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Escape, Hiding, and Freezing Behaviour Elicited by Electrical Stimulation of the Chick Diencephalon

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Abstract. A largely medial escape, hide and freeze (EHF) system has been plotted in the chick diencephalon, from which escape or freezing can be elicited peripherally as alternative responses. Supra-threshold stimulation in the core of the system yields escape mixed with freezing and hiding.

The EHF system starts in the rostral anterior hypothalamus and runs back through the medial dorsal hypothalamus. A lateral extension occurs at the entry of the hypothalamic component of the occipito-mesencephalic tract. Posterior to this point the system shows a ventralward extension which does not include the putative nu. ventromedialis, but coincides instead with medial and periventricular fibres. The pre-optic area, lateral hypothalamus and mamillary area are all free of e, h and f sites.

The EHF system thus corresponds well in distribution with the mammalian defensive threat-escape system. In both mammal and bird, similar behaviour is elicitable from both the diencephalic escape system and the central mesencephalic grey. The two are probably connected in the bird also by periventricular routes, some part of which can already be identified by e, h and f sites. Other properties of the EHF system, such as its role in the control of vocalisation, and its activation by non-reinforcement have been discussed elsewhere.

Key Words
Diencephalic
stimulation
Chick
Escape
Hiding
Freezing

Introduction

The pioneer work of HESS [1957], followed by that of HUNSPERGER [1963], demonstrated a functional system in the diencephalon and mesencephalon of the cat from which defensive threat, attack and fleeing could be obtained by brain stimulation. More recently CHI and FLYNN [1971a, b] have shown that the hypothalamic sites from which such behaviour can be elicited are connected by descending fibres to the central mesencephalic grey (CMG).

A comparable functional system with a similar distribution is described here in the domestic chick. The resemblances between bird and mammal are close and it seems likely that the two systems are homologous. In the chick the system has an anterior and a posterior division within the hypothalamus. The posterior division appears to receive the hypothalamic ramus of the occipito-mesencephalic tract (HOM) but it is here argued that the remainder of the system, in particular its mesencephalic portion, is not directly associated with the tract.

Threshold activation of the system evokes escape or freezing, the two patterns being mutually exclusive. More complete activation yields hiding, in which elements of both escape and freezing are combined. The involvement of the system in vocalisation, which has been discussed elsewhere [ANDREW, 1973] adds a further resemblance to mammals.

Materials and Methods

Male domestic fowl chicks (Warren Sex-link, Southdown, Uckfield) were used throughout. They were separated on arrival from the hatchery (day 2) into individual living cages, which had a floor space 20×20 cm and contained chick starter food spread thinly over a white absorbent paper floor, and a water dish. The cage sides were 30 cm high. Three were black and a fourth of perspex; the latter faced a window in the wall of the cooled incubator (25 °C) in which each cage was housed so as to allow video recording. The incubator was lit through a window cut in its roof. Electrodes were implanted on day 2 under barbiturate anaesthesia (0.025 ml 60 mg/cm³ nembutal for a 40-gram bird) and stimulation was carried out on days 4, 5 and 6 either in the home cage itself or in a cage and chamber of identical appearance.

Stainless steel insect pins insulated with Formvar varnish (diameter: 0.29 mm uninsulated, 0.40 mm insulated), with the pointed tips ground down flat so as to expose a circular conducting tip of approximately 0.20 mm diameter, were used as electrodes. A female amphenol micro-miniature connector was attached to each to allow connection to the stimulating lead. They were implanted, usually in pairs, to stereotactic co-ordinates taken from an atlas prepared for this strain of chick, the relevant planes of which are figured in this paper. The co-ordinates shown are for 2- to 3-day-old chicks, weighing 40-42 g and are accurate to ±0.05 mm in the better explored parts of the brain; they apply reasonably well on days 4 and 5 and up to weights of 45-50 g.

A modified mouse stereotactic instrument was used, with a bite-bar whose cross-section was an inverted triangle. The lower edge of the upper bill rested on the upper edge of the bite-bar at a point immediately below the posterior edge of the external naris. The upper edge lay 1.50 mm below the ear-ear line. When the head is so placed, the bite bar can be moved 1mm or more in the antero-posterior plane without rotating the head about the ear-ear line. This placement also has the advantage that sites back to the mid-brain are accessible without passing through

the tentorium. The ear-ear line was taken from a line engraved on a removable brass rod with a depression at either end into which fitted the rounded tips of the ear bars. The vertical and antero-posterior axes were fixed, after the scalp had been deflected to expose the skull, from a point in the midline of the skull roof and in the ear-ear plane. The midline was taken as the midpoint of the narrow space between the medial approximated edges of the still largely cartilaginous frontal bones (such a skull roof point is usually 11.05–11.08 mm above the ear-ear line). Before the chick was placed in the apparatus, the down was clipped from around each meatus, so that they could be clearly seen. The ear bar was inserted into the meatus firmly, but without unnecessary force, since the skull is elastically deformable at this age. Small windows were cut in the skull roof with very fine scissors to allow the insertion of electrodes which were secured by dental cement after the skull roof had become completely dry. Two other short lengths of pin were inserted at a divergent angle to each other at the rear of the skull and were also included in the cap of cement to give additional security. Skull growth caused movement of the electrode tips and eventually loosening of the skull cap in the second week of life; this was rarely a problem in the present study, in which birds were sacrificed on day 6. When tip movement had occurred it was obvious from the histology.

