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Brain plasticity in Diptera and Hymenoptera

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1. ABSTRACT

To mediate different types of behaviour, nervous systems must coordinate the proper operation of their neural circuits as well as short- and long-term alterations that occur within those circuits. The latter ultimately devolve upon specific changes in neuronal structures, membrane properties and synaptic connections that are all examples of plasticity. This reorganization of the adult nervous system is shaped by internal and external influences both during development and adult maturation. In adults, behavioural experience is a major driving force of neuronal plasticity studied particularly in sensory systems. The range of adaptation depends on features that are important to a particular species, so that learning is essential for foraging in honeybees, while regenerative capacities are important in hemimetabolous insects with long appendages. Experience is usually effective during a critical period in early adult life, when neural function becomes tuned to future conditions in an insect's life. Changes occur at all levels, in synaptic circuits, neuropile volumes, and behaviour. There are many examples, and this review incorporates only a select few, mainly those from Diptera and Hymenoptera.

Table 1. Definitions of some key terms

Plasticity	The latent capacity of a brain to become altered with time, and to generate changes that exhibit some level of permanence
Developmental plasticity	Plasticity that is manifest in some stage of development, chiefly in the larval and adult forms of Diptera and Hymenoptera. Changes that are evoked in larval or pupal stages may only appear in the adult nervous system
Morphological plasticity	Changes in a nervous system that show some form of morphological sign. Other changes that are structurally cryptic may not have been examined at sufficient resolution
Activity-dependent plasticity	Changes in a nervous system that depend on neural activity, chiefly interpreted to mean its electrical activity. Other forms of activity have rarely been assayed.
Synaptic plasticity	Changes in the structure or transmission at synaptic sites. Different stimulus paradigms distinguish many types of synaptic plasticity electrophysiologically, including various forms of potentiation
Hard-wired	Circuits or nervous systems that do not exhibit activity-dependent plasticity are said to be hard-wired. This may mean that an appropriate test designed to reveal possible plasticity has not yet have been applied
Phenotypic plasticity	Among social insects such as Hymenoptera, two forms of plasticity are manifest among individuals: polyphenisms and age polyethism
Polyphenisms	The occurrence of morphological phenotypes, as in the division of labour into reproductive and non-reproductive phenotypes
Age polyethism	Plasticity within worker caste members of the Hymenoptera (e.g. in bees) that enables workers to undergo different behaviours at different ages

2. INTRODUCTION

Despite earlier views to the contrary, insects exhibit a range of examples indicating that their brains are plastic. Plasticity is based on the latent capacity of a brain to modify itself with time as a result either of functional experience or other perturbations, such as injury, and to generate changes that exhibit some level of permanence. The term itself is widely used, and the changes produced may be considered from the perspective of the stimulus provoking the change, their time course, and the level of analysis (genetic, cellular, circuit or behavioural) at which the changes are documented (1). The phenomena encompassed by plasticity are hard to delimit, and as a result the subject is constituted by a rather loose assemblage of interesting findings on many species. At their widest, these encompass strictly developmental phenomena, especially axon guidance and its responses to lesions, events such as sensory adaptation, long-term potentiation and learning that are essentially synaptic in origin, and the functional tuning of synaptic circuits. These phenomena start with the obvious feature that nervous systems have the capacity to adjust their functional organisation depending on its use and experience. Yet, only a narrow subset of all possible changes manifest by brains, those that can be proved to be functionally adaptive, are judged by some to be true plasticity (e.g. 2). Indeed, the meaning of plasticity itself has changed as the reported range of these phenomena has increased, revealed in particular by studies on the vertebrate visual system (e.g. 3). In fact, the term plasticity may no longer have mechanistic depth insofar as most changes in brain function are now viewed as some sort of "plasticity". Nevertheless, we may delineate formally several major features and phenomena of plasticity, as used here (Table 1).

This review draws from many such examples, incorporating only the few that are particularly well studied, that derive mainly from two groups of insects, Diptera and Hymenoptera, and that in most cases exhibit a clear morphological basis. The metamorphic life-cycles of Diptera and Hymenoptera provide examples of plasticity not only in the adult but also in the larva, often not manifest (or examined) until the adult. Many changes clearly occur post-eclosion, however, and are driven by the experience of the newly emerged adult insect. Some such changes occur during a critical period, shaping the nervous system either during late development or early in adult life according to its experience (4). Yet further examples occur earlier, during development, and plasticity as a whole recycles many of the mechanisms deployed in normal development of the central nervous system (CNS), in response to internal and external stimuli. Finally, many cases of behavioural plasticity, such as associative learning in honeybees (5-6), or olfactory learning and memory in fruit flies, *Drosophila melanogaster* (7-8; for reviews see e.g. 9-11), are well established, underpinned by a large and growing body of cellular and molecular details (e.g. 10). Most of these will not be considered further.

3. PLASTICITY DURING LARVAL AND PUPAL DEVELOPMENT

Plasticity amongst neurons during larval or pupal development may be assayed both from their outcome in the adult, or at earlier stages during development. In practice it is often difficult, and also of little value, to distinguish such events by the time at which they become apparent, as it is to separate the changes themselves from the events of normal development.

3.1. The neuromuscular junction

At the larval neuromuscular junction (NMJ) of *Drosophila*, the number of neuromuscular varicosities increases continuously in an activity-dependent fashion (12). This form of morphological plasticity represents an adaptive response to regulate transmission to the growing larval muscles, yet is really just an aspect of normal development. Various local regulatory signals are involved, *Fas 2* (13), *highwire* (14), *hangover* (15) and others. These

act in concert with bi-directional morphogens, a muscle-derived BMP signaling pathway (reviewed in 16), and a motor neuron-derived morphogen, probably *wingless*, that acts both pre- and postsynaptically (17). A large literature on this topic has been recently reviewed (18-19), but given that these are peripheral synapses, and that their changes are properly considered part of normal development, we will not consider them comprehensively here.

The axons of motor neurons are also highly plastic, and exhibit various forms of sprouting in the periphery. They are sensitive to loss of target muscles in *Drosophila*, and form collateral terminals on foreign muscles without inducing changes in the shapes of terminal arbors on intact muscle targets (20). They form synapses autonomously, without competition with other motor neurons (20), but if a motor neuron is ablated prior to synaptogenesis, collateral sprouts form from its neighbours to provide alternative terminals, implicating a muscle attractant (21). Activity prior to the onset of neuromuscular synaptogenesis promotes motor collateral sprouting (22).

3.2. Sprouting of central neurons

After losing their inputs, central neurons may also undergo reactive changes that can include sprouting, remarkable both because it occurs in undamaged neurons and because it involves morphological restructuring of neurons. Some optic lobe neurons sprout dramatically when deprived of photoreceptor inputs, either experimentally (23-24) or genetically (25), changes that have no clear time of onset but are assumed to occur in the pupa.

3.3. Sensory neurons

In contrast to the examples above, neither the olfactory receptor neurons in *Drosophila*, nor their projection neuron target cells, sprout to neighbouring glomeruli and are transformed after the other is ablated either by genetic means, or by de-antennation in the adult, indicating the stability of circuits formed by these neurons. On the other hand, the density of contralateral olfactory receptor neuron terminals does increase in the corresponding ipsilateral glomerulus, when the ipsilateral glomerulus is denervated (26).

3.4. Synapse formation

Flies in particular provide many examples of plasticity in synapse formation. Reactive synaptogenesis is reported, for instance, amongst synaptic populations of the first optic neuropile, or lamina, in the fly but is quantified as an outcome in the adult fly, and thus considered below.

