

A8–OLFACTION AND GUSTATION

Organised by P. Newland, L. Holden-Dye and G. Poppy for the Neurobiology Group and sponsored by Cambridge Electronic Design Ltd, Nikon UK Ltd, Pfizer Ltd, Syngenta, Universal Imaging Corporation, Carl Zeiss Ltd and British Neuroscience Association.

A8.1–Fruitfly maggots: olfactory processing with only 21 receptor neurons

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Drosophila larvae possess an extremely simple olfactory system, with only 21 receptor neurons in each of two olfactory organs and rudimentary integration in the brain. In the adult, there are approximately 1200 receptor neurons on each antenna, converging on clear glomerular structures. The number of receptor molecule types per larval neuron is unknown: so far, none of the 60-odd receptor genes that are expressed in the adult antenna have been found in larval structures. Despite the relative simplicity of the larval olfactory system, maggots respond to a wide range of odours (> 50). The organisation of this simple olfactory network and its underlying coding principles can be inferred from behavioural and genetic studies. Cross-adaptation reveals that maggots can detect functional groups and chain length and that the network involves odour equivalents, inhibition between receptors and integrative functions. Genetic studies show that specific anosmias exist, indicating the presence of structures involved in processing different odours. Behavioural, genetic, anatomical and functional data will be presented and compared with results from other Dipteran species.

A8.2–Olfactory coding in moths – periphery and CNS

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Moths rely to a large extent on olfactory information in their search for mates, food and oviposition sites. During a number of years we have investigated the peripheral and central olfactory system of a number of different moths. At present an EU supported project aimed at using the moth olfactory system as a model for guiding chemotactic unmanned flying vehicles to odour sources in nature is under way.

The moth antenna houses a large number of olfactory receptor neurons (ORN). These were among the first insect ORNs to be characterised electrophysiologically and a large amount of data is present. In a major investigation we have ventured into an investigation of the neural coding patterns of moth ORNs. How similar is

the olfactory code in the same neuron over time and between different neurons?

In the moth antennal lobe ORN input is received in spherical neuropil, glomeruli. We have shown that these glomeruli are functional units. Presently we are investigating amplification phenomena and coding patterns in the lobe using intracellular and calcium imaging methods. The signal from the lobe is processed and integrated in the mushroombodies. One of the main neuron types of these are the Kenyon cells, extremely densely packed neurons with very small diameter. These neurons are now investigated using whole cell *in situ* patch clamping. Olfactory responses have been recorded and a number of cells have been stained.

The perception of an odour is manifested in a heart response of the moth. This response can most likely be seen as mirroring attention of the animal. We have been able to record responses to extremely low doses of pheromone and host-related odours, equivalent to approximately 25 molecules reaching the antenna.

A8.3–Olfaction in *Drosophila*: From Receptors to Perception

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How do insects recognize and discriminate hundreds of distinct odorous ligands in their environment and respond to these stimuli with appropriate behaviors? We have used a molecular approach to identify the receptor genes that underlie the process of odorant recognition in the fruit fly, *Drosophila melanogaster*. These candidate *Drosophila* odorant receptor (*DOR*) genes bear no sequence relatedness to odorant receptors of vertebrates or the nematode *C. elegans*, but share the common structural motif of seven membrane-spanning domains that is a hallmark of chemosensory receptor proteins. Each *DOR* gene is selectively expressed in a small non-overlapping subset of adult olfactory neurons in either the antenna or maxillary palp. The spatial distribution of neurons expressing a given receptor is conserved between individuals and generates a topographic map on the surface of the antenna. Neurons are likely to express only a single *DOR* gene along with a *DOR* gene that is expressed in all olfactory neurons, rendering them functionally distinct. Our goal is to use a molecular genetic approach to link the function of individual odorant receptors to identified ligands and the stereotyped behaviors that these odorant stimuli elicit.

A8.4—Functional expression of *Drosophila* taste receptor

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Taste is an extremely important sensory modality in the behaviour of insects. Chemically-mediated behaviours include detection and feeding on highly caloric food-stuffs, avoidance of harmful and toxic substances and the recognition of mating partners and mating sites. Gustatory processes begin with the activation of specialised receptors in the apical membranes of the taste neurons housed within taste sensilla. Ligand binding triggers signal transduction cascades leading to the formation of action potentials transmitted to and decoded by the insect brain. Recent molecular and physiological studies have revealed that gustatory receptors belong to the superfamily of G protein-coupled receptors (Clyne et al., 2000). To date close to 60 *Gr* genes have been molecularly characterised (Scott et al., 2001; Dunipace et al., 2001). One of these genes, *Gr5a*, expressed in labellar and tarsal taste neurons, is required for the behavioural response to trehalose, a major *Drosophila* feeding stimulant (Dahanukar et al., 2001; Ueno et al., 2001). We have heterologously expressed *Gr5a* and measured responses to trehalose and a number of other disaccharides by monitoring ligand-evoked intracellular calcium (Ca^{2+}) release. Our results show that *Gr5a* functions as a narrowly tuned trehalose receptor, responding only weakly to other naturally occurring disaccharides and to glucose, a common monosaccharide component of all disaccharides tested.

