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EFFECTS OF LIGHT REVERSAL ON THE CIRCADIAN PATTERN OF MOTOR ACTIVITY AND VOLTAMMETRIC SIGNALS RECORDED IN RAT FOREBRAIN

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SUMMARY

1. To investigate the functional relationships between the circadian changes in rat motor activity and changes in the extracellular concentration of ascorbic acid and homovanillic acid (HVA) monitored in the striatum and nucleus accumbens, reversal of the light/dark cycle was used to disturb the pattern of motor activity.

2. Microcomputer-controlled linear sweep voltammetry with carbon-paste electrodes was used to continuously monitor circadian changes in the ascorbate signal and the HVA signal simultaneously in nucleus accumbens and striatum over a 13 day period in unrestrained rats; total motor activity for each animal was also recorded.

3. Regression analyses were carried out on each day's data to investigate the relationships between motor activity and the two voltammetric signals. During days 1–5, the lighting was on normal 12/12 light/dark cycle and high correlations were observed.

4. Reversal of the light/dark cycle on day 6 caused an immediate change in the pattern of motor activity and electrochemical signals; by days 7–8 after light reversal the relationships between lighting, ascorbate, HVA and motor activity were re-established under the new lighting conditions.

5. During the intervening period, however, there was a complete breakdown in some of the correlations. The findings are discussed in the light of the hypothesis that changes in brain extracellular ascorbate reflect changes in the release of excitatory amino acids, and in terms of a recent model of the role, in the control of motor activity, for cortical and mesencephalic inputs to forebrain subcortical regions.

INTRODUCTION

The functional role of the mammalian caudate putamen and nucleus accumbens in the control of movement is not clear at present. Both structures receive a dopaminergic projection from the mid-brain and inputs from the neocortex and allocortex which are thought to release excitatory amino acids (Walaas, 1981). The importance of dopamine is demonstrated by the movement disorders which result in animals from selective neurotoxic lesions of dopamine terminals in the caudate

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putamen (Makanjuda & Ashcroft, 1982) and from the degeneration of these neurones in Parkinson's disease in humans (Da Prada, Keller, Pieri, Kettler & Haefely, 1984). However, on the basis of electrophysiological studies, it has been suggested that the cortex is responsible for the initiation and maintenance of movement (Penney & Young, 1983; McGeer, Staines & McGeer, 1984). In contrast, the substantia nigra pars compacta cells show little variation in discharge frequency during gross changes in motor activity for both the cat (Trulson, Preussler & Howell, 1981) and rat (Miller, Farber, Gatz, Roffwarg & German, 1983). This has led to the suggestion that dopamine in the striatum acts as a neuromodulator with a tonic action, rather than as a classical excitatory or inhibitory transmitter (McGeer *et al.* 1984).

Because of the technical difficulties in monitoring changes in the release of neurotransmitters in behaving animals, little is known about the relationships between motor activity and transmitter release in the striatum and accumbens; furthermore, neuronal discharge frequency is not an index of release since there is evidence for extensive modulation of release by presynaptic receptors (de Belleroche, 1982). A number of techniques designed to detect changes in the chemical composition of brain extracellular fluid have been developed recently (Marsden, 1984); these include push–pull perfusion, dialysis, cortical cup, and *in situ* electrochemical techniques. Microcomputer-controlled linear sweep voltammetry with carbon-paste electrodes is one of the electrochemical methods now available (O'Neill, Fillenz, Albery & Goddard, 1983*b*). Carbon-paste electrodes implanted in discrete brain regions detect changes in the extracellular concentration of homovanillic acid (HVA) (O'Neill & Fillenz, 1985*a*) and ascorbic acid (O'Neill *et al.* 1983*b*). HVA is a methylated metabolite of dopamine and, although drug-induced changes in metabolite concentration cannot be used as a reliable index of the activity of dopaminergic neurones, in the absence of drugs, release rate and metabolite concentration show parallel changes, albeit with different time courses (Commissiong, 1985).

