EPSPs. These results rule out the rather remote possibility that the alterations of DR EPSPs by 4-AP are due to its action on the postsynaptic membrane. The effects of small concentrations were reversible, while after long exposure to large concentrations of 4-AP we could not achieve the complete restoration. The rate of recovery of antidromic spike was clearly much faster. A similar dramatic increase of the RF EPSP by 4-AP was found (figure 1), although the latter were much smaller in amplitude and could be detected only in few motoneurones in contrast to electrotonic DR EPSPs which were present practically in all impaled cells.

The electrotonic VR EPSPs can be subdivided into 2 separate groups: early and late. The former are characterized by very short latency (0.5–2.0 msec) and relatively long duration. The subtraction of the extracellular response from the intracellular one demonstrates that the early VR EPSPs always begin before the peak of the antidromic spike. After 4-AP treatment, the early VR EPSPs were enhanced and prolonged in a similar way as DR and RF EPSPs (figure 2, A). This fact and the short latency of early EPSPs suggest that they are produced by monosynaptic connections between recurrent collaterals of motor axons and motoneurones.

In standard saline, the delayed VR EPSPs were present only in a few cells. They had a very fast time course, but always appeared after the peak of the antidromic spike (latency 2.5–4.8 msec). When 4-AP was applied, the delayed VR EPSPs appeared in most cells and their height could reach up to 8–12 mV. However, the duration was only slightly altered and they preserved the rapid spike-like shape (figure 2, B). The long latency of delayed VR EPSPs and their sudden appearance after 4-AP treatment are consistent with the prolongation of the soma-dendritic spike and suggest that this type of activity is transferred from motoneurone to motoneurone via electrically operating dendro-dendritic synapses.

Our results show that 4-AP is an efficient potentiator of excitatory transmission in different electrotonic junctions and that this effect is produced at the presynaptic level. The observed enhancement of electrotonic EPSPs by the substance blocking K^+ conductance further indicates that they are not caused by K^+ accumulation in perineuronal space. The differential effect of 4-AP on different electrotonic junctions may reflect the distinct structural organization of relevant synapses (axo-dendritic and dendro-dendritic), but may depend also on the peculiarities of molecular architecture of K^+ channels in different electrically excitable presynaptic membranes. Therefore, this substance may be used as a probe for analysis of presynaptic events which, as a rule, are not subject to direct electrophysiological investigation.

Projections from nucleus accumbens to globus pallidus and substantia nigra in the rat[1]

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Summary. Stimulation of nucleus accumbens produces inhibition and facilitation of neuronal activity in the ipsilateral globus pallidus and substantia nigra. Small lesions in the accumbens reduce pallidal but not nigral GABA content.

Recent studies using the orthograde transport of labelled amino acids or the retrograde transport of horseradish peroxidase have shown that neurones of the nucleus accumbens send efferent projections to the globus pallidus and substantia nigra[2–4]. This report provides electrophysiological confirmation for these projections and discusses the possible neurotransmitters released.

Materials and methods. Experiments were performed in 10 male albino rats lightly anaesthetized with urethane (1.2 g/kg, i.p.). The effect of nucleus accumbens stimulation was examined on spontaneous neuronal activity in the ipsilateral globus pallidus and substantia nigra. The centre barrel of a multibarrelled micropipette was used to record extracellular action potentials. Other barrels contained aqueous solutions of substances for electrophoresis; pontamine blue, GABA, dopamine, 1 M NaCl (current control). Methods for recording and analysis have been described[5].

In other animals (11) unilateral electrolytic lesions (300–400 μA for 20 sec) of the nucleus accumbens were made. The concentration of GABA[6] was estimated in globus pallidus and substantia nigra 14 days afterwards.

Results. The effects of nucleus accumbens stimulation are summarized in the table. In the globus pallidus 39 of 62 cells tested were activated. The predominant response was inhibition of firing (33 cells) (figure 1). This occurred alone with relatively short and constant latency (3.3 msec), but in other cells the latency was longer (13.0 msec) more variable and inhibition was of shorter duration (table). In 2 cells short latency inhibition was followed by a single phase of rebound excitation. In 3 cells facilitation preceded a period of inhibition (figure 1) and 6 cells responded by facilitation alone. Facilitation was characterized by a burst of action potentials (figure 1) and no evidence was obtained for antidromic activation. The latency of these excitations was similar (2.4 msec) but the duration of excitation occurring alone was longer (45.0 msec) than that which preceded a period of inhibition (15.7 msec). Histological examination revealed that most cells affected (22 inhibited, 5 excited) were localized rostromedially in the ventral two-thirds of the pallidus. Both GABA and DA (5–75 nA) depressed all cells tested in the pallidus (26 cells).

