

A9—COMPARATIVE NEUROBIOLOGY (POSTER SESSION)

Organised by M.R. Elphick for the Neurobiology Group

A9.1—Metabotropic Glutamate Receptors In *C. elegans*

Dillon J, Pilkington B, Cook A, Willson J, Holden-Dye L and O'Connor. V, SOBS, University of Southampton

The neurotransmitter glutamate acts to co-ordinate animal behaviour by binding to evolutionary conserved ionotropic (iGluR) or metabotropic (mGluR) receptors. The *C. elegans* genome project has revealed both classes of glutamate receptor exist in this model organism. The experimental tractability of *C. elegans* has provided important insight into ionotropic glutamate receptor function.⁽¹⁾ However, less is known about the role of mGluRs in *C. elegans*. In silico analysis identified 3 mGluR genes in *C. elegans* and preliminary analysis predicts these receptors display the archetypal mGluR structure, consisting of an extracellular N-terminal domain, 7 transmembranous helices and an intracellular C-terminal tail with distinct splicing variants. Such splicing suggests the C-terminal is important for organisation of mGluRs function. This is reinforced by studies of mammalian mGluRs, where the C-terminal and receptor interacting proteins are known to scaffold function by directing receptor targeting, anchoring and signalling in neurons. Our analysis of existing transgenic worms expressing mGluR-GFP fusion proteins⁽²⁾ has shown potential scaffolding of these receptors within distinct populations of neurons. Furthermore the reversible dose-dependent inhibition of pharyngeal action potential frequency by mGluR agonists (trans-ACPD 100 μ M) implies glutamate receptors contribute to network activity that regulates pharyngeal function.

We are currently using a yeast two hybrid method to identify mGluR interacting proteins that might scaffold their function. In parallel we are developing behavioural, electrophysiological and optical assays to provide insight into how these interacting proteins contribute to receptor function.

1. Bargmann, C.L. et al Annual Review of Neuroscience 21. Pg 279

2. Ishihara, T. et al Worm Breeders Gazette 14(3) pg 40

A9.2—Nitric oxide-dependent cytokine induction in a new in vitro model of inflammation

Cuttle, M., Blond, D., Pringle, A.K. and Perry, V.H., University of Southampton

Among the factors that are involved in the development of brain damage and neuronal degeneration are the pro-inflammatory cytokines tumor necrosis factor (TNF- α

and interleukin 1- β (IL 1- β), cytokines with pro-inflammatory and cytotoxic properties. We have utilised an in vitro acute brain-slice model to investigate intrinsic pathways in the brain that generate pro-inflammatory cytokines and compared the results to an established in vivo model. Cytokine synthesis in vitro was robustly and reproducibly initiated by rapid temperature elevation to 35 °C after sectioning. The time course and magnitude of cytokine synthesis in this model mimics in vivo studies, making the system potentially of great value for rapid screening of anti-inflammatory drugs. Our investigation of the potential mechanisms underlying cytokine production demonstrated that Interleukin 1 *beta* (IL-1 β) production was dramatically reduced when nitric oxide (NO) synthesis is inhibited by L-NAME. Using confocal microscopy and the NO sensitive probe 4,5-Diaminofluorescein diacetate we observed an increase in NO levels prior to cytokine production. When this NO 'burst' was inhibited, cytokine production was equally inhibited, suggesting that NO is a trigger of cytokine production and not a product of cytokine production in the in vitro model.

This work was supported by the EU grant 'Neuril' and the Royal Society.

A9.3—Imaging dynamic changes in nitric oxide within the crayfish central nervous system

Cuttle, M., Schuppe, H., Chad, J. and Newland, P.L. (University of Southampton)

Local circuits in the central nervous system are subject to continuous modulation that matches their outputs to the specific requirements of an ever-changing environment. The terminal abdominal ganglion of the crayfish coordinates tailfan movements in locomotion and escape responses. Many studies have shown that the free radical nitric oxide (NO) plays a major role in circuit modulation. In the crayfish NO upregulates synaptic transmission to specific ascending intersegmental interneurons that innervate water motion-sensitive hairs on the tailfan, while down-regulating or depressing synaptic inputs to another class of intersegmental interneurons (Aonuma and Newland, J Exp Biol 204:1319–13, 2001). We have investigated the NO synthesis in the terminal abdominal ganglion using the fluorescent probe 4,5-Diaminofluorescein diacetate, DAF-2 DA. Following DAF-2 loading, reproducible cell-specific patterns of fluorescence appeared in the ganglia, with strongly fluorescent cell bodies in specific anterior, central and posterior regions. We found that pre-incubation with the nitric oxide syn-

these inhibitor L-NAME reduced DAF-2 fluorescence levels in the ganglia, whereas the inactive isomer D-NAME had no effect. Washout of pre-incubated L-NAME caused increased cell-specific fluorescence due to endogenous NOS activity. Application of the NOS substrate L-arginine also resulted in an increase of DAF-2 fluorescence. Bath application of the NO donor SNAP resulted in DAF-2 fluorescence increases in most cells. We therefore presume that the cell-specific pattern of DAF-2 fluorescence indicates the distribution of neurones actively synthesising NO.