A8.5—Gustatory processing in the thoracic ganglia of the locust

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Chemosensory basiconic sensilla are located over all the surface of the legs of locusts. These sensilla contain four chemosensory and one mechanosensory neurone, which project to their local thoracic ganglion somatotopically, such that the location of the sensilla on the leg is preserved in the relative location of the arborisations of the sensory neurones in the central nervous system. Both mechanosensory and chemosensory neurones project to the same region of neuropil, and no modality or sensitivity-specific segregation of neurites could be observed. Chemosensory neurones make monosynaptic connections onto the same spiking local interneurons in the thoracic ganglia that receive mechanosensory sensory inputs from the leg. We have developed a behavioural assay of chemosensory responsiveness as mediated by the chemosensilla on the legs, in which the probability of a locust withdrawing its leg from a droplet of a chem-

ical solution applied to the hind leg was measured for concentration series of various chemicals. All tested chemicals, even nutrients, could elicit withdrawal responses but critically the concentration at which different stimuli became effective stimuli in eliciting the behaviour varied over several orders of magnitude. The relative size of the response of spiking local interneurons to these same solutions as well as their outputs onto leg motor neurones closely correlated with the probability of eliciting a withdrawal response. We suggest that chemosensory processing in the thoracic ganglia directly assesses a chemosensory quality, that of aversiveness, rather than individual chemical identities and concentrations.

A8.6—Neural processing and plasticity underlying odor-modulated behavior in moths

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A principal goal of our research is to understand the neurobiological mechanisms through which information about specific, behaviorally significant olfactory signals is encoded, processed, and integrated with inputs of other modalities in the brain of the moth *Manduca sexta* and how the message ultimately initiates and controls the insect's characteristic behavioral responses. We seek to help advance fundamental knowledge about olfaction and at the same time to contribute to understanding of insect biology.

We have focused on two odor-dependent behaviors: the anemotactic, odor-modulated flight of a male moth in response to the conspecific female's multicomponent sex pheromone, and the behavioral responses of female moths to the blends of volatile compounds emitted by the flowers and foliage of living hostplants. We aim to elucidate neural mechanisms underlying central processing of semiochemical information.

We focus mainly on the antennal lobe (AL), the primary olfactory center in the moth's brain, and on the functions of glomeruli. In male *Manduca*, the sexually dimorphic glomeruli of the male-specific macroglomerular complex (MGC) are responsible for processing sensory information about sex-pheromone components. Sexually dimorphic glomeruli in the female's AL also have been identified, and their functions are beginning to be understood. We have characterized many individual uniglomerular projection neurons (PNs) associated with MGC glomeruli in the male AL and one female-specific glomerulus in the female's AL. Our findings support the idea that olfactory glomeruli are organized chemotopically. The morphology, physiology, and developmental plasticity of these glomeruli and the neurons innervating them will be emphasized in this presentation.

A8.7–NO-cGMP signalling in the olfactory pathway of insects

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A number of studies have analyzed the NO signalling architecture of insect brains, particularly in the antennal lobes (AL) and the mushroom bodies (MB) where NOS is expressed at high levels (Müller, 1997; *Prog Neurobiol* 51:363). However, important questions regarding the anatomical sources and targets of NO remain unanswered, mainly due to methodological constraints. Here we present new findings based on novel approaches including (1) NADPH diaphorase staining after methanol/formalin fixation as a marker for NOS (Ott & Elphick 2002; *J Comp Neurol* 448:165); (2) use of YC-1, an NO-independent sensitizer/activator of vertebrate sGC, which we find to be effective in insects, improving significantly the localization of sGC activity; and (3) infiltrating the living animal with gaseous NO prior to fixation and cGMP immunostaining, exploiting the direct gas supply in insects provided by the tracheal system. While previous studies have identified local neurons as the sole targets for NO in the locust AL, we find intense NO-induced cGMP-immunoreactivity in virtually all olfactory afferents. In the MB of locust, cricket and cockroach, NADPHd derives predominantly from subsets of Kenyon cells. It is therefore Kenyon cells that account for the tubular NOS expression in the locust α -lobe and not, as reported previously, extrinsic neurons. Moreover, NO-induced cGMP occurs in Kenyon cells whose axons reside in the cores of the NOS-positive tubes. This expression of NOS and NO-induced cGMP in complementary subsets of Kenyon cells supports a theoretical model of NO signalling in locust MB proposed by O'Shea et al. (1998; *Neuroreport* 9:333).

A8.8–Natural antisense, RNAi and gene silencing: novel regulatory mechanisms in NO signalling

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Nitric oxide (NO) has an important role in the chemosensory activation of feeding behaviour in the snail *Lymnaea stagnalis*. Also the NO-cGMP signalling pathway is required for the formation of long-term memory (LTM) following single-trial chemical conditioning of the feeding response. Thus a single pairing of sucrose (the US) with amyl acetate (the CS) results in a conditioned feeding response to the CS which can last for up to 14 days. We have shown that the LTM formation requires NO signalling in the first five hours following training and also depends on transcription and translation during this period. Experiments on the regulation of the NO synthetic enzyme nNOS show that there is a

transient up-regulation of nNOS gene transcription following training. Also we show that the expression of nNOS in the cerebral giant cell (a modulatory neuron involved in feeding) is highly variable and correlated with a sporadic expression of the nNOS transcript. This may be explained by a novel natural antisense mechanism involving the transcription of an antisense containing nNOS pseudogene. Antisense mechanisms may be involved in the physiological regulation of the NO signalling pathway. We are currently trying to establish a role for nNOS gene regulation in LTM formation and one of the tools we have recently developed uses dsRNA to induce targeted silencing of the nNOS gene by RNA interference (RNAi).

