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A novel approach to reveal spike coding in rat oxytocin cells

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Mean spike frequency as a single measure of neural activity does not represent the full extent of coding. Similarly, description of instantaneous frequency as a Poisson process is not fully adequate. It assumes that adjacent spikes occur independently which is not true, because for example of the absolute refractory period.

The methods proposed here adopt a direct approach to describe spike interval patterning because this constitutes the information that reaches the axon terminals. An information model has been developed in which spike patterning increases the predictability of interspike intervals (ISIs). Log ISI histogram probability distributions were used to evaluate the entropy, an absolute measure of the disorder of the interval distribution. Very regular intervals exhibit minimal disorder and convey only frequency information.

The ISI histogram provides a useful representation of the mean spike frequency variability of coding but neglects the order in which the ISIs occurred. Plotting the duration of adjacent ISI pairs against each other as a scatter reveals statistical relationships between neighbouring intervals. The strength of association is given by the mutual information: the reduction in entropy of one ISI given the duration of its neighbour. To describe the scatter distribution, a probabilistic (Bayesian) approach has been adopted and random (Markov) simulations have been undertaken to detect non-stationarity in spike activity.

Oxytocin cell activity in female Wistar rats were tested under four experimental conditions: supraoptic neurones of virgins *in vitro* and *in vivo*, and in lactating rats in the absence of presence of suckling pups. All experiments were undertaken in accordance with the Animal (Scientific Procedures) Act, 1986, and experiments *in vivo* were conducted under urethane anaesthesia (1.1 g kg^{-1} I.P.); all animals were killed humanely. Although there were no significant differences in mean spike frequency between the different groups, the ISI entropy was significantly lower *in vitro* ($P < 0.02$, ANOVA with *post-hoc* Student's *t* test) than in each of the *in vivo* conditions; the impoverished coding was possibly a result of the loss of connections *in vitro*. The mutual information was significantly higher ($P < 0.001$) in suckled rats than in the other recordings *in vivo,* reflecting the increased extent of spike patterning.

Appropriate parameters of neural activity thus revealed significant differences in coding between cells in the different experimental groups. Reduced synaptic input in slices decreased the complexity of coding and spike patterning was increased during reflex milk ejections. The study highlights the limitations of mean spike frequency to represent neural activity.

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All procedures accord with current UK legislation.

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Inputs to the suprachiasmatic nucleus from the arcuate nucleus are modulated by melatonin

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There is a good evidence for an input to the hypothalamic suprachiasmatic nucleus (SCN) from the retrochiasmatic area including the arcuate nucleus (ARCN), which may carry non-photic neuroendocrine cues to the SCN. The present study was undertaken to determine if melatonin influenced the spontaneous activity of cells in the SCN or their responses to stimulation of the ARCN. The experiments were carried out in accordance with the Animal (Scientific Procedures) Act, 1986. Animals were killed humanely. Conventional extracellular recordings were made from single units in the SCN in 500 μm hypothalamic slices from male Wistar rats. Responses of the SCN neurones to the ARCN stimulation were assessed by peristimulus time histograms. Twenty-seven of 47 SCN cells tested responded to single pulse stimulation of the ARCN. Both excitatory ($n = 10$) and inhibitory ($n = 11$) responses were observed. Additionally in some cases ($n = 6$) complex responses (excitation followed by inhibition or vice versa) were seen. These may reflect both excitatory and inhibitory projections. Typically the responses had a short latency (< 20 ms) after the stimulus pulse. Excitatory responses were usually short ($\leqslant$ 25 ms) and inhibitory responses were longer ($\geqslant$ 40 ms).

