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Partial adaptation to simulated time-zone shifts

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Two or three days after a time shift we have placed subjects under constant conditions for 24 hr and made hourly measurements of temperature and urinary excretion. These have been cross correlated with similar observations before time shift in four different ways:

(1) with varying time shift as if the phase of the rhythm had altered;

(2) with a mixture of the rhythm in the old and new phases in varying proportions as if two oscillators were additive, one in the old, the other in the new phase;

(3) with a mixture of the unadapted rhythm and a rhythm adapted by a variable number of hours;

(4) with a mixture of a fully adapted rhythm and a rhythm adapted by a variable number of hours.

The highest correlation has been accepted as the best descriptor of the state of partial adaptation.

Inferences about the process of adaptation to time shift can be drawn from these different descriptions of the state of partial adaptation. Since 3 or 4 was, in 173 out of 192 tests, the best descriptor, adaptation can best be described as involving two oscillators.

How do rhythms adjust to time shifts?

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When circadian rhythms adapt to a time shift it is difficult to be certain whether the circadian clock has adjusted its phase or whether the measurable responses result simply from exogenous factors masking the influence of the clock. To throw light on this problem we have exposed twenty-four subjects to artificial time shifts of 8 hr advance or retard. One to three days later we have maintained these subjects under constant conditions for 24 hr, producing a urine sample and measuring rectal temperature hourly, taking small identical food and fluid intake hourly, and remaining awake and sedentary throughout the 24 hr. The data have been examined by cross-correlation (Mills, 1976).

On current hypotheses a partially adapted rhythm would be one in which the rhythms have moved less than the full eight hours of the time shift. For a westward shift this would represent a gradual lengthening of the period of the circadian rhythms, while for an eastward shift it would represent a gradual shortening. It is, however, conceivable that adaptation might take place by a shift of 16 hr 'in the wrong direction'.

Results suggested that adaptation commonly included a lengthening of the cycle, so that after the westward shift the phase gradually moved through eight hours, whereas after the eastward shift a lengthening by sixteen hours would be necessary before adaptation was complete.

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Dopaminergic influence upon the cerebral circulation in the anaesthetized baboon

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The level of cerebral blood flow appears to be adjusted to the metabolic demands of cerebral tissue (Olesen, 1971; Risberg & Ingvar, 1973), although the mechanisms which ultimately trigger the changes in cerebrovascular resistance are still controversial. There have been few attempts to determine what influence dopamine exerts upon the cerebral circulation despite the reported importance of dopamine to cerebral function (Carlsson, 1972). It has also been suggested that cerebral blood vessels, particularly those in regions such as the caudate-putamen, may be innervated by dopaminergic neurones of an intracerebral origin (Hartman, Zide & Udenfriend, 1972). In the present study we examined in the baboon the effect upon cerebral blood flow and metabolism of both stimulation and blockade of dopamine receptors using apomorphine and pimozide respectively.

The surgical preparation of the baboon, anaesthesia and the measurements made, have been described in detail elsewhere (MacKenzie, McCulloch, O'Keane, Pickard & Harper, 1976). Cerebral blood flow (CBF) was determined from the externally monitored clearance of ^{133}xenon following its injection into the internal carotid artery. The cerebral metabolic rates for oxygen and glucose utilization ($CMRO_2$ and CMR_{glu}) were estimated from the product of CBF and the arterio-venous differences of oxygen and glucose contents. Cerebral venous blood was obtained from the superior sagittal sinus.

Apomorphine (0·02–0·5 mg.kg^{-1}, I.V.) administration resulted in highly

significant increases in CBF which were accompanied by increases in both indices of cerebral metabolism. At a dose of apomorphine of 0·1 mg.kg^{-1}, CBF was increased from 57 ± 6 ml. 100 g^{-1} min^{-1} (mean ± S.D.) to 90 ± 21 ml. 100 g^{-1} min^{-1} ($P < 0·01$): $CMRO_2$ increased from 3·10 ± 0·17 to 4·21 ± 0·79 ml. 100 g^{-1} min^{-1} ($P < 0·02$): CMR_{glu} from 4·47 ± 0·60 to 7·68 ± 2·08 mg.100 g^{-1} min^{-1} ($P < 0·02$). Neither blood pressure nor arterial CO_2 tension was changed significantly.

Pimozide (0·5 mg. kg^{-1}, I.V.) produced no significant change in either cerebral perfusion or metabolism. The vasodilator response of the cerebral circulation to induced hypercapnia was unaffected by dopaminergic blockade. However, the ability of apomorphine to stimulate cerebral metabolism and increase flow was totally abolished by pimozide administration.

The results suggest that, although stimulation of dopamine receptors can have a profound effect upon cerebral metabolism and perfusion, endogenous dopamine is not involved in the normal maintenance of hemispheric cerebral metabolism and blood flow, in contrast to the role which has been suggested for noradrenaline (MacKenzie, McCulloch & Harper, 1976).

