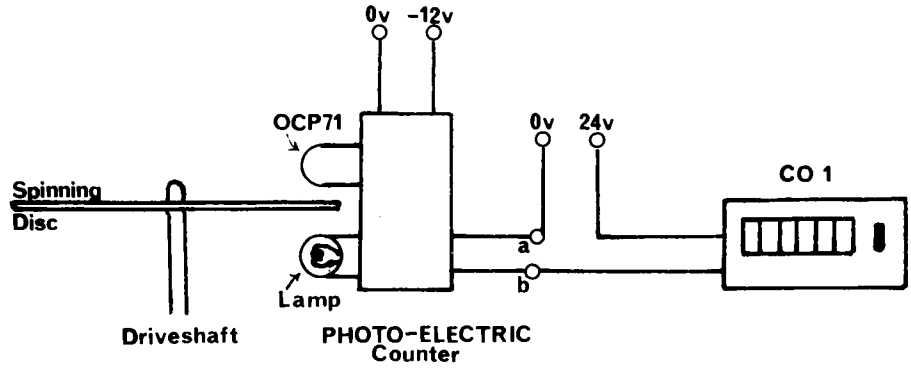
B) Layout Diagram

C01 24volt, 6 digit resetable counter. 180ohms coil, and made by Lancashire Dynamo Electronic Products Ltd.

A) Circuit Diagram



B) Layout



a maximum output signal of several volts it is provided with a variable attenuator (R35) so that the wave-form displayed on the cathode ray tube can be reduced to a reasonable amplitude. (For circuit and layout diagrams see Fig. A4.2)

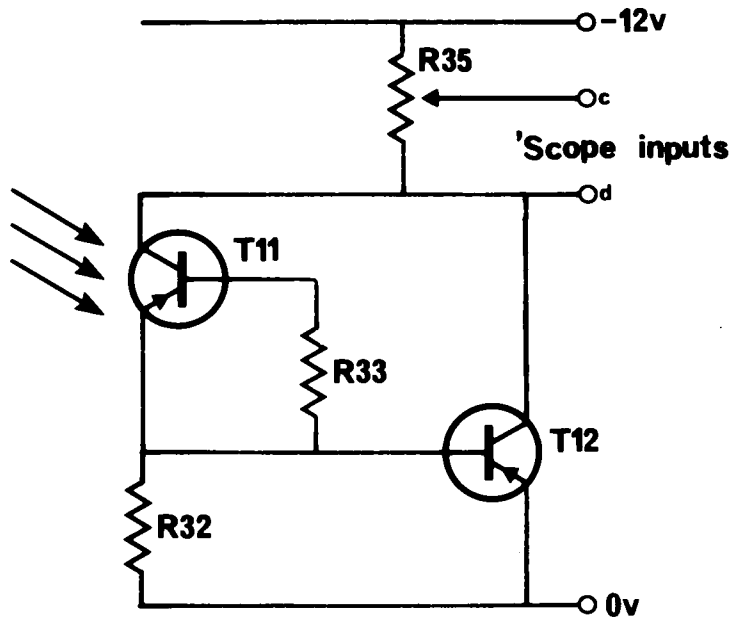
FIGURE A4.2 A Flicker Monitor

A) Circuit Diagram

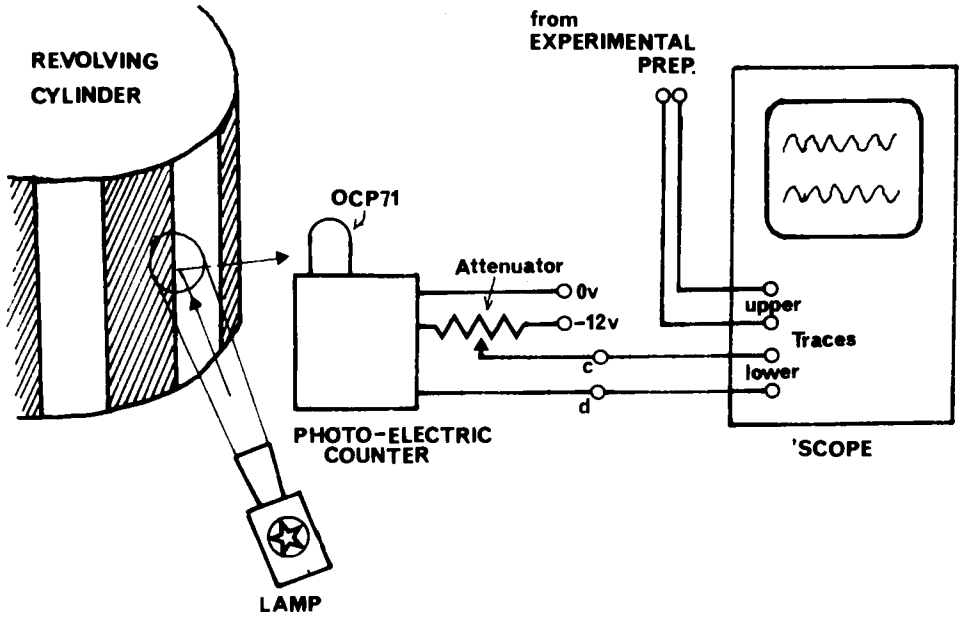
components as for Figure A4.1

B) Layout Diagram

A) Circuit Diagram



B) Layout



B) The Day-Callow Directional Counter

The circuit was originally designed by Mr. D.J. Day, Institute of Sound and Vibration Research, Southampton University and subsequently modified and improved by Mr. G. Callow, Department of Physics, Southampton University. The working requirement is that the counter should be able to monitor movement along a single pathway (a tube) independently in both directions and give continuous independent readout for each direction of movement. Thus all ants travelling one way are monitored and all ants travelling the other way are also separately monitored.

Due to the small size of ants, the only transducer acceptable for this purpose is a photo-electric device, no other methods being practical or sensitive enough.

A single photocell is not able to indicate direction of movement and thus a simple logic sequence must be built into the circuit.

With three photocells labelled A, B and C, a circuit can be designed in such a way that if an ant passes the three photocells (obscuring them) in the sequence A then B then C, then one relay will be triggered. Alternatively an ant obscuring the photocells in the sequence C then B then A will trigger another relay.

This logic sequence can be carried out with only two photocells A and B, thus simplifying the circuitry, assuming that it is possible to obscure both photocells simultaneously. Thus the sequence will be:

A, A + B, B or B, B + A, A

where for A + B and B + A both photocells are obscured. In addition, safety factors can be built into the system whereby if an ant obscures A, A + B then A again, it does not trigger a relay, similarly B, B + A then B again. Thus ants would have to complete the sequence A, A + B B or B, B + A, A before they were counted, preventing an ant moving backwards and forwards in front of the photocells and being counted many times.

Basically the counter consists of two identical circuits linked together in such a way that they affect each other, either in preventing transmission of signals or in providing summation of signals. Fig. A4.3 shows a block wiring diagram. The Photocell unit contains an amplification stage, transistor T9 and sensitivity adjustment at resistor R31. For circuit and construction diagrams see Figs. A4.5 and A4.12. Fig. A4.4 is a complete circuit diagram which can be split down into the separate units as in Figs. A4.5 - A4.10.

Description of Circuit Operation

This applies to the cycle A obscured - A + B obscured - B obscured - both illuminated. For the reverse cycle, for A read B and for B read A.