Pairs of 1 msec rectangular pulses of opposite polarity and equal magnitude, which were generated by Tektronix pulse generators and passed through Grass RF stimulation-isolation units so that they were free-floating with respect to ground, were used in stimulation at a rate of 100 pairs/sec. Current was monitored across a 100- Ω resistance in series with the bird and is given as a peak-to-peak value; flow was between the tip of the monopolar electrode and an indifferent return provided by a broadly exposed wire running between skin and neck muscles.

Flexible leads were used, which were suspended from an overhead counter-balanced arm which allowed free vertical movement and also continuous rotation; the last was made possible by using contacts dipping in circular mercury filled tracks. Free feeding and locomotion was possible after the leads had been connected to the electrodes before stimulation and chicks rarely showed any disturbance as a result.

Three schedules of stimulation were carried out at each site in each bird (by R. D. O.) except when this was occasionally impossible, usually because of electrode loosening.

1. *Main survey.* Three stimulation trains of 2.0 sec duration and 0.25 mA intensity were administered at a rate of one every 10 sec. This test was carried out on different days at 25 °C and at 12 °C.

The lower temperature caused the chick to give a series of loud peeps, and occasionally to show spontaneous escape behaviour. It was used in order to reveal sites which interrupted such behaviour. In general, data from this series of stimulations have not been used in classifying sites (below).

2. *Threshold survey.* The lowest current at which a clear response of some sort (e.g. head or body movement, calls) could be obtained was found; this was commonly 0.06–0.04 mA, but might be as high as 0.2 mA or as low as 8 μ A. Stimulation trains of 2.0 sec at this threshold value were then administered at a rate of one every 10 sec for a period of 20 min at a temperature of 25 °C (i.e. that of the home cage). Three stimulations at the beginning and three at the end of the session were recorded.

3. *20-sec threshold survey.* A threshold train of 20 sec duration was given at 25 °C.

Four tests in all were thus administered at each site. Tests were separated by at least 1 h for any particular bird and, except for test 1 at 12 °C, periods when the chick was disturbed were avoided. Data from a study of intracranial self-stimulation (ICS), which used different subjects, have also been considered here in the first part of the results section. The only forced stimulation test administered to such birds was test 1 at 25 °C with the complication that at some sites currents of 0.2 or 0.3 mA were used in place of 0.25 mA, usually because such a current had proved effective in ICS.

Birds were perfused on day 6 under deep barbiturate anaesthesia with electrodes still in place. The dorsal aorta was clamped, and the right ventricle laid widely open; 5 cm³ of bird Ringer's solution, followed by 20 cm³ of Susa's fixative were injected into the left ventricle. Sections were stained with cresyl violet (echt). Electrode tracks were exposed to the tip, which was readily localised.

All test sessions were video recorded (Shibaden VTR), and then transcribed during replay on to an Esterline-Angus event recorder and punched paper tape. Complex acts such as fleeing were identified by the same observer throughout (R.J.A.). A number of simple parameters of behaviour were also scored by pairs of observers (usually R.J.A. and R.D.O.); two of these, 'moving the feet' and 'immobility', were used to measure the duration of escape (fleeing) and freezing.

Sites were classified by the following rules: in general when fleeing, hiding or freezing was elicited it was consistently the same for each of the three stimulations recorded. The main exception was that at sites rated as freezing the first stimulus might elicit a dash into a corner, which was followed by sustained freezing with no further locomotion during subsequent stimuli. The behaviour was also usually the same at any one site in both the series of stimulation at 25 °C and that at 12 °C. At 14 sites there were differences of intensity between the two tests. In one type of instance a forced movement was rated as dubious fleeing in one session (usually 12 °C) and clear fleeing in the other. In another, the behaviour during stimulation was rated as immobility rather than definite freezing in one session, and as freezing in the other. The rating from the 25 °C session has been used in all cases except one, because the 12 °C session was more difficult to evaluate, due to the frequency of escape behaviour induced by the cold. The one exception was a site in plane Q which has been rated as freezing based on the 12 °C session, because the behaviour was so well-developed in that session.

Results

High Currents. Distribution of an Escape-Hide-Freeze (EHF) System

The behaviour to be discussed here has been classified as follows:

None (0): No recognisable fleeing, hiding or freezing. Forced movements of head and body were common, and there might be changes in calling, including the appearance of peeps, which usually accompany fleeing both in normal behaviour and when it is elicited by central stimu-

lation (e sites, below). Movement (m): Violent forced locomotion during stimulation which might have included low-intensity fleeing in a masked form. Escape (e): Escape leaps at side of cage and/or trying to burrow through the side (with movements of bill tip up and down or side to side). Sustained intense running is common. Hide (h): Rush in crouch to corner and burrow into it in a sustained crouch. Crouch (c): Move slowly in deep crouch – here fleeing is probably almost inhibited by freezing. Freeze (f): Immobile in crouch on spot, which is then sustained between stimulations.