In the substantia nigra 13 of 30 cells tested responded. Only simple inhibition (7 cells, figure 2) or simple facilitation (6 cells) was observed. The latency of inhibition (6.7 msec) or excitation (5.2 msec) was similar as were the respective durations of the evoked effect (table). Of the responsive neurones, most (5 inhibited, 5 excited)

1 Acknowledgment. This work was supported by an MRC grant to Prof. D. W. Straughan.
2 B. S. Bunney and G. K. Aghajanian, Brain Res. *117*, 423 (1976).
3 L. C. A. Conrad and D. W. Pfaff, Brain Res. *113*, 589 (1976).
4 L. W. Swanson and W. M. Cowan, Brain Res. *92*, 324 (1975).
5 A. Dray, T. J. Gonye and N. R. Oakley, J. Physiol. *259*, 825 (1976).
6 I. P. Lowe, E. Robins and G. S. Eyerman, J. Neurochem. *3*, 8 (1958).

Effects of nucleus accumbens stimulation on the activity of neurones in the ipsilateral globus pallidus and substantia nigra

	Inhibition Latency (msec)	Duration (msec)	Facilitation Latency (msec)	Duration (msec)
Globus pallidus	n = 19 3.3 ± 0.4	28.5 ± 4.3	n = 6 2.4 ± 1.2	45.0 ± 16.4
A 5.9–6.8	range 2–7	7–90	range 2–10	10–100
L 2.5	n = 9 13.0 ± 0.9	18.3 ± 2.4		
D +0.5 to –2.0	range 10–17	10–35		
Substantia nigra				
A 1.6–2.6	n = 7 6.7 ± 0.9	14.6 ± 2.5	n = 6 5.2 ± 0.2	10.8 ± 2.3
L 2.0	range 5–10	5–25	range 5–6	5–20
D –1.5 to –3.0				

Stereotaxic coordinates[16] for the recording micropipette are indicated. Stimulation of the accumbens (coordinates A 9.4–9.8, L 1.0, D –0.5) was performed with a bipolar electrode (tip separation 0.25 mm insulated to 0.25 mm of the tips) using single square wave pulses of 50–250 μA, duration 0.2–0.3 msec at 1–2 Hz. The position of the stimulating electrode and micropipette tip position (dye ejection) was varified histologically after each experiment. See text for further details.

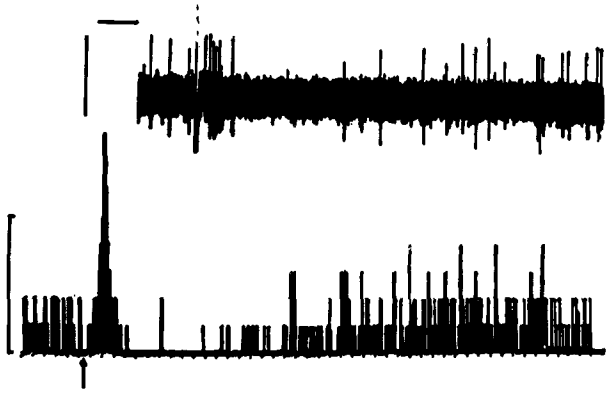

Fig. 1. Inhibition of a globus pallidus neurone evoked by stimulation of the ipsilateral nucleus accumbens. Spike record also shows preceding excitation, characterized by a burst of action potentials (2 sweeps, calibration 10 msec; 100 μV). Below is the poststimulus histogram of 100 consecutive sweeps from the same neurone (sweep duration 250 msec, calibration 10 impulses per bin).