When photocell A is obscured, schmitt trigger A fires (Fig. A4. 11,1) and 2)) and drives the collector of transistor T5A, in the lockoff bistable, positive (Fig. A4.11,3) whilst the collector of transistor T5B drops to a low voltage (Fig. A4.11,4) thereby

FIGURE A4.3 Block Wiring Diagram of the Day-Callow Directional Counter

(A.O.T. = Adjust on test)

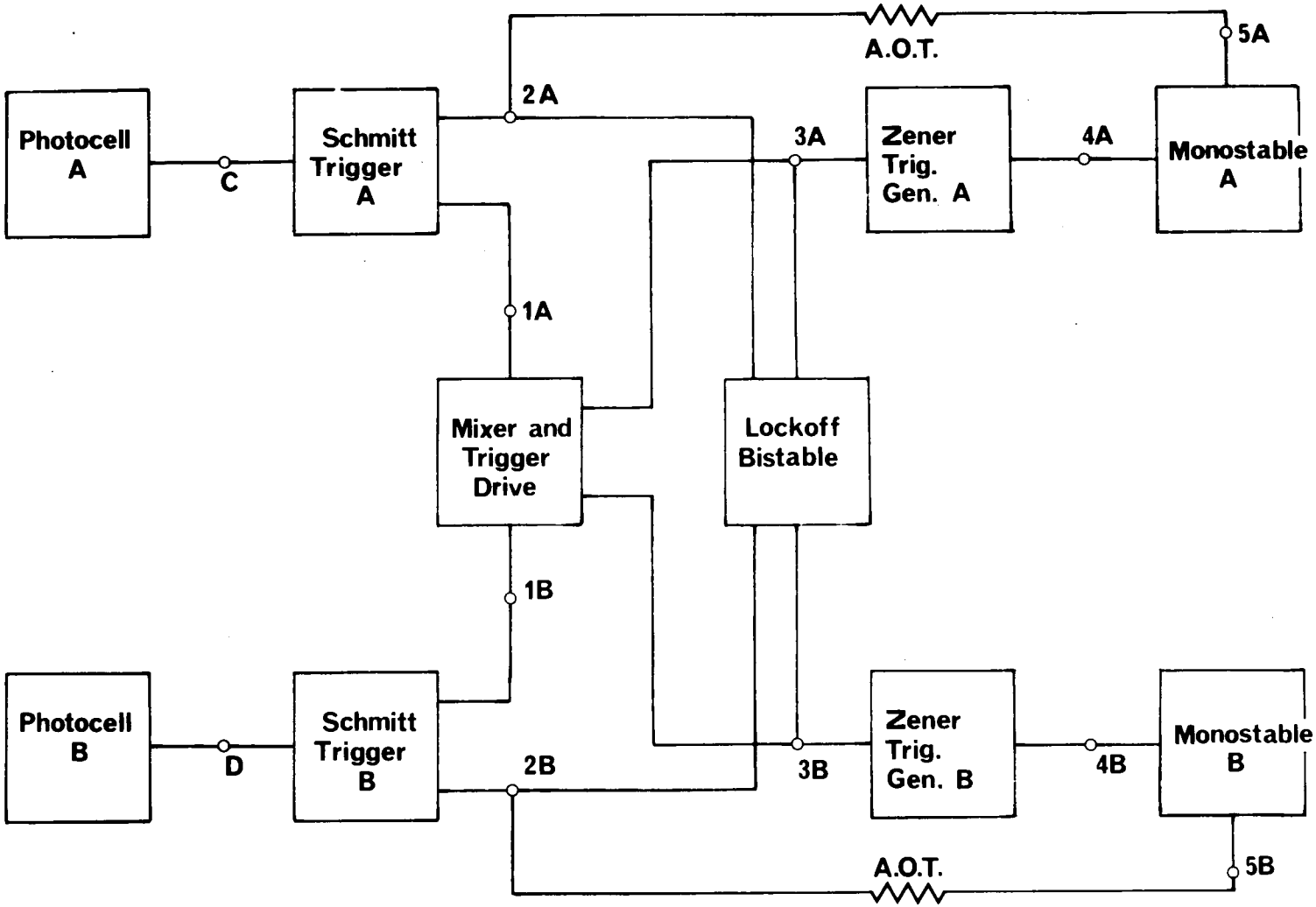

A.O.T.
5A
2A
Photocell A
C
Schmitt Trigger A
3A
Zener Trig. Gen. A
4A
Monostable A
1A
Mixer and Trigger Drive
Lockoff Bistable
1B
Photocell B
D
Schmitt Trigger B
2B
3B
Zener Trig. Gen. B
4B
Monostable B
A.O.T.
5B

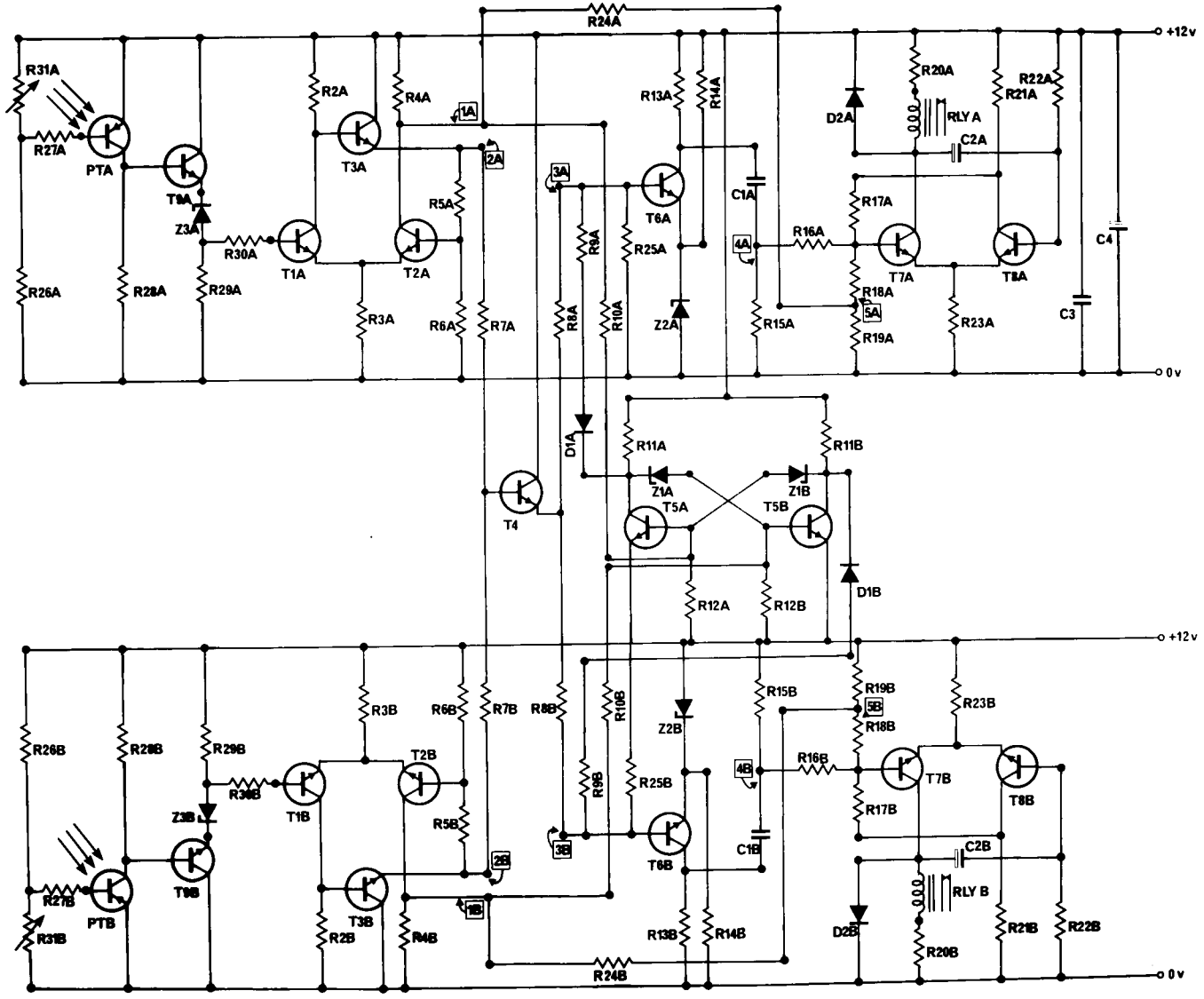
FIGURE A4.4A Complete Circuit Diagram of the Day-Callow Directional Counter

* R8A and R8B may need altering to make transistor T6 bottom only on receipt of a second input step function

** R20A and R20B, approximately 100ohms, are selected for optimum relay operation without excessive current in transistor T7

*** R24A and R24B, 10K - 27K, selected starting at high values and reducing by preferred value steps until the appropriate relay just triggers reliably. Too low a value holds the relay energised or causes continuous pulsing

FIGURE A4.4B Component Values


R24A
+12v
R31A
R27A
PTA
T9A
Z3A
R30A
T1A
R2A
T3A
R4A
1A
2A
R5A
T2A
R26A
R28A
R29A
R3A
R6A
R7A
3A
R9A
R8A
R10A
R13A
R14A
T6A
R25A
Z2A
C1A
4A
R16A
R15A
R20A
D2A
RLY A
C2A
R22A
R21A
R17A
T7A
T8A
R18A
5A
R19A
R23A
C4
C3
0v
D1A
R11A
R11B
Z1A
Z1B
T4
T5A
T5B
D1B
R12A
R12B
+12v
R26B
R28B
R29B
R30B
T1B
Z3B
T9B
PTB
R27B
R31B
R3B
R6B
R7B
R8B
T2B
R5B
2B
3B
1B
T3B
R2B
R4B
R10B
R9B
R25B
Z2B
T6B
C1B
4B
R15B
R16B
R19B
5B
R18B
R17B
T7B
T8B
C2B
RLY B
D2B
R20B
R21B
R22B
R13B
R14B
R23B
R24B
0v

COMPONENT VALUES FIGURE A4.4B

Resistors				Capacitors		
R	Value(ohms)	R	Value(ohms)	C	Value(F)	V. Wkg.
1	68K	17	6K8	1	100nF	20v.
2	10K	18	3K3	2	25uF	16v.
3	220ohms	19	10K	3	1nF	16v.
4	3K3	20**	100ohms	4	500uF	25v.
5	3K3	21	10K			
6	1K	22	10K	Zener Diodes		
7	33K	23	220ohms			
8*	18K	24***	A.O.T.	Z	Voltage	W. max.
9	3K3	25	18K			
10	10K	26	22K	1	7.5v.	400mw.
11	3K3	27	4K7	2	3.3v.	400mw.
12	3K3	28	10K	3	4.7v.	400mw.
13	4K7	29	4K7			
14	560ohms	30	100K			
15	47K	31	220ohms (preset)	All Diodes = OA95 (germanium)		
16	18K					

All resistors $\frac{1}{4}$watt.

