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THE ROLE OF NEUROSECRETION IN THE PHOTOPERIODIC CONTROL OF POLYMORPHISM IN THE APHID *MEGOURA VICIAE*

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SUMMARY

The location of the photoperiodic mechanism controlling the production of the sexual and parthenogenetic morphs by apterous parents was examined by selectively injuring the brain with an R. F. microcautery. Lesions destroying the Group I neurosecretory cells (NSC) in the protocerebrum abolished the response to changed daylength. Extensive damage to other NSC Groups, to the compound eyes and optic lobes was without effect. It is concluded that the Group I NSC are the effectors, secreting a virginopara-promoting substance; in its absence only oviparae are produced. Areas slightly lateral to the Group I NSC are also required for the long-day response, indicating that this is the probable site of the neuronal photoperiodic clock which regulates the release of neurosecretory material (NSM) from the Group I cells.

INTRODUCTION

Photoperiodic systems in animals include several components: a photoreceptor and clock which measure the day or night length and an effector which controls the response. Since photoperiodic responses generally govern developmental changes involving the whole organism, it is usual to find that they are mediated by the endocrine system and that the effectors are hormones.

In insects, studies on the 'brain' or prothoracotropic hormone in *Antheraea pernyi* have demonstrated the role of an endocrine effector in the photoperiodic control of diapause (Williams & Adkisson, 1964; Williams, 1969). In general, however, little is known either of the identity of the effectors or of the way they are regulated by the output of the clock and photoreceptor. The physical location of these last components is also uncertain.

Previous work with the aphid *Megoura viciae* Buckton has shown that the photoperiodic mechanism is maternal and controls the determination of female embryos within the abdomen of the pregnant parent virginopara, causing them to develop either as virginoparae (parthenogenetic, viviparous females) or as oviparae (egg-laying females fertilized by a male). The photoperiodically sensitive areas were

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mapped out by attaching light-conducting filaments to the adult aphid through which localized supplementary long-day photostimulation was supplied (Lees, 1959, 1960, 1964). The results showed that ovipara-producing (short-day) parents only 'switched over' to the production of the alternative morph when the head received long-day illumination. As the most sensitive areas proved to be the centre and anterior parts of the dorsum, which are devoid of organized epidermal photoreceptors in the aptera, it was inferred that the response depended on a 'deep' photoreceptor within the brain. However, a more precise localization of the photoreceptor was prevented by the light scattering which occurs when the beam traverses the cuticle and enters the head. Moreover, this method provided no information on the nature of the endocrine effectors which were assumed to transmit the instructions from the photoperiodic clock to the embryos developing within the abdomen.

The cytological evidence of neurosecretory activity in the brain of *Megoura* has recently been analysed in detail (Steel, 1976*a*). Five anatomically separate groups of neurosecretory cells (NSC) have been identified in the protocerebrum. The axons from certain of these cells (Groups I and II) are engaged in the 'directed delivery' (see Scharrer, 1972) of stainable neurosecretory material (NSM) to the abdomen. The axons, which are swollen at intervals with NSM, form substantial 'axonal reservoirs' and can be traced through the suboesophageal and thoracic ganglia into the abdomen.

In the present paper we describe the effects on photoperiodic switching of a wide variety of anatomically defined lesions to the brain and other parts of the head. An indication that this experimental approach might prove useful in identifying the areas essential for the photoperiodic response was obtained in previous blinding experiments (Lees, 1964); when the compound eyes were destroyed bilaterally, some aphids failed to switch over after exposure to long photoperiods, even though others continued to respond normally. We present evidence that the effector of the photoperiodic mechanism is a neurosecretory product of a small, localized region of the protocerebrum. This neurosecretory effector promotes the production of virginoparae under long-day conditions. The question of whether these neurosecretory cells also constitute the photoreceptor and clock is discussed.

METHODS

Experimental stocks

Apterous virginoparae were reared in sparsely populated colonies on germinating tick beans (*Vicia faba*) in long-day conditions (16L, 8D) at 15 °C. After surgery, aphids were isolated in well-ventilated gauze cages and were transferred to fresh seedlings every few days to facilitate the sequencing of the progeny. Larvae were dissected at an early stage of development to establish their morph type so that any change in morph production by the parent could be detected rapidly while the parent was still alive. Young oviparae were distinguished by the presence of growing eggs, virginoparae by the short chains of embryos in their ovarioles and males by the presence of testes and accessory glands.

Surgical procedures

Operations were performed under light ether anaesthesia at the times detailed below. Small, localized lesions were produced by a pulse of 60 ms duration and regulated amplitude from a radio frequency (2·0 MHz) microcautery. The pulse was delivered to the brain through specially prepared electrolytically tapered tungsten electrodes (Steel, 1976*b*). As the dorsal head cuticle of *Megoura* is quite soft, electrodes could be inserted directly through the cuticle. The location of the insertion point was obtained from a reference map of the head of *Megoura* (Steel, 1976*b*) and, as the head capsule is sparsely pigmented, the accuracy of electrode placement could also be confirmed visually. Most of the operations required ablation of small groups of cell bodies immediately underlying the neural lamella and efforts were therefore made to minimize damage to underlying neuropile. In these instances, the electrode tip was aligned with the neural lamella overlying the area to be eliminated, but was not inserted through it. If deep lesions to regions of neuropile were required (as, for example, in the optic lobes) the electrodes were inserted into the brain itself. The R.F. pulse could be seen to produce a small white patch in otherwise translucent brain tissue, although the size of this patch was less than the area of damage revealed by histology (see below). On withdrawing the electrode a hole was left in the dorsum about 10 μm in diameter which sealed rapidly without loss of haemolymph.

All operated aphids were maintained under the appropriate experimental conditions until the progeny sequence had clearly revealed the parental responsiveness to photoperiod, as judged by the criteria described below. Since the fecundity and longevity of the aphids was entirely normal, the required post-operative survival period of at least four weeks was usually achieved (see Table 1).

Assessment of lesions

Histological autopsies were performed on all operated aphids. Near the end of reproductive life, each was fixed in a modified Bouin's fluid. Transverse serial sections were cut at 5 μm and stained with paraldehyde fuchsin (PAF) as described previously (Steel, 1976*a*). In sections, lesions were visible as areas of complete electrocoagulation surrounded by a smaller zone of more or less cytologically abnormal tissue which has been interpreted elsewhere as a zone of 'damage' (Steel, 1976*b*). The size and position of lesions was reconstructed from the serial sections and drawn on the map of the head. The number, distribution and cytological appearance of surviving PAF-positive NSC and axons was added for comparison with data derived from normal insects (Steel, 1976*a*).

Photoperiodic regimes

In this paper LD and SD refer to long day and short day respectively. When preceded by numerals, L and D refer to the hours of light and dark.