A major question centres on whether synaptogenesis is activity-dependent (see below). Some sensory receptors may not exhibit activity-dependent synaptogenesis, at least for some of their synapses. This has been nicely shown in decapitate flies, in which stimulation of individual bristle mechanosensory inputs elicits a grooming reflex that is plastic. Small patches of sensilla can be made mutant for a temperature-sensitive allele of *shibire*, which encodes dynamin that acts during clathrin mediated endocytosis at nerve terminals (27). In such flies, sensory axons from the mosaic patches of *shibire* mutant sensilla project into their target neuropile and form terminals that look normal (28), but brief exposure to a non-permissive temperature temporarily blocks their reflex responses to stimulation, irreversibly so if the heat pulse is 8 hours long (28). Despite the irreversible reflex blockade induced by heat pulses of long duration, terminals of the corresponding non-transmitting mechanoreceptor axons fail to change their shape, indicating that the structure of a terminal arborisation is maintained even when the terminal is non-functional. This result is amplified in mosaic patches of mutant sensilla with altered excitability. Sensilla double-mutant for *para* and *nap*, have blocked axonal conduction, but nevertheless form normal terminals (29), indicating that blocking activity exerts no action on pathfinding and terminal arborisation. Sensilla double-mutant for *eag* and *Shaker*, on the other hand, are hyperexcitable and exhibit spontaneous activity, but nevertheless also have unaltered terminals (30).

3.5. Plasticity during metamorphosis

With successive moults, hemimetabolous insects accommodate progressive incremental additions to their sensorium. More radical changes are seen in the nervous systems of holometabolous species, during metamorphosis (31-32). The imaginal reorganization of persistent larval neurons has been reported among interneurons of ventral ganglia in flies (e.g. 33) and persistent serotonin-immunoreactive larval neurons such as LBO5HT in the fly's optic lobe (34). Further examples are reported for *Drosophila* thoracic neurosecretory cells (35). The changes are probably all under direct influence of ecdysteroids, and have been analysed in other Holometabola (36, reviewed in 37-38) as well as *Drosophila* (39).

In *Drosophila* (32, 35) similar changes recycle remodelled persistent larval neurons, and these contribute about 7% of the cells of the thoracic nerve cord (40). Although small, this number includes many motor neurons and wide-field aminergic and peptidergic neurons that are likely neuromodulators (35). In the ventral ganglion of the fly *Sarcophaga*, for example, seven pairs of metamorphic leucokinin-like immunoreactive neurons receive a further three pairs in the adult (41) while all six FMRFamide-like immunoreactive neurons survive from the larva into the adult, with only one undergoing a change in position (42). The neuromuscular innervation of metamorphic larval motor neurons is also remodeled, first retracting, the larval muscles disappearing, and then sprouting to adult muscles. A retrograde stimulus from the larval muscles is required for the retraction, whereas elongation during sprouting requires ecdysone. Signalling between motor neuron and muscle during pupal metamorphosis in *Drosophila* is revealed either by developmentally retarding or permanently eliminating synaptic partners during the formation of the indirect flight muscles (43-44). It reveals that the size of the myoblast pool and early myogenesis depend on the presence of the motor nerve, while the later development of the motor neuron is synchronised with the developmental state of the muscle (reviewed in 45). Growth and remodeling of dendrites is especially clear

among larval motor neurons, either MN1-4, which undergo dendritic regression followed by regrowth, or MN5, which undergoes differentiation *de novo*, during metamorphosis (46).

A dramatic instance of metamorphic neuronal remodelling occurs in the mushroom bodies, or corpora pedunculata extensively studied in flies and honeybees (e.g. 47-52). These morphologically diverse sensory integration centres are involved in high-order computations such as learning and memory (e.g. 5-6, 53-55). Bilaterally symmetric clusters of intrinsic neurons, the Kenyon cells, are located in the dorso-posterior brain; their number in the adult brain is taxon specific. Many adult holometabolous insects inherit larval mushroom bodies, with architectures that vary considerably between species at hatching (reviewed in 51). In Drosophila, the number per mushroom body increases from about 700 at the beginning of larval life, to about 2,100 at its conclusion (56). At pupariation the fibres and dendritic arborizations of many of these (Class II Kenyon cells) start to degenerate and their axons to regrow (57). This regrowth drastically changes the morphological structure of the fibres (58). In Apis, there is also evidence for degeneration of some Class II Kenyon cells during metamorphosis, but their rewiring is less radical than in Drosophila (59). Genetic studies in Drosophila have revealed the many genes that guide mushroom body development. For example, two early reports identified the structural mutants mushroom bodies deranged and mushroom body defect (mud), which perturb the normal axonal regrowth of Kenyon cells at early pupal stages. In these mutants, regrowing axons are unable to re-enter the protocerebral neuropile to form the adult lobes, and mis-target to form a disorganized mass around the input neuropile, the calyx (57, 60).

Retention of learning through metamorphosis is documented in *Drosophila*. Conditioned odour-avoidance behaviour induced in fly larvae by pairing electrical shocks with a specific odour is still present in the adult fly eight days afterwards, and thus survives metamorphosis (61). The single-gene memory mutants *dunce* and *amnesiac* fail to learn as larvae and to retain memory in the adulthood. Such learning tasks are believed to involve circuits in the mushroom bodies (62-63), and it is therefore remarkable that memory retention survives metamorphosis, given the radical restructuring of this brain region.

Other influences on brain development are seen on the volumetric growth of the brain or on effects in the number of synaptic complexes in the brain of insects reared under different conditions during development. Fruit flies, for example, reared at higher larval densities have larger mushroom bodies after emergence than flies reared at lower larval densities (64). In female honeybees, pupal rearing temperature affects the number of synaptic complexes in the adult calyces (65-66). In addition, bees that develop at low pupal rearing temperatures perform less well in olfactory learning tasks as young adults (67-68) suggesting that developmentally induced differences in the number of calycal synaptic complexes may affect adult olfactory behaviour.

4. PLASTICITY IN ADULT INSECTS

The structure of the adult nervous system is plastic, not universally so, but widely so. Even if some changes are relatively minor, they include various phenomena. Two obvious examples illustrate this range. First are changes in the number of cells with increases occurring through continued neurogenesis, documented in the mushroom bodies of certain insect species (see below). These are aspects of normal development, and if modified by external events would constitute forms of plasticity in the nervous system. Second, there are also volumetric changes. Individual cells change in volume, for example during daily or circadian rhythms (69). At least in part such size changes among cells must lead to volumetric changes in an entire neuropile (64). For example, the axon calibre of L1 and L2, two classes of interneurons in the lamina, contribute to the increased neuropile volume seen in flies reared in constant light (70). Many other aspects of neural growth are however also to be suspected, if not at this site then elsewhere. These include increased complexity in: neuronal arborisations, the envelopments of glial cells; and dendritic spines, such as at Kenyon cells (see below), contributions to overall changes in neuropile volume that have yet to be separately quantified. Given their strategic location as bridges between an input axon and the dendrites of its target neurons, together with their concentration of filamentous (f-) actin, dendritic spines have been suggested as primary sites of synaptic plasticity in vertebrates (for reviews see 71-73), and also recently demonstrated in insects (65, 74-75).

4.1. Behavioural plasticity

Pre-imaginal and imaginal effects differ in the role that adult behaviour may play in shaping the final nervous system. Behavioural plasticity in adult insects is well known, for *Drosophila* in vision, courtship behaviour and mate selection (reviewed in 76). Various sensory inputs are involved. For example, female *Drosophila* prefer to copulate with males raised in the same light regime (77-78). These differences are adaptive: dark-reared males are at a disadvantage when they compete with males reared in a light-dark cycle for light-reared females (77). Further details are reviewed by Hirsch *et al.* (4).

More mundane, grooming reflexes are redeployed to different limbs if usual ones are lost. Thus a housefly will groom off a covering of latex paint over one eye with its prothoracic limbs; if these are amputated, it will adapt within two days and do the same using its metathoracic limbs (79). Postural reflexes can be conditioned associatively in headless insects, as shown in a leg learning preparation first developed by Horridge (80). Leg position can also be conditioned in *Drosophila*, in which the underlying mechanisms may be dissected by genetic means (81).