Transistors

PTA and PTB .....Mullard OCP71 p-n-p phototransistor
T1 to T8 .....Texas BSY65 n-p-n medium gain silicon
T9 .....Texas 2N1302 n-p-n low gain germanium

Relays

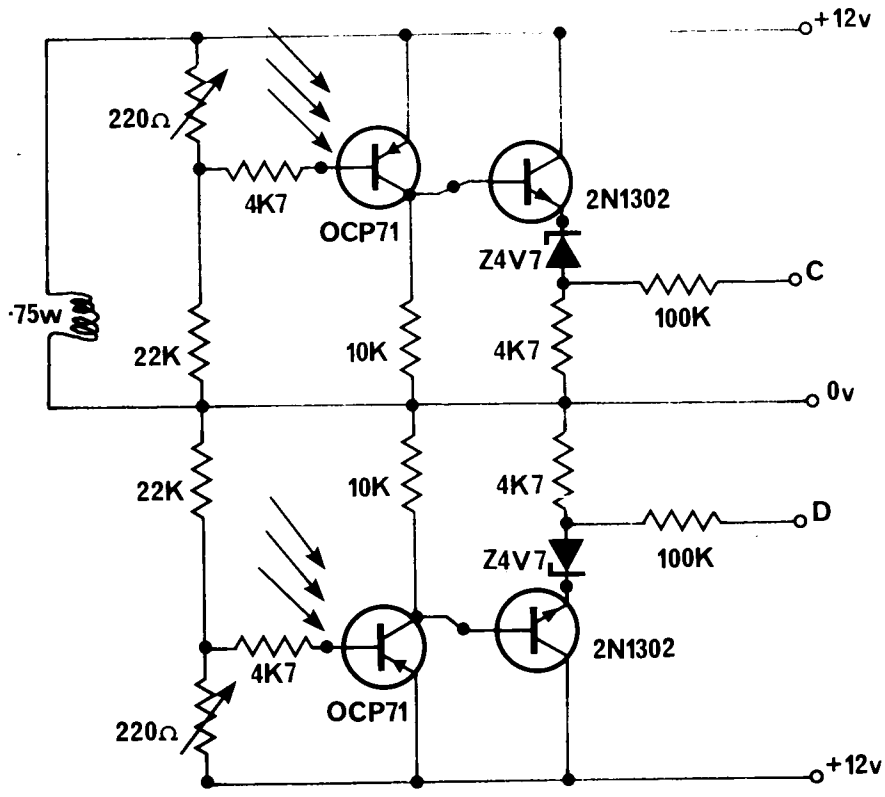
Radiospares miniature open type. Single pole, 6volt D.C., 335ohm coil, normally open.

L1 .....L.E.S. pilot bulb, 12-14volt, 0.75watts

FIGURE A4.5 Photocell Head

(sensitivity adjustable at 220ohm preset)

FIGURE A4.6 Schmitt Trigger


+12v
220Ω
4K7
OCP71
2N1302
Z4V7
C
100K
·75w
22K
10K
4K7
0v
22K
10K
4K7
D
Z4V7
100K
2N1302
4K7
OCP71
220Ω
+12v

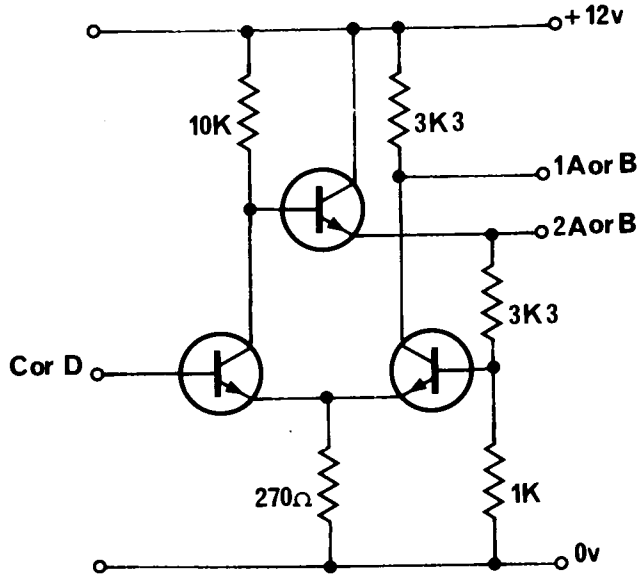
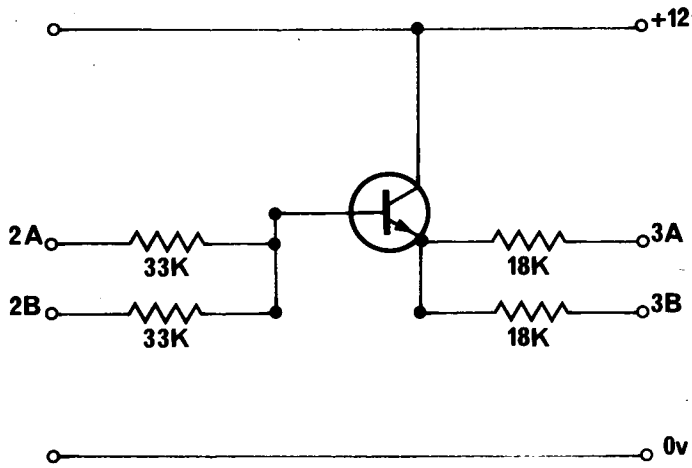
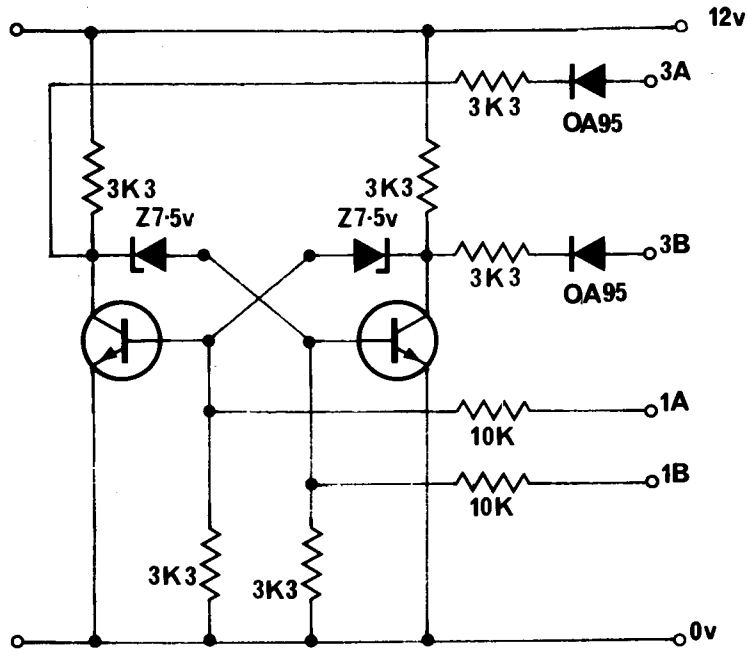