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A8.9–Nitric Oxide and cGMP in the Processing of Olfactory Information in *Manduca sexta*

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Gaseous messengers such as nitric oxide (NO) are thought to play a role in the processing of olfactory information in both vertebrate and invertebrate species. The expression patterns of both nitric oxide synthase (NOS) and a target of NO, soluble guanylyl cyclase (sGC), vary between species but generally suggest a neuromodulatory role. Using molecular and electrophysiological methods, we have investigated the role of this signaling system in the olfactory system of *Manduca sexta*. NOS is found in the axons of the olfactory receptor neurons (ORNs) and sGC expressed in a subset of antennal lobe (AL) neurons that includes GABAergic interneurons (LNs), serotonergic interneurons, and projection neurons. This expression pattern suggests that NO produced in the ORNs upon odor stimulation could modulate the subsequent response of AL neurons. Using two NO-sensitive dyes, we find that NO is produced in the antennal lobe in response to stimulation with a variety of different odors including pheromone and host-plant volatiles. Intracellular recordings from LNs show that interference with NO signaling has profound effects on the ability of these neurons to respond to antennal stimulation. Interestingly, LNs are affected even if they do not express sGC. The importance of NO signaling on inhibitory LNs is further supported by multi-unit recordings that show overall activity levels in the AL increase dramatically in the absence of NO. These results suggest that NO can profoundly modulate the output from the antennal lobe and that these effects may be mediated in part by NO-sensitive targets other than sGC.

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A8.10—Molecular mechanisms of olfactory memory formation

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In organisms as diverse as molluscs, insects, birds and mammals memory exists in a variety of temporal domains ranging from short-term memories in the range of minutes to long-term memories (LTM) lasting a lifetime. Evidence from invertebrates and mammals shows that distinct molecular mechanisms contribute to the multiphasic process of memory formation. Although it is a general characteristic that the sequence or the temporal succession of the stimuli during training is critical for induction of a particular memory, little is known about how these training features modulate the molecular processes and how this affects memory formation. Associative olfactory learning in honeybees provides a perfect system for studying the highly dynamic modulations of molecular processes during the training procedure and the processes of memory formation. Our analysis revealed that the second messenger cascades involving protein kinase A (PKA) and protein kinase C (PKC) partake in different processes during learning and memory formation. We demonstrate a tight connection between the training procedure, the temporal activation pattern of the cAMP/PKA cascade and the induction of olfactory LTM. Only a training procedure that induces a distinct PKA activation pattern in the range of minutes leads to a LTM. While this temporally defined activation of the PKA during training is critical for LTM, the proteolytic cleavage of the PKC during the training procedure leads to an autonomous PKM that is required for the maintenance of a mid-term memory in the range of hours.

A8.11—The odour world inside and outside of the bee brain

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Honeybees discriminate a large range of odours, and learn odours as signals for food. We have used optical imaging and intracellular recording techniques to examine the neural substrates of odour coding and memory formation in the primary and secondary neuropil of the honeybee olfactory system.

Odours are encoded in combinatorial spatial patterns of activity in the glomeruli of the antennal lobe (AL). The local network of the AL leads to sharper glomerular activity patterns, less dependence on odour intensity and complex odour-specific temporal patterns of the projection neurons (PN) linking the AL with the mushroom body (MB). The two tracts of PNs leaving the antennal

lobe differ with respect to the temporal patterns of odour-induced action potentials and in their respective response profiles for many odours.

It is concluded that neural processing in the antennal lobe results in two parallel streams of outputs that code odours with different temporal parameters in a spatially-distributed combinatorial activity patterns. Odour learning leads to an enhanced activity pattern for the learned odour that also becomes more different from that of non-learned odours. In the MB odours are recoded in partially overlapping, odor-specific patterns of Kenyon cells that show a high dynamic with odour repetition and learning. Some of the Kenyon cells are recruited, others are lost and others appear unchanged in the course of odour learning. Statistical analysis reveals complex learning-related changes of activity patterns at the level of MB intrinsic neurons.

A8.12—Flexibility, constraint and opportunism in flower foraging by hawkmoths: lessons from interdisciplinary studies of *Manduca sexta*

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Manduca sexta, a model organism for the study of insect olfaction and development, feeds from a broad spectrum of flowers across the Americas. We examined 3 potential sources of feeding modification by manipulating larval diet, flower morphology and nectar presence. (1) Since less than 30% of lab-reared *M. sexta* feed normally, we proposed that moths raised on a leaner diet should feed more as adults. Moths reared on low-sucrose diet or tobacco leaves carried significantly lower fat stores than did those reared on control, low water and low protein diets. However, only moths whose larval diet was spiked with beta-carotene showed greater interest in floral arrays, suggesting Vitamin A deficiency of control diet. (2) We constructed artificial flowers that decoupled visual, tactile and gustatory guides to learn which cues moths use during the final stages of floral approach and feeding. Striking differences in the shape of 'learning curves' (discovery time and feeding success) were associated with these manipulations, especially when floral texture or nectar trace were removed from the proper context. Both visual contrast and tactile guides are crucial to proboscis placement. (3) We used nectarless flower arrays to test for habituation in a nectar-feeding context. Two cohorts were presented with flowers on consecutive days, empty in one group, nectar-filled in the other, and empty for all moths on day 2. Contrary to expectations, unrewarded moths continued to probe empty flowers, while moths that fed successfully probed

longer at empty flowers on day 2, suggesting the primacy of positive reinforcement.