A key feature of social insects, like honeybees, is their ability to evolve polyphenisms: the occurrence of morphological phenotypes accompanied by physiological, neural and behavioural plasticity (e. g. 82-83). In honeybee societies, division of labour into reproductive and non-reproductive phenotypes – the queen and the worker caste

– is induced by larval environmental factors rather than by genetic predisposition (84-85). Within the worker caste a range of tasks is performed that depends on worker age, a phenomenon referred to as age polyethism (82). During their adult life, workers undergo two major phases (first as nurses inside the hive and second as foragers) that differ vastly in sensory input and motor activities. This age-related shift is typically highly stereotyped. In addition to the behavioural plasticity of its individuals, the composition of the colony is also plastic: workers are able to alter their activity in response to changes in task demands, adjusting the proportions of worker stages or worker tasks to ensure the correct division of labour within a colony. Such changes can occur in response to external factors (if workers of one type are removed from the colony, for example), and because they occur through behavioural changes in individual workers, age polyethism and task allocation are a plasticity of individual behavioural plasticities, and thus forms of hyperplasticity (for reviews see 86-87). Several studies in honeybees and other social Hymenoptera have addressed the correlation between reproductive polyphenism and age polyethism and morphological differences in brain structure (see below).

Plasticity in adults is well documented in sensory systems, especially the visual and olfactory systems, and in higher brain regions, notably the mushroom bodies, which we will now consider in turn.

4.2. The visual system

Flies exhibit a period of post-eclosion plasticity in visual behaviour. They will normally walk towards an illuminated screen, by phototaxis, and discriminate patterns on the screen depending on their prior visual experience. *Boettcherisca*, for example, normally prefers, in particular, a star to an oblique bar, but develops this ability only over the first four days of adult life and fails to do so when dark-reared past the fourth day (88). The effect thus has a critical period (see below), the fly requiring visual experience during a period until the end of day 4 to develop normal pattern discrimination. A clear case of pattern discrimination plasticity is manifest by flies exposed to bars of differing orientations. Flies exposed to horizontal bars prefer horizontal over vertical bars, and vice versa; whilst flies exposed to right down-oblique bars choose these over left-down oblique bars (88). These examples all illustrate preferences that are relative, and favour the patterns to which the fly is exposed in early adulthood.

These effects also persist to varying degrees. Although pattern discrimination fails to develop within 25 days, if a fly is dark-reared for 5 days after it emerges (89), even a one hour exposure in this period to a particular pattern leads a fly to discriminate some (horizontal stripes) but not other (vertical, oblique) patterns. Normal pattern discrimination can be rescued in a fly that receives such treatment, depending on the visual experience it receives after 5 days post-eclosion (89). The effects of visual pattern deprivation persist longer in flies that are dark-exposed than in those that see light, and that receive longer post-eclosion periods of exposure to the original pattern. These findings reveal the importance of the first 4 days of adult visual experience (see below). The effects of selective pattern deprivation are diminished after parts of the compound eye are covered, and fail to transfer to the contralateral side, suggesting that the substrate for these effects is the optic lobe itself, rather than the central brain (90). Cytochrome oxidase activity in the optic lobe mirrors these behavioural results (91).

Related findings are also available in *Drosophila*. Dark-reared flies presented with a simple test of visual preference (moving towards vertical stripes of different widths) are more attracted to wider stripes than are flies reared in a normal light/dark cycle (92). Their preference is not the result of darkness, but of the timing of exposure to it, being significant only after four or more days of dark-rearing, again suggesting the importance of visual deprivation during the first days of adult life (92).

In addition to plasticity in pattern discrimination, some preferences are innate. Honeybees, for example, have an innate preference for patterns containing radiating elements (93).

4.2.1. Spectral sensitivity

Worker honeybees readily learn to associate spectral light with a food reward (e.g. 94), and their spectral sensitivity also reveals plasticity. Reared in selected light spectra, worker bees are differentially changed in their sensitivity to other wavelengths, spontaneous positive phototaxis being, for example, reduced to wavelengths in which they were selectively deprived. Reared in ultraviolet (UV) light, the bees are less sensitive to light of longer wavelengths (95). The terminals of photoreceptors that signal these wavelengths show corresponding synaptic changes (see below). Flies, *Lucilia cuprina*, are able to associate spectral light with a food reward (96), but whether their sensitivities to different spectra can be influenced by differential rearing is not known.

4.2.2. Motion detection

Detecting wide-field movement in flies provides a further example of plasticity, when the direction of movement is experimentally inverted by reversing the feedback signals in a closed-loop apparatus (97). Each alteration in flight torque causes the body to move in the wrong direction, producing a progressively greater alteration in visual input to drive the flight motor. Devastating for the fly, this situation lasts 20-30 minutes before adjustments in the fly's torque response are observed. Flies also show plasticity in leg posture when this is used to control the visual panorama (98). There are many more subtle or short-term examples of visual learning and plasticity. Those in the visual behaviour of *Drosophila* have been reviewed elsewhere (97, 99). They include plasticity in landing (100-102), one of the most rapidly occurring examples of plasticity involving visual input.

4.2.3. Eletroretinogram

The electroretinogram (ERG) shows long-term sensitivity shifts after differential rearing. Thus, bees rendered less sensitive to long wavelength light through rearing in UV have reduced ‘on’ and ‘off’ lamina transients of their ERG (95). These are thought to arise from altered photoreceptor synaptic populations in the lamina (see

below). Also considered in greater detail below, daily changes are also reported in the ERG transients in the blowfly *Calliphora vicina* (103). Light and contrast sensitivity, as measured from ERG recordings in the housefly, *Musca domestica*, increase with age after eclosion and after dark-rearing during the first five days post-eclosion. Corresponding light increment thresholds are smaller in dark-reared flies. These effects are still detectable three weeks after the flies are brought back to normal light conditions (104), and are compatible with the existence of a critical period.

4.2.4. Synaptic changes

A clear early example of synaptic changes was reported in the UV-reared honeybees discussed above. The terminals of photoreceptors with maximal sensitivity in the green, in which the bees were selectively deprived, have 50% fewer presynaptic profiles than control bees (105). Individual synaptic contacts are unchanged in size, so that the change is in the total numbers of synapses. The numbers of synaptic profiles in the other terminals is unchanged, and there is no information on possible changes among other lamina synapses, which might either offset or augment those of the photoreceptor inputs.

The size of photoreceptor terminals also changes in UV-reared bees, doubling the overall surface density of synaptic contacts (105). Many issues await further study: the effect of exposure duration to UV; the duration of a developmental sensitive period for the synaptic changes; the temporal characteristics of their possible reversibility with white light; whether the differences are primarily a rearing effect or an effect of UV deprivation on the adult eye; and whether the differences are those of synapse formation during development or the possible loss of synaptic contacts prior to establishing the adult synaptic population size (in *Musca* half the synaptic contacts initially formed during synaptogenesis are later lost: 106). Analysis in the bee, with a fused-rhabdome eye, is especially instructive because terminals of photoreceptors with different spectral sensitivity maxima are co-occupants of a single cartridge (107), making it easier to compare their relative inputs to the monopolar cell dendrites that collectively service all postsynaptic sites (108). Thus light exerts a clear influence on photoreceptor synaptogenesis, even though photoreceptors release neurotransmitter tonically, even in the dark (109).

Fly photoreceptor synapses also reveal synaptic plasticity, as reported in the lamina of *Musca*.