This work was supported by the Royal society and the BBSRC.

A9.4–Nitric oxide modulates presynaptic inhibition of mechanosensory afferents in crayfish

H. Schuppe and P.L. Newland, School of Biological Sciences, University of Southampton

In crayfish imposed movements of the terminal appendages, the uropods, cause deflection and activation of sensory hairs located on their surface. The actual movements of the uropods are monitored by proprioceptive chordotonal organs within the tail fan whose activation leads to inhibitory inputs in sensory hair afferents. This presynaptic inhibition thus controls the effectiveness of the exteroceptive sensory channel over long time periods. Here we investigate whether inhibitory inputs, elicited in response to stimulation of the protopodite-endopodite chordotonal organ (PEncO) are subject to modulation by the gaseous neuromodulator nitric oxide (NO).

Inhibitory inputs, in the form of primary afferent depolarizations (PADs), were recorded intracellularly from sensory hair afferents and elicited by mechanical stimulation of PEncO. NO levels were altered by bath-application of the NO-precursor L-arginine, the NOS-inhibitor L-NAME, the NO donor SNAP and the NO scavenger PTIO, respectively, while changes in PAD amplitude were measured. Application of L-arginine or SNAP consistently resulted in a decrease in PAD amplitude, whereas L-NAME and PTIO induced increases in PAD amplitude.

The results suggest that NO may decrease inhibitory inputs to exteroceptive neurons thus enhancing transmitter release at their output synapses. NO is thought to be generated in response to stimulation of sensory hairs and NO mediated decreases of PAD amplitude may result in enhanced excitation of second order neurons over longer periods of uropod deflection.

This work was supported by a grant from the BBSRC (UK)

A9.5–Successive differentiation of neuron classes during postembryonic neurogenesis in *Drosophila*

H. Schuppe, School of Biological Sciences, University of Southampton; L. Nicholson, Biology, Yale University; D. Williams, Zoology, University of Washington; D. Shepherd, School of Biological Sciences, University of Southampton

In holometabolous insects like *Drosophila* the transition from larva to adult is accompanied by a dramatic increase in behavioural complexity which mirrors significant changes in neural design. Here we focus on the composition of the *Drosophila* ventral nerve cord in third instar larvae and examine the processes in neural development that occur during this stage.

Neurogenesis in the larval CNS was investigated using Mosaic Analysis with a Repressible Cell Marker (MARCM), an analysis based on the GAL4 technique. In our study a pan-neuronal GAL4 driver was used to drive expression of Green Fluorescent Protein (GFP). MARCM generates somatic mosaics in which GFP is expressed in identifiable clones of neurons and glia, or individual cells.

With mosaics induced 0–3 hrs after egg laying less than 5% of all central neurons labelled in third instar larvae were fully differentiated, most of which were motor neurons, some were neurosecretory cells, and only 8% were interneurons. Most of these were intersegmental, and labelling of fully differentiated local interneurons was rare. In flies grown to adulthood fully differentiated local and intersegmental interneurons were labelled in large numbers, indicating that these neurons can be revealed by MARCM.

Our results suggest that in the larval CNS most fully differentiated neurons are either motor neurons or sensory neurons. Most interneurons are only partially differentiated. Thus most neural processing in the larva is performed by sensory and motor neurons, whereas most interneurons only become functional in the adult.

This work was supported by a grant from the BBSRC (UK)

A9.6–Modelling tauopathies in *Drosophila*

Mudher, A.K., Lovestone, S., Newman, T. and Shepherd, D. (Institute of Psychiatry and University of Southampton)

Tau protein is a microtubule associated protein that forms the building block of the neurofibrillary pathology in neurodegenerative diseases that are collectively called the ‘tauopathies’. The role that tau plays in these neurodegenerative diseases is unclear and it is to shed light on this that we have established a *Drosophila* model of the tauopathies. We have previously demonstrated

that over-expression of tau in neurones causes neurodegeneration in this model without forming filamentous aggregates (Williams, Tyrer and Shepherd 2000). We are now using *Drosophila* to analyse how tau abnormalities cause neurodegeneration. By selectively co-expressing human 0N3R tau and a Green Fluorescent Protein marker of transport vesicles we have examined the consequences of tau over-expression on axonal transport in vivo. The results show that over-expression of tau disrupts axonal transport causing vesicle aggregation and a reduction of mobile vesicles without affecting the vesicle velocity. Disruption of axonal transport is associated with loss of locomotor function in larvae and adults. All these effects occur in the absence of neurone death. The data show that tau abnormalities significantly disrupt neuronal function before classical pathological hallmarks are evident. To investigate the mechanism by which tau overexpression mediates these effects, we are currently over-expressing the tau kinase Glycogen Synthase Kinase 3 beta (GSK-3 β) in this system. These studies shed light on the importance of tau containing pathological hallmarks in tauopathies and also demonstrate the usefulness of *Drosophila* as model organisms in these types of studies.

