peptide), since they are blocked by the partially-selective PAC_1 receptor antagonist, $PACAP_{6-38}$. Moreover, our data indicate that leptin-induced effects on feeding and body temperature are significantly attenuated by pre-treatment with the PAC_1 antagonist.
Semi-quantitative *in situ* hybridisation histology reveals that PACAP mRNA expression in the VMN, but not elsewhere in the forebrain, is regulated according to metabolic state. Either fasting or genetic leptin deficiency, both states of hunger and hypometabolism, are accompanied by a significant reduction in the relative expression of VMN PACAP compared with controls. Conversely, both high-energy diet and exogenous leptin treatment increase PACAP mRNA. Furthermore, mice with genetic VMN-specific deletion of leptin receptor also show reduced PACAP in the VMN, along with a modestly obese phenotype. To further investigate the direct action of leptin on VMN PACAP neurons we are examining endogenous PACAP and leptin receptor expression, and c-Fos induction using dual *in situ* hybridisation histological techniques. Together, these data reveal a highly plastic system which may be essential for maintaining normal energy homeostasis and modulating the central actions of leptin.

Dhillon H et al. (2006). *Neuron* **49**, 191-203.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C3 and PC13

Noradrenaline differentially regulates neuronal excitability in hypothalamic arcuate nucleus neurones *in vitro*

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The hypothalamic arcuate nucleus (ARC) is a key central neural component of the homeostatic feedback systems responsible for maintaining energy balance. Function-specific neural populations within the ARC respond to signals of central and peripheral origin indicating shifts in energy status, including noradrenergic inputs originating from brainstem nuclei. We have utilized whole-cell patch clamp recording techniques in vitro, to investigate the role of noradrenaline (NA) in regulating neuronal excitability in these neurones.
Adult male Wistar rats were humanely killed by cervical dislocation, in accordance with UK guidelines, and whole-cell recordings obtained from ARC neurones in hypothalamic slices as described previously (van den Top et al., 2004).
Brief (5-15s), bath application of NA (40μM) induced membrane depolarisation and increased electrical excitability in 51% (88/172) of ARC neurons, including orexigenic NPY/AgRP neurones (n=9), responses that persisted in TTX (n=12) suggesting a direct effect. NA-induced excitation was associated with increases (n=7; reversal potential -84.1 $\pm$ 5.3mV), decreases (n=5; reversal potential -24 $\pm$ 2.9mV) or no change (n=10) in conductance indicating inhibition of resting potassium and activation of non-selective cation conductances underpin these responses. Depolarising responses to NA were mimicked by phenylephrine (10μM; n=14), completely blocked by prazosin (200nM; n=16) and partly reduced by the α1a-adrenoceptor antagonist RS 100329 hydrochloride (100nM; n=14) suggesting excitation was mediated through α1-adrenoceptors, including α1a. 15% (26/172) of ARC neurones, including 4/9 putative anorexigenic cocaine-and-amphetamine regulated transcript (CART)-expressing neurones, responded to NA with hyperpolarisation and reduced excitability, the remaining CART neurones responding with excitation. 7.5% responded to NA with biphasic inhibitory/excitatory responses. NA-induced inhibition was characterised by an increase in conductance, reversal potential close to that for potassium (-83$\pm$7mV), that persisted in TTX. NA-induced inhibition was mimicked by UK-14,304 (10μM; =12) and suppressed by idazoxan (200nM; n=4), indicating a mechanism involving activation of α2-adrenoceptors coupled to a potassium conductance.
Taken together these findings suggest an orexigenic role for NA in the ARC, through activation of α1 on NPY/AGRP and in part through inhibition of anorexigenic CART-expressing neurones. The functional significance of differential regulation of CART neurones requires further clarification.

van den Top M, Lee K, Whyment AD, Blanks AM & Spanswick D. (2004). Nat Neurosci 7, 493-494.

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C4 and PC14

Thyrotropin-releasing hormone excites hypocretin (orexin) cells of the hypothalamus

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The neuropeptide thyrotropin-releasing hormone (TRH) was originally described as the hormone that regulates the hypothalamus-pituitary-thyroid axis, but its additional role as a neurotransmitter is now widely recognised. Central effects of TRH include the regulation of energy balance and cognitive arousal (1), though the mechanisms involved are not yet clear. A few areas in the brain that express TRH send their projections to the lateral hypothalamus, e.g. the dorsomedial hypothalamus (2). The latter is critical for the temporal organisation of food entrainment of circadian rhythms. On the other hand, the lateral hypothalamus is the only brain region to contain hypocretin (orexin) cells. These cells are essential for cognitive arousal and feeding behaviour; their loss leads to obesity and narcolepsy, whereas their activation promotes wakefulness (3). Considering that both TRH and hypocretin have a role in the regulation of sleep and feeding, and that some of the afferents to the lateral hypothalamus (where hypocretin cells are located) arise from TRH-expressing areas, we tested the effects of TRH on the activity of hypocretin cells.