Ascorbic acid, like excitatory amino acids, is found throughout the brain (Kuo, Yonehara, Hata & Yoshida, 1978). The spontaneous and drug-induced changes in the ascorbate signal and their variation with brain region (O'Neill & Fillenz, 1985*b*; Louilot, Gonon, Buda, Simon, le Maol & Pujol, 1985) suggest that they may reflect some aspect of neuronal activity. Experiments with synaptosomes showed that ascorbic acid was released by excitatory amino acids; the effect was stereoselective for glutamate-evoked release and was Na^+, but not Ca^{2+}, dependent. Further pharmacological tests suggested that the release was mediated by a carrier-mediated hetero-exchange mechanism (Grunewald & Fillenz, 1984). The possibility that changes in the extracellular concentration of brain ascorbate *in vivo* might serve as an index of excitatory amino acid release was supported by microinjection, lesion and electrical stimulation experiments (O'Neill, Grunewald, Fillenz & Albery, 1983*c*; O'Neill, Fillenz, Sundstrom & Rawlins, 1984*b*).

We have recently shown that there are high correlations between changes in motor activity and changes in both the HVA (O'Neill & Fillenz, 1985*a*) and ascorbate (O'Neill & Fillenz, 1985*b*) signals recorded in the striatum and nucleus accumbens. However, no conclusions about functional relationships can be drawn from this since the three parameters, although parallel, could be independent. It is known that reversal of the light/dark cycle disrupts the circadian pattern of motor activity. In

the present study, in order to test the functional significance of the correlations, we have monitored motor activity and the two voltammetric signals simultaneously over a 13 day period during which the light/dark cycle was reversed. A preliminary report of some of these results has been published (Fillenz & O'Neill, 1985).

METHODS

Carbon-paste electrodes were prepared and implanted as described previously (O'Neill, Grunewald, Fillenz & Albery, 1982). Briefly, carbon paste (2·8 g carbon powder to 1 ml silicone oil) was packed into 300 μm (o.d.) teflon-coated silver wire. Male Sprague–Dawley rats (initial weight 270–400 g) were stereotaxically implanted, under chloral hydrate anaesthesia (7 ml/kg, 5% (w/w) solution I.P.), with these electrodes; silver wires, placed in the cortex, were used for the reference (O'Neill *et al.* 1983*c*) and auxiliary electrodes. Rats were implanted with carbon-paste working electrodes in nucleus accumbens and striatum. The coordinates, with the head level between bregma and lambda, were as follows: accumbens, AP 1·5 (from bregma), L 1·5 (from bregma), DV 7·0 (from skull); and striatum, AP −0·5, L 3·0, DV 4·8. The animals were given at least 2 days to recover before being placed in the recording cages and connected to the microcomputer-controlled equipment (O'Neill *et al.* 1983*b*).

Linear sweep voltammograms were recorded at 12 min intervals at a rate of 5 mV/s between 0 and 650 mV. The background current for each electrode was measured *in situ* before each experiment as described previously (O'Neill, Fillenz & Albery, 1983*a*). Two rats were scanned simultaneously and the voltammograms stored on magnetic disk. Voltammograms recorded from the two brain regions consisted of three separate peaks whose height was measured as described previously (O'Neill & Fillenz, 1985 *a*) (inset, Fig. 4). Peak 1 is due to the oxidation of ascorbic acid (O'Neill *et al.* 1983*b*), peak 2 to uric acid (O'Neill, Fillenz, Grunewald, Bloomfield, Albery, Jamieson, Williams & Gray, 1984*a*), and the dopamine metabolite HVA is responsible for peak 3 (O'Neill & Fillenz, 1985*a*). Between the electrochemical recordings (for approx. 9 min duration) the total motor activity of each rat was monitored using a Doppler-shift microwave device linked to the PIO of the interface (O'Neill & Fillenz, 1985*a*). The lighting in the windowless room was also controlled by the microcomputer. During days 1–5 the lighting was normal: light on during 08.00–20.00 h; light off during 20.00–08·00 h. The light/dark cycle was reversed on day 6 at 12.00 h and recording continued for a further 8 days.