+12v
10K
3K3
1A or B
2A or B
3K3
C or D
270Ω
1K
0v

FIGURE A4.7 Mixer and Trigger Drive

FIGURE A4.8 Lockoff Bistable


+12v
2A
33K
2B
33K
3A
18K
3B
18K
0v


12v
3A
3K3
OA95
3K3
Z7·5v
3K3
Z7·5v
3B
3K3
OA95
1A
10K
1B
10K
3K3
3K3
0v

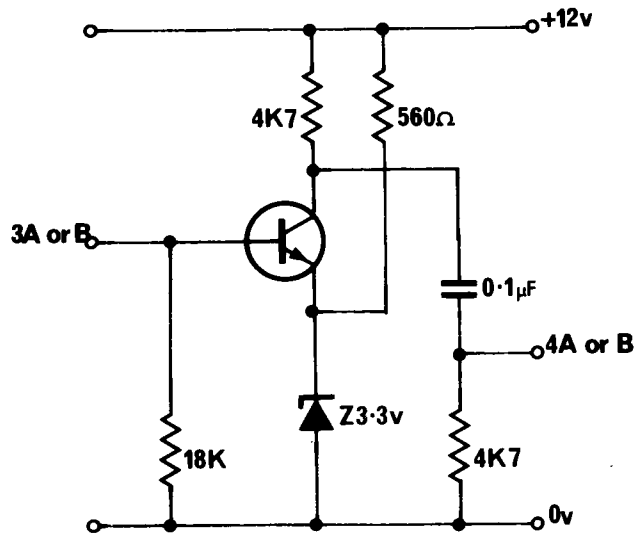
clamping the B zener offset trigger generator transistor T6B firmly off. Simultaneously a positive step function appears at point 1A (Figs. A4.4 and A4.11,2)) and brings points 3A (Figs. A4.4 and A4.11,5)) and 3B each up to between 3 - 6 volts. Point 3A applies this voltage to the A zener offset transistor T6A but 3B has negligible effect on the B zener offset stage transistor T6B because it is locked off by the lockoff bistable. Also simultaneously point 2A applies a negative lockoff pulse to the A monostable via R24A (Fig. A4.11,9)) which can thus not fire prematurely.

When photocell B is obscured and schmitt trigger B fires, the negative pulse applied to the base of transistor T5B in the lockoff bistable (cf. 2A) does not cause the bistable to change state because this base is firmly clamped by the 7.5 volt zener diode from the collector of transistor T5A (Fig. A4.11,3)). Hence no step function can occur on the B zener offset stage input, but the emitter follower mixer and trigger driver transmits the positive step function to the A zener offset stage which now fires (Fig. A4.11,5)) producing a negative step at the collector of transistor T6A (Fig. A4.11,6)) and a sharp negative pulse at point 4A by differentiation in the network C1A, R15A (Figs. A4.4 and A4.11,7)). This pulse has no effect on the A monostable which fires only on positive changes of input voltage.

Now when photocell A is re-illuminated, schmitt trigger A returns to the quiescent state and the A zener offset stage transistor T6A switches off (Fig. A4,11,5) and 6)) giving a sharp positive pulse from the differentiator (Fig. A4.11,7)). Simultaneously the lockoff

FIGURE A4.9 Zener Offset Trigger Generators

FIGURE A4.10 The Monostable


+12v
4K7
560Ω
3A or B
0·1μF
4A or B
Z3·3v
18K
4K7
0v

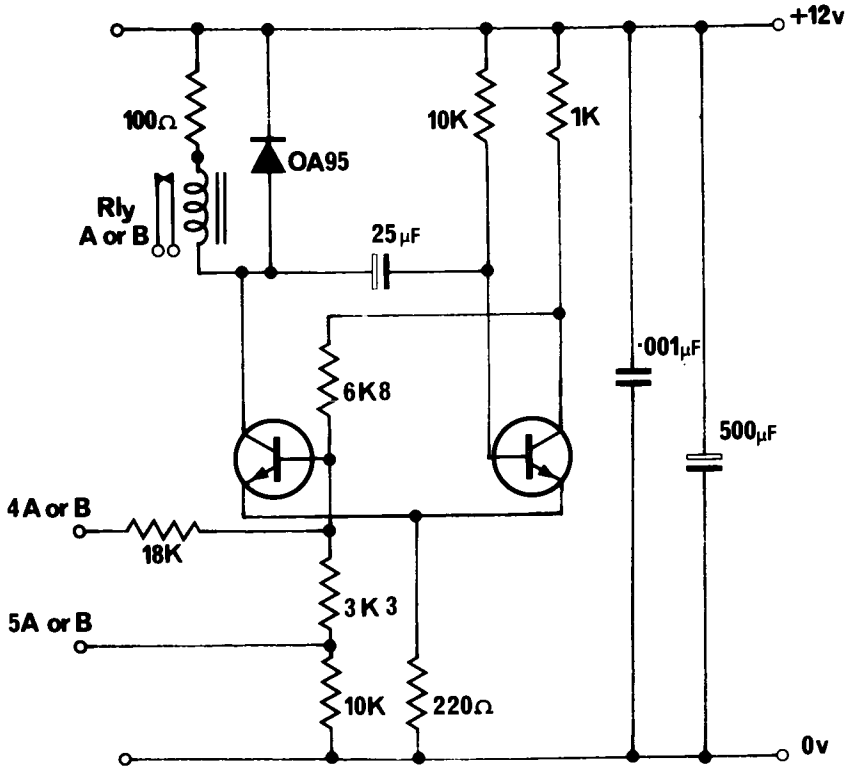

+12v
100Ω
OA95
10K
1K
Rly
A or B
25μF
·001μF
6K8
500μF
4A or B
18K
3K3
5A or B
10K
220Ω
0v

FIGURE A4.11 Pulses observed in the circuit.

1) Schmitt A output pulse to Lockoff Bistable and A.O.T. resistor

2) Schmitt A output pulse to Mixer and Trigger Drive

   point 3A has a very small positive voltage change and point 3B has a small negative change, the lockoff pulse

3) The A collector in the Bistable is blocked from the A Zener Offset Stage by diode D1A

4) The B collector in the Bistable transmits to the B Zener Offset Stage via diode D1B causing the base of T6B to stay at a low voltage

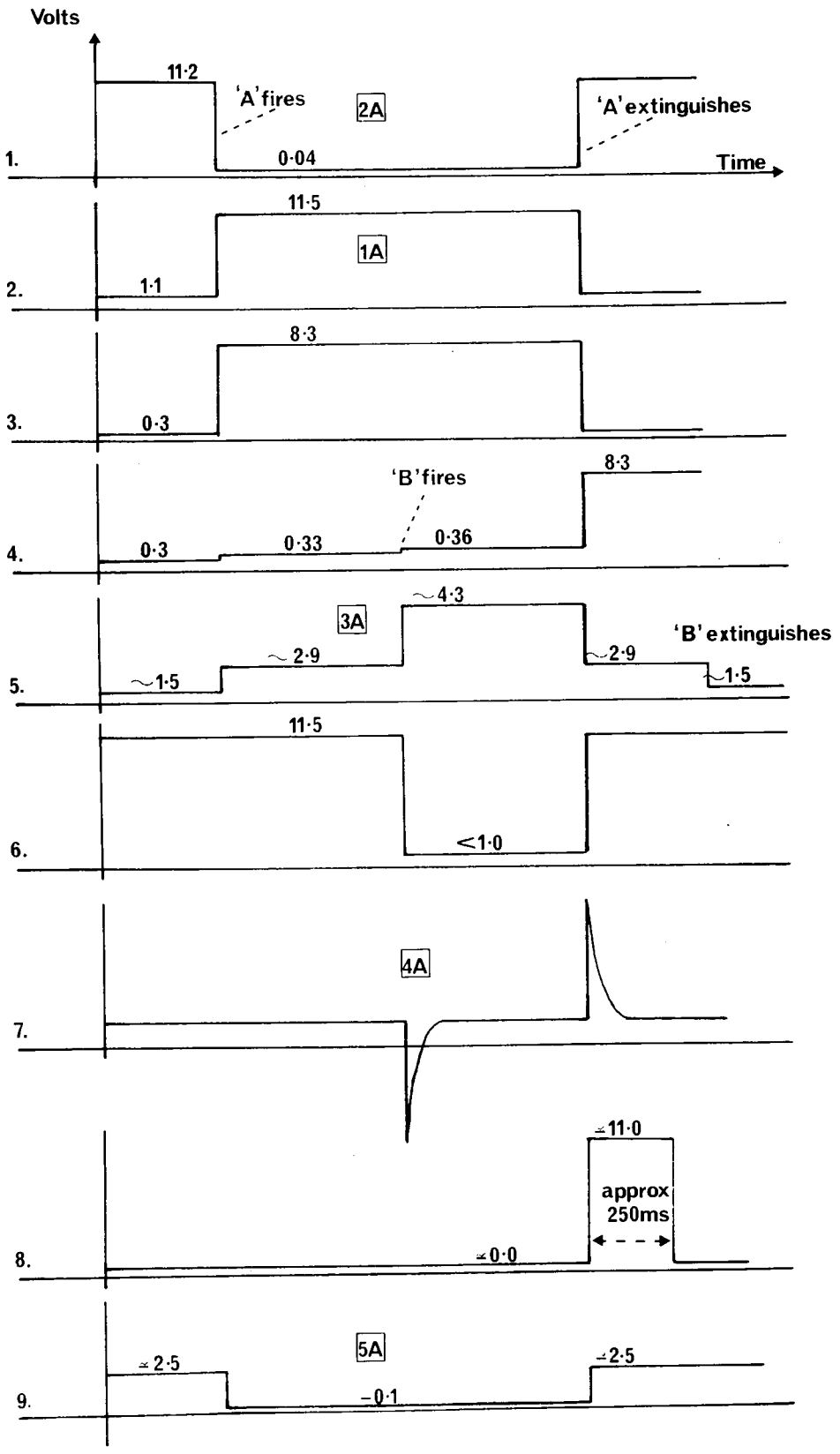
5) The 4.3volt positive pulse at point 3A exceeds the zener voltage and causes the pulse in 6) below