A9.7–Nicotinic acetylcholine receptor ligands from the Egyptian Milkweed, *Asclepias sinaica*

S. Elbanna, S. Zalut, Zoology, Suez Canal University, Egypt; Bhupinder Khambay, Rothamsted Research; Ian R. Mellor, Ian R. Duce, Francis Gilbert, Life and Environmental Sciences, Nottingham

We have used whole-cell patch-clamp of TE671 human muscle cells to identify ligands of muscle type nicotinic acetylcholine receptors (nAChR) from the milkweed, *Asclepias sinaica*. The plant is toxic to mammals and insects, and the bug, *Spilostethus pandurus* (Hemiptera: lygaeidae), sequesters toxins from the plant for its own protection. It is possible that neuroactive components of the milkweed may be useful as lead structures for pesticides or pharmaceuticals, as well as playing a role in chemical ecology.

A methanol extract of the aerial parts of the plant induced whole-cell currents when applied to TE671 cells. The crude extract was fractionated into petroleum ether (F₁), diethylether (F₂), ethylacetate (F₃), chloroform (F₄) or methanol (F₅). No active compounds were found in F₁, F₂ or F₄. F₃ contained three components when analysed by HPLC (I–III). F₃-I was inactive. F₃-II and F₃-III were antagonists of nAChR. This antagonism was non-competitive and voltage (V_H)-dependent, indicating that they are open channel blockers at nAChR. The methanol fraction (F₅) contained six components (I–VI). Only F₅-III and F₅-VI were active at

nAChR. F₅-III antagonized responses of nAChR to ACh in a V_H-independent and ACh concentration-dependent manner. F₅-VI evoked mimicked acetylcholine when applied alone to TE671 cells.

In summary, *A. sinaica* contains both nAChR agonists and antagonists in its aerial parts. Given that other nAChR ligands are pesticides (e.g. imidacloprid) or antihelmintics (e.g. levamisole, pyrantel), the components described here may have similar uses. S.E. was supported by a British Council studentship.

A9.8–Role of sensory inputs in aimed leg movements of a locust

K.L. Page and T. Matheson, Zoology, University of Cambridge, Cambridge, U.K.

Locusts respond to tactile stimulation of their wings with repetitive scratching movements of their hind legs that are accurately directed towards the site of stimulation. We have sought to understand the role of sensory inputs from the hindwings and legs in controlling this targeting. The likelihood of eliciting a scratch is dependent on the region of the wing being stimulated. This regional sensitivity can be explained by the distribution patterns of three types of sensilla on the hindwing. Basiconic sensilla comprising both chemosensory and mechanosensory afferents are evenly distributed over the wing surface, whereas purely mechanosensory sensilla are restricted to the wing veins. The longest mechanosensory sensilla are present solely along the anal vein (A1), which defines the dorsal fold of each wing, and is the most effective site in eliciting a scratch. The long hairs stand upright, making them ideal for detecting tactile stimulation from above. A sexual dimorphism in the number of mechanosensory sensilla may partly explain sex differences in scratching behaviour.

Ablation of a hindleg proprioceptor (the femoral chordotonal organ) was used to remove a key source of information encoding leg position. Locusts were unable to fully compensate for the absence of proprioceptive feedback caused by the ablation, thus the targeting accuracy of both anterior and posteriorly directed scratches was markedly reduced.

These findings may help us to understand better the roles of exteroceptive and proprioceptive sensory inputs that must be integrated to produce accurate targeting.

A9.9–Effects of temperature on crustacean behaviour and neurophysiology

J.S. Young; L.S. Peck* and T. Matheson; Zoology, University of Cambridge, and *British Antarctic Survey, Cambridge, U.K.

The effect of temperature on the neuronal control of behaviour was assessed for four marine crustacean spe-

cies from stable or variable thermal environments: *Carcinus maenas* and *Ligia oceanica* from the British coastline, where temperature varies between 2 and 24 °C annually, and *Glyptonotus antarcticus* and *Paraceradocus gibber* from Antarctica, where the temperature is a constant –1.8 °C.

The temperate species produced coordinated behaviour up to at least 20 °C, but the Antarctic species became uncoordinated above 5°C and died at approximately 8 °C.

There were positive linear relationships between temperature and neuronal conduction velocity in the pereopodal (leg) nerves of all four species between 0 and 22 °C. In contrast, measurement of how these action potentials are converted into post-synaptic potentials at the neuromuscular junction (NMJ) showed that the temperature at which electrical stimulation of the pereopodal nerve failed to elicit potentials in the muscle depended on the temperature tolerance of the species. Intracellular recordings from *C. maenus* showed an exponential decrease in the time constant of decay of excitatory junction potentials (EJPs) between 5 and 10 °C, with EJP failure as the temperature exceeded 20 °C. Recordings from *P. gibber* showed similar declines in both facilitation and the time constant of decay of EJPs as temperature increased above 0 °C, with EJP failure occurring between 8 and 12 °C. Experiments to determine why *P. gibber* cannot produce EJPs above 8 °C showed that the resting potential of muscle fibres remained constant or decreased (i.e. became more positive) as the temperature was raised, rather than increased as predicted.