The rats were given no drugs and were used to record simultaneously the circadian variation in motor activity, and the ascorbate and HVA signals in accumbens and striatum. To reduce the fluctuations inherent in recording these small currents (< 1 nA for peak 3) and to clarify the systematic relationships between the variables, a weighted moving average of the original data was calculated (O'Neill & Fillenz, 1985*a*). Correlation coefficients for motor activity *vs.* the ascorbate signal and for motor activity *vs.* the HVA signal were calculated for each 24 h period (120 points). Cross-correlation analysis was carried out to find the time of maximum correlation. The average value of the time-shift needed for maximum correlation between motor activity and the ascorbate signal was approx. 60 min (O'Neill & Fillenz, 1985*b*), whereas that for motor activity *vs.* the HVA signal was 30 min (O'Neill & Fillenz, 1985*a*, *c*); in both cases the voltammetric signals lagged behind the changes in motor activity. Data were recorded from each rat for a period of 13 days. Mean ± S.E. of mean are given; the n values quoted are the number of determinations (rats × days). Omission of the n value implies n = number of rats = 3.

RESULTS

Motor activity. Changes in the levels of motor activity over 24 h periods are illustrated in Fig. 1: *A*, under normal 12/12 light/dark conditions; *B*, for day 1 following reversal of the light/dark cycle; and *C*, for day 8 after light reversal. The circadian pattern, which is characterized by low levels of activity during light-on periods and large blocks of movement during the hours of darkness, was immediately

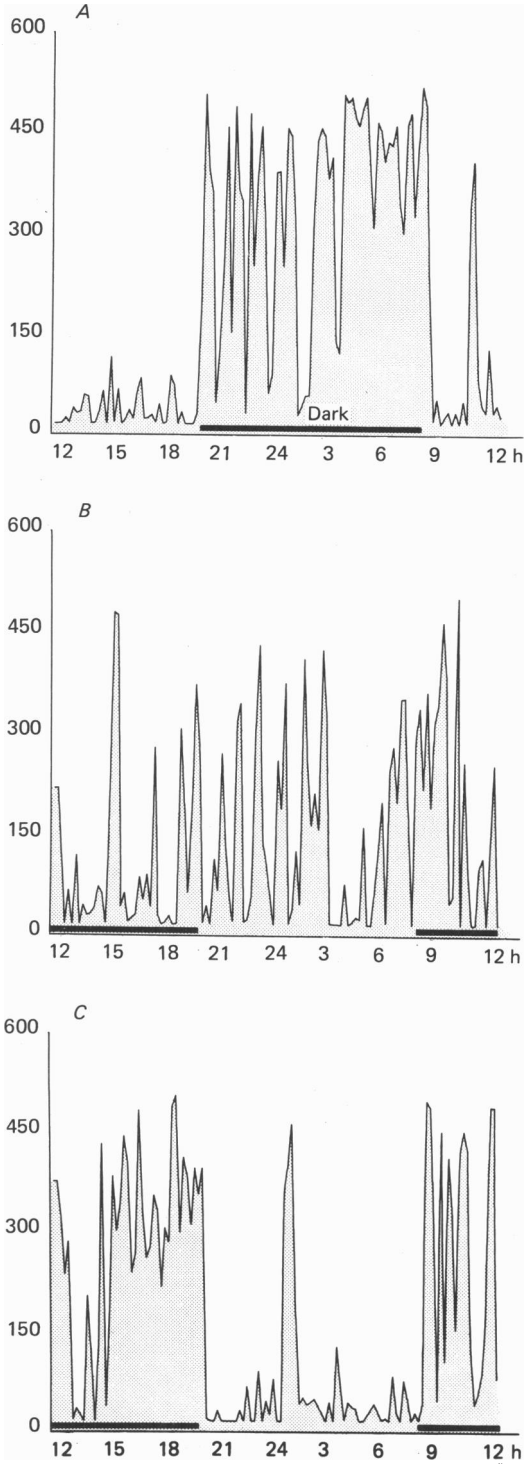

Fig. 1. An example of changes in the total motor activity of a rat over 24 h periods. Motor activity was recorded at 12 min intervals using a Doppler-shift microwave device. *A*, normal lighting (08.00–20.00 h, lights on). *B*, day 1 following reversal of the light/dark cycle. *C*, day 8 following light reversal.

disrupted by reversal of the light/dark cycle; this pattern was re-established 8 days after light reversal. To quantify these changes and to examine their time course, we analysed each day's motor activity data in a number of different ways (Fig. 2):

A. The total number of counts registered by the Doppler-shift detectors in each 24 h period did not change throughout the 13 days: 19600 ± 600 counts/day.