Photoreceptor tetrad synapses that provide input to the lamina do not show activity dependence (110), and are usually rather stable under steady-state physiological conditions. But they both form and disappear rapidly in the adult, even in minutes, in response to two types of transient reversal in the photoreceptors: 1) Light exposure after dark rearing gives rise to a short-lived burst of light-evoked synaptogenesis speculated to constitute a mechanism for light-adaptation at the first synapse (111). Although any physiological correlates are lacking, the reciprocal case of dark recovery after a prolonged light-adapting stimulus causes reduced transmitter output (112), correlating with a reduced number of tetrad synapses (111). The rapidity of this light-evoked synaptogenesis, within minutes, indicates that new synaptic sites must assemble through polymerisation of existing presynaptic proteins in the cytoplasm and of filamentous, or f-actin in the dendrites, rather than under transcriptional control. 2) Tetrad synaptogenesis is also seen after another reversal, during warm recovery after cold-exposure (113). The physiological significance is also not clear, but the rapidity of this synaptogenesis again suggests the lack of transcriptional mechanisms, but is associated with the dynamic restoration of synaptic organelles from a molecular machinery that already exists. Synaptogenesis like this, in the adult, reveals that insect neurons previously thought to bear a fixed population of synaptic contacts (e.g. 114), are nevertheless capable of rapid, dynamic change.

Photoreceptor feedback synapses from L2 reveal clear evidence of synaptic plasticity too. They are more numerous in young dark-reared adult flies and beneath an eye that has been monocularly occluded for one to two days, than beneath an eye receiving normal vision (79). Their number also increases during the night phase of a day/night cycle and during the subjective night phase in flies held under constant darkness (115). A possible correlate is the change in lamina cell spines, which also exhibit a daily rhythm (75, see below).

These examples provide clear evidence for structural plasticity at more than one class of synaptic sites in the lamina. Interpreting such phenomena is made difficult by the different experimental conditions and systematic study of further examples may not be useful. Examination of a single form of plasticity (for example, the action of light deprivation on feedback synapses) will be easiest using genetic approaches in *Drosophila*, although it is possible that such plasticity may be more highly developed in other species.

4.2.5. Reactive responses to loss of inputs or targets

L2 feedback synapses in *Musca* also exhibit reactive synaptogenesis when they lose their chief targets, the R1-R6 photoreceptor terminals. After photo-ablating R1-R6, existing feedback synapses are lost, but concurrent reactive synaptogenesis occurs that generates extra presynaptic sites where the presynaptic ribbons are smaller than before (116). These lack the R1-R6 terminal – now degenerated -- as their postsynaptic element, but instead provide augmented input upon the normal second element of the postsynaptic dyad, the transmedullary T1 cell (116).

4.2.6. Neuropile volumes

Entire optic neuropiles also exhibit volumetric changes, although the underlying cellular bases are mostly not yet resolved.

Optic neuropile volumes increase differentially in *Drosophila* depending on their visual experience (70), as part of the volumetric changes seen in other brain regions under various rearing conditions (64). At least for the optic neuropiles, the changes can be attributed to a well-defined population of cells (117), but only in the lamina can it truly be said to result from increased cell volume (hypertrophy) rather than cell number (hyperplasia). Monocular occlusion decreases optic neuropile volumes. In the lamina, these

changes, up to 30%, result largely from the enlarged terminals of R1-R6. Lamina volume increases in the 24 hours after eclosion more in the light than in darkness. The relative effects of light and dark are reversible during the first few days of adulthood, when flies kept in the dark are brought back to the light. Dark shifts after day 4 are less effective, suggesting again a critical period for lamina development that is maximal during the first day after eclosion. Visual stimulation is needed to maintain lamina size during the first 5 days. Dark-rearing for $\geq$1 day during this period decreases lamina volume to the level in flies raised in constant darkness. Once reduced in size, lamina volume seems not to return to normal (70). The difference in lamina volumes is absent in the phototransduction mutant *norpA*, which lacks light-evoked photoreceptor responses, but is not absent in the null mutant for *histidine decarboxylase* (*hdc*) synthesisis of photoreceptor transmitter, which blocks transmission to the lamina (118). This difference suggests that the effect of vision on the lamina arises mostly from changes in the terminals of R1-R6 and not their target cells (70).

An interesting cycle of volumetric changes also occurs in the lamina, where L1 and L2 change the calibre of their axons and their dendritic spines during a daily cycle (69, 75, 119) that has a circadian basis. No claim is made that such volumetric changes *per se* manifest neuronal plasticity, as opposed to osmotic changes resulting from ionic redistributions, but they presumably reflect other dynamic events that do.

A related finding is reported in the ant *Harpegnathos*, in which the optic lobes shrink dramatically in workers that become reproductives and are thus confined inside their dark nest, compared with workers who undergo normal foraging, with its reliance on intense visual experience (120). Disproportionate shrinkage of the brain occurs in the medulla and other parts of the visual system during the early adult life of queen carpenter ants, relative to virgin females. This reduction appears to be adaptive insofar as mature queens live in the dark and, unlike virgins, do not rely on vision. Julian and Gronenberg (121) suggest that queens increase their fitness by reducing metabolically costly neural tissue.

4.3. The olfactory system

Neuropile volumes also change in the first neuropile, the antennal lobe, of the insect olfactory system. The structure of these regions is illustrated for the worker honeybee brain in Figure 1. The antennal lobe neuropile of the worker honeybee increases in volume for example during the first four days after eclosion (122). Individual glomeruli each have a specific volumetric growth pattern (123) and in specific cases change according to the behavioural duties performed by the worker bee (122). In specific glomeruli, a significant increase in volume is accompanied by a significant increase in the total number of synapses (123). Using various manipulation techniques and by applying the juvenile hormone (JH) analogue methoprene, Sigg *et al.* (124) confirm that changes in glomerular volume do indeed depend causally on the bee's behavioural experience, coinciding with improved associative learning of floral odours.

Biogenic amines may mediate at least some of these changes (124). Different brain regions of adult worker honeybees have concentrations of dopamine (DA), serotonin (5-HT), and octopamine (OA) that vary with age-related divisions of labour (125-126). Thus, foragers have more biogenic amines in their antennal lobes than nurses, regardless of age, the difference for OA being largest (125, 127), suggesting that in honeybees antennal lobe OA may be important in controlling both temporal polyethism (see below) and changes in antennal lobe neuropile volume in foraging workers. Behavioural change may correlate with changing patterns of D2 dopamine receptor expression, which is up-regulated with age in the mushroom body calyces of honeybee workers and drones (128). An important extrinsic factor influencing the maturation of glomerulus volume must also emanate from the queen bee, examined by removing the queen from its colony. Thus, when the queen is present 4-day old adult workers have two specific antennal glomeruli that are larger than in bees from a colony which lacks a queen (129). There are also behavioural correlates, the number of young bees responding to a conditioned olfactory stimulus after a single learning trial increases, but more slowly among queenless bees (129).

Aversive olfactory memory seems to be localized to the mushroom body in *Drosophila*, where different subpopulations of Kenyon cells are involved in acquiring and stabilizing olfactory memory (reviewed in 10). Activation of Kenyon cell nicotinic receptors by cholinergic inputs from projection neurons causes intracellular calcium increases that are experience-dependent and could contribute to these adult behavioural changes (130). Many further examples of learning and memory, especially in Hymenoptera, have been analysed at an exclusively behavioural level and will not be considered further in this summary.

4.4. Mushroom bodies

The mushroom bodies integrate a number of sensory modalities, and in flies are predominantly higher olfactory centres (e.g. 131) but in honeybees and other Hymenoptera receive both olfactory and visual projections, along with other sensory input (e.g. 132-133). They demonstrate perhaps more clearly than any other brain region the important influence of young adult behaviour on the final circuits of neurons established in the adult brain. Two types of plastic response are reported for the adult insect CNS, changes in cell number through taxon-specific persistence of neurogenesis, and changes in neuropile volume --and in synaptic numbers -- that in most groups does not depend on altered cell numbers.