In all cases except f, it is only the behaviour during stimulation which is considered. Fleeing locomotion elicited during stimulation sometimes persisted for 0.5–1 sec afterwards.

An EHF system can be plotted from the distribution of e and h sites, which will for the moment be considered together. Freezing sites are mentioned where relevant and considered in more detail later. The anterior-most part of the system as so far plotted is represented by a cluster of sites in the anterior hypothalamus (AH) and at the base of the pre-optic area (fig. 1Q, R). There is some indication that the ventral half of the rostral AH does not support escape and hiding behaviour (2 sites, fig. 1Q), but such sites are present more posteriorly, distributed from the dorsal edge of the AH down to its base.

The EHF system extends caudally on either side of the ventricle (fig. 2T, U) into the dorsal hypothalamus as a backward prolongation of the AH; the nu. paraventricularis magnocellularis (PVM) runs just above it as a backward continuation of the base of the pre-optic area. The escape system continues along the same line through the dorsal hypothalamus for some distance further (fig. 2V, W); thereafter its full caudal extent is not yet mapped. Medial zone 5 may mark its posterior boundary (2 negative sites, fig. 3Y, Z). There is evidence (Discussion) for connections of the system with the central mesencephalic grey, but it is likely that these involve fibres which do not run directly caudad. It should be noted that the medial areas of the dorsal hypothalamus which have so far been discussed include medial and periventricular fibre systems from plane T caudad; zone 3 can be seen to be involved (fig. 2V).

A downward extension of the EHF system begins in plane T with a single medial site. The medial area which is probably equivalent to the mammalian nu. ventromedialis is not involved (1 site, fig. 2T; 4 sites, fig. 2U). Instead, at this level and at no other, e and h sites appear in the lateral hypothalamus and its dorsal and lateral borders (6 sites, fig. 2U, 1

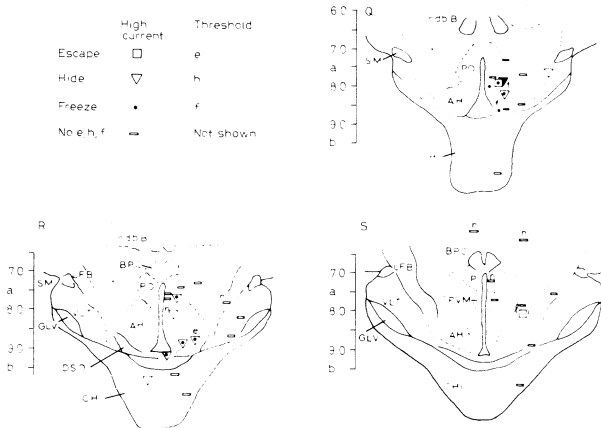


Fig. 1. The absence of e, h and f sites in the PO and the clustering of h sites in the AH should be noted. The numbers used in figures 1-4 indicate the following: (1) field of fibres which includes the medial forebrain bundle; (2) zone of medial and periventricular fibres; (3) ventralward continuation of 2; (4) ventralmost part of 2; (5) zone of fibres which probably correspond with mamillo-thalamic tract; (6) lateral part of mamillary area which contains many fibres, and (7) medial part of mamillary area.

Abbreviations to Figures 1-4 (see also General Note, p. 208)

AC	Commissura anterior	OVD	Nu. ovoidalis
AH	Anterior hypothalamus	P	Area of transition between PO and PVM
BPC	Bed nucleus of commissura pallii	PO	pre-optic area
CHI	Chiasma opticum	Pe	Nu. periventricularis
DL	Nu. dorsolateralis anterior thalami	PH	Posterior hypothalamic area; at some levels no clear demarcation of LH within this area is possible
DM	Nu. dorsomedialis anterior thalami	PLC	Commissura pallii
DSO	Decussatio supraoptica	PMI	Nu. parameianus internus thalami
EM	Nu. ectomamillaris	PVM	Nu. paraventricularis magnocellularis
EP	Area of specialised ependyma	ROT	Nu. rotundus
GLV	Nu. geniculatus lateralis, pars ventralis	SM	Tr. septomesencephalicus
HL	Nu. habenularis lateralis	SpM	Nu. spiriformis medialis
HM	Nu. habenularis medialis	VLT	Nu. ventrolateralis thalami
LFB	lateral forebrain bundle		
LH	lateral hypothalamic area		
ndbB	Nu. of diagonal band of Broca		

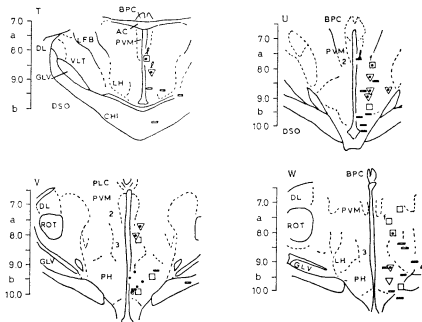


Fig. 2. E, h and f sites continue to be present at the base of the PVM as a prolongation of the AH sites. A cluster of h sites in U marks the entry of the HOM. In V, f and e sites mark the anterior face of the posterior zone of the EHF system. The numbering is explained in figure 1.