We performed whole-cell recordings from hypocretin cells using brain slices taken from 13-22 day-old mice. These mice expressed enhanced green fluorescent protein under the hypocretin promoter, thus allowing us to unambiguously identify hypocretin cells as described previously (4). TRH was applied extracellularly at concentrations of 100, 250 or 500 nM while cells were recorded in voltage- or current-clamp mode.
TRH depolarized and increased the firing rate in all hypocretin cells tested (n=22) in a dose-dependent manner. Membrane depolarization persisted in the presence of tetrodotoxin (n=6 cells) or in a low Ca^{2+}/high Mg^{2+} extracellular solution (n=4 cells), suggesting a post-synaptic mechanism. When we analysed the effects of TRH on action potentials, we found that their width increased (control 1.15±0.05 ms, TRH 1.40±0.10 ms, p=0.006, n=11), whereas their firing threshold remained unchanged (control -36.1±0.9 mV, TRH -35.4±1.2 mV, p>0.1, n=11). We also measured the effects on cell excitability by quantifying input-output gain using frequency-current plots, and found that TRH significantly increased gain in 9 of 10 hypocretin cells. Finally, our voltage-clamp recordings revealed that the TRH-induced depolarization involved the activation of an inward current that depended on extracellular Na^{+}. In addition, we found that the peptide also increased post-synaptic voltage-gated K^{+} and Ca^{2+} currents.
These results show that TRH directly and potently stimulates hypocretin neurones. The actions of TRH on hypocretin cells may contribute to the physiological regulation of rhythms in feeding behaviour and cognitive arousal.

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C5 and PC15

Characterisation of a 'non-starvation' food withdrawal response in the nematode *C. elegans*

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Food withdrawal provides a very powerful way of adapting behaviour of the model organism *C. elegans* (Hill *et al.*). Indeed, previous work has highlighted that worms are considered starved when cultivated in the absence of food for 2 hours. We have recently noted an adaptive response of the muscle organ, the pharynx, following withdrawal of food. Investigation of developmentally staged worms (L4 plus 16 hours) that were deprived of food showed differential regulation of feeding behaviour to the absence of food over time. Feeding behaviour was quantified by counting the pumping, i.e. the movement of a grinder (visible as a black line in the pharynx). This paradigm sees three phases of behaviour i. the initial cessation in pumping is followed by a slow increase in pumping until it reaches a ii. steady state pump rate (~50 pumps/min) after 70mins. After this initial plateau, which persists for approximately one hour, the worms undergo a series of erratic pumps in which they are relatively quiescent or pump at the high frequency similar to seen in the presence of food. This latter phase is quantified by the increased variation in pump rates. Although, previous investigations have defined removal from food for 5 hours as starvation (Avery *et al.*), our investigations of the nutritional status show that the worms retain their lipid stores during the initial 5 hours (Sudan Black staining), indicating that they may not be starved. However, after 10hrs there is a decrease in fat staining in the head region of the worms as measured by pixel density, from 1180862 (n=14) to 739853 (n=19) pixels (P<0.0001, t-test). The microcircuit that controls pharyngeal pumping is made up of 20 embedded neurons and a single neuron contact to the extra-pharyngeal nervous system. This circuit is underpinned by a number of fast and neuromodulatory transmitters, whose homologues regulate feeding behaviour in mammals. Analysing mutants that define key transmitters within this circuit helps define the locus for the neuroadaption and the cellular and molecular determinants of the behaviour. E.g., although serotonin is a key regulator of the worm's response to the presence of food, it does not play a role in the adaptive response to the food deprivation. This work should provide a framework to investigate microcircuits that define model feeding behaviour and the modulation to varying nutritional states of the organism.

Hill *et al.*(2004); *J. Neurosc.* **24(5)**, 1217-1225.

Avery *et al.*(1990); *J. Exp. Zool.* **253**, 263-270.