B. The variation in the level of activity, expressed as the standard deviation over each 24 h period, showed an immediate increase (by $17 \pm 5\%$, $P < 0{\cdot}05$) which slowly returned to the pre-reversal value.

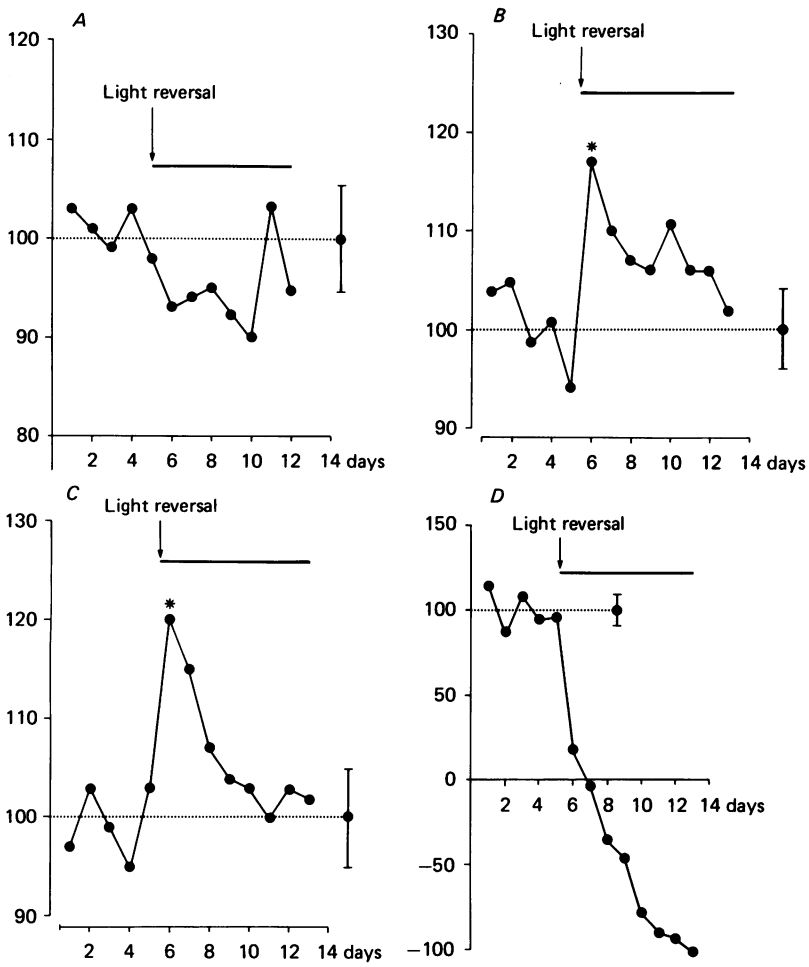

Fig. 2. Four analyses of the effects of light reversal on the circadian pattern of motor activity. There was normal lighting on days 1–5. The results are expressed as a percentage of the pre-reversal value; for clarity, only the average S.E. of mean is shown. *A*, total activity in a given 24 h period; there was no significant change in this parameter. *B*, the standard deviation for each 24 h period; maximum increase = $17 + 5\%$, $P < 0{\cdot}05$. *C*, the average difference between successive readings; maximum increase = $17 \pm 2\%$, $P < 0{\cdot}02$. *D*, the difference between the total activity for 08.00–20.00 h (original light-on period) and 20.00–08.00 h.