Administration of melatonin (1 nM) to the bath solution changed neither the mean spike frequency of SCN cells nor mean log interspike interval (ISI). However, more sensitive indices of neuronal coding, e.g. the entropy of the ISIs ($P < 0.05$, paired *t* test) and the mutual information between adjacent ISIs ($P < 0.02$, Wilcoxon signed rank test; Bhumbra & Dyball, 2002) were significantly increased. The input to the SCN from the ARCN was significantly altered by melatonin in 19 of 47 experiments. Both excitatory ($n = 5$) and inhibitory ($n = 6$) responses were significantly reduced by addition of melatonin. In six cases excitatory ($n = 3$) and inhibitory ($n = 3$) responses became evident only in the presence of melatonin; in one case melatonin enhanced an inhibitory response and, in one additional case, an initial excitatory response became inhibitory. The effects of melatonin developed relatively slowly (> 15 min) and once established were not usually reversible.

The present results showed that melatonin at a very low concentration can modulate the input to the SCN from the ARCN. Melatonin also had significant effects on the spontaneous firing of SCN neurones that involved changes in ISI coding without significant changes in firing rate. Differences in responsiveness of individual SCN cells to the ARCN stimulation may reflect the heterogeneity of the neurones of the ARC but it is clear that melatonin can affect the behaviour of cells in the SCN and probably also daily endocrine rhythms.

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PC40

The effect of melatonin on the pituitary response to lower body negative pressure in man

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Melatonin modulates neurohypophysial hormone release in response to elevated plasma osmolality in both man and rat (Forsling, 2000). A study has been performed on the effect of melatonin on pituitary hormone release in man in response to an effective reduction in central venous volume induced by lower body negative pressure (LBNP). A randomised double-blind study was performed on eight male subjects with approval of the local ethics committee. The subjects, aged 20–21 years, gave informed consent and refrained from taking heavy exercise, alcohol and from smoking prior to the study. Each subject participated on two occasions, receiving either placebo or 5.0 mg melatonin. After lying supine for 30 min, they were subjected to LBNP of −50 mmHg for 30 min followed by a recovery period of 30 min. Blood pressure and heart rate were monitored throughout and blood samples taken at intervals for determination of packed cell volume (PCV) and plasma osmolality, vasopressin, oxytocin and cortisol.

After 30 min LBNP mean heart rate increased from 68 ± 3.4 to 89 ± 5.4 beats min^{-1} (S.E.M., ANOVA, $P < 0.01$) following placebo treatment, with a similar increase following melatonin. Blood pressure, which was initially 97 ± 5.6 and 95 ± 7.7 mmHg, respectively, in the placebo and melatonin-treated groups, did not change significantly. Plasma osmolality also remained constant throughout, while PCV showed a transient increase on LBNP ($P < 0.05$). Vasopressin concentrations were increased from 1.2 ± 0.43 pmol l^{-1} to a peak of 2.2 ± 0.38 pmol l^{-1} ($P < 0.05$) at the end of 30 min of LBNP. Following melatonin treatment vasopressin concentrations showed a greater increase to 6.2 ± 12 pmol l^{-1} ($P < 0.02$). Oxytocin increased from 3.7 ± 0.4 to 6.3 ± 0.4 pmol l^{-1} at the start of LBNP. A peak of 11.0 ± 1.0 pmol l^{-1} was seen during LNBP following melatonin. The area under the curve for cortisol release was also significantly greater after melatonin ingestion. Thus the pituitary response to LBNP is enhanced by melatonin in doses similar to those recommended for the prevention of jet lag.

Forsling ML (2000). *Exp Physiol* **85**, 179–186S.

All procedures accord with current local guidelines and the Declaration of Helsinki.