The type of treatment used most frequently was designed to test the ability of aphids with brain lesions to respond to a change from short to long photoperiods. It is known that in *Megoura* the maternal photoperiodic mechanism controlling the determination of embryos as oviparae or virginoparae begins to function some 2 days before the birth of the parent and continues until the last embryos have been

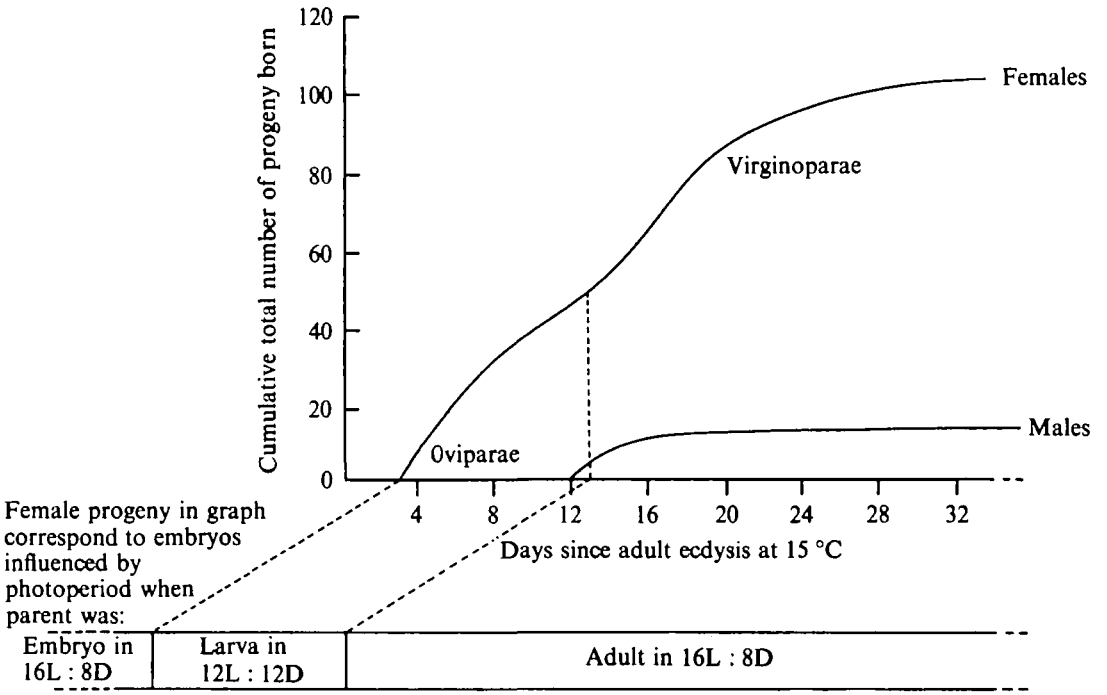

Fig. 1. Relation between number of progeny, their morph type and the rate of parturition, as observed in a typical virginopara reared in the regime (below) used for testing photoperiodic switching.

determined in the adult stage (Lees, 1959, 1963). Since the embryos are determined sequentially as they reach the critical growth stages, the progeny sequence reflects the past photoperiodic treatment. In normal insects exposed to short photoperiods (12L, 12D) (SD) during the prenatal and larval stages, and to long photoperiods (16L, 8D) (LD) after the adult ecdysis, a sequence of some 60–70 oviparae can be expected before the parent begins to produce virginoparae. However, this sequence of oviparae is considerably shortened (to about 50, see below) if the aphid has also been exposed to LD during the prenatal period (when the embryos experience the photoperiod through the abdominal wall of the mother (Lees, 1964)). This is due to the cumulative effect of LD photostimulation, an effect which is largely independent of the number of intervening SD cycles (Lees, 1972). As a result, fewer LD cycles are needed in the adult to produce a switch. It should be noted that prenatal LD treatment is not enough by itself to induce virginopara production; consequently, all the female progeny are oviparae unless additional LD cycles are given later in life.

These considerations have been taken into account in devising a suitable test regime for operated aphids. Adult uncrowded virginoparae were first exposed to LD conditions for 2–3 days until parturition was imminent. The photoperiod was then changed to SD. Larvae born during the night of the first SD cycle were retained and were reared in SD until the imaginal ecdysis some 14 days later. Surgery was performed during the photophase of the cycle in which imaginal ecdysis occurred. The operated insects were immediately placed under LD conditions which were continued without further change throughout adult life. This regime, which tests the ability to switch

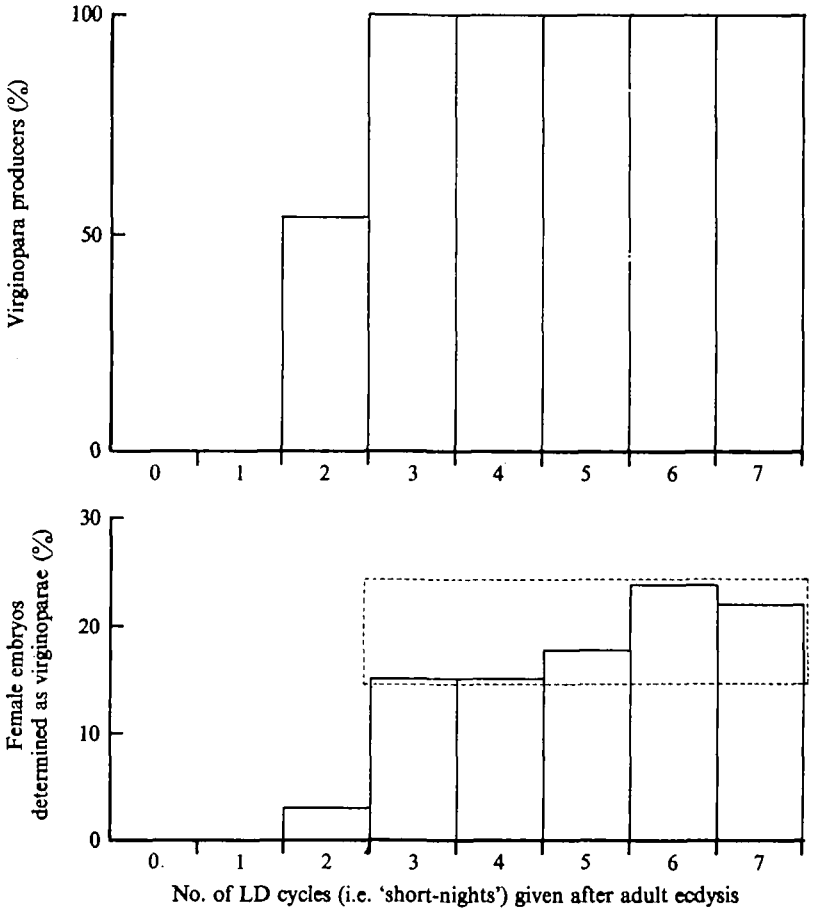

Fig. 2. The number of LD cycles required during adult life to induce parent aphids to switch over from ovipara to virginopara production. Parent aphids were exposed to LD prenatally and reared to the adult ecdysis in SD. Results are expressed in two ways: as a maternal response (above) and as the proportion of the total female progeny that exhibited the switch (below). Note that the proportion of virginoparae determined by seven LD cycles is not significantly greater than the proportion determined by three. The dotted box encloses columns between which no significant variation is revealed by analysis of variance.

from ovipara to virginopara production in response to increased daylength, is depicted diagrammatically in the lower part of Fig. 1. In addition, some aphids were operated on in the fourth instar and a few in the third but these were less suitable for surgery for reasons of size.

In a second type of experimental regime the prenatal photoperiod was again LD but thereafter it was held constant throughout the larval and adult periods, being either LD or SD. Thus, no change in photoperiod was applied following surgery which was again carried out on the day of the adult ecdysis. The object of this treatment was to test whether aphids with brain lesions showed any tendency to switch morph production spontaneously without any change of photoperiod.