6) This pulse on the Zener Offset Stage collector is differentiated to the two pulses in 7) below

7) Voltage at point 4A. The negative pulse has no effect on the circuit, the positive pulse triggers the Monostable as shown below

8) Voltage across relay A

9) Point 5A. The negative pulse on the A Monostable input prevents early firing and a similar pulse on the B Monostable prevents the possibility of it firing when A extinguishes


Volts
11·2
'A' fires
2A
'A' extinguishes
1.
0·04
Time
11·5
1A
2.
1·1
8·3
3.
0·3
8·3
'B' fires
4.
0·3
0·33
0·36
~4·3
3A
'B' extinguishes
~2·9
~2·9
5.
~1·5
~1·5
11·5
6.
<1·0
4A
7.
≃11·0
approx
250ms
8.
≃0·0
5A
≃2·5
≃2·5
9.
−0·1

pulse from point 2A to monostable A is removed (Fig. A4.11,9)), thus allowing the positive pulse to fire the monostable (Fig. A.4.11,8)) completing the count as the relay latches during the astable state of monostable A.

The lockoff bistable switches over at this point (Fig. A4.11,4)) allowing the step function from B schmitt trigger to affect the B zener offset stage. This however is only one of the two necessary step functions needed to fire monostable B on re-illumination and consequently when photocell B is re-illuminated the circuit resets and no B count follows. Each monostable is set to latch its relay for approximately 250 milliseconds (Fig. A4.11,8)).

The four counter circuits (see Appendix III) are run from a single 12 volt stabilised power supply. A fully stabilised supply is necessary because otherwise, interference between the channels can cause erroneous counting. The power supply, counter circuits and digital readout counters are mounted together on a Dexion frame, the photocell heads being discrete components attached to the main circuits via screened cables. The eight relays are connected to eight 1000 counts per minute digital electro-magnetic counters. As is common with inductive sources, the digital counters when switched on or off produce a back E.M.F. in the form of positive or negative spikes of several milliseconds duration and up to 100 volt peak. These, due to the close proximity of the digital counters to the sensitive relay circuits, at first caused erroneous counting in all circuits. This has been prevented by the addition of a diode across the counter contacts and also by smoothing the 24 volt

counter supply with capacitors across the relay contacts and across the counter contacts (Fig. A4.13). These additions to the circuit clip the back E.M.F. to a level low enough not to upset the balance of the counter circuits.

Fig. A4.14 shows the complete counter system block wiring layout.

C. A Simple A.C. Amplifier (see Section 2.24)

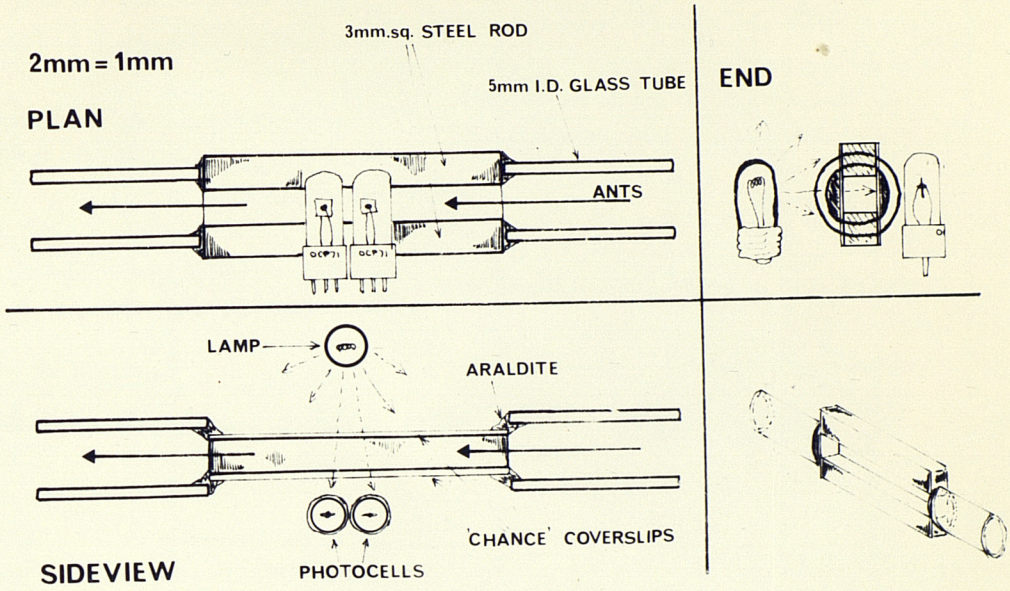
A general purpose amplifier - the circuit as shown in Fig. A4.15 is a conventional cascade three transistor configuration in which each transistor is directly coupled to the next without use of the usual coupling capacitors. This direct coupling enables very low frequency signals to be passed.

Negative feedback is provided by resistor R39 which lowers the gain and gives a flatter frequency response. R36 is variable to provide variable gain from 100 - 1000x according to the relationship GAIN = R39/R36. The maximum output is 8 volts peak to peak.

FIGURE A4.12 Construction Diagram of the Photocell Counting Chamber

FIGURE A4.13 Wiring Diagram of Diode D4 and Capacitors C5 and C6 to prevent back E.M.F.s set up when the digital counters latch

Components	
C5	10nF, 400volt working
C6	12.5μF, 40volt working
D4	10D8
C02	Ex-G.P.O. 4 digit counter, 500ohms coil, 1000 counts per minute


2mm = 1mm
PLAN
3mm.sq. STEEL ROD
5mm I.D. GLASS TUBE
ANTS
END
LAMP
ARALDITE
'CHANCE' COVERSLIPS
PHOTOCELLS
SIDEVIEW

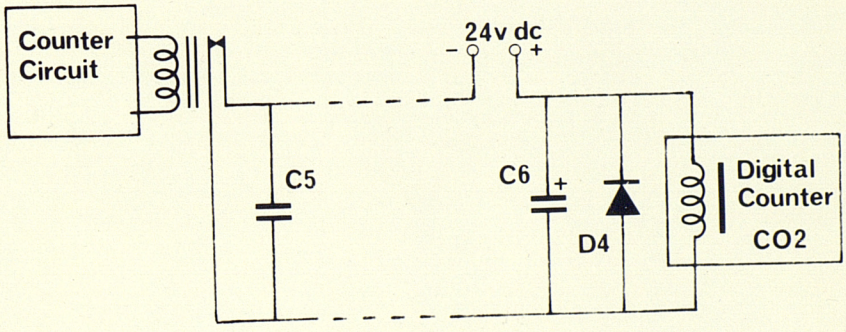

Counter Circuit
24v dc
C5
C6
D4
Digital Counter
CO2

FIGURE A4.14 Block Diagram of the complete counter system.