site, fig. 2V). These sites coincide with the area of entry into the hypothalamus of fibres of the hypothalamic component of the HOM and are probably associated with these fibres (Discussion). Medial sites appear ventrally in plane V. Here a medial cluster of freezing sites coincides with the appearance of fibres in the posterior face of the putative nu. ventromedialis. Medial e and h sites continue to be frequent in the posteriormost part of the posterior hypothalamic area (PH) (3, fig. 2W; 5, fig. 3Wa). The sites are probably to be associated with vertically running fibres which become conspicuous in the PH at this level, apparently as a continuation of zone 3. It is not certain whether nu. periventricularis, whose appearance has been taken as the posterior boundary of the PH, is really part of the EHF system, since non-escape sites occur close by it. E, h and f sites (1, fig. 3X; 3, fig. 3Y) continue to be present medially until the medial fibre systems disappear with the beginning of the mamillary area (fig. 3Z). The evidence thus suggests that the EHF system posterior to the

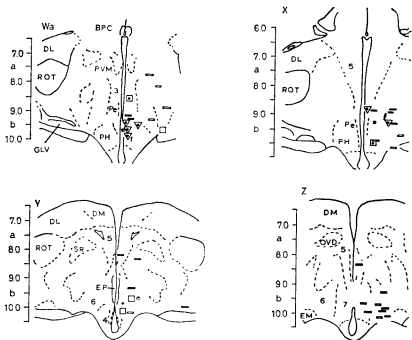


Fig. 3. In Wa a cluster of h sites defines the centre of the posterior zone of the EHF system. E and f sites occur on its posterior face, and the mamillary area (Z) is free of sites. The numbering is explained in figure 1.

AH involves medial fibre systems and their zone of interaction with HOM fibres.

E, h and f sites are almost completely absent in the mamillary area *sensu lato* (^{8/8}, fig. 3Z; ^{10/13}, fig. 4AA). It is possible that scattered sites in the lateral mamillary area, coinciding with fibre tracts running back to the tegmentum, represent posterior connections of the EHF system. However, lesions in the posterior ventral part of the EHF system have not yet resulted in fibre degeneration (see below) at any distance, which can be unequivocally shown to originate within this area. It seems probable that its main commerce is with the adjacent lateral hypothalamus.

The lateral boundaries of the EHF system are poorly plotted as yet. None of the few placements in thalamic nuclei yielded e, h or f responses (3, nu. ventrolateralis thalami, VLT; 2 in or near nu. geniculatus lateralis, pars ventralis, GLV; 4, nu. ectomamillaris, EM); the same was true of the optic radiation (8 sites) with one inexplicable exception (fig. 1R). Three e

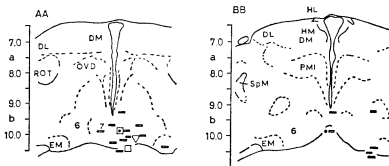


Fig. 4. Almost all sites are negative. Scattered e, h and f sites may coincide with tracts connecting the EHF system with the mesencephalon. The numbering is explained in figure 1.

or h sites on the lateral edge of the dorsal hypothalamus and lateral hypothalamus (LH) (fig. 2V) probably mark the entry of HOM fibres, as has already been noted; further sites might therefore be expected lateral and dorsal to these. The lateral forebrain bundle (LFB) has one definite negative placement within it, so that one e site on its inner face (fig. 1S) may again involve HOM fibres which are difficult to distinguish from LFB at this level. Apart from these probable HOM sites, sites on or just beyond the lateral edge of the dorsal hypothalamus are all free of e, h or f sites (9 sites). With the exceptions in planes U and V, which have already been noted, the LH is also free of e, h and f sites throughout its extent from plane T back to plane X (11 sites). It is probable that, in birds as in mammals, this zone is largely occupied by the medial forebrain bundle (MFB). ZEIER and KARTEN [1971] suggest that the MFB may be plotted in birds as degenerating fibres in the LH following a lesion in the *lobus parolfactorius*. When this is done in the chick (Fink and Heimer staining, 7- to 9-day post-operative survival), a clearly demarcated zone of fibres appears running from field 1 through the LH and lateral mamillary area to a corresponding zone in the anterior tegmentum. All placements in this zone, including field 1 (fig. 1Q, R) as well as more posterior planes (fig. 2W, 3Wa-Z) failed to yield e, h or f responses. It will be shown in a subsequent paper that such MFB sites support intracranial self-stimulation.

The dorsal boundary of the EHF system is well-defined through much of its extent. There is a clear absence of escape and hiding sites in the pre-optic area and anterior PVM (approximately $8/_{11}$ no escape, fig. 1Q,

R, S). Further, the exceptions (fig. 1Q, R) are all on the ventral border of the pre-optic area or PVM, and clearly are to be associated with the upper edge of the EHF system.