A8.13–Host Location Mechanisms in Insects

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Volatile plant semiochemicals play a key role in host location by phytophagous insects. The detection of these plant volatiles is largely mediated by olfactory cells located on the antennae and information from these peripheral receptors is then conveyed as nerve impulses to the central nervous system, where it is processed, and further transmitted to motor neurones, resulting in behavioural responses. A key question in olfactory research relates to understanding the mechanisms by which information relating to complex mixtures is encoded by the peripheral receptor system. Although this question has, to a large extent, been addressed for pheromone mixtures, with perception being mediated by highly specific olfactory cells tuned to the detection of one key compound, our understanding of the olfactory coding mechanisms for plant odours is limited.

The use of high resolution capillary gas chromatography coupled with electrophysiological recordings, particularly single cell recordings, has allowed the identification of plant semiochemicals and the investigation of peripheral coding of complex odour information. These studies, using physiologically relevant stimulus concentrations, have shown that perception of plant semiochemicals, as with pheromones, is mediated by highly specific olfactory cells keyed to the detection of individual compounds. For many insects, these cells are associated with the perception of ubiquitous plant compounds, even where mono- or oligophagous interactions are involved. Associated behavioural studies suggest that host recognition in insect/plant interactions can be conferred by ubiquitous components with specific blend characteristics.

A8.14–The role of contact-chemoreception in insect–plant interactions

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Olfaction is believed to be the major sensory modality mediating host–plant selection. This is true in many species, but definitively not in all. As a rule different sensory modalities including contact-chemoreception do interact during the perception of plants. Some contact chemoreceptor sensilla have been shown to contain neurones sensitive to plant volatiles. Thus, olfaction and contact-chemoreception are not always clearly separated as the different CNS pathways do suggest. Ablation studies and electrophysiological recordings revealed the

presence of contact-chemoreceptor sensilla on all body parts that come in close contact with potential host-plants. Contact chemoreceptor neurones perceive primary and secondary plant metabolites mediating stimulation or inhibition of feeding or oviposition. In general secondary plant compounds stimulating oviposition or feeding seem to be most important. In accordance, the identified receptor neurones are highly specific and sensitive. Receptor neurones can also be rather specific for some inhibitory (deterrent) compounds. As is the case with plant volatiles, non-volatiles seem rarely to be active singly but act as mixtures. Therefore different types of receptor neurones must be involved in the coding of quality and quantity of the stimulating and deterring compounds, a conclusion that has been confirmed in some species. Receptor neurones of isolated sensilla can interact and thus influence the neural code transmitted to the CNS. Closely related species have been found to differ in the type of compounds perceived and in the coding mechanisms. Thus, in an evolutionary context, the sensory organs seem to be rather variable allowing the colonisation of new food plants.

A8.15–Olfactory learning in parasitoid wasps: from neuron to behaviour

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Parasitoid hymenopterans possess well-developed capabilities for associative learning. The unconditioned stimuli are compounds on or inside the host organism encountered just prior to or during oviposition (unconditioned response), whereas volatile kairomones derived from the plant of the herbivorous host have been shown to act as conditioning stimuli. We have previously documented the behavioural ecology of host searching of two closely related braconid wasp species, *Cotesia glomerata* and *C. rubecula* (Braconidae: Hymenoptera). These two species consistently differ in the degree of plasticity of their searching behaviour. *Cotesia glomerata* needs only one oviposition experience to establish associative learning, whereas in our preference flight bioassays *C. rubecula* was not found to change its innate preference for a plant–host complex offered as an odour source. We recently adopted a sensitisation-controlled no-choice flight bioassay which demonstrated that *C. rubecula* also shows associative learning which shows significantly shorter memory retention (1 day), however, as compared to *C. glomerata* (5 days). To analyse the neuronal basis of the clear-cut difference in learning capacity a three-dimensional map of the glomeruli of the olfactory lobe of both wasp species was constructed. Glomeruli were stained by retrograde axon tracing of all axons in the antennal nerve and observed by confocal

laser scanning microscopy. A set of glomeruli that corresponds between the two species could be identified. This approach, combined with immunocytochemical and gen-expression studies, offers opportunities to pin down which neurobiological differences between both species underly the differences in olfactory learning behaviour.