A9.10–Neuronal plasticity in the visual system of solitary and gregarious locusts

T. Matheson, H.G. Krapp and S.M. Rogers, Zoology, University of Cambridge, Cambridge, UK

Desert locusts *Schistocerca gregaria* exist in a range of forms between two morphologically and behaviourally distinct extremes, the solitary and gregarious phases. One difference in behaviour is that solitary animals fly individually whereas gregarious locusts fly in dense swarms. We show that changes in the response properties of an identified visual interneurone, the descending contralateral motion detector (DCMD), can be related to this change in behaviour.

In gregarious locusts there was little habituation in the response of DCMD to looming objects presented at 1 min intervals. In solitary locusts, however, DCMD showed a pronounced habituation to 30% of the value recorded in gregarious locusts.

A spike in DCMD elicited a monosynaptic excitatory postsynaptic potential in the metathoracic fast extensor

tibiae motoneurone (FETi), the amplitude of which was approximately 150% larger in solitary locusts than in gregarious locusts. Thus although fewer action potentials were elicited in DCMD by visual stimuli in solitary locusts, each carried a greater weight at the FETi synapse. This tunes the visual pathway in solitary animals so that it is maximally sensitive to infrequent visual stimuli. In gregarious locusts the pathway is tuned so that it continues to function effectively even when the visual environment surrounding the locust contains moving objects. This may help gregarious locusts avoid collisions with conspecifics when swarming, or it may serve to maintain the sensitivity of the escape circuit to the sight of an approaching predator even when surrounded by many other locusts.

A9.11–Phase differences in the walking behaviour of the desert locust *Schistocerca gregaria*

Blackburn, L., Rogers, S. M. and Burrows, M. (University of Cambridge)

The desert locust, *Schistocerca gregaria*, displays a form of phenotypic plasticity ranging between two extremes: a cryptic solitary phase and a swarming gregarious phase. These phases differ extensively in their morphology, physiology, and behaviour, according to their particular ecological requirements (Simpson 1999). Walking patterns are significantly different between the two phases. The co-ordinates of the coxal, femoro-tibial and tibial-tarsal joints and the end of the tarsus were calculated for each leg over a stepping sequence. The angle of the femoro-tibial joint of the hind leg was calculated, along with the angle of the body to the floor during walking and the antennal angle to vertical. Gregarious locusts step significantly faster than solitary locusts but they take larger steps in the horizontal and vertical dimension. The range of the femoro-tibial angle is larger, the body is held at an angle with the floor during walking and they hold their antennae up. Solitary locusts drag the thorax and abdomen along the ground when walking and hold the antennae lower. There are also differences in the thoracic anatomy between the two phases. The cuticular apodemes in the thorax of the solitary locust are larger and affect the attachment points of coxal muscles of the legs. The neuronal differences that underly this behaviour are currently under investigation.

References: Simpson, S.J. et. al. (1999) A behavioural analysis of phase change in the desert locust. Biol. Rev. 74:461–480

A9.12—The temporal resolution of cuttlefish photoreceptors changes with growth

L. Nelson and R. Williamson, Biology, University of Plymouth & Marine Biological Association, Plymouth

The speed with which photoreceptors respond to changes in light level is important in determining the overall performance of an eye. This is particularly true for species, such as the cuttlefish, which are visual predators operating in a wide range of light environments. Previous studies have examined the spatial resolution of the eye, but here we investigate its temporal resolution. This can be determined by measuring critical flicker fusion frequency (CFF), which is the transition point where an intermittent light ceases to flicker and appears continuous. CFF is dependent on light intensity and so is often reported as maximum CFF (mCFF), the frequency at which increases in light intensity no longer lead to an increase in CFF.

Using the electroretinograms from isolated pieces of retina, we determined that the mCFF for juveniles was approximately 42 Hz, but that this was almost twice that of adults, approximately 24 Hz. This difference in mCFF is most likely due to the increase in photoreceptor size with growth, for cuttlefish eyes increase considerably in size with growth. Differences in eye temporal resolution are often correlated with the light level in the animal's natural environment. Thus, the apparent reduction in mCFF with growth in the cuttlefish may indicate that the adults experience a different range of light environments from the juveniles, and given that longer photoreceptors are generally more sensitive than shorter photoreceptors, may imply that the adults are more active in darker environments than the juveniles, i.e. either at night or in deeper waters.