Studies using adult fowl [PHILLIPS and YOUNGREN, 1971; PUTKONEN, 1967] confirm in broad outline much of the distribution of the EHF system which has just been described for the chick. Scattered hiding and attack sites are shown in the AH, in the dorsal medial hypothalamus, and ventrally in the PH.

Distribution of Different Sites in the EHF System

The differences in distribution between e and h sites within the EHF escape system are of interest. The former tend to occur on the edges of the system, whilst h sites form its central core. Thus, anteriorly, e sites occur at the base of the pre-optic area and PVM, and also on the upper margin of the escape system or on its lateral boundaries (fig. 1Q, S, 2T, U). The e site in plane V is a possible exception, but descent of the main system may already have begun. This distribution of e sites continues more posteriorly: as h sites descend, e sites remain above them (fig. 2W). However, they are not confined to the dorsal margin of the system. Ventrally, e sites lie both on the anterior and posterior face of the zone of h sites (fig. 2U, V, 3X). Two sites yielding violent forced movements (m) in the plane of X may be equivalent to two further e sites with this distribution. The e sites in the mamillary area and zone 7 may well be peripheral to restricted areas or tracts, capable of supporting h behaviour, which have not yet been identified at this level (above). An m site at this level (fig. 4AA) may be equivalent.

It will be remembered that h sites yield fleeing followed by hiding. Table I shows that their stimulation, unlike that of e sites, is typically followed by an after-response of freezing ($0.01 > p > 0.001$). This usually consists of a sustained crouch in the corner to which the bird has fled. It is thus possible to regard h sites as ones which yield the highest intensity or most complete behaviour, involving fleeing, hiding and freezing, whilst e sites give fleeing alone, because of less complete activation of the escape system. This is confirmed by the absence of h behaviour at threshold currents (below).

However, the above interpretation is complicated by the fact that f sites are associated with e sites, and also appear to be peripheral in the escape system. F sites occur with e sites at the base of the pre-optic area (fig. 1Q). There is a clear cluster, again with closely associated e sites, on

Table 1

Plane	After-response			
	freeze during stimulation		no-freeze during stimulation	
	hide (h)	escape (e)	hide (h)	escape (e)
Q, R	5	1	1	0
S, T	1	1	0	1
U, V	6	1	0	4
W, Wa, X	7	3	1	4
Y, Z, AA	0	1	1	3
Totals	19	7	3	12

Sites at which freezing occurred during stimulation are not considered here; the single h site in the CHI is also excluded as aberrant.

The differences in distribution of a freezing after-response between e sites and h sites is significant: $0.01 > p > 0.001$ (χ^2 , corrected for continuity).

the anterior face of the ventral part of the EHF system (fig. 2V: 4 f and 2 e sites). Another f site occurs amongst e sites on the anterior edge of the mamillary area (fig. 2V). A final site in the interpeduncular recess (through which the oculomotor nerve enters the mesencephalon) probably affected zone 7, which it must have touched, and so may be equivalent to a posterior diencephalic site.

The most likely explanation is that freezing and fleeing (escape) are alternative responses, either of which may be produced when the EHF system is only partially activated by an electrode which lies peripherally within it. The very close association in space of f and e sites at levels V and Y certainly suggests that much the same population of neurons may be effective in producing or facilitating either.

There is no doubt that within the peripheral part of the system f and e sites are to some extent mutually exclusive. Not only are there no freezing components during escape (with the interesting exception of the one c site) but as already noted, e sites are much less likely than h sites to show freezing as an after-response.

It is probable that normally evoked behaviour is similarly organised, although experimental proof seems to be lacking. Fleeing and freezing tend to be alternative responses in the chick when a moderately frightening stimulus is presented, whereas they alternate or are superimposed (in

the sense of fleeing in a sustained crouch) following a very effective stimulus.

It should finally be noted that the clustering of four f sites in the medial PH at level V (the posterior ventromedial area) is sufficiently close as to suggest that freezing is unusually likely here, although two e sites also lie within the same restricted area. The ventromedial area as a whole is also unusual in that it yields no calls on electrical stimulation. The predominance of freezing sites may be related to an actual inhibition of movement and other responses.

Threshold Currents

F and e sites were identified as before; it is significant that no hiding responses were obtained at threshold currents (above). A category of 'lying' sites (l) was added at which the bird crouched or lay at first stimulation, and then sustained this position for some time, as in incipient freezing. Sites at which any of these three responses were elicited, either during the series of short stimulations or during the 20-sec stimulation, are shown in the text figures by a letter (e, f or l) just above the site. It may be significant that no low threshold sites fell in the zone at the base of the pre-optic area; otherwise such sites were found within all parts of the EHF system, from the AH (two f, fig. 1Q; one e, fig. 1R) caudad. Low threshold sites traced out the anterior dorsal core of the system (two l, fig. 2T; one l and one f, fig. 2U; one f, fig. 2W), and also occurred within its ventral extension into the PH (one e and one f, fig. 2V; one f and one e, fig. 3Wa). More posteriorly two sites (one l, fig. 3X; one e, fig. 3Y) in the same small ventrolateral area once more suggest midbrain connections.