C. The variation in activity, expressed as the average difference between successive readings, also showed an immediate increase (by $17 \pm 2\%$, $P < 0{\cdot}02$) which returned to the pre-reversal value by day 4 after the change in lighting.

D. Finally, the difference in the level of activity between 08.00–20.00 h (original light-on period) and 20.00–08.00 showed an immediate decrease followed by a complete inversion by day 6 after light reversal.

Correlations between motor activity and voltammetric signals. Throughout the 13 days of recording there was no change, in a given 24 h period, in the average ascorbate signal in accumbens ($1{\cdot}8 \pm 0{\cdot}4$ nA) or striatum (4 ± 1 nA). Similarly, there was no change in the average HVA signal in accumbens ($0{\cdot}35 \pm 0{\cdot}06$ nA) or striatum ($0{\cdot}37 \pm 0{\cdot}03$ nA); $n = 39$. An example of the variation in voltammetric signal associated with the circadian changes in motor activity under normal lighting conditions is shown in Fig. 3.

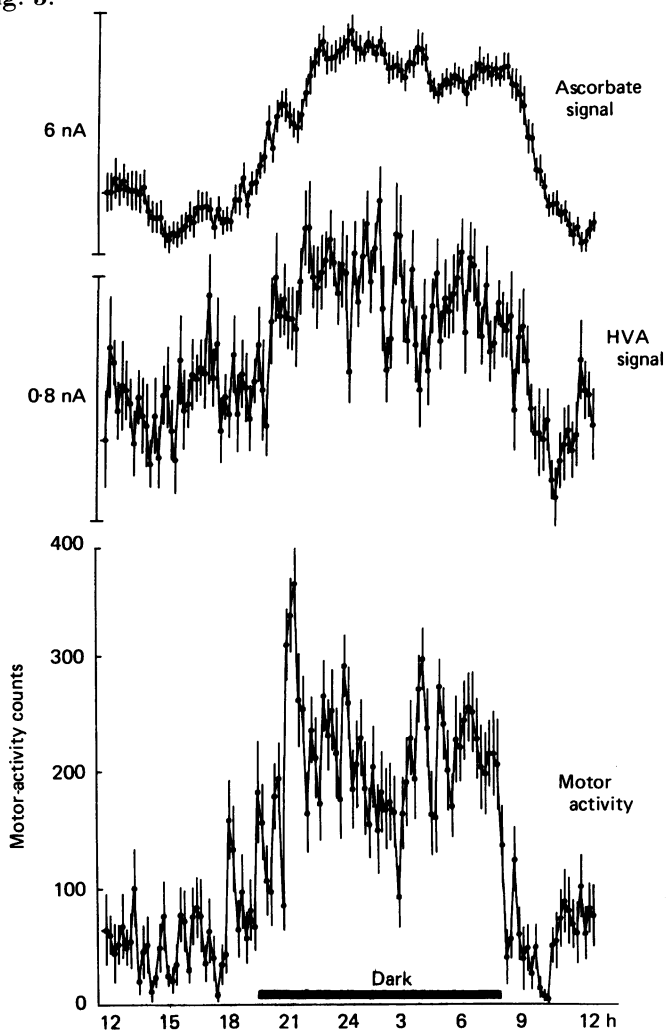

Fig. 3. The circadian pattern of the ascorbate signal and the HVA signal recorded in nucleus accumbens over 24 h periods; simultaneously recorded total motor activity is also shown. Mean ± S.E. of mean; twenty-two determinations using ten rats.

TABLE 1. Correlation coefficients calculated for circadian changes in motor activity *vs.* the ascorbate (A) and HVA (B) signals recorded simultaneously in nucleus accumbens and striatum. Lighting was on the normal 12/12 light/dark cycle for days 1–5; during days 6–13 the cycle was reversed

		Days		
	Brain region	1–5 ($n = 15$)	7–8 ($n = 6$)	12–13 ($n = 6$)
A	Nucleus accumbens	0·80±0·08	0·66±0·18	0·81±0·09
	Striatum	0·85±0·03	0·82±0·04	0·87±0·02
B	Nucleus accumbens	0·70±0·01	−0·2±0·1	0·70±0·03
	Striatum	0·72±0·04	0·22±0·16	0·68±0·08