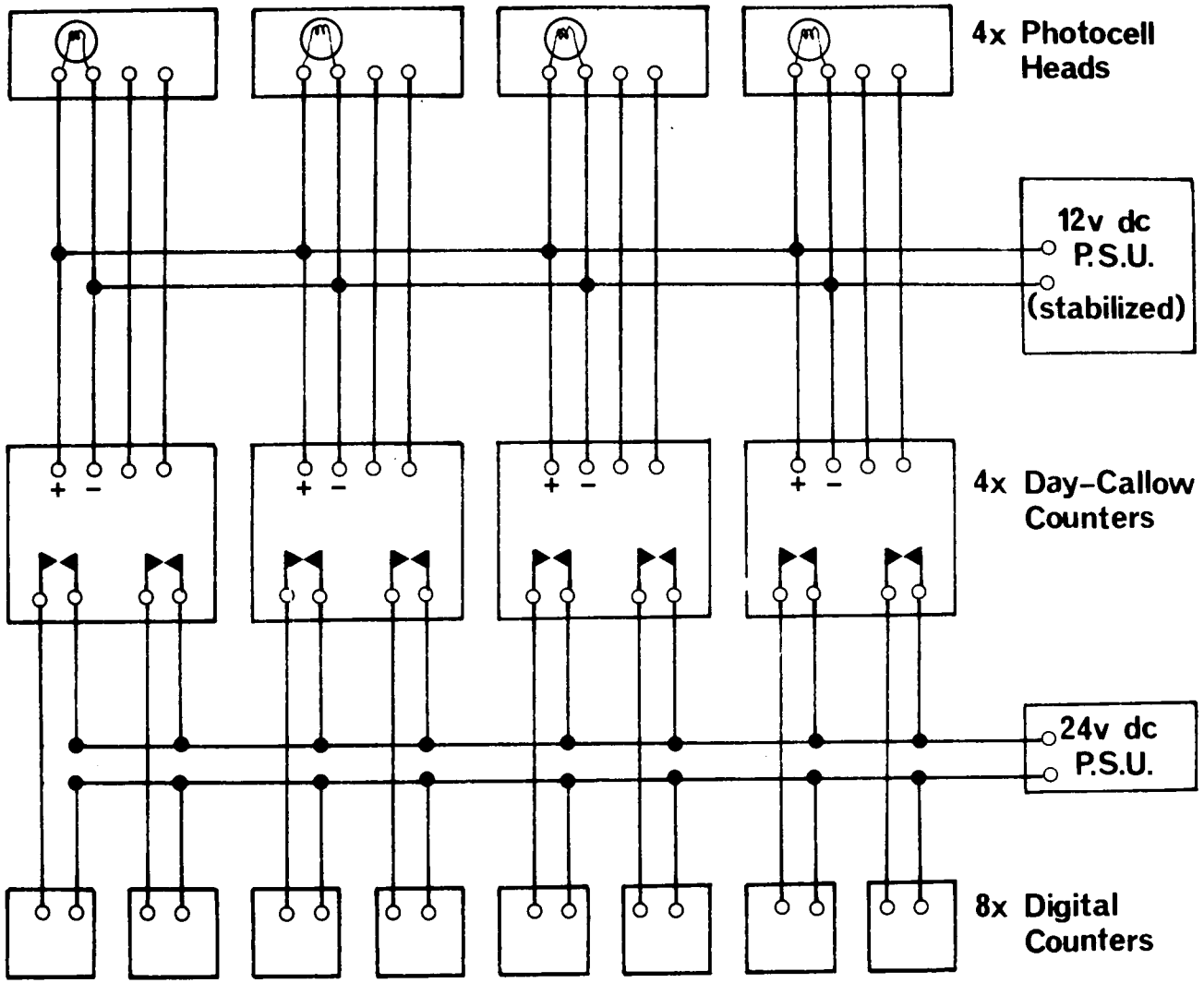
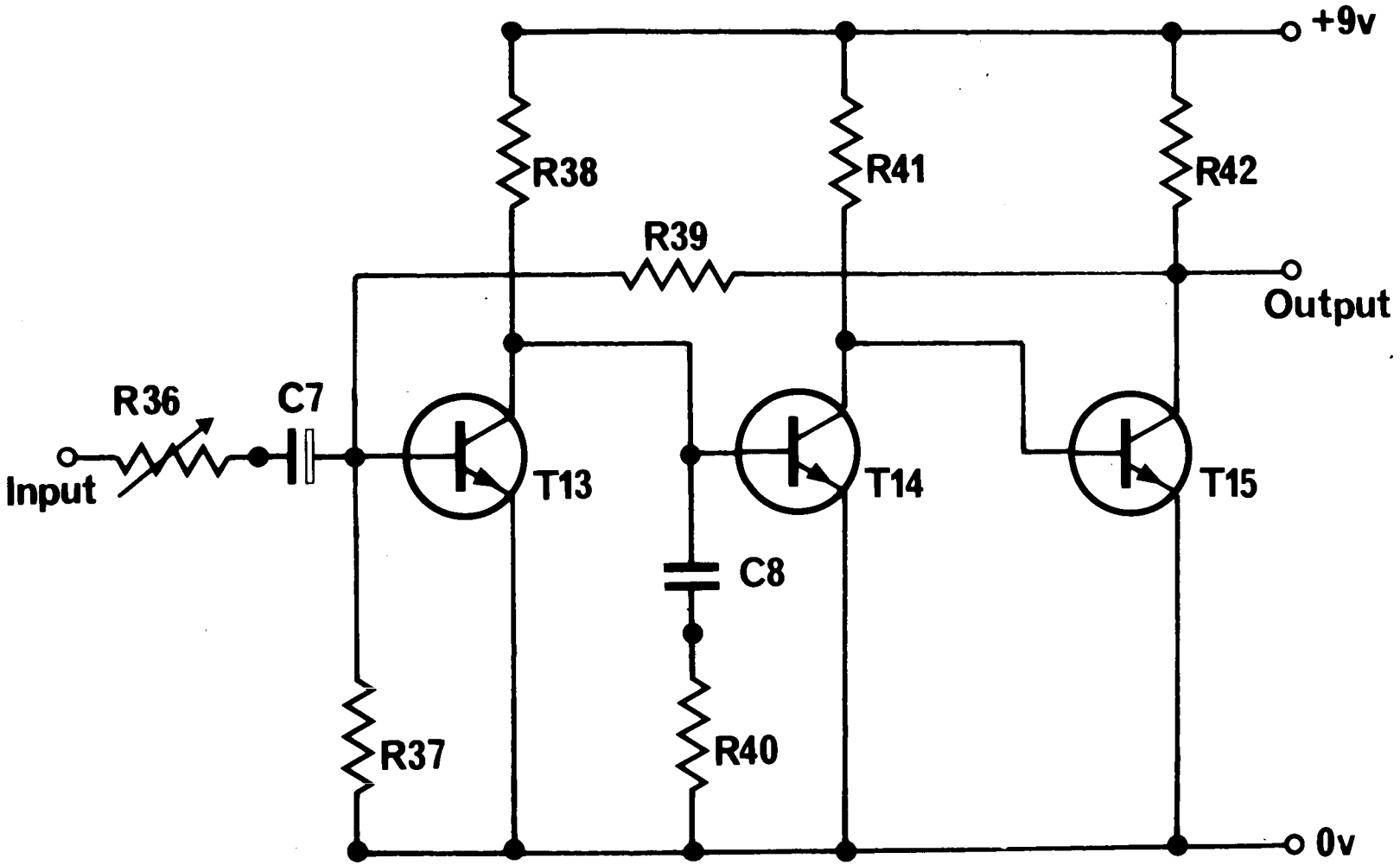

4x Photocell Heads
12v dc P.S.U. (stabilized)
4x Day-Callow Counters
24v dc P.S.U.
8x Digital Counters

FIGURE A4.15 Circuit Diagram of A.C. Amplifier

Components	
R36	15K variable
R37	75K (2x 150K)
R38	100K
R39	470K
R40	100ohms
R41	15K
R42	1K8
C7	25uF 15volt working
C8	100nF 250volt working

Transistors all S.G.S. A1670/1, nearest commercial equivalent is S.G.S. C764 n-p-n high voltage amplifier


+9v
R38
R41
R42
R39
Output
R36
C7
Input
T13
T14
T15
C8
R37
R40
0v

APPENDIX V

Electrophysiological Apparatus

(Schematic diagrams)

A List of Apparatus used in the Electrophysiological experiments

TABLE A5.1

A) Preamplifier: Tektronix type 122, A.C. coupled, three-stage battery operated amplifier. Gain 100x or 1000 x, with allowable input of 0,02 volts or less p-p at maximum gain. Single or double ended input.

B) Beck Binocular Microscope: Fitted with very low power objective lenses and a slide manipulator.

C) Intense Microscope Lamp: 24 volt, 150 watt Quartz Iodine lamp made by Projectina A.C., Heergebrugge, Switzerland.

D) Camera Shutter: As fitted to C).

E) Micromanipulators: Made by Prior.

F) Light-tight Box: Made of thick black card, light-tight, but providing easy access to the preparation.

G) Wire Cage: To screen the preparation from stray electrical signals.