The same sites gave e, h or f responses in the threshold survey, as in the main survey at higher currents (table II). Low intensity freezing ('freeze', 'interrupt') was more common behaviour at such sites at threshold stimulation than mild fleeing (e.g. intention leaps, running to corner without staying there). However, numbers are too low for it to be possible to conclude that freezing has a lower threshold than fleeing. It is more important that freezing occurred in some cases at sites which yielded fleeing in the main survey, thus providing some independent evidence that the two responses can be elicited under different circumstances from much the same population of neurones.

One new finding from these data is the existence of a topographically restricted area containing sites at which stimulation produced looking up and around, with restless locomotion, as though the chick were nervous.

Table II. Behaviour in threshold survey

Main survey	Threshold survey					
	freeze	interrupt	flee	alert	feed	forced movement
e	2	0	1	5	0	0
f	4	1	0	2	1	0
H, f and h	2	2	2	7	1	0
Still	0	3	1	4	0	0
Forced movement	0	0	1	22	4	11
Other	0	1	0	0	1	0
e, h, f, still	18			18	omitted	0
Forced movement	1			22	omitted	11

The categories of sites in the threshold survey were as follows: freeze – overt freezing in crouch, or sustained immobility in a normal posture; interrupt – cease ongoing behaviour without becoming immobile; flee – as in main survey; alert – wake up or begin to look around and/or up; feed – peck food or look down persistently at floor; forced movement – a stereotyped movement which by its nature (e.g. circus) is clearly distinguishable from fleeing. The category 'crouch' in the main survey has been omitted because of its small membership. A new category 'still' (sustained immobility in a normal posture) has been included, which was not separated from 'none' in the previous analyses; it is almost certainly low intensity freezing.

$\chi^2=26.2; p<0.001$ for the comparison between e, h and f sites, on the one hand, and the main control category of sites (forced movement), on the other. The same sites thus tended to yield e, h and f responses both in the main survey and at threshold stimulation. Only sites for which full data from both surveys were available have been included.

These are shown as 'n' in the figures. They lie near the dorsal edge of the ventricle and between it and the base of the septal area (2 and 1 more lateral, fig. 1R; 3, fig. 1S). One or two other such sites are scattered through the escape system. However, no such distribution of sites at which obvious looking up occurred was found during the continuous 20-sec stimulation; instead $\frac{4}{8}$ such sites occurred in plane Wa (not shown in fig. 3), perhaps by chance, since they did not coincide with a particular single structure. Further, the 'nervous' sites mostly coincided ($\frac{4}{8}$) with sites which did not give e, h or f responses at high currents. They clearly have no functional relations with the EHF system, and may prove not to be a genuine category of sites. However, sites yielding escape have been found in the septal area in a number of birds [PHILLIPS, 1964; PUTKONEN, 1967]. It may be that the 'nervous' sites which have just been mentioned lie at the base of a septal escape area.

Discussion

A comparison of the EHF system of the chick with the mammalian defensive threat system, which was first described by HESS [1957] for the cat, is illuminating. The main difference between the behaviour associated with stimulation of the two systems is that stimulation in the centre of the system yields defensive threat or attack in the cat and hiding in the chick. However, in the adult fowl [PHILLIPS and YOUNGREN, 1971] threat and attack can be obtained from sites within the EHF system as here defined and their absence in the present study probably simply reflects the absence of an appropriate stimulus object during test. In both chick and cat more peripheral sites yield fleeing.

HUNSPERGER [1956, 1963] defined more exactly a diencephalic zone in the cat within which defensive threat could be obtained centrally and flight peripherally. The zone appears to run from below and behind the pre-optic, through the subthalamus, dorsal hypothalamus and the top of the nu. ventromedialis, into an ill-defined area (flight only) above, but excluding the mamillary area. HUNSPERGER identified the zone as perifornical, but emphasises that such responses are not obtainable from the fornix itself. Flight responses were also obtained from the rostral hypothalamus within a zone closely corresponding to that described here in the chick. Escape and defensive threat were rare or absent in the pre-optic area, just as is true of responses in the chick. Miaows were obtained instead. Again the chick is similar in that calls are evoked from the pre-optic area without escape behaviour [ANDREW, 1973]. A ventral extension of the system similar to that in the chick can be seen just in front of the mamillary area. This is further confirmed in the opossum [ROBERTS *et al.*, 1967] where a defensive threat area was found to centre on nu. ventromedialis. It will be remembered that, if the nucleus is correctly identified in the chick, its anterior portion at least is not part of the EHF system, but that effective sites lie immediately posterior to it.

The LH is free of escape sites in the cat just as in the chick. So apparently are periventricular zones (as opposed to medial zones) throughout the cat diencephalon, although it is not clear how extensively these zones were explored. The same is true in the chick in the dorsomedial and ventromedial areas of the PH, but not more posteriorly in the PH. Nu. periventricularis (Pe) was also only doubtfully part of the EHF system in the chick. Further work is needed in mammals as well as birds before the periventricular distribution of effective sites is properly understood.