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A9.13—Changes in cuttlefish visual performance with growth

G. Groeger, R. Williamson, and P. Cotton (University of Plymouth and MBA, Plymouth)

The eyes of the cuttlefish, *Sepia officinalis*, although similar to vertebrate eyes in gross morphology, are more comparable with other invertebrate eyes at a cellular level. To understand fully visual processing in cuttlefish, some factors that may influence the retina's responses to different light intensities (i.e. its sensitivity) were investigated using recordings of the electroretinogram. All experiments were carried out on retinas excised from humanely killed animals. Retina pieces (1 cm²) or slices (300 µm thick) were stimulated using an LED, with white light added as background illumination when required. The intensity of both light types was varied using neutral density filters. The factors investigated were the size of

the animal, the wavelength of the stimulating light, and the intensity of illumination.

No significant differences were found in the absolute sensitivities and the V/log I curves obtained from the retinas of different sized cuttlefish. This was surprising, but not unprecedented. Further experiments found that the cuttlefish retina was 7 times more sensitive to blue (peak emission=500 nm) than to yellow (peak emission=590 nm) monochromatic light. This was expected as the peak absorbance of *Sepia* rhodopsin is 492 nm. When the background light was quadrupled, the V/log I curve shifted 2.5 log units to the right. A similar pattern has been found in other animals. With further experiments into the basic mechanisms of cuttlefish vision a model of their visual processing may be constructed.

A9.14—Neuronal regeneration in echinoderms and sea-squirts: cellular and genetical studies of regeneration

Dahlberg, C.M.O., Thorndyke, M.C. and Hallbook, F. (Göteborg University, Kristineberg Marine Research Station and Uppsala University, Sweden)

We are trying to elucidate the molecular background of the phenomenon of neuronal regeneration in the adult *Amphiura filiformis* and *Ciona intestinalis*. *A. filiformis* has naturally occurring neuronal regeneration following loss of arms subsequent to fish predation. *C. intestinalis* on the other hand has a protected neural complex not accessible to predators, but their neural tissue has a remarkable ability to regenerate after excision. We have been doing bioinformatical studies on the recently sequenced *Ciona* genome trying to find homologues to the neurotrophic receptors (Trk). Interesting candidates that might be the ancestral Trks have been found. We are also using in situ hybridisation to establish expression patterns of various genes in the regenerating neural complex in the adult *Ciona*. In the case of *A. filiformis*, we are characterising proliferative cell groups in the regenerating arm with BrdU incorporation. We are using neuronal markers to identify which cells will become the new neurons in the regenerating blastema. In addition, preliminary studies including sequencing of degenerate PCR products show putative Trk receptor homologues in the Ophiuroids as well. Functional studies of physiological roles for these genes are planned through over-expression and/or under-expression.

A9.15—An integrated approach to assess nociception in the rainbow trout

L.U. Sneddon, Welfare Biology, Roslin Institute, UK; current address: School of Biological Sciences, University of Liverpool, UK

Nociception is the detection and reflex response to a tissue-damaging stimulus and is distinct from pain perception since pain also comprises of adverse behavioural reactions. To demonstrate nociception in a fish, techniques in neuroanatomy and electrophysiology were adopted to examine the trigeminal nerve, which conveys nociceptive information from orofacial areas in higher vertebrates, for the presence of A-delta and C fibres that may act as nociceptive neurons. The trigeminal of the rainbow trout (*Oncorhynchus mykiss*) did possess A-delta and C fibres (25% and 4% total fibre type respectively). To investigate if these had a nociceptive function, single unit recordings were made from afferent cell bodies in the trigeminal ganglion. A variety of somatosensory receptor types were located on the head of the trout and 31% of these were polymodal nociceptors preferentially stimulated by noxious mechanical, thermal and chemical stimuli. A further 7% of these receptors were mechanothermal nociceptors that only responded to noxious mechanical and thermal stimuli. To assess pain perception, behavioural responses to administration of acutely acting noxious substances, bee venom and 1% acetic acid, were assessed and compared with handled controls and saline administered fish. The fish administered with venom and acid performed anomalous behaviours, did not feed until the venom and acid effects had subsided and also showed an almost double fold increase in respiration rate. These effects were not seen in the controls or in fish that had been treated with an analgesic, morphine. These results suggest the potential for pain perception in the rainbow trout.

A9.16—Molecular cloning and characterisation of the genes encoding urotensin I and II in the European flounder, *Platichthys flesus*

Lu, W., Gumusgoz, S., Brierley, M.J., Dow, L., McCrohan, C.R., Balment, R.J., and Riccardi, D., School of Biological Sciences, University of Manchester, Oxford Road, M13 9PT