A8.16—Plant signalling in a multitrophic world

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Since Peter Price and colleagues first highlighted the need to consider multitrophic interactions (Price et al., 1980), many groups have risen to the challenge of such research. During the last decade our proximate understanding of herbivore induced plant signals has increased to the extent that we now know the genes and chemicals involved and we can even simulate the signalling by applying caterpillar saliva to man-made wounds to plant tissue (Pickett and Poppy, 2002). Unfortunately, our adaptive understanding of the precise role of these plant signals has not progressed to the same extent and there is still a debate as to how important such signals are outside of the lab environment. I will use a range of studies including those in our lab on aphid parasitoids (Powell et al., 1998) to outline our attempts to bring together the proximate and adaptive approach to elucidating plant signalling in a multitrophic world. I will pay particular attention to recent advances in genomic and chemical assays which will allow us to answer previously intractable questions, but will also highlight that we must not lose context with our quest to understand the relevance of such signals, and how much they have shaped ecological interactions and could help us manage agroecosystems to our advantage.

Pickett, J.A. & Poppy, G.M. 2001. Trends in Plant Science 6, 137–139

Price, P. et al., 1980. Annu. Rev. Ecol. Sys 11, 41–65.

Powell, W. et al., 1997. Biological Control 11, 104–112

A8.17—The Underwater World of Chemical Signals and Senses

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The astounding diversity of chemoreceptor organs across animal phyla can be reduced to a few principles, which link them to the physics of chemical signals. The chemical properties of these signals provide nearly endless possibilities for specificity and thus precise identification of their source of release. However, the physical dispersal of these signals by diffusion and advection poses serious limitations on their use in locating signal

sources. As a result, mechanoreceptive senses are often linked to chemical senses. The best-studied example may be the vertebrate tastebud innervated by gustatory cranial nerves and branches of the mechanoreceptive trigeminal nerve. The two nerves project onto parallel receptive sheets in the brain where they form a joint spatial map of the mouth or body, each point connecting peripherally to corresponding chemotactile tastebuds. The behavioral function is to locate edible particles in the mouth.

The sense of smell is faced with a similar but more difficult problem. Its behavioral function is to identify important chemical signals in the medium where the medium is in constant chaotic motion. This makes identification of a spatial gradient difficult except when considerable temporal or spatial averages can be obtained. Moving animals can use the sequence of odor patches to help determine a spatial gradient, but robot simulations show that using chemical dispersal information alone is insufficient for efficient tracking of an odor plume. I will present examples of the variety of chemical senses in aquatic animals and their interesting adaptations for linking environmental constraints to behavioral tasks.

A8.18—Layers of antagonistic signals that regulate social vs. solitary feeding behavior in *C. elegans*

Mario de Bono, MRC Laboratory of Molecular Biology, Cambridge, England

Wild isolates of *C. elegans* can feed either alone or in groups. We have been studying the molecular and neural networks that regulate this simple social behavior. Our data implicate multiple antagonistic pathways that induce or repress social feeding. Signals from nociceptive neurons that detect adverse conditions upregulate aggregation behaviour, and genetic or laser ablation of nociceptive neurons transforms social animals into solitary feeders (de Bono et al., 2002). Upregulation of social feeding by nociceptive neurons may involve antagonizing inputs from other sensory neurons, since nociception-defective animals can be restored to social feeding by disrupting a subset of *C. elegans* chemosensory neurons. Another pathway that represses aggregation behavior acts in neurons exposed to the body fluid of the animal, and is mediated by the neuropeptide receptor *npr-1* (Coates and de Bono, 2002). There are two NPR-1 isoforms in the wild. These two isoforms differ in their ability to suppress social feeding, and are associated with natural variation in social feeding behavior (de Bono and Bargmann, 1998). NPR-1 may suppress social feeding partly by antagonizing a pathway mediated by a cGMP-gated ion channel that is required for social feeding (Coates and de Bono, 2002). Together,

our data suggest a model for regulation of social feeding by integration of multiple opposing sensory inputs.

Coates, J., and de Bono, M. (2002). *Nature* 419, 925–929.

de Bono, M., and Bargmann, C. I. (1998). *Cell* 94, 679–689.

de Bono, M., Tobin, D., Davis, M. W., Avery, L., and Bargmann, C. (2002). *Nature* 419, 899–903

A8.19—Arthropod semiochemicals: sealice and biting flies

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Arthropods such as insects and crustacea that feed on vertebrate hosts including man, transmit devastating diseases, cause great distress to their hosts and highly significant economic damage. Dealing with such pests traditionally is based on treatment of the host after biting/infection and control of the vector with pesticides. However, knowledge of the chemical ecology of these invertebrates is revealing the complexities of chemical signalling mechanisms in host location, mate location and oviposition, the components of which may be manipulated for semiochemical based control. Although lagging behind detailed knowledge for insect–plant interactions arthropod host interactions are revealing fascinating parallels in behavioural processes, chemosensory mechanisms and chemicals involved.

Flies (midges and mosquitoes) that bite man, animals and birds and sealice (copepod crustacea) that feed on salmon have been investigated by entrainment of air or water, extraction and distillation, followed by GC-MS analysis to identify components that switch on different behaviours. The sex pheromone of the midge *Culicoides nubeculosus* coupled with host blood volatiles in mating behaviour; host location cues of the Scottish biting midge *Culicoides impunctatus*; the mosquito vector of dengue fever *Aedes aegypti*; the sealouse *Lepeophtheirus salmonis* and oviposition cues of the filariasis vector *Culex quinquefasciatus* will reveal the processes involved in chemical signalling and how such cues may be used in developing push-pull control strategies.