Recognition of switch-over points in controls and in operated insects

Fig. 1 also illustrates the relationship between the parturition rate and the number and type of each morph produced by a typical aptera when reared in the first test regime described above. The total progeny produced during the 30 days of adult life ranges from about 70 to 130. Although many individuals reach the switch-over point after about 50 oviparae have been born, this figure varies between 35 and 75, depending on the rate of parturition. It is a striking feature, however, that the switch-over point, which is extremely sharp, regularly occurs 13–14 days after the imaginal ecdysis, irrespective of fecundity. We therefore attach less significance to the absolute number of progeny produced prior to the switch-over point than to the time factor. The use of this criterion is supported by the position of males in the progeny sequence (Fig. 1). It will be noted that the rate of parturition falls off steadily with time but, in addition, undergoes a temporary lag at the point in the sequence when male progeny are born. Under the present conditions this occurs at almost the same time as the transition to virginopara production. The appearance of males therefore provides an additional marker which is of value in deciding whether operated aphids have passed the point in the progeny sequence when a switch-over would be predicted. The number and position of males in the progeny sequence is unaffected by any of the photoperiodic or surgical procedures used here.

In summary, the switch-over point in normal insects is defined by three criteria:

(1) Time: 13–14 days after adult ecdysis.

(2) Position with respect to male progeny.

(3) Proportion of total progeny produced (not absolute number).

The same criteria were applied to operated insects. But in some individuals with brain lesions a fourth criterion was occasionally employed if the expected switch-over point was greatly delayed: the aphid was simply maintained under the experimental conditions until 75 oviparae had been born. This is the latest point at which a switch-over was ever observed; indeed, some of these aphids would have been exposed to nearly 30 LD cycles after the adult ecdysis. The data of Fig. 2 show that under the regime used here, only 2–3 LD cycles during adult life are required to elicit the production of virginoparae in normal insects. Thus, it seems that the photoperiodic mechanism in operated insects need only remain functional for 2–3 cycles for virginopara production to result.

Examples of the range of variation in the progeny sequences obtained in normal and operated insects are given in Table 1.

RESULTS

Role of organized photoreceptors

Earlier experiments designed to identify the photoperiodic receptors by direct illumination of the head of intact aphids did not completely exclude the possibility that organized photoreceptors, such as the compound eyes, were stimulated by internally scattered light (Lees, 1964). The results of previous blinding experiments were also inconclusive; in a series of 20 aphids bilaterally blinded with a cautery, five showed a normal photoperiodic response, 10 showed no response and in five it was delayed (Lees, 1964)

Table 1. *Representative progeny sequences of normal and operated aphids to illustrate the criteria used for the recognition and prediction of photoperiodic switching*

Type of response	Days after surgery: 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30	No. ♀ progeny prior to actual or predicted switch
Normal, unoperated		
a	58 ov, 7 ♂ | 28 v, |	58
b	52 ov, 6 ♂ | 1 v, 1 ♂, 27 v |	52
c	38 ov, 8 ♂ | 11 v, 2 ♂, 37 v |	38
d	41 ov, 7 ♂ | 26 v |	41
Normal, operated		
a	60 ov, 13 ♂ | 27 v |	60
b	51 ov, 7 ♂ | 10 v, 3 ♂, 41 v |	51
c	62 ov, 5 ♂ | 4 v, 5 ♂, 21 v |	62
d	39 ov, 9 ♂ | 20 v |	39
Delayed		
a	41 ov, 5 ♂ | 21 ov | 22 v |	62
b	38 ov, 5 ♂, 1 ov, 3 ♂ | 22 ov | 5 v |	61
Transient		
a	51 ov, 8 ♂ | 7 v | 18 ov |	51
b	61 ov, 6 ♂ | 9 v | 7 ov |	61
c	59 ov, 5 ♂ | 6 v, 1 ♂, 11 v | 15 ov |	59
No response		
a	74 ov, 2 ♂ | 33 ov |	74
b	64 ov, 8 ♂ | 27 ov |	64
c	42 ov, 7 ♂ | 39 ov |	42
d	54 ov, 2 ♂ | 7 ov, 6 ♂, 14 ov |	54

Vertical lines along the time axis indicate points of switching or predicted switching (interrupted lines) of female morph production and the days on which progeny collection was terminated. All animals alive at end of experiment.

Table 2. *Effects on the photoperiodic mechanism of lesions involving the compound eyes and optic lobes*

Type of operation	No. aphids operated	No. died or ceased reproduction prior to switch point	No. survivors in which photoperiodic response was		
			Normal	Delayed	Abolished
Cautery of compound eyes	29	8	20	—	1
Mechanical disconnexion of eyes from optic lobes	29	11	18	—	—
Cautery of distal optic chiasma	16	2	14	—	—
'Superficial' lesions to perikarya of optic lobes	18	4	13	1	—
'Deep' lesions to neuropile of optic lobes	12	2	7	2	1

Having refined the surgical procedures, we have now repeated these experiments and confirm that the organized photoreceptors play no part in photoperiodic sensitivity. Several methods were employed to produce visually blind aphids (Table 2). In all cases, the characteristic behavioural responses of the intact insect proved to be lacking. In initial experiments, in which the electrode was inserted directly into the compound eye, it proved difficult to determine histologically whether all the retinulae had been destroyed. Moreover, the three receptors of the 'larval eye' or triommatidium (Pflugfelder, 1936) are connected to the optic lobes by a separate tract of axons and these were destroyed only by very large lesions. However, the tracts from the triommatidium and compound eye merge at the distal optic chiasma. Bilateral ablation of this chiasma, accomplished either by inserting the cautery electrode through the dorsum of the head or by severing the chiasma, was therefore adopted as a more satisfactory technique for producing visually blind aphids. As can be seen in Table 2, cauterization of the chiasma resulted in much lower mortality and less ambiguous results, even when lesions as severe as that shown in Fig. 3(*b*) were produced. These operations were completely ineffective in preventing the normal photoperiodic response (Table 2).

Serial sections of the head of normal virginoparae failed to reveal evidence of other organized photoreceptors, either associated with the cuticle (ocelli are absent) or deeper in the head tissue. In the earlier experiments with blinded aphids (Lees, 1964) the high frequency of response failures at that time was attributed to widespread damage to other tissues. The single individual that failed to respond in the present series of eye operations (Table 2) was found to have sustained substantial damage which extended from the optic lobes into the lateral protocerebrum. Further attention was therefore given to these areas of the brain.