4.4.1. Adult neurogenesis

Adult neurogenesis in the mushroom bodies has been described for several hemimetabolous species (134). An outstanding instance involves mitotic activity of persistent mushr neuroblasts, first reported in the mushroom bodies of adult crickets, where many new Kenyon cells can be added per day (135-136). In holometabolous insects, except several coleopteran species (134, 137), post-embryonic neurogenesis ends in larval or mid-pupal stages (e.g. 49, 134, 137-138). Neurogenesis of

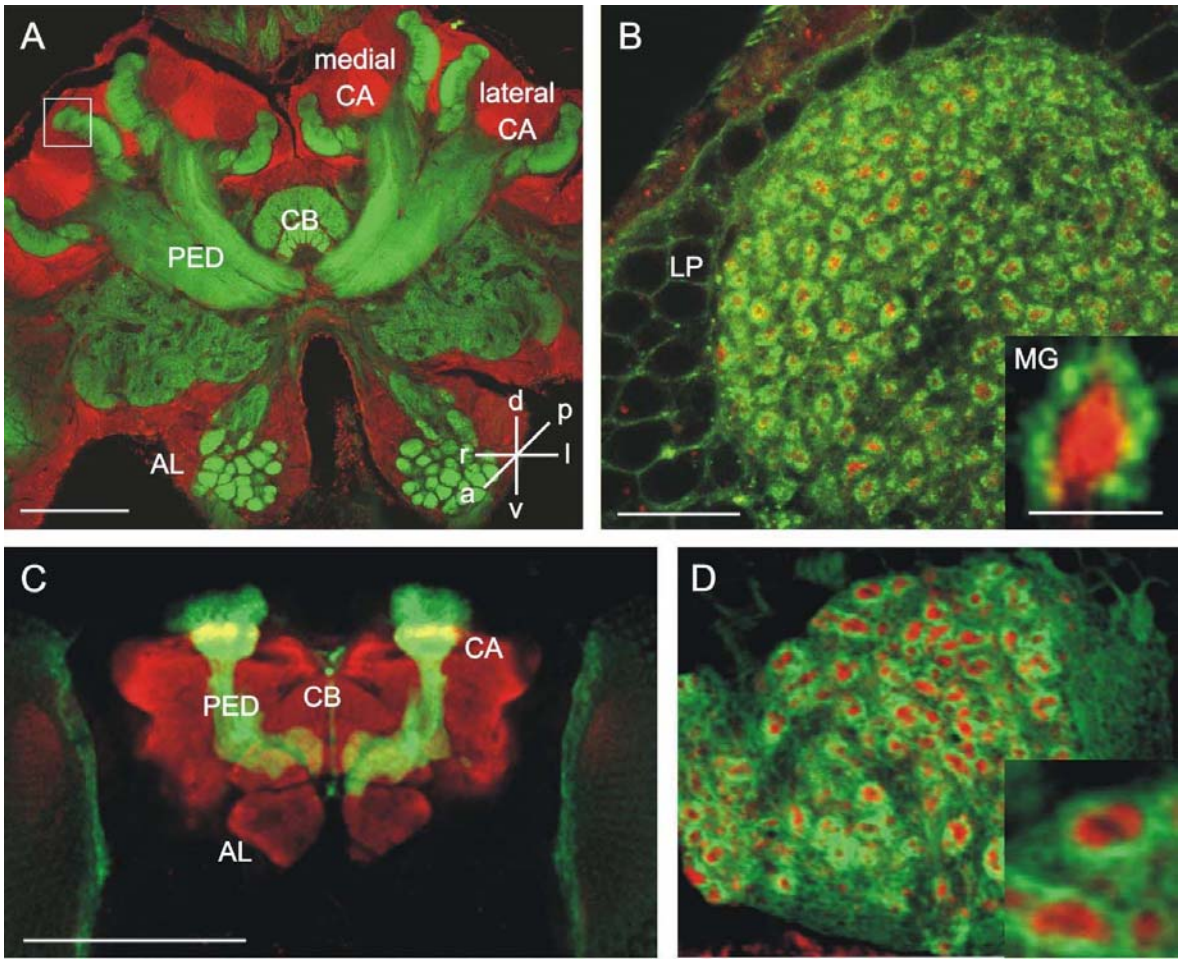

Figure 1. Neuropile regions of the worker honeybee brain and of the fruit fly. Frontal vibratome slices from the brains of both species. A: Mid-depth of the honeybee brain. Antennal lobe (AL) glomeruli are the first relay of projection neurons in the olfactory system that project to the mushroom bodies. Each of these has a lateral and medial calyx (CA), two input neuropiles, and corresponding output regions, the peduncles (PED), which straddle the midline neuropiles of the central body complex (CB). B: Pre- and postsynaptic labelling of microglomeruli (MG) of the lip region of the bee calyx. Each microglomerulus (inset) comprises a presynaptic bouton from a projection neuron at its centre (red), surrounded by postsynaptic Kenyon cell spines (green). LP: lip. C: Central plane of the fly's brain, anterior relative to that in A, showing the antennal lobes (AL), the calyces (CA), and the peduncle (PED). Expression of green fluorescent protein (GFP) in the mushroom body is driven by means of the Gal4/UAS system, using OK107-Gal4 and UAS-LimK to target GFP to the membranes of most Kenyon cells. D: Pre- and postsynaptic labelling of microglomeruli of the fly's calyx, as in B, with individual microglomerulus (inset). Scale bars: A, (200µm); B, (15 µm; inset: 3µm); C, (200µm); D (10 µm; inset: 2 µm).

mushroom body neuroblasts is reported in the adult mushroom bodies of neither *Drosophila* nor *Apis* (47, 49, 139-140). Proliferative activity is absent in adult brains, judged both from neuronal BrdU incorporation in the honeybee (49) and histological stains in the carpenter ant (141). This evidence implies that volumetric increases (see below) are the sole outcome of hypertrophy among individual cells.

Such instances of adult neurogenesis are part of normal development, but unexpected insofar as they signal the continuation of neuronal proliferation into imaginal life. Formally they provide instances of plasticity only when the proliferation can be modified through imaginal exposure. This indeed appears to be the case. Proliferation might be predicted to increase mushroom body volume, perhaps by adding new Kenyon cells as in the beetle *Aleochara* (137), but might also be offset by apoptotic events. In other holometabolous groups, Kenyon cell numbers and mushroom body volume certainly do exhibit suggestive changes related to behaviour. In curculionid Coleoptera, Kenyon cell number correlates with differences in the behavioural habits of the sexes (142), a correlation emphasised with particular clarity in studies on Hymenoptera.

4.4.2. Neuropile volumes

The rich behavioural repertoire of social insects has encouraged correlations between environmental, age- and status-dependent effects and the volume and/or size of

the mushroom bodies (e.g. 141, 143-146). The early literature on behavioural development and its plasticity has been reviewed for the honeybee (147). In the carpenter ant *Camponotus floridanus*, foraging activity may lead to a 50% increase in mushroom body neuropile volume (141), while the brains of mature queens are significantly smaller than those of virgins (121). In a reversed case, brain volume in the ponerine ant *Harpegnathos saltator* decreases in the absence of foraging experience, when workers become cloistered within their colonies as reproductives (120). In a third case, mushroom body volume seems to correlate with the dominance rank among workers of the primitive eusocial paperwasp *Polistes instabilis*, and thus with social aggression, more strongly than it does with the foraging experience of the worker (146). Unlike examples from other groups, adult volume changes in hymenopteran mushroom bodies are not a consequence of adult neurogenesis (see above).