PC41

Regulation of bursting activity in vertebrate neuroendocrine cells by cholinergic agonists, nicotine and oxotremorine

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Migration between tidal estuaries and open sea depends on a wide-ranging osmoregulatory capacity. This is achieved by few fish species (e.g. the flounder, *Platichthys flesus*) and provides increased access to rich feeding grounds. The caudal neurosecretory system (CNSS), located in the terminal eight vertebral segments of the spinal cord, contributes to the neurohormonal control of osmoregulation (Winter *et al.* 2000). Dahlgren cells secrete two osmoregulatory peptides (urotensins I and II) while acetylcholine (ACh) is synthesised within the CNSS in large quantities (Conlon & Balment, 1996). Dahlgren cells generate four types of electrical activity pattern (Brierley *et al.* 2001; unpublished data from this group). Transitions between quiescence, tonic, phasic and bursting activity patterns are likely to be important in maintaining appropriate circulating levels of neuropeptide. Here we examined the role of ACh in triggering such transitions.

Isolated CNSS dissected from fish (UK Home Office protocol), fully adapted to either seawater (SWA) or fresh water (FWA), were mounted in a cooled (9–11 °C) interface chamber and continuously superfused (0.5 ml min^{-1}) with aerated Ringer solution. Intra- and extracellular (multi-unit) recordings were made for up to 6 h. The selective ACh receptor agonists, nicotine (NCT, nicotinic AChR) and oxotremorine (OXT, muscarinic AChR) were superfused (100 μM in Ringer) over the CNSS for 10 min. Spontaneous transitions between firing patterns occurred in 20 % of Dahlgren cells. Superfusion with NCT or OXT triggered transitions in 44 % of cells (32 % of SWA cells and 63 % of FWA cells). Superfusion with NCT had no effect on the activity pattern of SWA bursting cells ($n = 11$) but triggered transitions to bursting activity in 8/17 non-bursting cells. In contrast, NCT triggered bursting in only 3/13 FWA cells and inhibited all activity in 7/9 spontaneously bursting neurones. Superfusion with OXT led to short-term inhibition of all cells leading to cessation of firing activity in 6/18 SWA and 7/11 FWA cells; the remaining cells were unaffected. Intracellular recordings showed a marked increase in membrane potential in SWA and FWA Dahlgren cells in response to OXT, leading to hyperpolarisation by 21.8 ± 5.7 mV ($n = 5$ cells, mean ± S.E.M.). In summary responses to muscarinic activation are largely inhibitory. However, Dahlgren cells show differential responses to nicotinic stimulation depending on their ongoing activity and adaptation state.

Brierley MJ *et al.* (2001). *J Exp Biol* **204**, 2733–2739.

Conlon JM & Balment RJ (1996). *Gen Comp Endocrinol* **103**, 36–44.

Winter *et al.* (2000). *Biochem Cell Biol* **78**, 193–203.

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PC42

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

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The flounder (*Platichthys flesus*) is capable of full adaptation to sea and freshwater environments. This involves major changes of function in osmoregulatory organs such as gut, gills, bladder and kidney. The neuropeptides urotensin I and II (UI and UII) are major players in this adaptive process (Marshall & Bern, 1981; Loretz *et al.* 1985). In mammals, UI and UII peptides have been associated with many vital functions such as regulation of

glucocorticoid synthesis and cardiovascular homeostasis. In fish, the source of these circulating neurohormones is the caudal neurosecretory system (CNSS), which is located in the terminal eight vertebral segments of the spinal cord. We have cloned the cDNAs encoding UI and UII peptides from flounder by the reverse transcriptase polymerase chain reaction (RT-PCR) and screening of a CNSS cDNA library. Flounder were obtained from Morecambe Bay (Cumbria, UK) and maintained at the University of Manchester in seawater tanks at 10–12 °C under natural photoperiod. Fish were humanely killed according to approved UK Home Office regulations (Schedule 1). RNA was extracted using the guanidine thiocyanate method and utilized for both RT-PCR and library construction. RT-PCR was carried out using degenerate oligonucleotide primers based on the amino acid sequence of flounder UI (Conlon *et al.* 1990*a*) and UII (Conlon *et al.* 1990*b*). A CNSS cDNA library was constructed into the phage vector λTriplEx2 and screened at high stringency using specific UI and UII probes obtained from degenerate RT-PCR. 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 as putative cleavage sites. 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. Using conditions that gave no cross-hybridisation with other genes, 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, including brain, spinal cord, intestine and bladder. The primary structure of UI and UII shows a close similarity between fish and humans. The cyclic region of UII, which is responsible for the biological activity of the peptide (McMaster *et al.* 1992), is fully conserved from fish to humans. From an evolutionary viewpoint these peptides may exert important physiological functions in both fish and humans.