Urotensin I (UI) and II (UII) are two of the neuropeptides released from the urophysis, the neurohemal organ of the caudal neurosecretory system (CNSS) of teleosts. The relationships between urotensin (I and II) and osmoregulation have been suggested for fishes. We have cloned the cDNAs encoding UI and UII peptides from flounder by the reverse transcriptase polymerase chain reaction (RT-PCR) using degenerate oligonucleotide primers, in conjunction with screening of a CNSS cDNA library. Results show that the UI precursor consists of 147 amino acid residues and the carboxyl terminus represents the 41 amino acid sequence of the mature peptide, preceded by Lys–Arg and followed by Gly–Lys. The UII precursor consists of 129 amino acid residues

and the carboxyl terminus represents the 12 amino acid sequence of the mature peptide, also preceded by Lys–Arg. Northern blot analysis of a range of tissues confirmed the CNSS as the sole major site of expression of UI and UII genes and also shows the possibility of multiple polyadenylation signals in the 3' untranslated region of both UI and UII. RT-PCR using specific primers indicated the presence of UII transcripts in all tissues tested. The primary structure of UI and UII shows a close similarity between fish and human. The cyclic region of UII, which is responsible for the biological activity of the peptide, is fully conserved from fish to human. From an evolutionary viewpoint these peptides may exert important physiological functions in both fish and human.

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A9.17—Modulation of the electrical output of the caudal neurosecretory system of the flounder

T.P. Craven, M.J. Brierley, J.R. Banks, R.J. Balment and C.R. McCrohan

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The caudal neurosecretory system (CNSS) is a neuroendocrine complex, located in the terminal segments of the spinal cord of all fish species. The neurosecretory magnocellular neurones within the CNSS (Dahlgren cells) release the osmoregulatory peptides, urotensins I and II (UI and UII). The European flounder is truly euryhaline, in that it can fully adapt its physiology to both fresh and seawater. In winter months fish remain in a seawater habitat, but they migrate into low salinity estuarine water in the summer. The CNSS therefore provides a valuable model for studies of neuronal plasticity, network function and neurohormone secretion in vertebrates.

In euryhaline fish like the flounder, plasma prolactin levels decrease rapidly after transfer from freshwater (FW) to seawater (SW), while plasma cortisol levels rise (Arnold-Reed et al., 1991). The reverse transfer (SW to FW) is associated with a fall in cortisol and rise in circulating prolactin concentration (Hazon & Balment, 1997). Prolactin has a role in adaptive functional changes in ion and water permeability of the osmoregulatory organs while cortisol is critical for changes in gill and gut ion transport capacity (Arnold-Reed et al., 1991). This study of Dahlgren cells investigates the neuromodulatory effects of cortisol and prolactin on electrical activity.

Electrophysiological recordings from an isolated CNSS preparation, taken from both SW- and FW-adapted fish, are coupled with superfusion of either prolactin or cortisol. This will allow us to determine the presence of membrane-bound receptors on the Dahlgren cells, and

also any differences in response depending on the adaptation state of the fish.

A9.18—Cholinergic modulation of electrical activity of Dahlgren cell in the flounder caudal neurosecretory system

M.J. Brierley, T.P. Craven, J.R. Banks, R.J. Balment, W. Lu, D. Riccardi and C.R. McCrohan. School of Biological Sciences, University of Manchester, UK

The caudal neurosecretory system (CNSS), located in the terminal segments of the spinal cord contributes to the neurohormonal control of osmoregulation. The CNSS is unique to fish and comprises mainly magnocellular Dahlgren cells. The flounder has a wide-ranging osmoregulatory capacity and can fully adapt to both sea (SW) and fresh water (FW). Dahlgren cells secrete two neurohormones (urotensins I and II) while acetylcholine (ACh) is synthesised within the CNSS (Conlon and Balment, (1996), *Gen. Comp. Endocrinol.* 103, 36–40). Our previous work has shown that, although Dahlgren cell activity is not significantly different between SW and FW adapted fish, plasma levels of urotensin II are significantly raised during SW adaptation. Here we examined the effect of ACh on Dahlgren cells *in vitro*, and whether ACh differentially modulates electrical activity in the two adaptation states. The muscarinic agonist, oxotremorine, was largely inhibitory, causing all FW cells (18 units/7 fish) and 26/28 SW cells (9 fish) to cease firing. Intracellular recordings confirmed that oxotremorine hyperpolarised Dahlgren cells (by 21.8 ± 5.7 mV; $n=5$, mean \pm S.E). Nicotine induced rhythmic bursting activity in nearly half of previously non-bursting SW Dahlgren cells ($n=8/18$ cells). In contrast, only 3/21 non-bursting FW Dahlgren cells generated burst patterns in response to nicotine. Nicotine had no effect on neurons with ongoing burst patterns, ($n=13$ spontaneously bursting SW & FW cells). In summary, the CNSS expresses both muscarinic and nicotinic ACh receptors, which have different roles depending on both the cells ongoing activity and the adaptation state of the individual.