Role of the optic lobes

Two kinds of optic lobe lesions were produced: 'superficial' lesions were confined to the external layer of cell bodies and were produced by placing the cautery electrode tip externally to the neural lamella (Table 2). 'Deep' lesions involved damage to the underlying neuropile and were produced by inserting the electrode tip through the lamella and into the underlying neuropile. In 10% of animals with bilateral optic lobe lesions, the switch to virginopara production was delayed by four days, and about 22

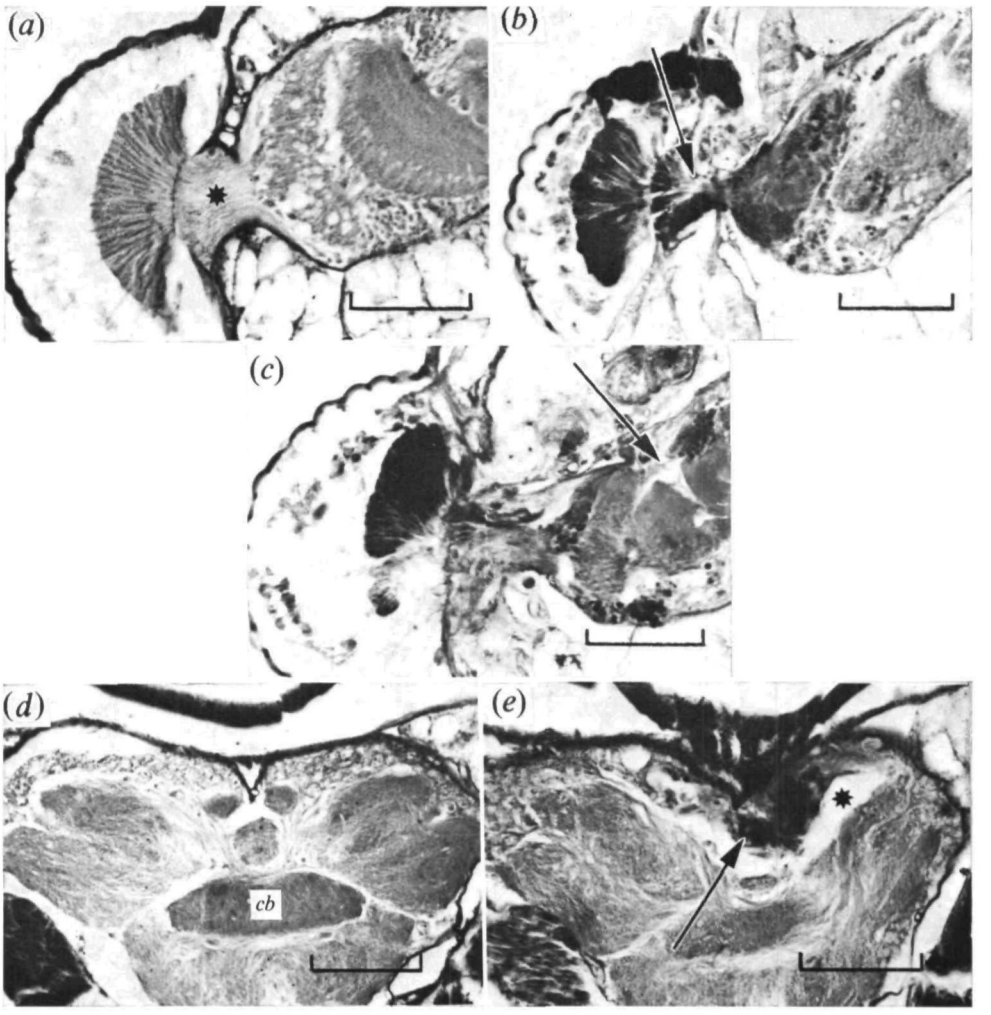

Fig. 3 (*a*) Transverse section of the head of a normal insect to show the compound eye at its connexion with the optic lobe. The distal optic chiasma is indicated by a star. Small vacuoles in the optic lobes are common fixation artefacts in unoperated insects. PAF and Halmi counterstain. Length of scale bar in all figures 50 μm.

(*b*) Region similar to that in Fig. 7 (*a*) following a lesion centred on the optic chiasma. Damage to the cuticle at point of electrode insertion is visible (arrow) above the chiasma. Note that damage has spread into the compound eye and also into the lateral portion of the optic lobe. Photoperiodic response normal.

(*c*) Similar region showing a massive lesion in the optic lobe produced by inserting the cautery electrode deep into the neuropile. Track of electrode insertion is visible at arrow. Note that damage extends laterally through the distal optic chiasma into the compound eye. Response to photoperiod was unimpaired in this insect.

(*d*) Transverse section of protocerebrum of *M. viciae* approximately 80 μm posterior to the Group I NSC. *cb*, central body.

(*e*) Section through the same region as in Fig. 3 (*d*) in an aphid with a deep and massive lesion in the dorsal protocerebrum. The Group II NSC were destroyed bilaterally together with a large mass of additional neurones and neuropile. Despite the extent of the damage, there was no effect on the photoperiodic response. In such lesions the zone of complete electrocoagulation (arrow) shrinks away from the underlying tissue leaving an intervening space (star). Note that this lesion was located too far posteriorly to involve the Group I collaterals.

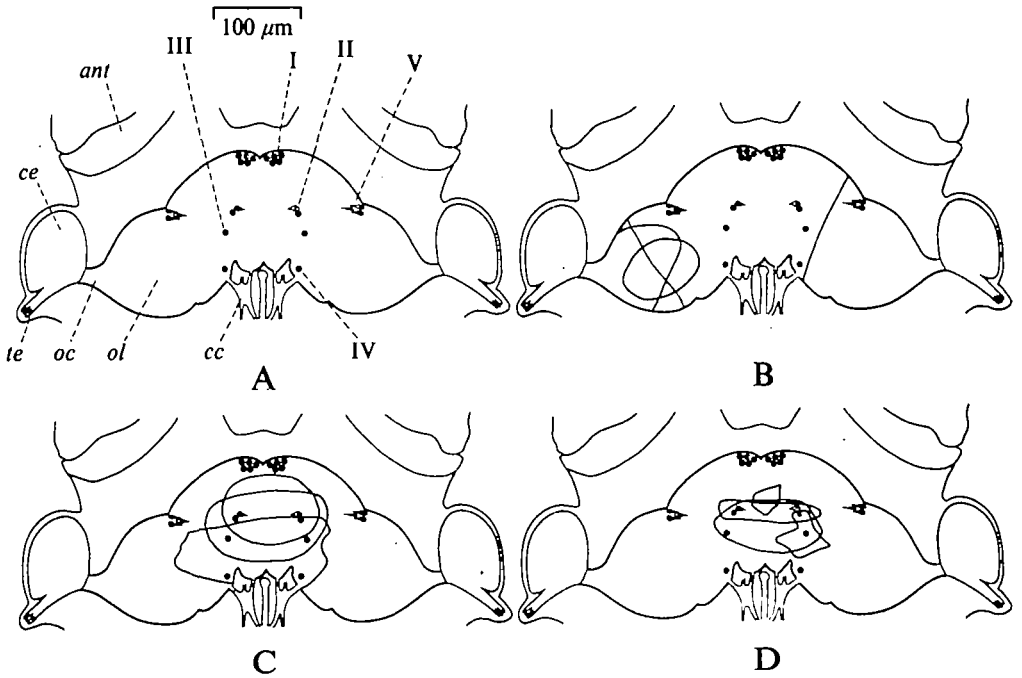

Fig. 4. Composite diagrams showing the location of various brain lesions which, with one exception (4B) were without effect on photoperiodic switching. (A) Intact brain from dorsal aspect, indicating the positions of NSC Groups I–V; *ant*, antenna; *cc*, corpus cardiacum; *ce*, compound eye; *oc*, distal optic chiasma; *ol*, optic lobe; *te*, triommatidial eye. (B) Lesions of the optic lobes. The large lesion involving the whole of the right optic lobe and the margin of the protocerebrum delayed switching. (C) Large superficial lesions involving NSC Groups II, III and IV. (D) Small superficial lesions involving selective damage to NSC Groups II or III.

additional oviparae were born after the predicted switch-point. But most significantly, in only one of 30 operated insects did the photoperiodic response fail (Table 2). Histological examination revealed that in these few cases of delay or failure of the response the lesions extended close to the junction between the optic lobes and the rest of the protocerebrum (Fig. 4B, right); signs of damage were also visible in the protocerebral neuropile which is known to contain the axons of the Group I NSC (Steel, 1976*a*). Massive lesions confined entirely to the optic lobes were invariably ineffective (Fig. 4B, left, and Fig. 3*c*). We therefore regard the optic lobes themselves as inessential for the photoperiodic mechanism and interpret these results as indicative of involvement of the protocerebral neuropile. This point is examined further below.