In the cat, defensive threat can be obtained from the amygdala and CMG as well as from the hypothalamic system which has just been described. Ablation experiments show that the amygdala acts through the hypothalamus, and the hypothalamus through the CMG; neither of the two more rostral structures are necessary for defensive threat to be evoked from the CMG [FERNANDEZ DE MOLINA and HUNSPERGER, 1959].

The anatomical distribution of the periventricular system of fibres [SZENTAGOTHAI *et al.*, 1968] (which is medial rather than periventricular in the narrow sense), that connects the medial areas of the hypothalamus with the mesencephalon and the medial areas with which it connects, shows a close resemblance to that of the defensive threat and EHF systems. Further, CHI and FLYNN [1971a, b] found that hypothalamic sites which yield attack with defensive threat components connect back to the mesencephalon by periventricular fibres.

The periventricular system falls into two divisions in mammals. The anterior division, which is chiefly efferent to the hypothalamus, arises in the AH and the anterior Pe, and runs dorsally and posteriorly in the medial hypothalamus to the CMG. The sites at the base of the paraventricular nucleus in the chick from plane R backwards to plane Wa coincide well with such a route. The posterior division, which is chiefly afferent to the hypothalamus, arises in the anterior CMG and runs medially down to the dorsomedial, ventromedial and pre-mamillary nuclei. This ventral zone is essentially the same as that in which e, h and f sites lie in planes V to Y. The medial areas in which both divisions of the periventricular system should lie posteriorly have not been explored from plane Y backwards in the chick. In the adult fowl, PUTKONEN [1967] found panic and attack sites running in a dorsomedial strip back to the transition to the mesencephalon, and this may well represent the anterior division of the periventricular system.

The CMG appears not to have been tested previously in conscious birds, although PHILLIPS and YOUNGREN [1971] found panic sites in the lateral midbrain. In the chick stimulation of the CMG (i.e. the medial periventricular grey below the posterior and tectal commissures, excluding its lateral extension below the tectal ventricle) produces typical e and h responses [ANDREW, 1973; DE LANEROLLE, 1972]. The parallel with mammals is thus complete.

In mammals, fibres connecting amygdala and hypothalamus are also closely associated with the defensive threat system in the diencephalon. This is clearest for the stria terminalis, sites along which almost all yield

defensive threat and associated responses [HUNSPERGER, 1963]. The stria terminalis is now known to be largely afferent to the amygdala [SZENTAGOTHAÏ *et al.*, 1968; ZBROZYNA, 1963]. The role of the direct amygdalofugal route, which connects the amygdala to most of the length of the medial hypothalamus in the defensive threat system, is less clear.

ZEIER and KARTEN [1971] have shown that the pars hypothalami of the HOM is the main route connecting those parts of the archistriatum, which correspond to the amygdala, with the hypothalamus. The coincidence of laterally placed hiding sites with the area of entry of HOM fibres, which has already been discussed, strongly suggests that the HOM forms part of the EHF system, again paralleling mammals.

PHILLIPS and YOUNGREN [1971] claimed that, in their study of the adult fowl, almost all sites which yielded escape responses (and some other behaviour such as calls) were closely associated with the HOM. The HOM itself is certainly associated with threat and escape sites along much of its length in the adult fowl. This may also be true of the ring-dove. HARWOOD and VOWLES [1967] obtained facilitation of escape, defensive threat and attack in the ring-dove at a number of sites which were classed as anterior hypothalamic, but which actually lay at the base of the forebrain, lateral to the tractus septomesencephalicus (SM) and medial to the lateral forebrain bundle; HOM fibres were probably involved. However, it is not clear that the whole of the diencephalic EHF system receives HOM fibres. The AH, for example, appears not to do so [ZEIER and KARTEN, 1971].

The mesencephalic division of the tract (OM) is an entirely different matter. It is thought on anatomical grounds by ZEIER and KARTEN [1971] to correspond with the outflow from the mammalian sensorimotor cortex. If this were true, e, h and f responses would not be expected to show any association with the OM. PHILLIPS and YOUNGREN [1971] found a broad zone of panic sites in the lateral midbrain which receives many OM fibres. However, there is some difficulty in the interpretation of this finding. One of the areas within the zone, the mesencephalic call area [ANDREW, 1973; BROWN, 1965] included panic sites in the study of PHILLIPS and YOUNGREN. Extensive stimulation of this area in the chick [ALLAN, 1970; DE LANEROLLE, 1972] never evoked any e, h or f responses even at low intensity. It is possible that problems of definition are responsible for the difference in findings. Panic involved wild running and/or flying, often running into walls and objects. Forced locomotion (e.g. running forwards or backwards) could be obtained from some call area sites in the

chick and was sometimes violent. However, it was never accompanied by components of escape behaviour (e.g. leaping at the wall, trying to burrow through the wall, hiding), and e, h and f responses never occurred as an after-response. It is possible, therefore, that some of the mesencephalic panic sites actually yielded only violent locomotion; if so, the fact that such sites occurred in the broad termination zone of the OM in the lateral mesencephalon ceases to be relevant to the hypothesis of PHILLIPS and YOUNGREN. Finally, perhaps the most telling argument against this hypothesis is that, according to ZEIER and KARTEN [1971], the OM does not supply the CMG (which yields intense e and h responses in the chick) but it gives an extensive contribution to the call area (which does not).

