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A15–COMPARATIVE NEUROBIOLOGY (POSTER SESSION)

Organised by Matt Brierley (Manchester)

A15.1 Differential expression of heat shock proteins during progressive anoxia and recovery in the turtle brain

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The turtle brain possesses an extraordinary capacity to survive anoxia. In an initial transition phase, lasting hours, ATP utilization pathways are decreased to basal levels, 1/10th of normoxic. In the second phase neural network integrity is maintained over days while the brain is in a deeply hypometabolic state. Because heat shock proteins (HSPs) have an important protective function against ischemia/anoxia in mammalian brain we investigated the expression of Hsp72 and Hsc73 in the anoxic turtle brain. Unlike the mammalian brain where baseline Hsp72 is almost undetectable, high levels of Hsp72 were found in the normoxic turtle brain. In anoxia levels of Hsp72 were significantly increased by 4h, remained constant until 8h and then decreased to baseline at 12h anoxia. At 24 hours of resumed air respiration the HSP levels had recovered to normoxic levels. The relatively short term rise and decline of Hsp72 points to a potentially protective role during the initial transition phase. By contrast Hsc73 was progressively and substantially increased throughout 12 hours of anoxia. This protein may be involved in maintaining the functional integrity of the neural network during the hypometabolic phase.

A15.2 Nitric oxide modulates rhythmic movements of the locust ovipositor valves

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Female locusts lay eggs by rhythmically extending their abdomens into appropriate substrates to depths of up to 7cm. The selection and digging of appropriate substrates involves the movements of two pairs of sclerotised valves. These valves are moved in a rhythmic, tri-phasic

motor pattern involving four pairs of muscles; the protractors, retractors, openers and closers to produce the digging movements.

The terminal abdominal ganglion contains the motor neurons that innervate the muscles producing the movements of the valves. Increasing the availability of the gaseous neuromodulator nitric oxide (NO) by bath applying the NO donor (SNAP), or the substrate for NO synthesis (L-arginine), increases the cycle speed of the motor pattern. Conversely, reducing the availability of NO by bath applying the NO scavenger, PTIO and an inhibitor of nitric oxide synthase, L-NAME, decreases the cycle speed of the motor pattern. To determine whether these effects could be mediated through a NO/cGMP pathway, we bath applied a membrane permeable analogue of cGMP, 8-Br-cGMP which increased the cycle speed of the digging rhythm. By contrast bath application of the soluble guanylate cyclase (sGC) inhibitor, ODQ, produced a reduction in the cycle speed of the digging rhythm. The bath application of the non-specific protein kinase inhibitor H-7 resulted in a decrease of the cycle speed of the digging rhythm.

These data suggests that NO modulates the motor output of the neural network via the enzyme sGC and the production of cGMP. This cGMP, in turn, appears to act on an as yet unidentified downstream protein kinase.

A15.3 Differential expression of neuropeptide genes in magnocellular neuroendocrine (Dahlgren) cells

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Teleost fishes possess a caudal neurosecretory system (CNSS) in the terminal eight vertebral segments where its anatomical arrangement appears analogous to the neurohypophysis. This organ has been shown to be responsible for secretion of two major neuropeptides, Urotensin I and II. Here we report cloning and sites of

expression of CRH, UI and II from the CNSS of the euryhaline flounder.

The cDNAs encoding CRH, UI and II were cloned from *Platichthys flesus* CNSS by the reverse transcriptase polymerase chain reaction using degenerate oligonucleotide primers, in conjunction with screening a CNSS cDNA library. Northern blot analysis of various tissues confirmed the CNSS as the sole major site of expression of all three CRH UI and UII genes and also suggests the presence of multiple polyadenylation signals in the 3' untranslated region, using conditions that gave no cross-hybridisation. In situ hybridisation analysis of the CNSS serial sections confirmed that CRH UI and UII peptides are predominantly expressed in Dahlgren cells, and each Dahlgren cell may express mRNA for none, one, two or all three peptides. A greater proportion of the cell population appear to express UI than UII, and UI and UII mRNA expression levels are stronger than CRH. Furthermore, using double immuno-gold labelling under EM, it has shown that individual nerve terminals can contain both UI and UII immunoreactive neurosecretory granules. In some terminals the same granules appear to co-localise UI and UII, while in others the peptides are in separate granules. Thus a more complex picture is emerging of the nature of neuropeptides available for expression and secretion.

A15.4 A NO/cGMP signalling pathway underlies modulation of local reflexes in crayfish

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The unorthodox messenger, nitric oxide (NO), has been implicated in the modulation of the local circuits and network properties of many integrative systems, including the mechanosensory system. In crayfish, NO is involved in modulating the activity of local circuits that control and generate movements of the appendages of the tailfan, at many different levels. In this study we have analysed the role of NO on the outputs of the local circuits in the terminal abdominal ganglion in response to stimulation of mechanosensory nerves. Electrical stimulation of sensory neurons that innervate water motion-sensitive sensilla on the tailfan evokes a reflex response of motor neurons that produce movements of the blades of the tailfan, the uropods. Bath application of compounds that elevate endogenous and exogenous NO levels such as L-arginine and SNAP increased the frequency of the evoked spike response. By contrast the application of the nitric oxide synthase inhibitor, L-NAME, and the NO scavenger PTIO, that reduce NO levels, decreased the frequency of response. To deter-

mine through what signalling pathway NO exerted these effects we bath applied ODQ, an inhibitor of soluble guanylyl cyclase (sGC), which decreased the frequency of response. To provide further evidence that NO acts via sGC, we elevated NO levels with L-arginine while simultaneously inhibiting sGC with ODQ. This application reduced the response to control levels, indicating that acts via sGC to regulate cGMP levels, which in turn modulates the responses of the uropod motor neurons.