Role of protocerebrum and neurosecretion

The protocerebrum contains five anatomically distinct groups of PAF stainable NSC, arranged as in Fig. 4A. The axons of many of these cells have been followed in detail through the neuropile of the brain, where stainable NSM was seen to accumulate in swollen axon reservoirs near the central body (Steel, 1976*a*). From these reservoirs NSM is distributed to the corpora cardiaca, or to abdominal tissues by axonal transport through the suboesophageal and thoracic ganglia.

Most of the NSC are unnecessary for the normal operation of the photoperiodic

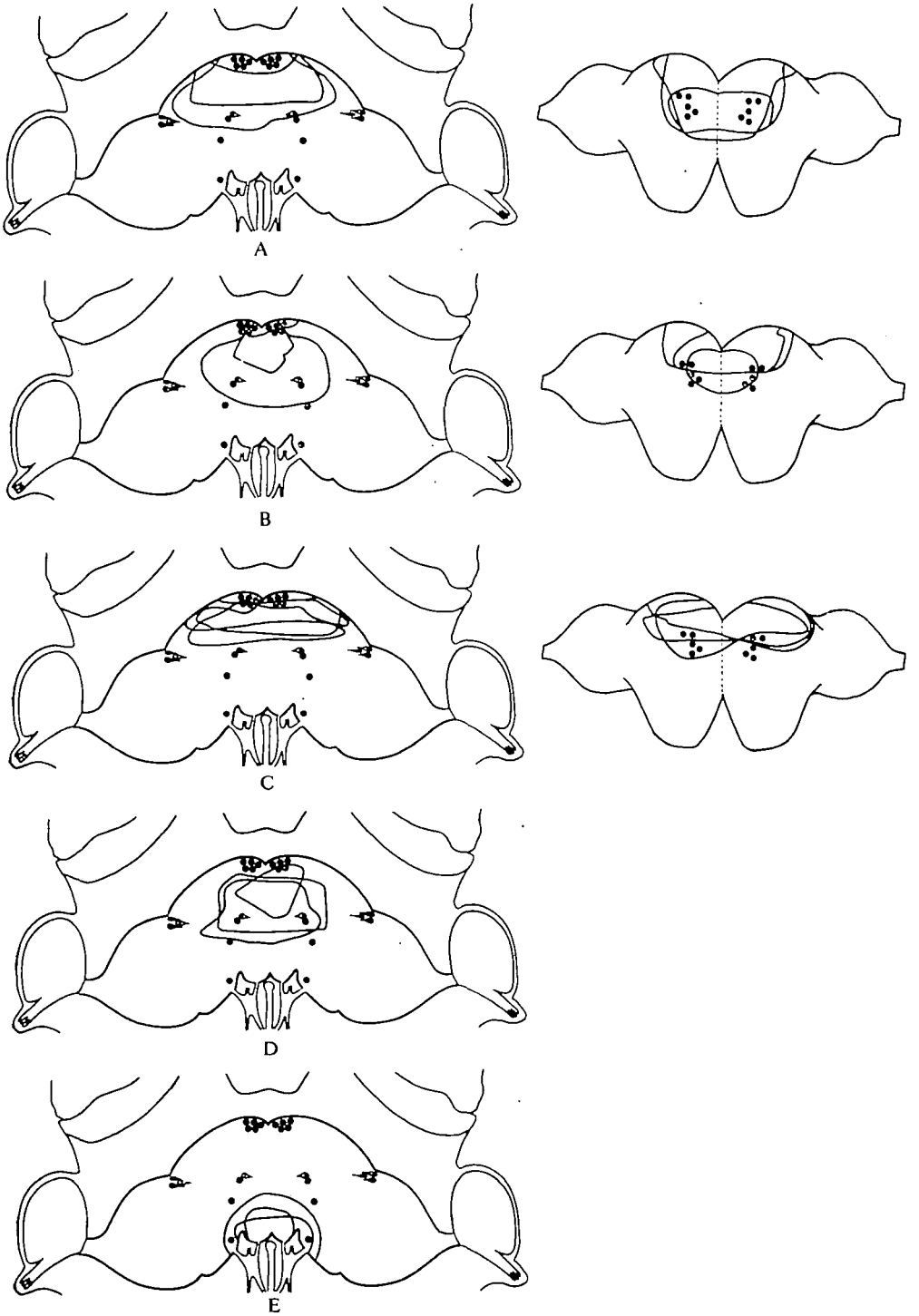

Fig. 5. Location of various brain lesions, shown both from the dorsal aspect (left) and in frontal view (right). (A) Lesions involving Group I NSC. Photoperiodic response failed in all cases. (B) Superficial lesions encroaching on NSC Group I. Loss of dorsal neurones and some NSC. Photoperiodic response normal. (C) Superficial lesions encroaching on Group I NSC but also spreading laterally. Photoperiodic response failed. Compare with (B). (D) Deep medial lesions involving damage to the NSC Group I axons and collaterals in the deep neuropile. Photoperiodic response failed. (E) Lesions involving NCC I. All switched normally.

mechanism. This point was most clearly established in the 25 insects which sustained massive (100 μm diameter) superficial protocerebral lesions, some of which resulted in destruction of several groups of NSC on both sides of the brain simultaneously (Fig. 4C, D). Bilateral ablation of NSC Groups II and III failed to prevent a normal response to photoperiod. In two individuals, Groups II, III and IV were all destroyed bilaterally and the photoperiodic response was again unaffected. It should be noted that these insects suffered the destruction of some hundreds of protocerebral neurones around the NSC (Fig. 4C), indicating that surgical trauma *per se* does not interfere with photoperiodic time measurement. Smaller lesions which destroyed individual groups of NSC (18 operated insects) were likewise ineffective (Fig. 4D). The destruction of NSC perikarya was invariably accompanied by the disappearance of stainable NSM from the axons and neuropilar reservoirs of the cells throughout the length of their passage through undamaged neuropile. For example, lesions involving the Group II NSC resulted in the disappearance of two of the 12 neurosecretory axons normally visible in sections of the thoracic and suboesophageal ganglia (Steel, 1976*a*). This confirms experimentally the observation (Steel, 1976*a*) that these axons are of cerebral origin.

Deep lesions similar in extent to those illustrated in Figs. 4C and 3(*e*) but extending inwards through the layer of perikarya into the neuropile caused the aphids to exhibit various behavioural abnormalities, such as impaired locomotor coordination; nevertheless, in these insects the photoperiodic response was again entirely normal, the transition from the production of oviparae to virginoparae occurring at the same time and at the same point in the progeny sequence relative to male progeny as in normal insects. No reduction in fecundity was encountered. Indeed, it is striking how much of the protocerebrum can be destroyed or damaged without impairing reproduction or longevity.