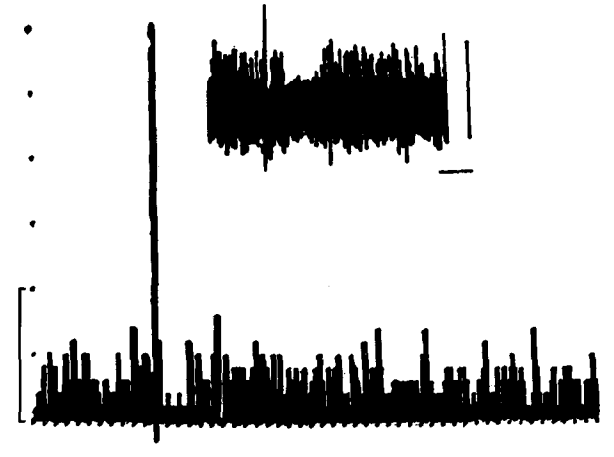

Fig. 2. Inhibition of a substantia nigra neurone by ipsilateral nucleus accumbens stimulation. Spike record of 20 superimposed oscilloscope sweeps showing inhibition of spontaneous firing (calibration 20 msec; 100 μV). Below is the corresponding PSTH from 100 consecutive stimuli. The high count preceding inhibition is the stimulus artefact (sweep duration 250 msec; calibration 10 impulses per bin).

were localized medio-caudally within the zona compacta-zona reticulata border.

Lesions of the accumbens produced a significant reduction of GABA in the globus pallidus (ipsilateral 5.16 ± 0.23; contralateral 6.47 ± 0.30 μmoles g^{-1}, n = 11, $p < 0.01$, t-test) but no changes in the substantia nigra GABA (6.23 ± 0.17 μmoles g^{-1}) of the same animals. All lesions were accurately placed in the medial portion of the accumbens and had damaged an area 500–750 μm in diameter. No obvious neurological disturbances were observed.

Discussion. The present observations confirm earlier anatomical studies[2–4]. In the pallidus, inhibition was the predominant response and for most cells was of constant latency. Excitation in the pallidus was also of constant latency but was somewhat shorter than the latency of inhibition. It is therefore possible that some inhibitions may be generated indirectly in the pallidus via the activation of an inhibitory interneurone. While it is also possible that the effects observed were due to stimulation of fibres 'en passage' the data are compatible with the existence of a mixed monosynaptic accumbens-pallidus pathway. However intracellular studies are required to confirm this suggestion. The longer latency and more complex responses may have resulted from activation of mixed or polysynaptic pathways. It is uncertain whether such pathways would be extrinsic or intrinsic to the pallidus. Only simple inhibition or facilitation was observed in the substantia nigra. These were of comparable and constant latency suggesting the possible monosynaptic nature of this pathway.

The distribution of neurons affected by accumbens stimulation was similar to the topographical distribution of accumbens projections observed in anatomical studies. Thus fibres from the medial accumbens (the site of stimulation in the present experiments) project to the medial and ventromedial pallidus[3,4]. Additionally fibres project to the medial substantia nigra[3] ending in the reticulata immediately subjacent to the compacta[4].

In agreement with other studies[7], GABA and DA depressed the activity of globus pallidus neurones though this was not necessarily correlated with evoked activity. High levels of GABA and GAD are found in the pallidus[8,9]

7 K. Obata and M. Yoshida, Brain Res. *64*, 455 (1973).
8 S. Fahn, in: GABA in nervous system function, p. 169. Ed. E. Roberts, T. N. Chase and D. B. Tower. Raven Press, New York 1976.
9 M. L. Tappaz, M. J. Brownstein and M. Palkovits, Brain Res. *108*, 371 (1976).

and some may originate from neurones in the accumbens[10]. The reduction of GABA in the pallidus following accumbens lesions suggests a possible role as the inhibitory transmitter in the pathway. That no significant changes in substantia nigra GABA were observed suggests that accumbens lesions were selective and did not damage the caudato-nigral GABA pathway[5]. The lack of biochemical changes in the nigra may also reflect the sparse projection from the accumbens or indicate that GABA is not involved in the pathway.

The nucleus accumbens thus appears to send inhibitory and excitatory projections to the pallidus and nigra. The identification of the neurotransmitters concerned requires further study. However since the accumbens is an important site for self stimulation[11] and for the actions of locomotor stimulants[12–15] it is reasonable to consider that such behaviour may be mediated in part through the pallidal and nigral projections.

10 G. J. Balcom, R. H. Lenox and J. L. Meyerhoff, J. Neurochem. *24*, 609 (1975).
11 E. T. Rolls, Brain Res. *31*, 275 (1971).
12 P. H. Kelly, P. W. Seviour and S. D. Iversen, Brain Res. *94*, 507 (1975).
13 A. J. J. Pijnenburg, G. N. Woodruff and J. M. Van Rossum, Brain Res. *59*, 289 (1973).
14 A. J. J. Pijnenburg and J. M. Van Rossum, J. Pharm. Pharmac. *25*, 1003 (1973).
15 C. Pycock and R. Horton, Brain Res. *110*, 629 (1976).
16 J. F. R. König and R. A. Klippel, The rat brain: a stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins, Baltimore 1963.