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A9.19—Kv ion channel transcription reversibly regulated by oxygen supply in turtle brain

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Voltage-dependent potassium channels are important determinants of brain electrical activity, rapidly opening and closing in response to membrane-potential changes. Any natural modifier of their behavior is likely to reg-

ulate neuronal firing properties. Using the anoxia tolerant turtle brain as a model, we found that brain Kv1 channel transcription is reversibly regulated by oxygen supply. Reverse transcription-polymerase chain reaction (RT-PCR) was used to analyze transcript abundance for Kv1 family members and for HIF-1 which were expressed as a percentage of actin transcript levels. We found that in turtle brains exposed to 4 hr anoxia Kv1 transcripts were reduced to 18.5% of normoxic levels and that Kv1 channel mRNA levels were restored to normal within 4 hr of subsequent reoxygenation. No changes were observed for actin or HIF-1 transcripts during anoxia and subsequent recovery. Our results indicate an important mechanism that matches brain activity to oxygen supply.

A9.20—Glucose transporters distribution in various brain regions of the Kir6.2 K-ATP knockout mice

Choeiri, C., Messier, C., Renaud, J.M., Miki, T. and Seino, S. (University of Ottawa, Canada and Chiba University, Japan)

K-ATP channels are widely distributed in the mammalian brain. Emphasis has been placed on their presence in the ventromedial hypothalamus where, along with glucose transporter (GLUT) 1, GLUT2 and GLUT4, they are proposed to play a role in glucose sensing. However, K-ATP channels have also been reported in memory-relevant brain regions such as the hippocampus and septum. Blocking of these channels has been shown to induce memory facilitation. Therefore, the aim of the present experiment is to test the working memory performance of the Kir6.2^{-/-} K-ATP knockout mice as well as assess the distribution of GLUT1, GLUT3 and GLUT4 in various brain regions. Relative to their corresponding wild type, working memory performance of the Kir6.2^{-/-} K-ATP mice is disrupted at age of 12 weeks but not at age of 5 weeks. Concomitant with these memory deficits is a relative decrease in GLUT1 levels in the hippocampus CA3 stratum radiatum. GLUT3 levels were not affected in the mice. However, relative to wild type, GLUT4 distribution in the Kir6.2^{-/-} K-ATP mice was increased in the motor cortex, CA1 and CA3 of the hippocampus. Considering that GLUT4 may be insulin sensitive in the brain, the increased insulin-induced cellular glucose uptake that was observed in the Kir6.2^{-/-} K-ATP mice may be correlated to the increase in GLUT4 levels in the brain.

K-ATP channels couple glucose metabolism to cellular activation. Therefore, their relevance to memory performance may be linked to local glucose uptake in the brain.

A9.21—Comparative neuroanatomy of fatty acid amide signalling: mutually exclusive patterns of fatty acid amide hydrolase expression in the ventricular epithelia of mouse and rat brains

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Fatty-acid-amides (FAAs) are a recently discovered family of neural signalling molecules, exemplified by the endocannabinoid anandamide and the sleep-inducing lipid oleamide. Physiological levels of FAAs in the brain are regulated by the enzyme fatty acid amide hydrolase (FAAH). Previously, we have investigated the distribution of FAAH in the rat brain using immunocytochemistry and, in addition to a widespread association with neurons, FAAH is localised in epithelial cells of the choroid plexi (Egertova et al., 2000; *Neurosci. Lett.* 282, 13–16). We have now examined FAAH expression in the mouse brain, using FAAH-knockout mice as negative controls. As in the rat brain, neuronal FAAH expression occurs in many regions of the mouse brain. Interestingly, however, FAAH-immunoreactivity is not present in epithelial cells of the choroid plexi of the mouse brain but occurs in ependymal cells that form the lining of the brain ventricles. Intriguingly, this pattern of expression is mutually exclusive with the pattern of FAAH expression in the rat ventricular epithelium, where FAAH is expressed in the choroid plexi but not in ependymal cells. The functional significance of this species-difference in the patterns of FAAH expression in the ventricular epithelia of the mouse and rat brains is not yet known. However, these differences in the chemical neuroanatomy of the mouse and rat brain may be important for future functional studies investigating the role of FAAH in regulating the formation of FAAs in the brain and the passage of FAA signalling molecules across the blood–brain barrier.

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A9.22—The expression of cyclic AMP response element binding (CREB)-like proteins in the CNS of *Lymnaea*

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The nuclear transcription factor, cAMP-responsive element binding protein (CREB) regulates a number of cellular functions by promoting the expression of specific genes. In the nervous system, the activation of CREB by the cAMP-activated protein kinase (PKA) is a highly conserved key step in the molecular cascade leading to long-term memory formation. In the present study, we

characterized this transcription factor in the *Lymnaea* central nervous system, which is extensively used in cellular and molecular studies of learning and memory. First, by incubating *Lymnaea* brain extracts with a radioactive CRE containing oligonucleotide probe we identified a CRE-binding protein that was also recognized by a mammalian anti-CREB antibody in a gel mobility supershift assay. Second, using polyclonal antibodies raised against the conservative site of the kinase-inducible region of phosphorylated (P-CREB) and non-phosphorylated mammalian CREB, we identified two, approximately 35 and 38 kDa molecular weight proteins by immunoblotting. Third, in *Lymnaea* brain sections, we found CREB-like immunoreactivity in the nucleus of most neurons. Only between twenty and forty percent of the CREB-like-immunoreactive nuclei displayed P-CREB immunoreactivity but after applying the adenylyl-cyclase activator forskolin this increased to between sixty and eighty percent. Densitometric analyses of immunoblots also revealed that forskolin treatment significantly increased the amount of P-CREB. This work has both established the presence of CREB-like proteins in the *Lymnaea* CNS and indicated the importance of the cAMP-PKA pathway in their phosphorylation, opening the way for an analysis of their role in long-term memory formation.