In contrast, lesions that invaded the anterior extremity of the protocerebrum frequently resulted in response failures. This region of the brain contains the paired group of five NSC described previously as Group I (Steel, 1976*a*). In all insects in which this group was completely destroyed, photoperiodic responsiveness was lost. This was true both for individuals with large superficial lesions involving extensive but 'inessential' areas of the dorsal protocerebrum in addition to the NSC, as well as for nine insects which sustained very much smaller lesions which destroyed the NSC more selectively (see Fig. 5A). We therefore conclude that a small area of the protocerebrum containing Group I NSC is indispensable for the operation of the photoperiodic mechanism. However, it has proved technically impossible to destroy the two groups of five NSC without some damage to other neurones among and between the NSC. The problem of whether these NSC are indeed the essential component of this small area of protocerebrum rather than the few other neurones located among the NSC is resolved by comparison of the effects of lesions which encompass only part of the area occupied by Group I NSC. Fig. 5B shows a selection of such lesions; in all these insects neurones lying between the NSC and adjacent to them dorsally were also destroyed, the precise numbers and location depending on the geometry of the lesion. But in all instances a few NSC remained undamaged. Such insects always responded normally, even if as few as two of the NSC remained intact. When all the ten NSC were eliminated, the response failed.

The above experiments provide the first direct evidence that the photoperiodic mechanism in *Megoura* contains a neurosecretory component. As mentioned previously, the cytological evidence suggests that the secretory product of the Group I NSC is delivered directly to the abdomen, and perhaps to the reproductive system itself (Steel, 1976*a*). However, Group I NSC were also regarded as forming part of a larger NSC Group, equivalent to the 'medial' cell group of other insects (Steel, 1976*a*). There is evidence in other species that the medial NSC may influence the reproductive system, either by the release from the corpus cardiacum of a hormone acting directly on the reproductive system or by influencing the secretory activity of the corpora allata (see discussion by Engelman, 1970). As both these mechanisms operate through the nervus corporis cardiaci I (NCC I) we have examined the possibility that Group I NSC in *Megoura* influence the reproductive system via the corpora cardiaca or allata. This was achieved by placing deep lesions in the posterior protocerebrum of 12 insects where the NCC I emerges from the brain (Fig. 5E). These lesions resulted in depletion of the stainable extrinsic NSM from the corpora cardiaca, although no direct damage to the corpora cardiaca was discernible. However, every insect still responded normally to photoperiod. We therefore infer that the influence of Group I NSC on morph determination is not achieved by way of the NCC I. This is consistent with the view that the NSM from Group I acts directly on the reproductive system to influence the determination of embryos as virginoparae or oviparae. It further indicates that the material is not released into the haemolymph from the corpora cardiaca.

It is concluded that Group I NSC constitute the effector of the photoperiodic mechanism in *Megoura* and that their secretory product is the effector substance. The question of whether the photoreceptor and clock are also located in these cells is examined below.

Photoperiodic regulation of neurosecretory cells

If photoreceptor, clock and effector are all contained within the same cells, the photoperiodic mechanism should function independently of other neurones in the brain. We have already seen that the mechanism is independent of very large areas of the protocerebrum, including neurones lying immediately dorsal and medial to the Group I perikarya. However, superficial lesions which encompassed the lateral parts of the anterior protocerebrum also resulted in consistent failures of the mechanism (14 insects) (Fig. 5C). These lesions were similar in size to those which destroyed the medial and dorsal neurones without impairing the response. In all autopsies of aphids with lateral anterior lesions, a few Group I NSC were found to be destroyed or damaged, but the extent of this damage to the NSC was considerably less than that sustained by the insects shown in Fig. 5B, in which the response was normal. Since, as is shown above, the response is retained when as few as two NSC survive, we conclude that the effects of lateral damage are due to the destruction of the neurones and not to the loss of NSC. The perikarya of the NSC in these insects remained cytologically normal, but the usually abundant NSM in the axons deep in intact neuropile was depleted or entirely absent. As this depletion occurred at sites remote from the lesion and in cells whose perikarya were intact, it cannot be a consequence of damage to the axons. Instead, it seems to point to a malfunction of the NSC brought about by removal of neurones in the lateral anterior protocerebrum.

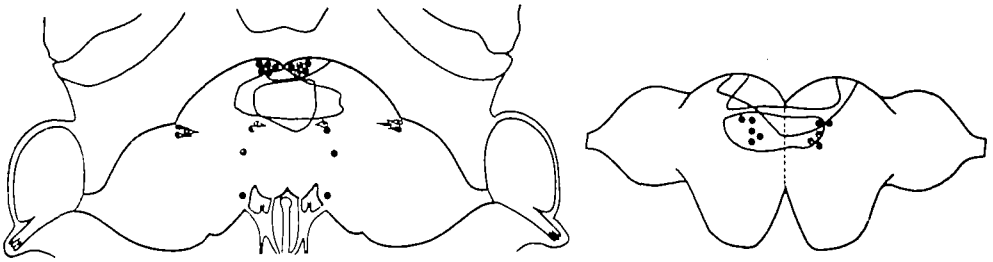

Fig. 6. Superficial lesions that produced transient switching. Right, frontal view. Note that the location of the lesions varies considerably although all are near the Group I NSC.

The possibility that these neurones are involved in the control of the NSC was examined further in experiments designed to disrupt synaptic input to the NSC. The axons of Group I NSC have been shown to possess collaterals which arise close to the perikarya and run posteriorly along the top of the central body (Steel, 1976*a*; see also Fig. 7A). It is reasonable to assume that any presynaptic input to the NSC from lateral neurones occurs at synapses along these collaterals (see Adiyodi & Bern, 1968; Maddrell, 1974). Deep lesions in this region of neuropile were produced by inserting the electrode into the brain in the midline from the dorsal side. This direction of insertion prevented injury to Group I NSC perikarya and electrodes were not inserted deeply enough to cause damage to the Group I axons. The layer of neurones overlying the neuropile was also damaged by this treatment, but these are cells which we have already shown can be eliminated without affecting photoperiodic switching. Results from nine operations all of which produced a response failure, showed that the NSM in the axon reservoirs was depleted, i.e. the effect was the same as when the perikarya of the lateral neurones were destroyed by superficial lesions. This suggests that these neurones provide some presynaptic input to the NSC which is essential to the maintenance of their normal secretory behaviour.

Transient switches

Twelve operated aphids produced a group of 7–17 virginoparae, commencing at the predicted time, and then reverted to ovipara production (see Table 1). This phenomenon did not occur in response to lesions localized in any particular area except that they were all close to Group I perikarya (Fig. 6). In these insects the axonal reservoirs were depleted as in the examples of response failure noted above, but in addition, the perikarya had lost their normal blue-staining flocculent material and had taken up the uniform green colour of the counterstain. The nuclei appeared normal and the cytoplasm contained numerous fine particles but none of these stained with PAF. These observations are interpreted as indicating subliminal damage to the NSC perikarya to an extent sufficient to impair the synthetic machinery of the cell but insufficient to produce gross damage. The occurrence of a short run of virginoparae in these aphids seems to indicate that the photoperiodic mechanism began to function normally but failed when the supply of previously synthesized NSM from the axonal reservoir was depleted (see Discussion).

Cerebral neurosecretion and morph type

The experiments so far described have served to define the capacity of aphids to perform the entire switching process in response to a change in daylength. They provide no information about the nature of the effect of the Group I NSM on embryonic differentiation. Here we examine whether this effect is on the differentiation of female embryos into oviparae, virginoparae or both.