H) Black-out Cloth: Forming a light-tight drape round the complete apparatus set-up.

J) Experimental Preparation: Specimen of Formica rufa worker.

K) Oscilloscope: Cossor model 1049 Mk.III, double beam.

L) Oscillograph Camera: Cossor model 1458. Fitted with a hood for either a 4" or a 5" screen.

M) Audio Amplifier: One valve low power, amplifier.

N) Speaker: 3 ohm, 6" diameter.

O) Camera Flash Synchronisation Switch.

P) Watson Binocular Microscope: Low power objective lenses and fitted on a swinging arm.

Q) Stroboscope: Griffin Xenon Stroboscope type L15-740.

R) Intense Microscope Lamp: 6 volt, 48 watt tungsten lamp made by Vickers Instruments Ltd.
R_1 Adapting and illumination lamp.
R_2 Illumination lamp for Flicker Monitor V).

S) Oscilloscope: Tektronix type 502 dual-beam.

T) Screen and Bay window for Stimulus Cylinder.

U) Stimulus Cylinder: 7.5 cm. diameter brass cylinder on ball races with a stimulus pattern round the circumference.

V) Flicker Monitor: See Appendix IV.

FIGURE A5.1 Schematic diagram of apparatus used to find the visual sensitivity curve of F. rufa. See Sections 2.28 - 2.31.

(Labelling as in Table A5.1)

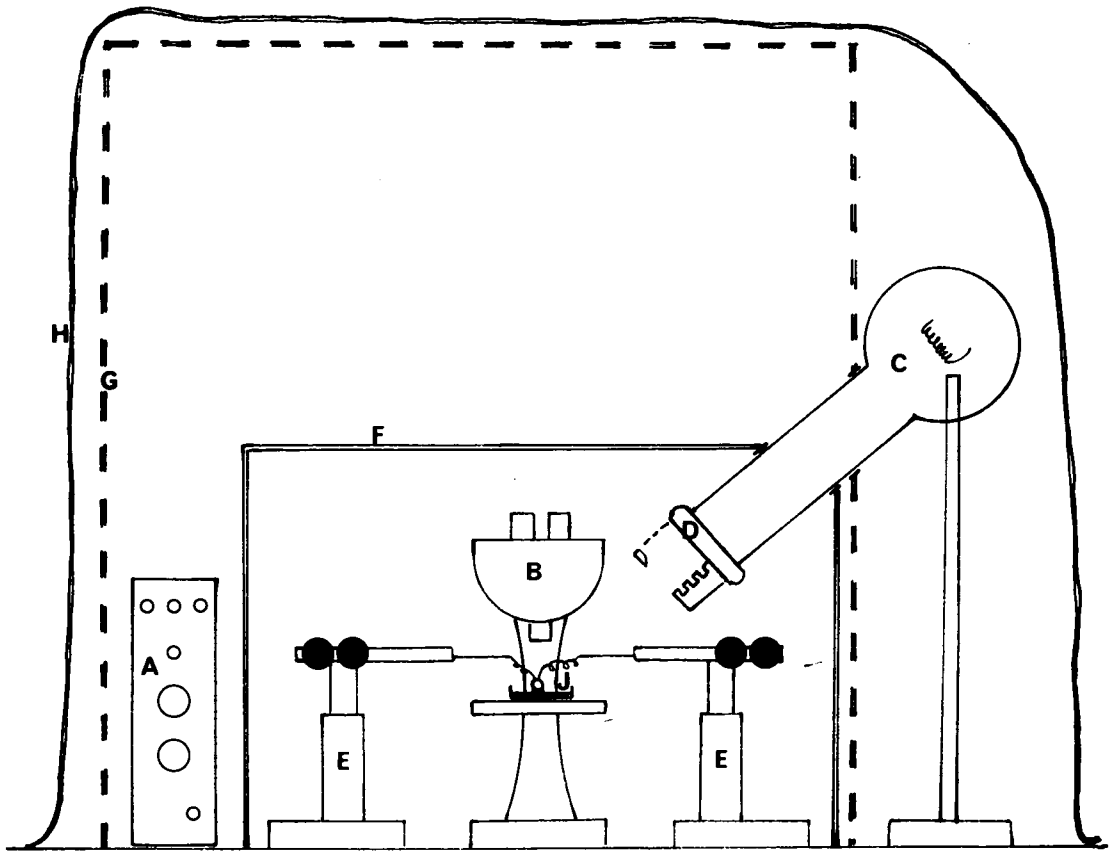
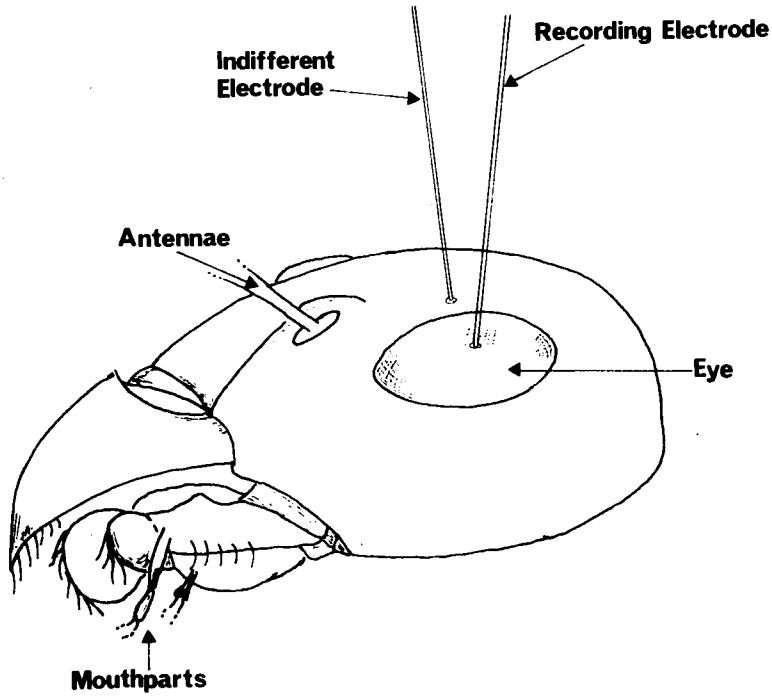

H
G
F
C
B
D
A
E
E

FIGURE A5.2 Diagram of the head of *Formica rufa* (worker) to indicate the positions of the recording and indifferent electrodes.

FIGURE A5.3 Block wiring diagram of the apparatus shown in Figure A5.1.

(Labelling as in Table A5.1)