Plasticity in honeybee mushroom body neuropile volumes is associated with the caste and age-based structure of the colony. In workers, progressive increases in mushroom body volume occur with foraging experience (e.g. 143, 144, 148). Similar volume changes within the mushroom bodies occur during the first two weeks of adult life in queens, even in animals without flight experience (83). In eusocial wasps age-related task specialisation in workers correlates with an expanded mushroom body volume (149). Even though mushroom body size increases may reflect the role of processing complex environmental stimuli, the effective sensory cues these contain are not known. In addition to Hymenoptera, similar reports on experience-dependent volume changes come from studies on Diptera. In *Drosophila*, flies reared under relative social isolation, or deprived of antennal input, have fewer Kenyon cell axons along the peduncle of the mushroom bodies than control flies that receive normal stimulation (139, 150). Even subtle cues can exert an effective influence, at least as registered by neuropile volume. Thus, the volume of the mushroom body calyx in a female fly is larger if it is partnered with a female that with a male (64). Vision influences the volumes of the calyces, which are larger in young flies reared for 4 days in constant light than in flies reared in constant darkness (151). The effect is found after monocular occlusion, the smaller calycal volumes found on the ipsilateral, deprived side exclude a role for the widespread bilateral action of a hormone or neuromodulator. The effect of visual experience on calyx volume is absent in the learning mutants *dunce* and *amnesiac*, indicating a role for cAMP. In an additional effect, social crowding exerts an enhancing effect on calyx volume, one not found in *dunce*, amnesiac or *rutabaga (rut)*, and is therefore c-AMP dependent (151).

4.4.3. Synaptic changes

In adult honeybees, adult volume changes in the mushroom bodies are not a consequence of adult neurogenesis (140, see above). Quantitative analyses by Farris *et al.* (152) suggest in fact that an increase in both the length and branching pattern of Kenyon cell dendrites contributes to volume increases in the calyx. In an early report of insect neuronal plasticity, Kenyon cell dendritic spines were shown to change at the time of the bee's first flight (153-154). Recently, intense phalloidin staining of f-actin has been reported in Kenyon cell dendritic spines within synaptic complexes (microglomeruli) in the insect calyx (65-66, 74). The location of f-actin in the insect calyx suggests that microglomeruli may constitute the sites of synaptic and structural plasticity that may partly account for volumetric changes. Indeed, phalloidin-positive profiles express remarkable developmental and adult plasticity in female honeybees (65-66). For example, the thermoregulation of sealed brood cells has important consequences on the synaptic organization within the calyx of the adult workers and queens that emerge from them. Even slight variations in the pupal rearing temperatures affect the number of microglomeruli in the olfactory lip region. Temperature effects were less pronounced in the visual collar region. In queens, the largest number of microglomeruli in the olfactory lip region develops after rearing at 1°C below the temperature that produces the largest number of microglomeruli in workers (33.5 vs. 34.5°C). This is surprising because the rearing temperature in natural queen cells is similar to that in worker brood cells (66, 155). In addition to the influence of brood care conditions, microglomeruli exhibit a striking long-term adult plasticity throughout the extended life-span of queens. Whereas the number of microglomeruli in the olfactory lip continuously increases with age, the number of microglomeruli in the adjacent collar decreases over a period of one year (66). In general, changes in the number of microglomeruli might be the underyling cause of volume changes in the mushroom body calyx and might include contributions from both pre- and postsynaptic elements, most likely caused by changes in their branching patterns, but these details have all to be confirmed.

Are these phenomena adaptive, leading to improved fitness? Cayre *et al.* (134) speculate that newborn Kenyon cells in orthopteroids, that are the product of imaginal neurogenesis, may provide flexibility in the acquisition of new information about the adult environment. Given that some proliferation is glial, and that species like honeybees and fruit flies fail to show such changes, this may not be the complete picture, but the broad correlation between behavioural experience and maturational growth in the mushroom bodies, either through increased Kenyon cell numbers or increased mushroom body size, suggests that they are experience-dependent. In addition, changes in neuropile volume reported in the honeybee anticipate experience (156), and these lend greater weight to the idea that such changes are preparatory and thus adaptive. Fahrbach and Robinson (147) propose that neural plasticity in the brains of worker honeybees is required to support the demanding cognitive task of foraging. Confirming this claim will first require that we know more about the cellular bases for volumetric changes in the mushroom bodies and their functional consequences, and also that we understand mushroom body function more clearly than at present.

4.5. Basis for changes in cell and neuropile volumes

Volumetric changes in an entire neuropile could result from changes in either the number of component cells (hypo- or hyperplasia) or their individual volumes (hypo- or hypertrophy). The former result from altered

patterns of cell proliferation or survival; the latter result from altered patterns of arborisation and/or changes in the dimensions of individual neurites and/or their terminals.

In honeybee workers, foragers have a larger mushroom body neuropile than nurses (143). Initial reports suggested a dependence on the titre of JH, because some aspects of volumetric plasticity that coincide with foraging are also found in workers treated with methoprene, and because plasticity seen after methoprene treatment occurs independent of actual foraging experience (148). High JH titres and enlarged mushroom body neuropile volumes are both characteristic of the forager, and increases in both of these coincide, leading to the proposal that JH may mediate mushroom body volumetric plasticity (147). However, subsequent study of worker bees reared in isolation found no correlation between increased JH titre induced by the solitary rearing and changes in neuropile volume (156). By contrast, a correlation exists with the three biogenic amines mentioned above (125). Of these, brain OA has the strongest and most consistent correlation with behavioural plasticity, independent of age (157). OA in the antennal lobes is of particular importance in controlling age-related division of labour (125), oral administration increasing the numbers of new foragers among bees old enough to forage (126-127).

We have seen that volume changes in the optic neuropiles are influenced by visual input. This influence on the central complex and mushroom bodies, and the influence of other sensory stimuli as well as of behavioural experience, have all been revealed in *Drosophila* by analysing mutants that block volumetric plasticity (151). The influence of vision revealed by the phototransduction mutant *norpA* is to be expected for the optic neuropiles, but is not expected for the mushroom bodies. Structural plasticity in the latter arises from both the volumetric differences in the neuropiles when flies are reared under different regimes (64, 151), which is sensitive to the mutant *rut*, and the outgrowth and retraction of Kenyon cell axons (150), which is *rut*-insensitive. Even though the cellular contributions to neuropile volume changes are most clearly resolved in the visual system, in the lamina (70), even at that site the contributions of the different parts of the monopolar cell interneurons are not yet resolved, especially with respect to the dendritic spines of lamina cells. Apart from rearing effects, the transient daily and circadian changes seen in L1 and L2 are reversible, with a mechanism that is probably not a simple osmotic change. Cyclical modulation of ion pumps on the membranes of lamina glia is a possible basis (158), and although the cartridge glia are involved (159), as is the vacuolar and plasma membrane proton-adenosine triphosphatase V-ATPase (160), direct evidence for the cyclical activity of ion pumps is incomplete.

5. PLASTICITY AND THE TIME COURSE OF EVOKED CHANGES IN THE ADULT INSECT BRAIN

5.1. Short-term synaptic changes

Nervous systems must adapt to changes in the external world. This need is felt first in sensory systems, and sensory adaptation may use mechanisms that are agents of longer-term plasticity. The insect compound eye and optic lobe undergo light adaptation (161), tentatively identified with which new photoreceptor synapses appear in the lamina of *Musca* of dark-reared flies after a brief light pulse (111). *Drosophila* exhibits an odorant-induced antenna-mediated startle response which habituates (162); this habituation is promoted by GSK-3/Shaggy (163).

Short-term synaptic changes include transient plastic phenomena such as facilitation and depression, as well as augmentation, a longer lasting form of facilitation, and the longer-lived potentiation are all reported at the *Drosophila* larval NMJ (164). Facilitation appears when the presynaptic nerve is stimulated either at high frequency (frequency facilitation) or when pairs of pulses are applied in rapid succession (165), in which case the second response is larger than the first. The NMJ exhibits paired-pulse facilitation, augmentation (with a duration of hundreds of milliseconds), and post-tetanic potentiation, for all of which calcium concentration is critical (165-166). These events have been analysed genetically, using ion channel and second-messenger learning mutants, and have been reviewed for the NMJ elsewhere (164).

5.2. Long-term synaptic changes

Longer-lived changes in synaptic transmission are also reported that are intermediate in duration between the short-term changes mentioned above and permanent changes in synaptic strength and anatomy that follow. As one example, associative long-term potentiation is reported for PE1, an identified alpha-lobe extrinsic neuron of the honeybee brain (167).