Virginoparae were taken from stock cultures which had experienced only a LD regime and the Group I NSC destroyed by cautery in 24 newly ecdysed adults. The operated insects, which were replaced in LD conditions, proceeded to give birth to virginoparae until that point in their progeny sequence at which a switch to oviparae would have been expected if they had experienced a change in daylength at the time of the operation. They then switched spontaneously to the production of oviparae although remaining in LD. Group I NSC are therefore required for the determination of embryos as virginoparae under LD conditions.

The converse experiment was performed in order to decide whether these NSC were also required for the determination of oviparae in SD conditions. In this instance the parent aphids were again exposed to prenatal LD (for reasons explained under Methods), but were reared from birth onwards in SD. Group I NSC were again destroyed by cautery in the newly ecdysed adult (16 operated insects). These insects continued to give birth to oviparae throughout life and showed no tendency to switch morph production spontaneously. Hence, we conclude that the NSC are unnecessary for ovipara production under SD conditions.

DISCUSSION

The object of the present series of cautery operations on the brain of *Megoura* was to identify the components of the photoperiodic mechanism which control the determination of embryos as virginoparae or oviparae. We have found that photoperiodic switching in response to a changed photoperiod is lost when a relatively small area of the brain is destroyed. This area contains the paired group of five NSC previously described as Group I (Steel, 1976*a*) which lies close to the cleft in the anterior protocerebrum. Together with the Group II NSC these cells are regarded as the probable homologues of the 'medial' NSC group found in the pars intercerebralis of other insects (Steel, 1976*a*). The axons of the Group I cells, with their abundant NSM, form a coherent neuropilar tract which can be traced in sections stained with PAF through the suboesophageal and thoracic ganglia towards the ventral abdominal nerve which in turn sends branches to the vicinity of the ovarioles. We regard these elements of the neurosecretory system in *Megoura* as the effectors concerned in mediating the photoperiodic response (see Fig. 7).

The extent of the brain structure which is unnecessary for the photoperiodic response (and for survival) is a striking feature. Redundant structures include the compound eyes, the optic laminae and indeed the entire optic lobes. A delayed response was only encountered with very deep lesions of the optic lobes which extended medially towards the Group I axons. Extensive superficial lesions involving NSC Groups II, III and IV, all of which lie posterior to Group I, have no detectable effect on the photoperiodic response of the adult aphid. Nevertheless, such operations

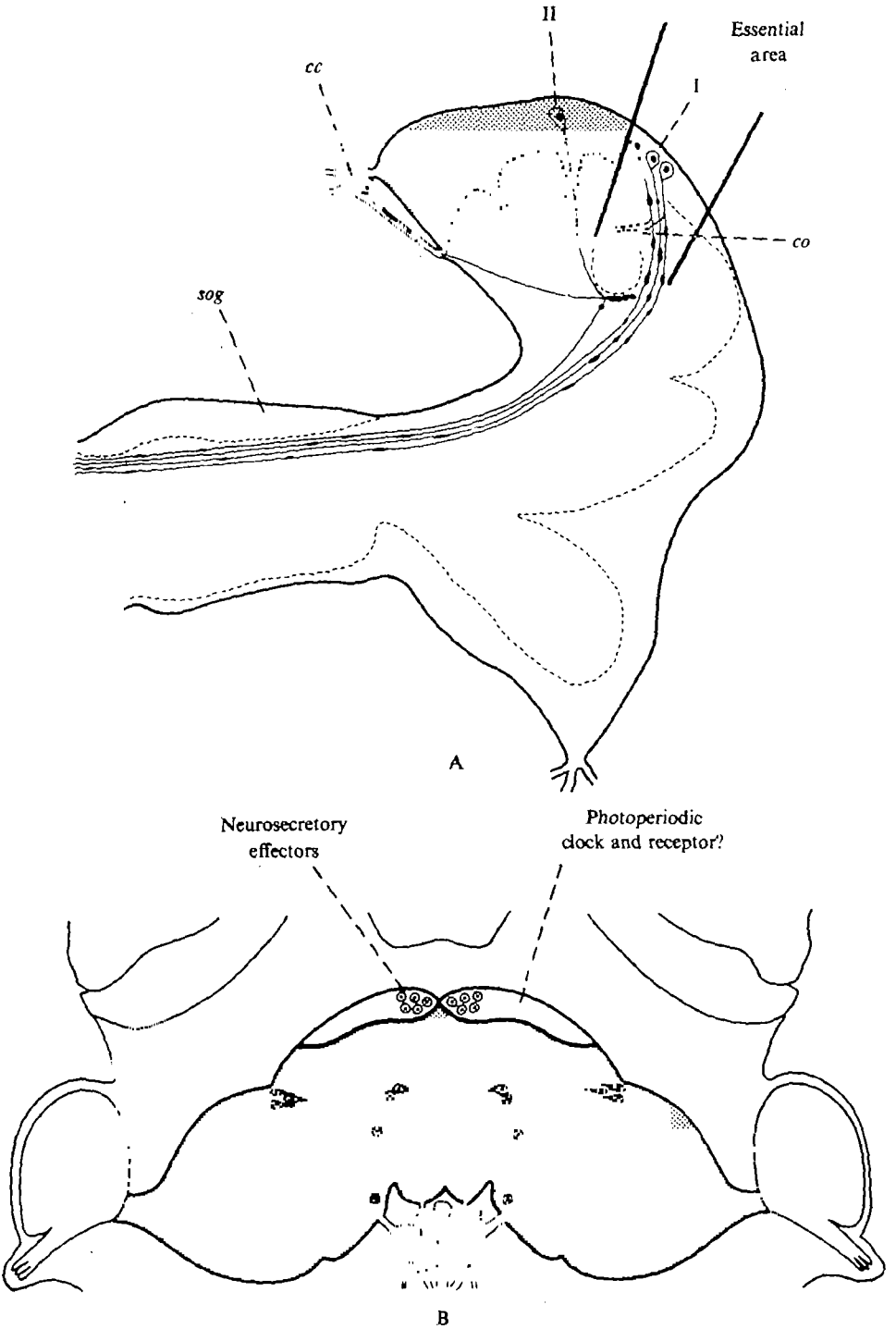

Fig. 7. Brain of *Megoura* indicating semi-schematically the anatomical and functional features associated with the photoperiodic control of polymorphism. (A) Near-sagittal section to indicate the axons of NSC in relation to parts of the brain necessary for the photoperiodic response. The region between the two heavy bars *cannot* be damaged without disrupting the photoperiodic mechanism. (I, II) Cell bodies of NSC Groups I and II; *cc*, corpus cardiacum; *co*, collaterals of Group I NSC; *sog*, suboesophageal ganglion. (B) Dorsal view to show the lateral regions of the protocerebrum which may contain the photoperiodic clock and photoreceptor. Stippled areas in (A) and (B) have been destroyed experimentally without impairing the mechanism.

can be shown to result in endocrine deficiencies since they inhibit moulting if carried out in the third or fourth instar larva and also prevent tanning of the teneral adult (Steel, 1976*b*). Posterior lesions of the protocerebrum which involve the NCC I, in addition to NSC Groups III and IV are also inconsequential. Disruption of the NCC I tracts by cautery results in the loss of stainable NSM from the extrinsic portions of the corpora cardiaca, without affecting photoperiodic switching. This is readily explicable in terms of the Group I NSC axonal projections which, as noted above, do not pass to the CC (Fig. 7A).