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C6 and PC16

Mechanisms of GIP secretion from primary intestinal K-cells

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Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone that promotes insulin release and coordinates the fate of dietary fat [1]. It is released from K-cells located in the duodenal and jejunal epithelia, which are thought to directly sense the presence of nutrients in the gut lumen. However, at the cellular and molecular level, little is known about how K-cells respond to stimuli. The aim of this study was to generate transgenic mice with fluorescently labelled K-cells and to use these to investigate pathways by which K-cells detect nutrients. Transgenic mice were generated in which the GIP promoter drives expression of a fluorescent protein, Venus. Fluorescent cells from intestinal tissue were purified by flow cytometry and analysed by quantitative RT-PCR. GIP secretion in response to

and add to our understanding of the complex neural networks modulating appetite.

Przydzial MJ, Heisler LK (2008). Nociceptin/Orphanin FQ peptide receptor as a therapeutic target for obesity. Mini-Reviews in Medicinal Chemistry 8(8), 796-811.

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PC5

Effect of chronic treatment with growth hormone in a murine model of accelerated aging

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Aging is associated with alterations in cardiovascular system and changes in several organs and tissues. The aging theory postulates that this process may be due to the accumulation of oxidative damage and inflammatory processes in cells and molecules (1-4). The purpose of this study was to investigate the effect of aging on different physiological parameters related to inflammation and oxidative stress in hearts from male senescence-accelerated mice (SAMP-8) and the influence of chronic administration of Growth Hormone (GH) on these animals, whose endogenous production is reduced by age (5). Thirteen old male mice of 10 months of age were used. Animals were divided into two experimental groups, one group that was treated with GH (2 mg/kg/day/subcutaneous). The second was treated with saline, and acted as the old control group. After 30 days of treatment, mice were sacrificed by decapitation, and hearts were collected. A group of six 2-months-old male mice was used as young controls. The expression of tumor necrosis factor-alpha (TNFα), interleukin 1 (IL-1), interleukin 10 (IL-10), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), were determined by real-time reverse transcription polymerase chain reaction. Results were submitted to a two way ANOVA statistical evaluation using the STATGRAPHICS program. Inflammation as well as oxidative stress in the heart were increased in the old SAMP-8 males. Pro-inflammatory cytokines (TNFα, IL-1), were significantly increased and anti-inflammatory IL-10 decreased in those animals ($p<0.05$). Increased age also diminished the levels of eNOS ($p<0.05$). Exogenous GH administration reverted the inflammatory status and recovered the levels of eNOS present in the young mice ($p<0.05$). In relation to expression of iNOS, no significant differences were found in it. Our results suggest that the inflammatory process could play an important role in the secondary cardiovascular alterations associated with aging and that GH may play a potential protective effect on the cardiovascular system during this period.

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PC6

Expression of nucleoside transporters in the primary culture of rat cortical astrocytes

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Nucleosides are important modulators of neural activity and survival. In the present study, we produced primary cultures of rat cortical astrocytes, as described earlier [1], and examined the expression of nucleoside transporters at transcript level by reverse transcription-polymerase chain reaction (RT-PCR) and by real time PCR; the sequence of the primers and the expected sizes of the products are presented in the Table 1. RT-PCR identified the mRNAs for the rat equilibrative nucleoside transporters (rENT)1, rENT2 and recently characterized rENT3, as well as for the rat concentrative nucleoside transporters (rCNT)2 and rCNT3, while the band corresponding to rCNT1 was faint (Figure 1). Real time PCR was used to explore the amount of mRNA for these transporters, relative to the amount of mRNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase(GAPDH). Results have revealed that mRNA encoding rENT3, a transporter mainly located in lysosomes, was the most abundant, with relative expression of 1.47 ± 0.23 (mean$\pm$SEM, n=4); the amounts of mRNA for rENT2 and rENT1 were 0.65 ± 0.26 (n=3) and 0.19 ± 0.06 (n=4), respectively. Amounts of mRNA for the two purine-preferring concentrative transporters, rCNT2 and rCNT3 were also relatively high, 1.22 ± 0.23 (n=4) and 1.11 ± 0.19 (n=3), respectively, while the mRNA for the pyrimidine-preferably rCNT1 was scarce, 0.14 ± 0.07 (n=3) relative to mRNA for GAPDH. These results demonstrate for the first time that rat astrocytes in primary culture express all six nucleoside transporters; however, relative expression indicates that concentrative transport of purine nucleosides across the cellular membrane, as well as the equlibrative transport within the intracellular compartment, may be important for homeostasis of nucleosides and nucleotides in these cells.