In summary then, it is likely that there is in both bird and mammal a functional system concerned with e, h and f responses and with threat and attack, which has two main diencephalic areas of maximum development in the AH and the posterior medial hypothalamus. The periventricular fibre systems form part of this functional system and connect it back to the CMG, whilst anteriorly it is connected to, and involves the amygdala. The mesencephalic representation of the system requires further study. The CMG is broadly connected with the lateral midbrain by the *radiatio grisea tegmenti* [ARIENS KAPPERS *et al.*, 1936], and some of the panic sites of PHILLIPS and YOUNGREN [1971] may be associated with this system of fibres.

Evidence has been given which suggests that activation of the EHF system tends to evoke both fleeing (escape) and freezing, which are mutually exclusive during moderate activation of the system. During intense activation both types of response tend to occur almost or quite simultaneously. The fact that in the adult fowl threat and attack can be obtained from sites within the EHF system suggests that these responses too may be facilitated during intense activation. Elsewhere [ANDREW, 1973], evidence has been given which shows that other responses (e.g. peep calls, behaviour associated with non-reinforcement) also result from activation of the EHF system. It seems likely that the EHF system will prove to be involved very widely in behaviour, including situations in which e, h and f responses are absent.

The close resemblances between bird and mammal which have been noted suggest that the EHF system may be of very ancient origin. Other workers have argued that the hypothalamus may be very differently organised in bird and mammal. Thus PHILLIPS and YOUNGREN [1971] claimed that only a limited range of 'complex sequences' of behaviour

can be evoked directly by brain stimulation in the fowl; namely fighting, threat and fleeing. E, h and f responses were by far the most readily evoked sequences in the chick also. However, it is not safe to conclude that the same range of behaviour will not be eventually evoked from the bird diencephalon as from the mammal. Feeding can be obtained from the LH in chicks [ANDREW, 1973] for example. Despite faults of interpretation (e.g. failure to exclude indirect effects of brain stimulation) and lack of any histological localisation, some of the very varied behaviour obtained by VON HOLST and VON ST. PAUL [1963] in the adult fowl may also be relevant.

General Note Concerning Figures 1-4

In general the nomenclature of the domestic fowl atlas of VAN TIENHOVEN and JUHASZ [1962] has been followed, with some references to that for the pigeon by KARTEN and HODOS [1967]. The accounts of general hypothalamus anatomy by CROSBY and SHOWERS [1969], and by SZENTAGOTHAI *et al.* [1968] have been used as more general guides. The following special points should be noted:

1. The posterior boundary of the AH is taken arbitrarily to lie just anterior to the plane in which the optic chiasma begins to divide.
2. A medial ventral area of distinctive cell bodies at levels S-U probably corresponds in part with nu. ventromedialis. It becomes indistinct in plane V, with the appearance of medial fibre systems, and this has been taken as the plane in which the PH begins.
3. The posterior boundary of the PH has been taken to be the plane of maximum development of Pe. So defined, the PH may well include pre-mamillary nuclei.
4. In general the extensive medial and periventricular fibre systems which make up the anterior periventricular nucleus of Szentagothai have been numbered to avoid premature identification.
5. The same applies also to the mamillary area, which has been taken to begin in the plane in which there is a characteristic zone of hypertrophied ependyma (EP). Zone 5 probably corresponds in part with the mamillo-thalamic tract. The posterior boundary of the area is taken as the plane (AA) in which decussate various lateral hypothalamic fibre systems (e.g. the infundibular tract). The system of stereotactic co-ordinates (given in mm) is discussed in the text. The horizontal axis of the figures is to the same scale as the vertical axis, co-ordinates along which are shown as mm below the level of the skull roof in the ear-ear plane. The horizontal plane of the figures, which is that for which histological studies have been made, is somewhat tilted in relation to that for which accurate co-ordinates are available. The antero-posterior co-ordinates thus increase by +0.125 mm with each mm increase in depth. They are tabulated below for depths a and b in each figure: values in front of the ear-ear plane are shown as positive, and those behind as negative.

	Q	R	S	T	U	V	W	Wa	X	Y	Z	AA	BB
a	+1.80	+1.65	+1.40	+1.15	+0.85	+0.60	+0.25	+0.10	-0.05	-0.12	-0.45	-0.70	-1.00
b	+2.10	+1.90	+1.65	+1.40	+1.10	+0.85	+0.50	+0.35	+0.20	+0.12	-0.20	-0.45	-0.75

It will be seen that an additional plane has been included at Wa, since the change in distribution of crucial structures is very rapid between planes W, Wa and X.

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