Detailed assessment of the lesions which include, or encroach upon, the Group I NSC has shown that the photoperiodic response disappears if the area of coagulation involves the whole group, but that the aphids switch over normally to virginopara production even if only two of the 10 cells escape with no cytological sign of damage. This observation has several implications. First, it suggests that all ten NSC are engaged in the same function since it is improbable that the same cells would survive in different operations. This inference is consistent with the cytological homogeneity of this group of NSC (Steel, 1976*a*). Secondly, it seems probable that surviving cells can compensate for the absence of others, perhaps by increasing their rates of synthesis and release of secretory product. A third implication concerns the mode of delivery of this product to effector tissues. If the NSM is transported intra-axonally to the abdomen, as cytological evidence suggests (Steel, 1976*a*), considerable branching of the neurosecretory axons must occur in the abdomen in order to provide all the ovarioles with a direct supply of NSM. A more conservative explanation would be that the endings of the neurosecretory axons may consist not of specialized terminations on the effectors but of 'leaky' release sites in their proximity, from which released NSM may diffuse over a wider area.

The small neurones lying between and dorsal to the Group I NSC are clearly not required for the photoperiodic response since the lesions which destroy some but not all the Group I NSC also eliminate these small neurones in varying combinations. In contrast, photoperiodic switching fails if an area lateral to the Group I perikarya is destroyed, even though cytologically normal Group I perikarya are still present at the time of autopsy several weeks after the operation. Evidently the Group I NSC cannot carry out all the functions of the photoperiodic mechanism, which must include photoreception and time measurement as well as production of an effector substance. We have noted that PAF staining has revealed short axonal branches near the Group I perikarya, apparently terminating in the deep neuropile. These are regarded as collaterals which are believed to form synaptic connexion with neurones that control the transport and release of the NSM. We have been unable to detect any cytological differences between the amount of NSM in the axon reservoirs of LD and SD aphids; but cytological evidence that such control exists is found in the depletion of the normally abundant NSM in the axon reservoirs which takes place when the areas lateral to Group I NSC have been damaged; and a similar effect is observed if the cautery is introduced from the mid-dorsal aspect and inserted deep into the neuropile containing the collaterals. It is inferred that Group I NSC receive some essential synaptic input through these collaterals. It is tempting to speculate that this essential synaptic input derives from a neuronal photoperiodic clock located laterally to the Group I NSC. These neurones do not appear to possess any special cytological

features. Previous experiments with localized light are consistent with the view that the photoreceptor could lie in this part of the brain (Lees, 1964) (Fig. 7B).

These results can be compared with Williams's (1969) observations on *Antheraea pernyi*. Using brainless diapausing pupae Williams showed that photoperiodic discrimination was at least partially restored by implanting whole brains or fragments of the protocerebrum containing both the median group of NSC (which secrete the 'brain' or prothoracopotropic hormone) and a more lateral area which in this insect also includes a lateral NSC group. We have seen that in *Megoura* the indispensable part of the brain is very similar although it does not appear to extend so far posteriorly; neither does it include lateral NSC, since the Group V cells which are situated close to the optic lobes are not a necessary part of the photoperiodic mechanism. Williams concluded that the lateral group of NSC (or other neurones in their vicinity) is responsible for the clock function in *Antheraea* which, in turn, was thought to regulate the transport of NSM along the axons of the cells of the medial NSC group. The present results with *Megoura* contrast with those of Williams in two respects. First, the photoreceptor and clock mechanisms in *Megoura* appear to reside in neurones with no apparent neurosecretory characteristics. Secondly, the effector mechanism, though neurosecretory in both species, may not be endocrine in *Megoura*.

Apart from operations that completely abolished photoperiodic switching, we have recorded a number of examples of 'transient switching' in which the aphid produced a short series of virginoparae before reverting spontaneously to ovipara production. This phenomenon clearly differs from the type of random switching which occurs when normal aphids are exposed to darkness or to photoperiods which are intermediate in length between LD and SD; this latter type of switching is presumably due to the failure of the clock mechanism to provide a decisive input to the effector (Lees, 1959, 1963). In transient switches the production of the few virginoparae always began at the expected point in the progeny sequence, suggesting that the clock and neurosecretory system at first respond normally to LD but fail to sustain the response. This effect is associated with the depletion of the axonal reservoirs of the Group I NSC and with cytologically recognizable changes in their perikarya. It seems possible that transient virginopara production may be caused by the emptying of the axonal reservoirs and by their subsequent failure to refill, perhaps because of some impairment of the synthetic processes in the perikaryon. If this inference is correct, the duration of virginopara production, which is usually about 3 days, should correspond to the length of time for which the NSM stored in the reservoirs can support this function. Since three LD cycles are sufficient to elicit virginopara production in normal insects (see Fig. 2), it is not immediately clear why short sequences of virginoparae are not recorded in all insects in which the photoperiodic mechanism is disrupted. A possible explanation is that in transient switches we are observing the effects of subliminal damage to the synthetic machinery in the perikaryon, while the processes controlling transport and release in the axons and terminals continue to function normally. Thus, the occurrence of transient switches reinforces the above conclusion that the receptor, clock and effector components of the photoperiodic mechanism are anatomically separate, as it is possible to interfere surgically with one component without disrupting the others.

Transient switching, according to the above interpretation, implies that the

neurosecretory product of the Group I NSC ultimately causes competent embryos to develop as virginoparae. This view is confirmed by the fact that ablation of Group I NSC destroys the ability to produce virginoparae. Previous indirect evidence, based mainly on the overt effects of photoperiodic switching, has also led to the view that LD conditions exerted positive (or 'promotive') effects whereas short days favoured ovipara production much less strongly and might indeed be entirely neutral (Lees, 1959, 1963). The evidence was based on the cumulative action of LD cycles which are much more potent in switching development than sequences containing the same number of SD cycles (Lees, 1972). A more extensive analysis using a wider range of non-circadian light cycles, also showed that the essential feature in photoperiodic time measurement is the length of the scotophase; and whereas the virginopara-promoting effect of the LD cycle is clearly due to its short night component, there are no indications that long nights (as in a SD regime) actively promote the formation of oviparae. A model was therefore proposed in which the determination of the two morphs was assumed to depend on the presence or absence of a hypothetical 'active product' or hormone, the formation of which depended on cumulative short-night stimulation (Lees, 1973).

The present observations provide direct evidence that Group I NSC are required for virginopara production in LD but not for ovipara production in SD. These results are the reverse of those of von Dehn (1969), who reported briefly that cauterization of the 'median' brain NSC in *Megoura* caused virginopara production to continue even under SD conditions. Although von Dehn also considered the scotophase to play a key role, she was of the opinion that ovipara production was actively promoted by the long night moiety of SD cycles. She therefore regarded her results as evidence that the maternal switching mechanism had been eliminated. We agree, for different reasons, that the Group I NSC are indeed the effectors but conclude that their role is to promote the differentiation of female embryos as virginoparae. Since the effect of NSM on the reproductive system may well be direct, it seems likely that the neurosecretion is itself virginopara-producing.

It should be emphasized that the proposed role of the Group I NSM is the *maternal* control of switching. The development of the progeny, once determined as virginoparae or oviparae by the mother, may well be under intrinsic endocrine control. If the role of the Group I NSM is indeed the manipulation of the endocrine system in the late embryo and neonate larva, local delivery of secretion to the reproductive system may have evolved as a device that prevents the interaction of the maternal factor with the programming of the parent's own endocrine system.

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