5.3. Daily changes

Each 24 hour cycle in an insect's life brings about recurrent, reversible changes that are manifest widely in the nervous system (e.g. 158, 168-170). Both diurnal and circadian changes, those persisting under constant conditions after a period of entrainment to a light/dark cycle, are forms of plasticity.

Changes in the visual system are widely reported, especially for insect photoreceptors. In the mosquito ocellus, for example, diurnal changes in rhabdome size (171) provided a clear early case of morphological plasticity, while in the blowfly *Calliphora vicina* there are daily changes in the ‘on’ and ‘off’ transients of the ERG (103). These increase during the subjective night, in antiphase to cyclical changes in the ERG’s photoreceptor component (103), and are possibly synaptic in origin.

In *Musca* the optic lamina exhibits two types of cyclical morphological plasticity, which in the night and subjective night show: reduced numbers of feedback synapses from one of the lamina cells, L2 (115); and decreased axon calibre in L2 and its partner, L1 (69). The changes in L1 and L2 axon sizes are offset by changes in size of the surrounding glia, and their regulation involves these cells (159). The photoreceptor terminals, by contrast, do not change their girth. The existence of these two types of change, synaptic and axonal, suggests that there may be

many more, and cyclical variations in axon size are also seen in *Drosophila* (119). There, they correlate with changes in the length of L2's dendritic spines, which are longest at the beginning of day (75). Changes in axon size also correlate with the pattern of the locomotor activity (172), and axon size can be further increased, by stimulating flight intensively (173). Details of the times at which peak changes occur differ between fly species, but these are analysed most completely in *Drosophila*.

Cyclical release of neuromodulators is postulated to drive the changes (174), from two classes of wide-field optic lobe neurons (169) that express immunoreactivity to either serotonin (175) or the neuropeptide PDH (PDF) (176). PDF released by 16 clock neurons is the sole confirmed output neuromodulator of the fly's circadian clock; a second candidate neuropeptide is IPNamide (IPNa), product of the gene *neuropeptide-like-precursor 1* (177, for a review see 178). PDF distributes information for circadian change throughout the optic lobe (158, 179). In *Drosophila* 4 of 5 small (s-LN_vs), and 4 large of the so-called ventrolateral neurons situated in the anterior cortex between the central brain and optic lobe express PDF; some -- especially the s-LN_vs -- are required to maintain behavioural rhythms in constant darkness (180, for many subsequent studies, see reviews: 178, 181), in particular to generate *Drosophila*'s biphasic locomotor activity rhythm (182-183). In *Musca* the varicosities of PDF-positive cells exhibit size changes compatible with cyclical release of PDF (184) and ultrastructural evidence for cyclical release of dense-core vesicles that contain the peptide (185). In *Drosophila* the arborisations of the s-LN_vs dorsal projections themselves are more complex during the day than night, and exhibit cyclical diurnal and circadian changes, being more complex in the day (186). *Drosophila* mutant for the *pdf* gene, and flies in which PDF cells are selectively ablated, exhibit normal diurnal rhythms in behaviour but are arrhythmic under constant conditions (187). Sites of PDF receptor expression (188-190) exhibit somewhat different patterns, but *in vivo* bioimaging of *Drosophila* brain cells indicates that receptivity to PDF, although widespread among clock cells, is not uniform (191).

Finally, no doubt correlated with their remarkable behavioural timing and marked diurnal rhythms, the honeybee protocerebrum also exhibits both age-related and diurnal changes in expression of the product of the clock gene *period* but no such changes in the expression of PDF (192).

5.4. Critical periods

Various neural responses exhibit critical periods in their development in response to either differential experience or experimental interference. Stimulating a small mosaic patch of *shibire* mechanoreceptors in *Drosophila* elicits a grooming reflex that is blocked at the non-permissive temperature less severely in young flies than in flies older than 4 to 5 days (28). Critical periods also exist in honeybee olfactory behaviour (193-194), but are revealed most clearly in the visual system of flies.

Probably all fly species exhibit the effects of differential visual experience during the first few days post-eclosion that are reported for selected species. In houseflies, exposure to flickering light depresses the number of L2 feedback synapses relative to monocular occlusion or dark-rearing (79), and dark-rearing increases the sensitivity of the ERG (104). In *Boettscherisca* dark-rearing for $\geq$ 4 days prevents the development of pattern discrimination when flies are later exposed to normal patterns (88-89). *Drosophila* walk towards vertical stripes, more readily to large stripe widths than narrow after dark-rearing, but not when this occurs after day 4 (92). Developmental increases in *Drosophila* optic neuropile volumes are susceptible to rearing in constant light. Only if *Drosophila* is exposed to light in day 1 after it emerges can lamina neuropile volume enlarge to its full adult size. After day 1 light exposure is ineffectual, although for $\geq$ 5 days post-eclosion the lamina remains sensitive to light-deprivation, which decreases lamina volume (70).

5.4.1. Duration

The duration of the critical period seems to be somewhat fixed, regardless of species. The first 4 or 5 days of adult life in three fly species of different size and longevity, *Musca* (79, 104), *Drosophila* (92), and *Boettscherisca* (88). Assuming that changes occurring during the critical period adapt the visual system to the anticipated visual experience during the rest of the fly's life, four days may be a minimum period (4-6 24 hour cycles) required to assess the fly's visual regime reliably (70, 195). Many insects may scarcely outlive this period, however (reviewed in 196). Peak susceptibility may of course vary. Thus, the difference in L2 feedback synapse numbers between occluded and seeing eyes is greater in flies exposed between 2 and 4 days (79), whereas light-depriving males for just one or two days after eclosion produces a near maximal increase in light contrast sensitivity (104). Other species may be long-lived, and temporal polyethism in social insects such as honeybees includes the important behavioural transition from nurse to forager at about 3 weeks of age. It is unclear if each phase of neural and behavioural development has its own critical period, or if different species may have different critical periods.

Different stimuli act over different time courses to influence the growth of different brain regions. Thus different brain regions in *Drosophila*, even the optic lobe, can exhibit volumetric plasticity between 8 and 16 days of age (64), in response to social and olfactory deprivation or to spatial confinement, rather than to visual stimuli.

6. POSSIBLE MECHANSIMS

Considerable overlap exists between plasticity during development and plasticity manifest in the adult, and there may indeed be little difference between the mechanisms used during development and in the adult. Goodman and Shatz (197) distinguish three sequential steps in neural development, axonal pathfinding, target recognition and synaptic wiring. Each category encompasses a wide range of cellular phenomena, and considerable attention has been devoted to the extent that each may show activity dependence.

6.1. Factors regulating dendritic growth

Various regulators of normal dendrite development have been identified. For example, Cut (198), Tricornered-kinase/Furry signalling (199, and many other genes in 200) regulate the sensory dendrites of embryonic abdominal neurons of the *Drosophila* peripheral nervous system. These neurons also use the immunoglobulin family cell adhesion molecule Dscam1 to mediate self-avoidance, a tendency for neurites from the same cell to repel each other so as to separate dendritic branches from each other and cover a receptive field uniformly (201-202). The product of a second Dscam gene, Dscam2 mediates the tiling of lamina cells L1 across the medulla, ensuring that members of this single class of neurons establish non-overlapping synaptic domains (203). The effect is specific to L1 cells; Dscam2 lacks obvious action in partner L2 cells, and in R7 and R8. Thus, in these specific examples Dscam2 mediates tiling whereas Dscam1 mediates self-avoidance (reviewed in 204). The role of Dscam's in more central neurons or during plasticity remains to be investigated.