A8.20—Odorant binding proteins and chemosensory proteins in *Drosophila* and aphids

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In insects odorant binding proteins (OBPs) are responsible for the solubilisation of volatile signal chemicals and their transport across the sensillum lymph before

they interact with olfactory receptors to trigger a behavioural response. The OBPs and their genes have been identified in many insect species, mainly by the presence of six, conserved cysteine residues and their high levels of expression in antennae. There is growing evidence that OBPs play a specific role in molecular recognition although there are only a few examples where their interactions with ligands have been demonstrated and characterised. Other chemosensory proteins (CSPs), with only four cysteines, have been found in a wider range of insect tissues although their function is even more controversial.

We have used database searches (using an in-house algorithm) to identify 49 putative OBPs in the *Drosophila* genome, including 16 not previously annotated. Several of these have been cloned and expressed into recombinant proteins using baculovirus/insect cell lines and bacterial systems. We have also expressed recombinant CSPs from genes cloned from a range of aphid species. Our current work is focusing on the identification of the pheromones/host volatiles which act as ligands for these binding proteins, using biochemical and chemical techniques. In the longer term this will lead to an understanding of how semiochemicals influence insect behaviour and to possible novel control methods for pest species.

A8.21—A comparison of the reproductive physiology of migrant and non-migrant populations of the true armyworm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae)

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The true armyworm, *Pseudaletia unipuncta*, is a seasonal migrant in North America and Europe. In contrast, populations of the Azorean archipelago, which presumably are derived from continental ones are non-migrants. A long term interdisciplinary project has been examining the reproductive biology of migrant (Quebec) and non-migrant (Azores) populations. In this species, female sexual maturation (ovarian development and pheromone biosynthesis) is regulated by Juvenile Hormone (JH), whose titers vary significantly in response to abiotic cues (temperature and photoperiod) associated with habitat quality. Azorean individuals become sexually mature at a significantly younger age following emergence than those from Quebec, and despite having a lower body mass they have a significantly higher lifetime fecundity. However, an examination of JH biosynthesis, as well as JH and JH esterase titers in the hemolymph, clearly shows that the difference between the two populations results from more than just a simple temporal shift for earlier, post-emergence, JH production in the non-

migrant population. The responses of the corpora allata from different-aged Quebec and Azorean females to a fixed dose of the neuropeptide allatotropin, as well as those from early and late maturing lines selected from the Quebec population, also support the idea that there are complex differences in the reproductive physiology of migrant and non-migrant populations.

A8.22—Induced and constitutive plant signalling

John A. Pickett, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, U.K.

When plants are attacked by insects, volatile chemical signals can be released systemically from other parts of the plant, and after cessation of feeding. These plant-derived signals are perceived by olfactory sensory mechanisms in both herbivorous insects and their predators and parasitoids, and can be characterised chemically by means of electrophysiological assays using the insect sensory system. Evidence is mounting that such signals can also affect neighbouring intact plants which are caused to initiate defence, either through the generation of mechanical or antibiotic mechanisms, or by the induction of further signalling systems such as those increasing parasitoid foraging. Furthermore, insect electrophysiology can be used to identify plant compounds having effects on the plants themselves. It has recently been found that certain plants release stress signals even when undamaged, and that these can also cause defence responses in intact plants. Again, these signals, released constitutively by intact plants, can influence insect behaviour at second and third trophic levels, which provides opportunities for further identification of semiochemicals acting directly on plants. Discoveries of related signalling within the rhizosphere seem set to extend the understanding of aspects of allelopathy, with again the possibility of using chemosensory acuity of invertebrates to identify the signals involved.

M.A. Birkett et al. (2000) New roles for *cis*-jasmonate as an insect semiochemical and in plant defense. PNAS 97, 9329–9334.

Z.R. Khan et al. (2002) Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp., and the mechanism defined as allelopathic. Journal of Chemical Ecology 28, 1871–1885.

A8.23—

Abstract withdrawn

A8.24—

Abstract withdrawn

A8.25—Position-dependent sensitivity of taste receptors on the locust leg

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Unlike vertebrates the taste receptors of insects are distributed over the mouthparts, body and legs. In insects the sense of taste is used to select and reject food, select appropriate egg-laying sites and to avoid noxious chemicals in the environment. Here we have analysed some of the coding properties of the taste receptors, or basiconic sensilla, on different regions of the legs of locusts and related them to behavioural responses evoked through chemical contact.

We analysed the responses of taste receptors to two behaviourally relevant chemicals, sucrose and sodium chloride (NaCl). In response to stimulation with 500 mM NaCl sensory neurones innervating receptors along the dorsal or ventral surfaces of the hind and fore legs progressively produced more spikes towards the tarsus, so that tarsal taste receptors responded at twice the frequency (approx. 20 Hz) of proximal sensilla (approx. 10 Hz). Similarly, 100 mM sucrose applied to distal receptors on the hind and fore legs evoked more spikes than when applied to proximal receptors. Basiconic sensilla tested with a range of NaCl concentrations were more sensitive on the tarsus compared to proximal sites. Behavioural studies showed that the frequency of leg withdrawal movements to droplets of chemicals applied to a leg increased with increasing NaCl and sucrose concentrations. For a given concentration the frequency of withdrawal was greater when it was applied to the tarsus compared to proximal femur. The foreleg was more responsive than the hind leg to NaCl whereas the frequency of withdrawal was similar for sucrose for both legs.