Indifferent
Electrode
Recording Electrode
Antennae
Eye
Mouthparts

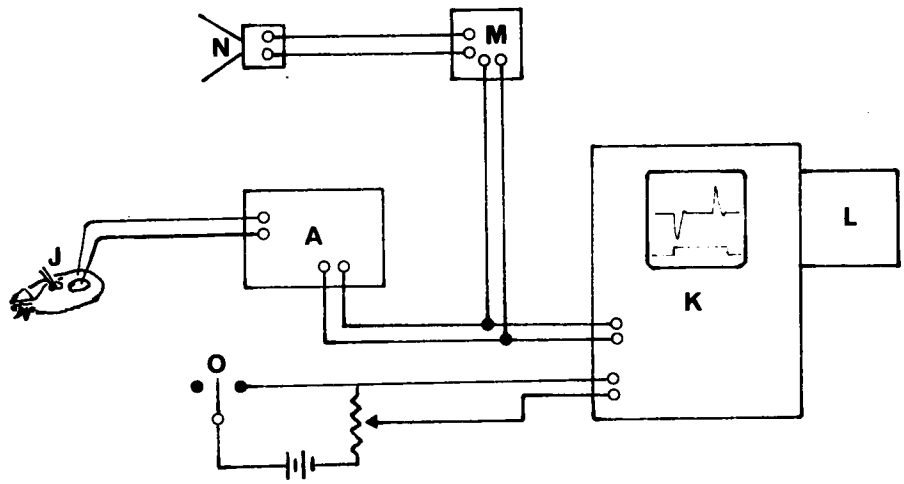
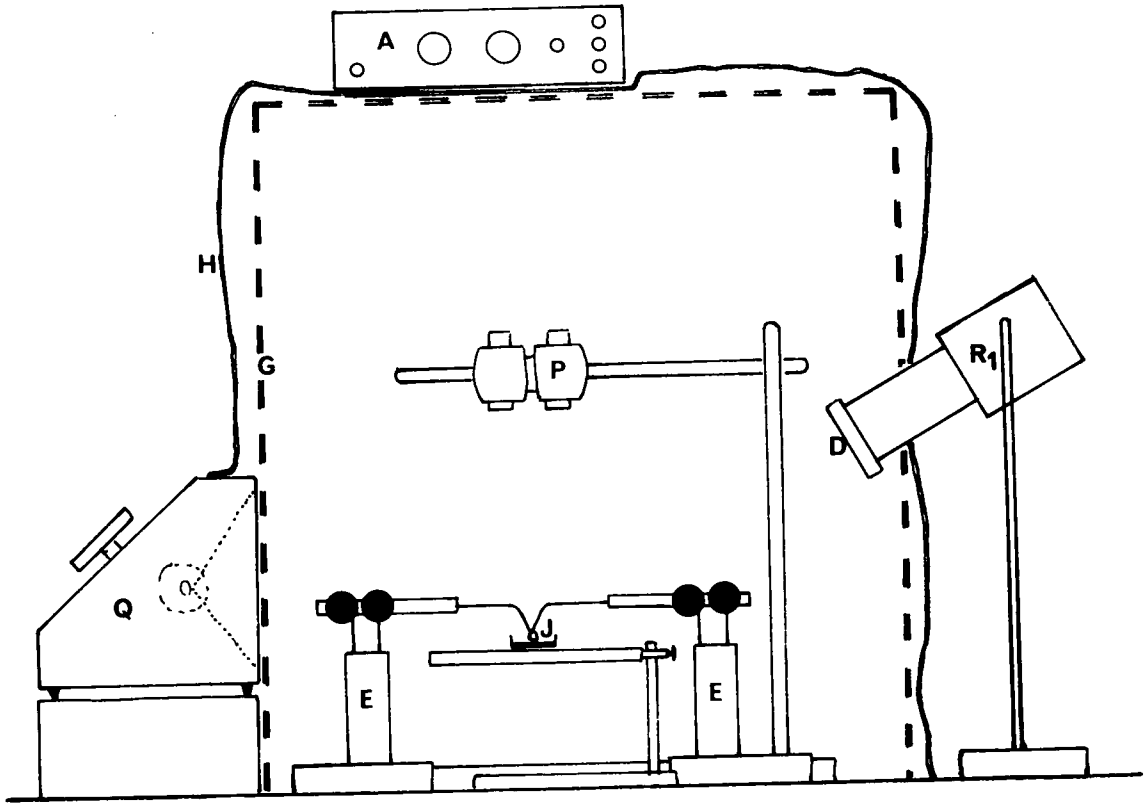

N
M
A
J
K
L
O

FIGURE A5.4 Schematic diagram of the apparatus described in Sections 2.32 - 2.34.

(Labelling as in Table A5.1)

FIGURE A5.5 Block wiring diagram of the apparatus shown in Figure A5.4

(Labelling as in Table A5.1)


A
H
G
P
R1
D
Q
J
E
E

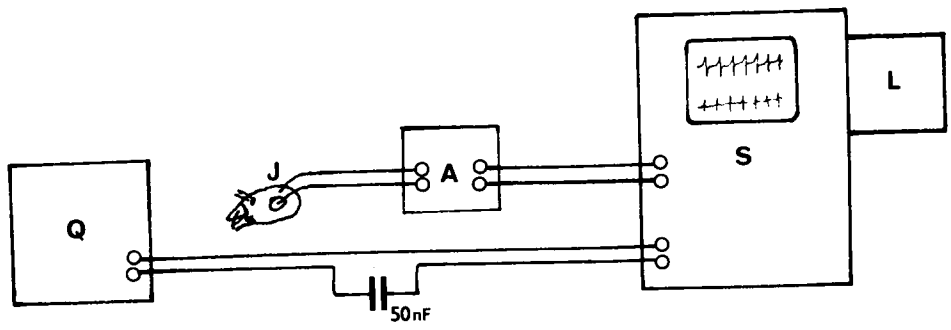
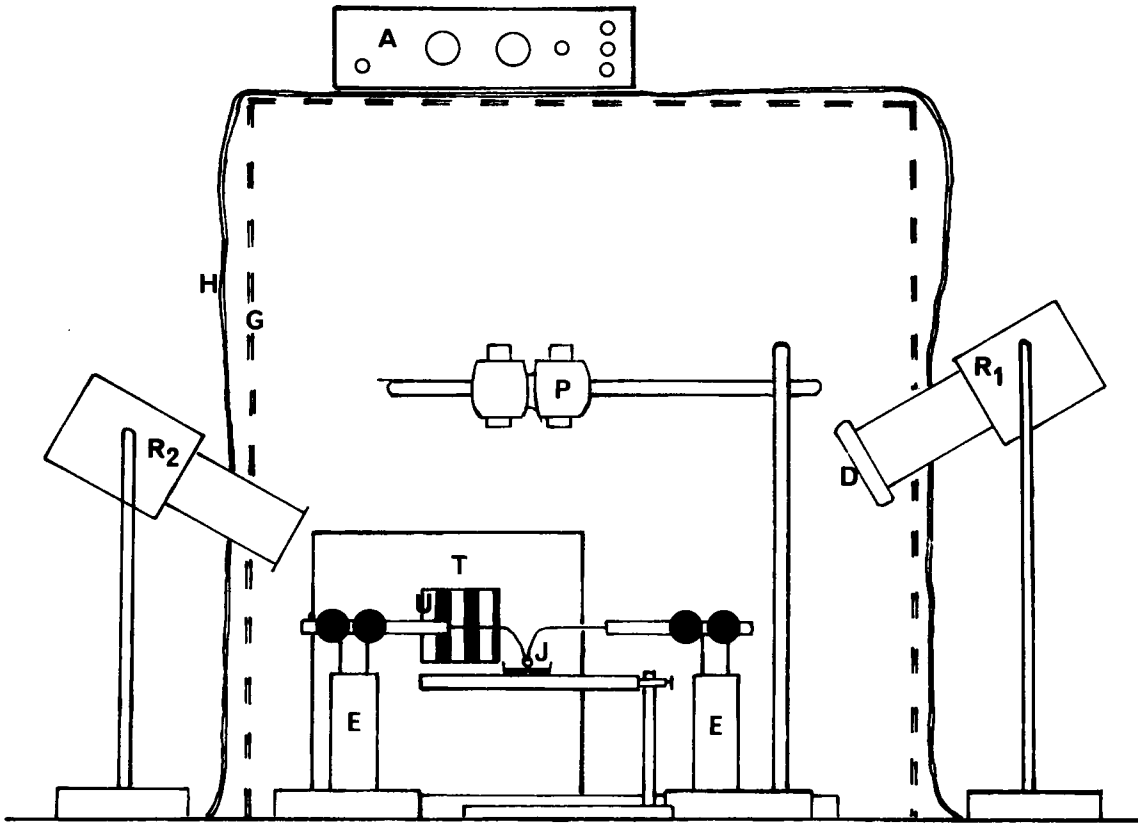

L
S
J
A
Q
50nF

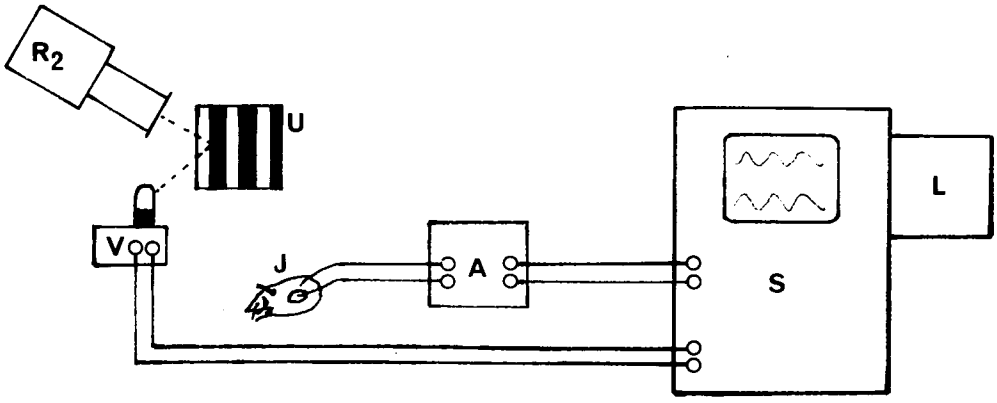
FIGURE A5.6 Schematic diagram of the apparatus described im Section 2.35.

(Labelling as in Table A5.1)

FIGURE A5.7 Block wiring diagram of the apparatus shown in Figure A5.6.

(Labelling as in Table A5.1)


A
H
G
P
R2
R1
D
T
U
J
E
E


R2
U
V
J
A
S
L

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