Temperature acclimation and learning in fish

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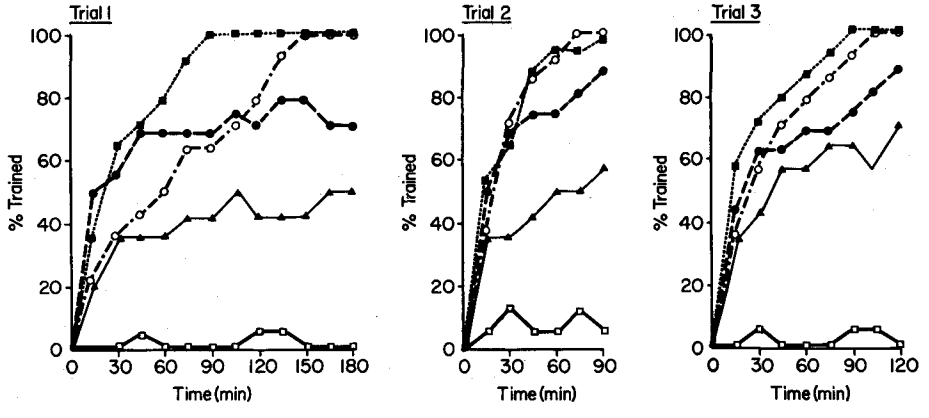
Summary. Control fish acclimated at 21 °C learned to swim upright following the attachment of floats to their ventral surfaces, while fish acclimated at both 5 °C and 33 °C failed to learn the swimming skill. Fish previously acclimated at 5 °C and then transferred to 21 °C 48 h before the task, learnt the swimming skill better than the control fish, but fish acclimated at 33 °C and transferred to 21 °C failed to acquire the new skill. Acclimation temperature, therefore, significantly modifies the learning behaviour of fish.

Fish have the ability to learn to swim in the upright position after polystyrene floats are attached to their ventral surfaces[1–5]. Shashoua[3] has shown that goldfish exhibit cyclic changes, with an annual rhythm, in their capacity to learn this swimming skill. Fish learned better during the winter months than during the summer months; the lowest levels of learning coincided with the onset of spawning[3]. These changes in learning patterns were thought to be due to cyclic hormonal and biogenic amine level changes[4]. The direct effect of temperature on learning was not, however, investigated.

We have investigated the effect of different acclimation temperatures on learning in fish using the same learning task as that used by Shashoua[1].

Materials and methods. *Cyprinus carpio* (L.) fingerlings weighing 14–18 g were used in all experiments. All experiments were performed during the autumn (May–June) and at the same time of day (evening). Groups of fish were acclimatized to 33, 21 and 5 °C for 3 weeks. They were kept in 40-l aquaria equipped with aerators, and the animals were fed regularly at fixed times. The fish were divided into 5 groups of 8 each for training. 3 groups were trained in their respective acclimation temperatures. The other 2 groups were removed from 5 or 33 °C and placed in a tank initially at the same temperature, which was then allowed to equilibrate to room temperature (21 °C). This transfer took place 48 h before training. Polystyrene floats 8.75% of the mass of the fish in 600 ml of water were made. The method of attaching these floats, as well as the method used for calculating the percentage trained, was the same as that used by Kaplan et al.[5]. The fish were assigned 3 stages of learning. During stage I the

1 V. E. Shashoua, Nature, Lond. *217*, 238 (1968).
2 C. J. Woolf, G. H. Willies, H. R. Hepburn and C. Rosendorff, Experientia *30*, 760 (1974).
3 V. E. Shashoua, Science *181*, 572 (1973).
4 V. E. Shashoua, Trans. Am. Soc. Neurochem. *3*, 121 (1972).
5 B. B. Kaplan, J. C. Dyer and J. L. Sirlin, Brain Res. *56*, 239 (1973).



Acquisition (trial 1) and retention (trials 2 and 3) of a learning task for fish acclimated to 21 °C (○–·–·–○), to 5 °C (□—□) and to 33 °C (●— — ●) for a period of 3 weeks. The results of fish transferred from 5 °C (■····■) and from 33 °C (▲—▲) after the same acclimation period to 21 °C 48 h before being subjected to the learning task in trial 1 are also shown. Each point represents the mean percentage trained for 8 fish at 15-min-intervals.