High correlations were obtained for motor activity *vs.* both the ascorbate and HVA signals in accumbens and striatum (Table 1). Reversal of the light/dark cycle did not significantly affect the movement/ascorbate correlation, although it was slightly reduced in the two brain regions during days 2 and 3 after light reversal (Table 1). For this initial period, however, there was a complete breakdown in the correlations for motor activity *vs.* the HVA signal; these returned to their pre-reversal values by days 7 and 8 following the change in lighting, i.e. days 12 and 13 of recording (Fig. 4 and Table 1 B).

DISCUSSION

Reversal of the light/dark cycle affected the circadian pattern of motor activity in a number of ways (Figs. 1 and 2). The increase in the standard deviation per 24 h seen in the initial period, indicates that the distribution of activity-count values tended away from the mean to the limits of the counter (0 and 535). The brief increase in the average difference between successive readings shown in Fig. 2*C* reflects the initial breakdown of the block pattern of activity shown in Fig. 1. Finally, although the total motor activity recorded in each 24 h period did not change throughout the 13 days, the difference between the total for 08.00–20.00 h (initial light-on period) and 20.00–08.00 h was reduced and, after 5 days, completely reversed.

During this 13 day period changes in the extracellular concentration of ascorbic acid and HVA were monitored at 12 min intervals in both the striatum and nucleus accumbens. The hypothesis that changes in the extracellular concentration of brain ascorbate reflect changes in the release of excitatory amino acid transmitters is based on studies *in vitro* (Grunewald & Fillenz, 1984) and is supported by a number of experiments *in vivo* (O'Neill *et al.* 1983*c*, 1984*b*). Although these techniques are by their nature indirect, in combination they provide strong evidence that released excitatory amino acids increase extracellular brain ascorbate concentration. However, there remains the question whether other factors affect the ascorbate signal. We have shown that this signal does not reflect changes in blood ascorbate concentration (Grunewald, O'Neill, Fillenz & Albery, 1983; Fillenz, O'Neill & Grunewald, 1985). The finding that neither kainic acid lesions of the striatum nor 6-hydroxydopamine lesions of the substantia nigra have an effect on the ascorbate signal recorded in striatum (Grunewald, 1983) indicates that the signal is not simply a reflexion of general neuronal activity. In studies with synaptosomes only the excitatory amino