A8.26—Chemosensory processing and learning in *Lymnaea*

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In gastropod mollusks, chemical cues can be used for appetitive conditioning. This is also the case in the pond snail *Lymnaea stagnalis*, a well-established model system for learning, which can be conditioned to associate sucrose (US) and amyl acetate (CS) leading to feeding in response to presentation of the CS alone. However, in *Lymnaea* relatively little is known about the processing of sensory information from the lips, the main chemosensory site. Using extracellular recording techniques we show here that the sensory nerves connecting the lips to the CNS respond with an increase in the overall firing

frequency to either the US sucrose or the CS amyl acetate. Furthermore, the sensory nerve responses to AA show no significant changes following conditioning. In contrast, activity in the cerebral-buccal connectives (cbc) that convey information between the cerebral and buccal ganglia, where the feeding central pattern generator is located, shows a significant change in response to amyl acetate presentation following conditioning.

These results suggest that chemosensory information in *Lymnaea* is processed in the cerebral ganglia, and that this processing is altered following appetitive conditioning. Based on these results, we propose a Hebbian model for plastic changes at synapses between chemosensory pathways and sensory integrating neurons including the feeding CPG interneuron N1M following appetitive chemical classical conditioning.

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A8.27—Localising sites of sensory integration involved in appetitive conditioning

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The cellular basis of learning and memory has been studied particularly successfully in gastropod molluscs such as the snail *Lymnaea* – where it is possible to record learning-induced changes in individual identified neurons. This snail exhibits long-term memory after one-trial appetitive conditioning as described in abstract A8.26. After such training, resultant changes in the animal's nervous system are likely to be at sites where the chemosensory information is integrated. Such integration sites are thought not to exist in the peripheral nervous system as biocytin fills of peripheral chemosensory nerves reveal no peripheral ganglia and extracellular recording of these nerves shows no learning-induced activity change to the conditioned stimulus. The pathway for chemosensory activation of feeding appears to enter the cerebral ganglion and then pass to the buccal ganglia, site of the feeding central pattern generator, via the cerebrobuccal connective (CBC). Cells that project into the CBC, for example the CA1, CV1a and N1M, are likely sites of integration as this nerve does show a significant change in response to the conditioned stimulus after training. Cells contributing to the CBC response to the unconditioned and conditioned chemostimuli are recorded intracellularly together with extracellular recording of the CBC. The nature of their activation (mono- or polysynaptic), contribution to the conditioned response seen in the CBC and role in activating feeding behaviour will be described.

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A8.28—The role of contact chemoreception in the oviposition behaviour of the desert locust

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Female locusts lay eggs by extending their abdomens rhythmically into appropriate substrates. Egg laying success depends on the water content, the physical and the chemical properties of the substrate. We have analysed the influence of chemicals on the choice of egg-laying sites and on the rhythmic oviposition movements of the abdomen.

We tested the effects of 7 chemicals on the selection of egg laying sites. These ranged from chemicals that represented those normally found in the diet (sodium chloride, sucrose and lysine glutamate) to noxious antifeedants (nicotine hydrogen tartrate (NHT), hydroquinone and tannic acid). All chemicals when present at sufficient concentration prevented egg-laying. Antifeedants were more effective than chemicals normally found in the diet at stopping egg laying. For example, 500 mM NHT stopped egg laying in contrast to 2 M sucrose.

The effects of chemicals on the oviposition rhythm were analysed by applying single droplets of chemicals to the ovipositor valves of rhythmically active isolated abdomens. All 7 chemicals stopped the rhythmic movements. For all chemicals, the greater the concentration the longer the duration the rhythmic movement stopped. For example, 2.5mM NHT stopped the rhythm for significantly longer durations (mean 39.6 ± 5.1 s) than 0.5mM NHT (mean 25.8 ± 2.9 s; $t = -2.34$, $p < 0.05$). By contrast 250mM sodium chloride stopped the rhythm for significantly longer durations (mean 38.3 ± 2.8 s) than 10mM concentrations (mean 30.2 ± 2.4 ; $t = -2.14$, $p < 0.05$). Antifeedants were again more effective at stopping the oviposition rhythm than chemicals found in the diet.

A8.29—A substrate for plasticity: sprouting neurons in an olfactory neuropile of the mature cricket brain

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In crickets, neurogenesis of local interneurons (Kenyon cells) persists throughout adulthood in the mushroom bodies, a high order olfactory neuropile of the brain, involved in learning and memory¹. These interneurons offer a natural in vivo system to study the establishment and layout of structural integration and synaptic coupling into a prominent, well defined columnar neuropile.

In *Gryllus bimaculatus*, we studied the site and geometry of mitotic neuroblasts, growing fibres and synaptic distribution in relation to fully differentiated extrinsic and intrinsic cell types in the mushroom bodies, using molecular marking of neurons, presynaptic sites, actin staining analyzed by confocal microscopy and by conventional electron microscopy.