6.2. Effects of neural activity

Based on the pattern of postnatal development of the vertebrate brain, a number of studies --most on sensory systems -- have sought evidence from insects for the activity-dependence of either the formation of synaptic connections in late development, or their adjustment in early adulthood. Activity is not always closely defined, nor is the aspect of neural development that may be affected by activity. Even so, it is clear that activity is highly influential at some synapses, such as larval *Drosophila* NMJs. A number of earlier studies (e.g. 165, 205-206) support the idea that neuronal activity activates the cAMP pathway in the motor neuron, to influence the arborisation of nerve terminals by regulating expression of adhesion molecules. Increased temperature also increases arborisation complexity, mediated by increased cell excitability (207). The central connections of *Drosophila* larval motor neurons also show activity-dependence. Transcription mediated by the neuronal Fos protein AP-1 is required for the growth of motor neuron dendrites, and is induced by neural activity (208).

In sensory systems, the effects are less clear, those on the visual system serving as an example. Synaptogenesis among the nascent tetrad synapses of photoreceptor terminals in the fly's lamina is not activity dependent (110). Since the release of transmitter from photoreceptor tetrads is tonic (209), darkness fails to extinguish its release, but this can be procured by genetic means. Hiesinger *et al.* (110) analysed a panel of mutants that disrupt: either the generation of electrical potentials (*norpA*, required for phototransduction; and trp mutant Ca^{2+} channels required for evoked as well as spontaneous generation of electrical potentials); or their conduction, $para^{ts1}$, a Na^{+} channel required to propagate graded potentials as well as tetrodotoxin injection during development; or the release of neurotransmitter in mutant *hdc* required to synthesize histamine, or synaptotagmin, a Ca^{2+} sensor required for release it. In all of these, the targeting of photoreceptor axons to the lamina and medulla, and the sorting of their terminals at the distal face of the lamina, were normal, providing no evidence for a requirement for evoked or spontaneous neuronal activity. Moreover, tetrad synapses form in normal numbers (110). These findings do not address activity dependence of the many classes of synapse other than tetrads, however, for which information is currently lacking.

Deeper in the visual pathway, visual experience is also not required adjusting the normal receptive field organisation of motion-sensitive neurons in the blowfly's lobula plate (210). These large identified neurons signal a fly's self-motion (211). Dark-reared flies that, 12 hours after emergence, are exposed for two days either to motion in one direction or to continued darkness, develop receptive field and motion sensitive properties indistinguishable from in flies exposed to normal control vision (210). Bearing out these findings, after dark-rearing the complex dendritic branching of the VS1 (vertical system neuron 1) motion-sensing neurons of the lobula plate in *Drosophila* are unchanged in complexity, spine number, spine density, and axon complexity (212). Dark exposure of course fails to eliminate all visual system activity in both these cases, but does remove motion stimuli. For these particular rearing parameters at least, sensory experience plays no role in the functional maturation of the neurons. Flies are adept at aerial navigation and must fly soon after emerging from the pupal case, in preparation for which these neurons at least are hard-wired.

The foregoing suggests that early experience plays little role in synaptic wiring. Later maturation of the fly's visual system may differ. The effects of selective pattern deprivation are mirrored by cytochrome oxidase activity within the optic lobe (91), and the establishment of pattern discrimination is susceptible to chilling, with a specific time course (213). Chilling for 30 mins after visual experience reduces pattern discrimination to a third, and 5 hours of normal activity prior to chilling are needed to consolidate visual experience into pattern discrimination. Various protein synthesis inhibitors mimic these results (213).

6.3. Neuromodulators

Neuromodulators have been either directly or intuitively implicated in plastic responses in the nervous system, and a number of instances are mentioned above: the role of PDH and 5-HT in diurnal and circadian changes in the visual system; the role of biogenic amines in honeybee olfactory learning (124); and the possible role of octopamine in regulating age-related division of labour (125-127, 157). As a possible morphogenetic action, 5-HT modulates the growth of *Manduca* antennal lobe neurons *in vitro* (214), but there is no direct evidence that changes in endogenous amine levels are required to change behaviour or brain morphology, and 5-HT seems an unlikely universal candidate to modulate task-specific neuropile volume increases in honeybees anyway, insofar as mushroom body 5-HT levels do not differ. In the ant *Harpegnathos*, different levels of intraspecific aggression involve no profound anatomical changes in serotonergic neurons (215).

DA in *Drosophila*, on the other hand, is a candidate neuromodulator regulating the branching of

arborisations to the gut that are immunoreactive to serotonin. In the mutant for the dopa decarboxylase gene *DfDdc*, which is able to synthesise neither 5-HT nor DA (216) 5-HT neurites reach their targets but have increased branching. The effect is specific for 5-HT immunoreactive neurites, not others, and can be rescued by feeding mutant larvae with DA, not 5-HT. Possibly DA regulates the cessation of branching among 5-HT immunoreactive neurites. Where are the endogenous sites of neuromodulator release? The varicosities of PDH cells are thought to release peptide for cyclical modulation in the fly's optic lobe (184), but the patterns and types of receptor expression and the alterations these may undergo in response to changes are more critical determinants of peptide action. As a result, exogenous application of neuromodulator can produce different actions at different sites. When 5-HT is injected into a fly's head, for example, it enhances the ERG's lamina transients. Injected into the eye it has the opposite effect, with a rapid onset interpreted to reflect a direct receptor action there, different from the receptor site for head injections (103). Not only the receptor site but also the receptor type determines modulator action. Two major isoforms of *Drosophila* ecdysone receptor (EcR) appear to regulate a different aspect of neuronal metamorphosis. Expressing EcR-A may allow a neuron to respond to ecdysteroids by sprouting and then forming synapses, whereas EcR-B1 is associated with loss of synapses and neurite retraction, which are regressive events (217).

7. CONCLUSIONS

The preceding library of examples readily confirms that the phenomena of plasticity in insect brains do not fall into clear-cut categories, but overlap to a considerable extent. The range of phenomena and their likely mechanisms is also very broad, reflecting the breadth with which the term plasticity itself is applied to nervous systems. Where known, the mechanisms of neuronal plasticity are simply those of normal neural development, recycled at a later stage in insect life and in response to some external stimulus. The chief difference between neural plasticity and neural development therefore lies in the physiological adaptation plasticity provides the insect with in the face of altered conditions, endowing the nervous system with a capability to shape itself, based on the insect's experience (4). Such adaptation enables the insect to respond most effectively to the range of stimuli it is most likely to encounter in the present, as much as to anticipate what it is likely to encounter in the future. Each species exhibits a specific range of adaptiveness that is particular to its habits, such as learning in honeybees. The examples we have considered have been restricted to Hymenoptera and Diptera partly because these holometabolous groups provide examples of a radical life shift between larval and adult forms that imposes major morphogenetic changes to the brain. The genetic manipulability of these phenomena in *Drosophila* offers one clear prospect to reveal the underlying mechanisms, but the generality of these will ultimately rely on comparisons with changes in many other species, because the phenomena of plasticity come not from a single species but from many. The final aim of studies that investigate plasticity should be to localize evoked changes in the nervous system to circuits of identified neurons, or to changes in the modulatory systems that regulate their operation. Only such functional studies and their behavioural outcome will reveal the prospective adaptiveness of the changes to the insect.

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Abbreviations: BMP: bone morphogenetic protein; BrdU: 5'-bromodeoxyunidine; DA: dopamine; Dscam: Down Syndrome Cell Adhesion Molecule; eag: ether a go-go; EcR: ecdysone receptor; ERG: electroretinogram; f-actin: filamentous actin; Fas 2: Fasciclin 2 or previously II; Fos: proto-oncogene; GSK-3: glycogen synthase kinase-3; hdc: histidine decarboxylase; JH: juvenile hormone; L1, L2: two classes of first-order lamina interneurons; nap: no-action potential; NMJ: neuromuscular junction; norpA: no receptor potential; para: paralytic; PDF: pigment dispersing factor, neuropeptide relative of crustacean pigment , dispersing hormone, PDH; rut: rutabaga

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