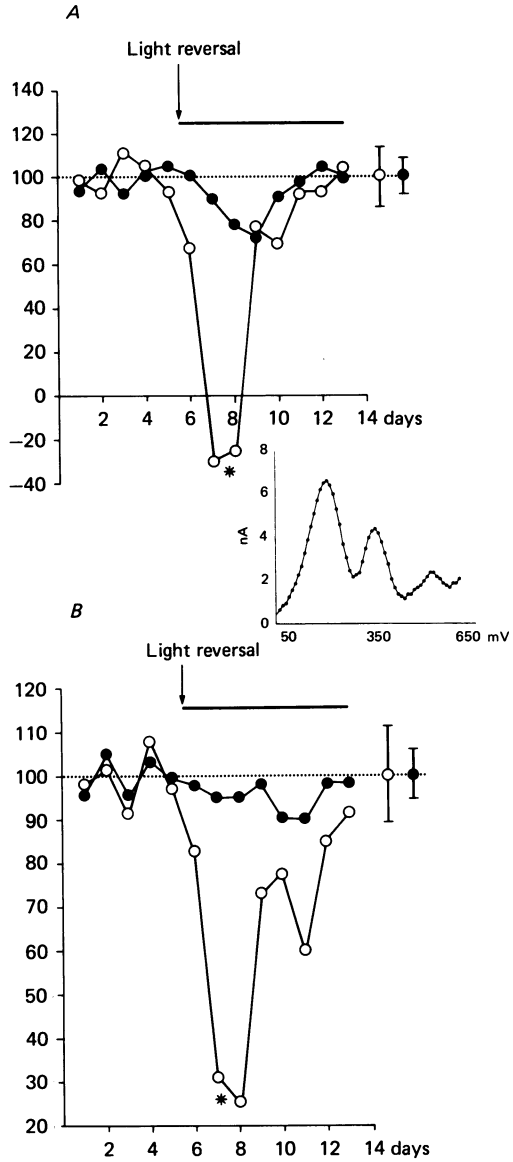

Fig. 4. Time course for changes in the correlation coefficients calculated for motor activity *vs.* voltammetric signals recorded simultaneously in nucleus accumbens (*A*) and striatum (*B*). ● = the ascorbate signal; ○ = the HVA signal. Results expressed as a percentage of the pre-reversal value. For clarity, only the average S.E. of mean is shown. There was no significant change in the correlation for the ascorbate signal; there was a complete breakdown in the correlation for motor activity *vs.* the HVA signals in both regions (* $P < 0{\cdot}02$ compared with pre-reversal values). Inset: example of a voltammogram recorded with a carbon-paste electrode in the striatum. Peak 1 is due to the oxidation of ascorbic acid, peak 2 to uric acid, and the dopamine metabolite, HVA, is responsible for peak 3. Changes in the height of each peak are proportional to changes in the extracellular concentration of the corresponding compound.

acids, and not γ-aminobutyric acid (GABA), glycine, monoamines or acetylcholine, caused ascorbate release (Grunewald & Fillenz, 1984). But perhaps the strongest support for the hypothesis comes from experiments using rats with unilateral lesions of the glutamatergic cortico-striatal pathway (O'Neill *et al.* 1983*c*). In these rats the nocturnal increase in the ascorbate signal recorded in the striatum on the side of the lesion was reduced by 80% compared with the control side. The data of Walaas (1981), which indicate that approx. 20% of the excitatory amino acid input to the striatum arrives via the fornix/fimbria, is consistent with the conclusion that the nocturnal increase in striatal ascorbate is due almost entirely to increased excitatory amino acid release.

Transmitter release in the synapse cannot be measured *in vivo* since detection would modify the release process through short- and long-loop feed-back mechanisms. Thus an indirect approach must be adopted. The use of metabolite levels as an index of the release of the parent neurotransmitter has been widely criticized (Commissiong, 1985). The objections are generally valid for pharmacological studies since drugs often interfere with metabolite production and clearance. However, in the absence of drugs, changes in the concentration of metabolites induced by electrical stimulation (Commissiong, 1985) or those accompanying spontaneous changes in transmitter release (O'Neill & Fillenz, 1985*a*, *c*) are likely to be a faithful reflexion of release. A more direct method to monitor release is to sample the extracellular fluid for transmitters. This has the advantage that there are no intermediate metabolite steps to reduce the time resolution. However, since high-affinity uptake mechanisms (whose kinetic parameters may vary with release rate (Nieoullon, Kerkerian & Dusticier, 1983)) keep the extrasynaptic concentration of transmitters low, small changes may be difficult to detect. Ascorbic acid, which is released during excitatory amino acid re-uptake, and the dopamine metabolite HVA are easily detected using *in situ* electrochemical techniques. In the light of the considerations outlined above, we have, under the present experimental conditions, used the ascorbate and HVA signals as an index of excitatory amino acid and dopamine release, respectively. The problem of the time lag between the recorded signals and the neurochemical events of interest (i.e. release) can be partly circumvented by the use of cross-correlation analysis.

We analysed the changes in the voltammetric signals (induced by light reversal) which accompanied those in motor activity in a number of ways and found that changes in the correlation coefficients were most sensitive to the effect of light reversal. The contrast between the correlation for motor activity *vs.* the ascorbate signal, which was essentially unaffected by the change in lighting, and that for motor activity *vs.* the HVA signal, which disappeared during days 2 and 3 of light reversal (Fig. 4), supports the recent suggestion (McGeer *et al.* 1984) that excitatory amino acid release in striatum plays a more direct role in the control of motor activity than does dopamine release; the results suggest a similar relationship in nucleus accumbens.

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