Sprouting Kenyon cells send a bundle of actin-positive fibres into the anterior mushroom body calyx cup with radial dendritic bundles spreading to marginal synaptic subcompartments, after passing a different proximal synaptic network. In the calyx periphery, a regular pattern of presynaptic boutons of deutocerebral antennal lobe projection neurons contacts Kenyon cell dendrites. Within the columnar stalk the central compact actin-positive fibre bundle appears mainly devoid of synapses. In the mushroom body α - and β -lobes, areas of these sprouting neuronal fibres display a weak synaptic marking compared to mature presynaptic fibre bundles, seen by confocal microscopy. Growing portions of Kenyon cells in the lobes occasionally show tiny synapses with rarely encountered extrinsic fibres.

¹Cayre et al., Comp. Biochem. Physiol.B 132:1–15 (2002)

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A8.30—Electric fields influence the walking behaviour of the cockroach *Periplaneta americana*

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Cockroaches detect air movements caused by approaching predators using highly sensitive filiform hairs on the paired terminal appendages, the cerci. These same hairs are deflected by electrical charge, raising the possibility that electric fields can influence the behaviour of cockroaches. Using a Y-pathway choice apparatus, with a small charged polytetrafluoroethylene (PTFE) plate on one arm, we analysed the choice of walking pathways taken by cockroaches.

Control experiments showed that during walking 97% of cockroaches avoided the charged arm of the choice apparatus ($n=35$). To understand how cockroaches detect electric fields we analysed the influence of a number of sensory structures during walking. We ablated different combinations of the cerci, antennae and palps from first and second instar cockroaches. These systems were chosen as they all showed movement when exposed to charge. For example, the filiform hairs on the cerci, and the antennae were deflected by electric fields. When cockroaches with ablated cerci were tested in choice experiments, 97% still avoided the charge ($n=$

35), indicating that cerci alone contribute little to the initial sensing of electrical fields. Further removal of sensory systems resulted in more errors. With no antennae, but intact cerci, 24% approached the charge ($n=41$); with antennae and cerci ablated, 41% approached the charge ($n=34$), and with palps, antennae and cerci ablated, 75% approached the charge.

We conclude that the cerci alone do not contribute to the detection of electric fields but in combination with more anterior sensory systems lead to the avoidance of electric charges.

A8.31—Electrostatic effects resulting from the interaction between walking House flies (*Musca domestica*) and dielectric surfaces

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House flies were used to model the electrostatic effects of an insect walking across different dielectric surfaces. The results could have important implications for the materials used in insect experimentation. Walking flies accumulated electrostatic charges via a combination of tribocharging and friction. The polarity and maximum saturation charge [$+54.1\text{pC}$ (Correx) to -14.9pC (Acrylic)], depended on the position of the dielectric material in the triboserries relative to the fly.

After i footsteps, the charge acquired per footstep (dq/di) was directly proportional to the difference between the fly's maximum attainable charge q_s on that surface and its charge ($q_{(i)}$): $dq/di = \alpha(q_s - q_{(i)})$ where α is constant.

The charge acquired by the fly (q) is related to the saturation charge level by: $q = q_s[1 - e^{-\alpha i}]$

Experiments on excised fly wings, resulted in increased pick up of dye particles to the fly surface, as the level of charge increased.

Walking flies were also shown to produce electrostatic foot prints on the dielectric surfaces, of the reciprocal charge to that which they pick up.

Dielectric materials are often used in the construction of insect cages or experimental surfaces; this work raises many questions including:

1. What are the effects of these small insect charges on insect behaviour and physiology, or on sensitive recording equipment?
2. Will the charge build up on dielectric surfaces due to insect movement, affect insect behaviour or physiology? Higher charges are known to deter some insects (see abstract A8.30).

3. Can this knowledge be used to manipulate particle pick-up by insects e.g. bees & pollen or enhanced pick up of insecticidal substances or using insects to vector particles.

A8.32–The influence of larval gustatory and olfactory experience on adult feeding and oviposition site selection in *Drosophila*

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Habitat selection in relation to feeding and oviposition is an essential behaviour affecting an insect's survival rate, signifying the importance of experience. We investigated the influence of larval gustatory and olfactory experience on adult preference for feeding and oviposition site selection.

Gustatory experience was analysed using *Drosophila* larvae reared on a medium with different nutritional pro-

files of yeast, sucrose and sodium chloride. Subsequent adult behaviour was observed in a choice chamber containing the medium experienced as larvae and a nutritionally balanced fly diet. Comparisons were made between adults that experienced the novel medium as larvae and those which experienced a standard diet. Results indicate that larval gustatory experience can influence adult feeding and oviposition behaviour.

Olfactory experience was analysed using two odours, carvone and citral in association with the normal diet. Control individuals showed a preference for citral associated food during feeding and oviposition, although no previous exposure had occurred. Larvae experiencing either carvone or citral in association with standard food demonstrated a distinct preference for feeding and egg laying on a standard diet associated with citral. In larvae reared on a standard diet with citral and subsequently given the choice of the larval medium encountered or a more optimal diet (0.025m NaCl) associated with an aversive odour, carvone, 81% of adults chose to consume food experienced as larvae with females laying significantly more eggs in this medium. Adaptive changes resulting from experience may result from pre-imaginal conditioning toward a specific food combination or odour.