Primer sequence

Primer	Sequence	Expected size (bp)
rENT1	sense 5′- TTGCTGATCTTCACCTGCC -3′ antisense 5′- AACTTGGTCTCCTGCTCTGC-3′	476
rENT2	sense 5′-CCGTCTGGTTCATCAATTCC -3′ antisense 5′- TGTGAAGACCAACACAAGGC-3′	509
rENT3	sense 5'-TCGCTAACTTCCTGCTTGTCA-3' antisense5'-GGCCTCATGTAGTACCTGGCAT-3'	433
rCNT1	sense 5′- GCACCGGCAGCTGTTTGGA-3′ antisense 5′- CCCCAGGACACAGCTCGCC-3′	399
rCNT2	sense 5′- GGAGCTC ATGGAAGTCGGAAC -3′ antisense 5′- CCCATGAACACCCTCTTAAGCCA-3′	390
rCNT3	sense 5'-CTGTCTTTTGGGGAATTGGA-3' antisense 5'- CAGTAGTGGAGACTCTGTTT-3'	361
GAPDH	sense 5′-GTCCTGTGGCATCCACGAAACT-3′ antisense 5′- TACTTGCGCTCAGGAGGAGCAA-3′	343

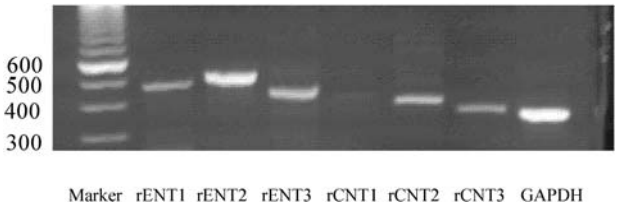

Figure 1. Expression of nucleoside transporters in rat astrocytes in the primary culture. Figure shows an ethidium bromide stained gel. Primer sequences and expected sizes of products are presented in the Table 1. Negative controls including both reverse transcriptase minus samples (RT-) and water samples were negative (data not shown).

Parkinson FE & Xiong W (2004). *J Neurochem* **88**, 1305–1312.

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PC7

Hypothalamic cytokine signalling pathways increase following recurrent hypoglycaemia

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Counterregulatory responses (CRR) to acute hypoglycaemia may become impaired following exposure to recurrent hypoglycaemia. Current evidence suggests that the predominant sites for hypoglycaemia sensing and/or triggering CRR are in the hypothalamus. We hypothesised that impaired CRR develops through coordinated changes in gene expression in hypothalamic pathways.

We created a rodent model of impaired CRR in catheterised (inserted surgically under isoflurane anaesthesia 1 week prior to studying) Sprague Dawley rats. One group (recurrent hypo [RH], n = 4) underwent 3 days of sc regular insulin injections (10, 8 and 6 units/kg on days 1 to 3) followed by a day 4 hyperinsulinaemic (20 mU/kg/min) hypoglycaemic clamp. A second group (acute hypo [AH], n = 4) had 3 days of sc saline followed by a day 4 hypoglycaemic clamp, while a third control group also had 3 days sc saline followed by clamped euglycaemia ([EU], n = 4). At 150 min, whole hypothalami were removed, frozen for later RNA extraction etc and expression analysis using Affymetrix rat 230 genome 2.0 chip. Genes showing significant change (using Genespring GX- Agilent) were subjected to pathway analysis (Ingenuity Systems).

We identified 116 and 103 genes which significantly increased or decreased expression respectively in AH compared with EU, a surprisingly high number considering that these groups were treated identically except for 120 min of exposure to different plasma glucose values on day 4 (fig below). Pathway analysis suggested significant decreases ($p<0.05$) in AH in glucocorticoid (GC), interleukin (IL) 6 and acute phase response signalling. Looking then at the comparison between acute and recurrent hypoglycaemia (AH vs RH), 143 genes increased and 158 decreased. As a striking comparison when compared with AH, pathways with significant increases in RH ($p<0.05$) included GC, IL-6, IL-10, IL-2 and acute phase response pathways.

Finally, given this analysis suggested coordinated changes in hypothalamic cytokine/ inflammatory signalling and previous data suggesting a role in the hypothalamus for the key inflammatory cytokine IL 1-beta in controlling peripheral metabolism, we quantified IL-1B expression with RT-PCR. In keeping with the broad patterns seen in microarray data, hypothalamic IL1B expression was significantly higher in RH than AH (figure below).

In summary, our data show that (1) even 120 min of hypoglycaemia results in robust coordinated changes in hypothalamic gene expression and (2) that marked differences exist in gene expression between acute and recurrent hypoglycaemia in cytokine/ inflammatory pathways. We speculate that that non-neuronal cells- astrocytes and/or microglia- may contribute to modulating neuronal glucose-sensing in the hypothalamus by locally modulating cytokine/ inflammatory signalling.