A15.5 Modulation of the olfactory response to calcium and sodium in the flounder (*Platichthys flesus* L.) Adapted to different salinities

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Several studies have shown that fish have olfactory sensitivity to calcium and sodium ions. The current study assessed whether this sensitivity is modulated by the environmental concentrations of these ions in the flounder, a species which regularly experiences large changes in environmental salinity. Extracellular multi-unit recording from the (upper) olfactory bulb of seawater-adapted flounders gave large, sustained increases in bulb activity in response to decreases in $[Ca^{2+}]$ of artificial seawater (ASW) flowing over the olfactory epithelium. This response had an apparent IC_{50} of 0.8 mM. Na^+ -free ASW had no such effect. Freshwater-adapted flounders (5% seawater) also gave large olfactory responses to decreases in external $[Ca^{2+}]$, similar in amplitude and form to those of seawater-adapted flounders. However, the IC_{50} of this response was much lower (0.015 mM) than seawater-adapted fish. Increases in $[Ca^{2+}]$ of 5% ASW had no effect. In contrast to seawater-adapted fish, freshwater-adapted flounders also responded to increases (but not decreases) in external $[Na^+]$ with an apparent EC_{50} of 122 mM. These results strongly suggest that the flounder is able to modify its olfactory sensitivity to these physiologically important ions in accord with their environmental concentrations. We suggest that the flounder is monitoring environmental $[Ca^{2+}]$ as a 'proxy' for external salinity; the role of the response to changes in external $[Na^+]$ seen in freshwater-adapted fish is less clear. The mechanisms by which this sensitivity is mediated, how it is modulated, and the physiological and behavioural responses to this olfactory input are currently under investigation. Financial support: Treaty of Windsor.

A15.6 Activity of monoamine systems: effects of social stress perception vs production of social responses

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Social stress evokes alterations in limbic system monoamine activity. It is unclear whether these changes result from *perception* of social stress or the *production* of behavioral stress responses. We utilized male green anoles (*Anolis carolinensis*) to explore the nature of monoaminergic alterations within stress-responsive forebrain limbic regions during brief social interaction. Subjects were shown a digital video stimulus of a size-matched opponent, manipulated to contain naturally high amounts of aggressive behaviour representative of a considerable social threat. Control stimuli comprised footage of an empty cage. Brains were collected from animals after 90 s of stimulus exposure, with monoamine activity measured using electrochemical HPLC. Subjects that remained motionless while viewing the simulated opponent showed increased striatal norepinephrine levels and NAc serotonergic activity relative to controls. In contrast, those producing a behavioural signal indicating motivation to engage in social contest exhibited increases in amygdalar and striatal dopaminergic activity, and in striatal epinephrine levels. This suggests that changes in limbic monoamine activity occur differentially according to whether behavioural responses are produced following social stress perception. As the regions sampled mediate behavioural readiness and contextual conditioning, variation in monoamine activity may reflect individual differences in assessment of social threat level relative to internal motivation, allowing an appropriate social response to be produced.

A15.7 Activation and recovery of monoamine systems during brief restraint stress in a lizard model

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Across vertebrates, stressors initiate an array of neural and endocrine changes to produce coordinated coping responses. Adaptive coping also requires that neuroendocrine activity recovers appropriately after stressor removal. How neural and endocrine systems coordinate during coping is not well understood. This study explored the neurochemical and hormonal correlates

associated with activation versus recovery of stress systems following brief restraint of male green anoles (*Anolis carolinensis*)-an established model of stress biology. Lizards underwent 90 seconds of restraint, and were immediately killed or left to recover in their home cage for 5 minutes before collection. Plasma catecholamine levels, and brain monoamine/metabolite levels within micro-punches of stress responsive regions, were measured by HPLC. Plasma corticosterone levels were determined via ELISA. Restraint increased activity of all monoamines within the amygdala and locus ceruleus (LC), with the exception of decreased amygdala dopamine activity. Recovery was only noted for LC serotonin, and amygdala dopamine and epinephrine. Restraint also increased plasma epinephrine and norepinephrine levels, which recovered at 5 minutes. Conversely, plasma corticosterone corresponded to control levels immediately after restraint, but had significantly increased 5 minutes later. Results suggest differential activation and recovery of peripheral stress hormones, and also differential recovery of individual brain monoamine systems. These may represent differing neuroendocrine strategies for coping during and following stress.

A15.8 Behavioral and neuroendocrine correlates of displaced aggression in rainbow trout

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In humans and other primates, violent actions performed by victims of aggression are often directed toward an individual or object that is not the original source of provocation. This psychological phenomenon is referred to as displaced aggression. We demonstrate that displaced aggression is either rooted in evolutionarily conserved behavioural and neuroendocrine mechanisms, or represent convergent patterns that have occurred independently in fish and mammals. Rainbow trout that briefly encountered large, aggressive fish reacted with increased aggression towards smaller individuals. There was a strong negative correlation between received aggression and behavioural change: Individuals subjected to intense aggression were subdued, while moderate assaults induced strong agitation. Patterns of brain serotonin turnover and plasma cortisol suggest that the presence of socially subordinate fish had an inhibitory effect on neuroendocrine stress responses. Thus, subordinate individuals may serve as stress reducing means of aggressive outlet, and displaced aggression towards such individuals appears to be a behavioural stress coping strategy in fish.