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A9.23—The role of Urotensin I in the adaptive physiology of the euryhaline flounder *Platichthys flesus*

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Urotensin I (UI) is a 41 amino acid peptide synthesised by the caudal neurosecretory system (CNSS), a discrete neuroendocrine system at the terminus of the spinal cord of teleost fish. Although the CNSS is absent in higher vertebrates the tetrapod orthologue of UI is expressed in the central nervous system. The function of the CNSS has therefore been questioned. The storage organ of the CNSS the urophysis drains directly into the renal portal system, this blood is delivered to the kidney, one of the main osmoregulatory organs in fish. This plus published peripheral actions of UI at osmoregulatory epithelia suggests a potential osmoregulatory role for the CNSS and UI.

The flounder is a euryhaline fish which performs seasonal migration, from a marine environment in the winter where it breeds, to the brackish estuarine water in the

summer months. This movement from one environmental extreme to the other is a huge osmotic challenge to the fish. Many endocrine factors have been identified as having an osmoregulatory function in this transfer, for example cortisol and prolactin, but the role of UI in this adaptation process is yet to be characterised.

In order to identify the role of UI in adaptation to different salinities as a result of environmental change, the circulating levels of the hormone need to be quantified. We report here the development of a homologous radioimmunoassay for UI to allow the measurement of circulating plasma levels in fish adapted to different salinities.

A9.24—Analysis of spontaneous EPSCs and IPSCs in the amacrine neurons of the optic lobe of cuttlefish and their modulation by FMRF-amide

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Using whole cell voltage-clamp recordings in a brain slice preparation of the cuttlefish optic lobe, we have identified and characterized spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs respectively) in amacrine neurons of the inner granule cell layer. We have recorded from 42 amacrine neurons, 43% of them had both sEPSCs and sIPSCs. 33% of these amacrine neurons displayed only sEPSCs and the remaining 24% of them exhibited only sIPSCs. Unlike medulla neurons that showed significant differences between sEPSCs and sIPSCs the amacrine neurons didn't show any significant differences. The sEPSCs in these cells were blocked by kynurenic acid suggesting that they are mediated largely by glutamate receptors. The sIPSCs in these cells were blocked largely by the GABA_A receptor antagonist, bicuculline, suggesting that they were mediated by GABA_A receptors.

The effect of the neuropeptide FMRFa on sEPSCs and sIPSCs on identified amacrine neurons in this newly developed slice preparation was examined using whole cell voltage-clamp technique. FMRFa reversibly reduced both the mean frequency and mean amplitude of sEPSCs in these neurons. By contrast, FMRFa had no significant effect on sIPSCs. In the presence of TTX, FMRFa had no effect on miniature EPSCs suggesting a presynaptic

effect of FMRFa. This neuropeptide also decreased the amplitude or even totally blocked the evoked EPSCs. In this optic lobe slice preparation, we have demonstrated that FMRFa didn't have any effect on GABA_A receptor-mediated sIPSCs, but decreased the glutamate receptor-mediated sEPSCs via a presynaptic mechanism. This work was supported by the Wellcome Trust.

A9.25—Transforming Growth Factor- β s in ophiuroid regeneration

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Echinoderms have long been utilised as model organisms for the study of molecular mechanisms in development. More recently, there has been increased interest in the use of this group to investigate the molecular regulation of regeneration.

Here, work has been conducted to identify putative neural growth factors from the brittle star *Amphiura filiformis* (Echinodermata: Ophiuroidea). Two novel genes encoding transforming growth factor-beta (TGF- β) super-family members have been identified from regenerating arms by degenerate primer RT-PCR and extended sequence attained using RACE (rapid amplification of cDNA ends). One of these genes, *afbmp2/4* belongs to the bone morphogenetic protein 2/4 subclass. Phylogenetic analyses of the other gene reveal highest sequence similarity to *univin*, a TGF- β family member expressed during sea-urchin development. This gene is tentatively named *afuni*.

In situ hybridisation studies show *afuni* expression in the radial haemal canal of regenerating arms extending into the regenerating tissue. This canal is a transport system closely associated with the radial nerve cord of the arm. Expression of this gene is also seen in the radial haemal canal of non-regenerating arms, however expression is not detected at the tips of non-regenerating arms.

These TGF- β encoding genes are potentially up regulated in regeneration and, based on evidence from developmental studies, may be involved in processes such as neural tissue identity and boundary regulation. At present however, experimental evidence of the functions of these genes in regeneration is lacking.