Conlon JM *et al.* (1990*a*). *Peptides* **11**, 891–895.
Conlon JM *et al.* (1990*b*). *FEBS Lett* **266**, 37–40.
Loretz CA *et al.* (1985). *Am J Physiol* **249**, G285–293.
McMaster D *et al.* (1992). *Gen Comp Endocrinol* **87**, 275–282.
Marshall NS & Bern HA (1981). *Gen Comp Endocrinol* **43**, 484–491.

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PC43

Corticosterone transfer across blood–brain barrier and distribution in rat brain

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In both humans and animals reduced early growth is a risk factor for cardiovascular disease, stroke, hypertension and type 2 diabetes. These associations involve changes in the hypothalamic-pituitary-adrenal axis (HPAA) activity, and regulated in part by glucocorticoids feedback to brain. One determinant of glucocorticoid activity in brain is the transfer of hormone across the blood–brain barrier. The aim of this study was to determine normal corticosterone delivery from blood to brain.

Brain penetration of [^{3}H] corticosterone was investigated using a bilateral *in situ* brain perfusion technique with [^{14}C] mannitol as the vascular marker, followed by capillary depletion analysis (Preston *et al.* 1995). Adult female Wistar rats (200–250 g) were anaesthetised I.P. with hypnorm (0.4 ml kg^{-1}) and hypnovel (0.4 ml kg^{-1}) and heparinized (100 000 units kg^{-1} I.P.). Both carotid arteries were perfused with Ringer containing dextran (39 g l^{-1}), [^{3}H] corticosterone and [^{14}C] mannitol for up to 30 min. A CSF sample was taken by puncture of the cisterna magna, the rat humanely killed by decapitation and the choroid plexuses and pituitary removed. Brain was homogenised at 4 °C and capillaries removed using dextran density centrifugation. Ringer and tissue samples including whole brain homogenate, capillary-free (supernatant) and capillary-rich (pellet) fractions were solubilized and counted (β-liquid scintillation counter).

A plot of d.p.m. in tissue/d.p.m. in Ringer per unit weight against time produces a straight line with slope K_{in} (the initial rate constant for unidirectional transfer) (Preston *et al.* 1995).

Penetration of [^{3}H] corticosterone was linear up to 30 min in adult rats and K_{in} values are summarized in Table 1. Greatest uptake was in the pituitary and choroid plexuses which lack the characteristically tight blood–brain barrier. However, even in the capillary-free brain samples and CSF, corticosterone K_{in} was at least 17 times greater than mannitol. These data demonstrate that corticosterone rapidly enters brain and CSF, despite presence of the blood–brain and blood–CSF barriers.

Table 1. Corticosterone uptake into CSF, choroid plexus and brain

	[^{3}H] corticosterone K_{in}(μl min^{-1} g^{-1})	[^{14}C] mannitol K_{in}(μl min^{-1} g^{-1})
CSF	24.0 ± 0.5	0.9 ± 0.1
Choroid plexuses	257.6 ± 8.0	7.7 ± 0.3
Pituitary	222.8 ± 7.0	8.6 ± 0.5
Whole brain homogenate	36.1 ± 0.5	2.9 ± 0.2
Capillary-free brain (supernatant)	36.2 ± 0.8	2.1 ± 0.3
Capillary-rich brain (pellet)	31.3 ± 1.2	2.3 ± 0.4

n = 6–8, means ± S.E.M.

Preston JE *et al.* (1995). *Dev Brain Res* **87**, 69–76.

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