These studies were supported by Tenovus (Scotland).

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Analgesia produced in cats by the C-fragment of lipotropin and by a synthetic pentapeptide

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After the C-fragment of lipotropin was isolated from porcine pituitary (Bradbury, Smyth & Snell, 1975) and found in *in vitro* experiments to have high affinity for the opiate receptors in brain (Bradbury, Smyth, Snell, Birdsall & Hulme, 1976), we showed (Feldberg & Smyth 1976) that it produced strong analgesia, measured by the tail-pinch method of Russell

& Tate (1975), when infused in a small volume (40 μl.) into the third cerebral ventricle of unanaesthetized cats. It also produced the other central effects of morphine similarly infused. On a molar basis, C-fragment was about 100 times more potent as an analgesic than morphine.

Since then, two further observations have been made with C-fragment: (1) The analgesia and other central effects produced on intraventricular infusion were reversed by intraperitoneal Naloxone (1 mg/kg). In one experiment, analgesia returned as the effect of Naloxone subsided. (2) Intravenously, C-fragment (200–250 μg/kg) produced some analgesia which lasted about 20 min. Again, C-fragment was more potent than morphine since 250 μg/kg morphine sulphate intravenously was insufficient to produce analgesia.

Lipotropin (150 μg) itself produced no analgesia when infused into the third ventricle.

Methionine enkephalin, known to interact with the opiate receptors in the guinea-pig ileum, mouse vas deferens and brain homogenate (for references see Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) produced either no analgesia when infused intraventricularly (Feldberg & Smyth, 1976), or very weak analgesia lasting a few minutes. As this happened sometimes after control infusions, it is uncertain whether the analgesia was a genuine effect of the methionine enkephalin.

An entirely different result was obtained after blocking the two ends of methionine enkephalin, the one by an *N*-methyl group, the other by an amide. It had been suggested that methionine enkephalin might be rapidly destroyed *in vivo* and be unable to produce analgesia (Hughes *et al.* 1975) but this might be prevented by blocking the two termini. The *N*-methyl-pentapeptide amide,* though less potent than C-fragment, certainly produced strong analgesia lasting over 4 hr when 150–180 μg were infused into the third ventricle of unanaesthetized cats, and a condition of deep stupor and catalepsy developed. Analgesia, stupor and catalepsy disappeared within minutes of an intraperitoneal injection of 1 mg/kg Naloxone.

Both ends of methionine enkephalin have apparently to be blocked for the pentapeptide to become a potent analgesic. Blocking the C-terminal end with an amide group* did not endow the pentapeptide with analgesic action; at least none occurred with 200 μg infused into the third ventricle. The peptide blocked at the other end by an *N*-methyl group* could be infused only in a dose of 70 μg because of its relatively low solubility, and this dose produced no analgesia.

* Synthesized by A. F. Bradbury, D. G. Smyth and C. R. Snell.

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The release of acetylcholinesterase into rabbit cerebrospinal fluid on cooling the substantia nigra and caudate nucleus

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The cooling of cerebral structures is known to depress their activity in an easily reversible way. Electrical stimulation of the caudate nucleus in rabbits causes release of acetylcholinesterase into the cerebrospinal fluid (c.s.f.) collected from the cisterna magna (Greenfield & Smith, 1976). If this acetylcholinesterase release is a result of electrical activity then cooling might be expected to depress the effect. Therefore, we cooled caudate nucleus and substantia nigra in an attempt to depress the release of acetylcholinesterase. We implanted cooling probes stereotaxically in caudate nucleus and substantia nigra of 12 rabbits anaesthetized with urethane and sampled c.s.f. continuously from cisterna magna.

Unexpectedly we found that cooling either caudate nucleus or substantia nigra below 14° C without electrical stimulation led to a marked increase in the concentration of acetylcholinesterase in cisternal c.s.f. (*ca.* 80 m-u./ml.) This increase was maintained throughout different cooling periods (from 30 min to 2 hr) and the enzyme activity reverted to pre-cooling levels within 30 min of rewarming. It was therefore unlikely to have been due to the actual process of cooling or warming but occurred throughout the period of low temperature. The activity of the cytoplasmic enzyme lactic dehydrogenase in the c.s.f. remained unchanged throughout, which suggests that cooling did not cause physical damage to cells. The effect appeared to be confined to caudate nucleus and substantia nigra, as cooling a neighbouring structure, the dorsal thalamus, which also contains a substantial amount of acetylcholinesterase, had no effect on cisternal acetylcholinesterase levels.

Furthermore the increase in acetylcholinesterase on cooling either substantia nigra or caudate nucleus was blocked by intravenous atropine

* M.R.C. scholar.