Spontaneous firing rate of neurones in the prefrontal cortex of the rat: evidence for a dopaminergic inhibition

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A mesocortical dopamine system which provides a dopaminergic input to the prefrontal cortex, mainly from the dopamine-containing cell group A10 in the mesencephalon, has recently been found in the rat using biochemical pharmacological and histochemical techniques4,5,10,15,20,21. Here we describe the first electrophysiological and pharmacological investigation into the possible role of this dopaminergic input to the prefrontal cortex. The prefrontal cortex is of interest at present because of its involvement in motivated behaviour12,17-19, as well as in relation to the dopamine hypothesis of schizophrenia12.

The plan of the experiments was to record simultaneously from a single neurone in the medial prefrontal cortex and from a single neurone in a region of the cortex thought not to receive a dopaminergic input to provide a control for non-specific effects, and then to administer intravenously pharmacological agents known to activate or block dopamine receptors, or to increase the release of endogenous dopamine from the presynaptic terminals.

The recordings were performed in male albino rats (Sprague-Dawley strain) weighing 250-300 g, using tungsten microelectrodes insulated with glass, and standard electrophysiological techniques described in more detail elsewhere18. The rats were anaesthetised with either 20% urethane (1.2 g/kg, 28 animals) or Equithesin (Jensen-Salsbury Labs Inc., 2.0 ml/kg, 14 animals). (The results reported here in this paper were found in both groups of rats independent of the anaesthetic used.) A catheter was introduced into the heart through a jugular vein to allow pharmacological agents to be administered and to act rapidly to facilitate the analysis of effects on neuronal activity. The rats were placed in a stereotaxic instrument and holes were trephined to enable access to the medial prefrontal cortex (level-head coordinates 2–5 mm anterior to bregma, and 1.1 mm lateral to the midline) and for control recordings to the somatosensory cortex (6.0 mm posterior to bregma, and 4.5 mm lateral to the midline)14. In some cases, where stated, control recordings were made from the hippocampus. The

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firing rates of the single units were plotted continuously on a polygraph recorder, and the neuronal activity was recorded on magnetic tape for subsequent additional photographic analysis (see Fig. 3). The drugs used were apomorphine (which stimulates dopamine receptors directly) at doses of 0.016, 0.06 and 0.1 mg/kg (expressed as the hydrochloride and dissolved in 0.9 % saline with 0.5 mg/ml ascorbic acid), and spiroperidol (which blocks dopamine receptors) at doses of 0.06 and 0.1 mg/kg dissolved in 0.01 M tartaric acid (kindly supplied by Janssen Pharmaceutica). Experiments were also performed with d-amphetamine dissolved in 0.9 % saline (which acts by releasing dopamine, as well as noradrenaline, and blocks their re-uptake) at doses of 1, 2 and 4 mg/kg (of the sulphate), and L-DOPA (which is converted to dopamine in the nerve terminals) dissolved in 5 % glucose and neutralised with NaOH before injection at the single dose of 100 mg/kg (cf., ref. 8). To avoid any possible residual drug effects the activity of only two cells (one in the medial prefrontal cortex and one control) was analysed in each animal. The baseline spontaneous firing rate of the cells was measured for at least 10 min before any solution was injected. Then, injections of saline or of the solvents to be used, if these were not saline, were given and the activity of the neurones was measured for at least a further 5 min to ensure stability of the firing rates. Thus, the spontaneous firing rate of the units was measured for 15–20 min before any pharmacological manipulation was performed. After this, selected pharmacological agents were administered in volumes of 0.2–0.5 ml over 5 sec through the catheter. Body temperature was maintained constant throughout the experiments. At the end of every experiment the recording sites were marked with lesions made through the recording microelectrodes (+30 μA for 50 sec) for subsequent histological verification (see Fig. 1 for examples of the recording sites).

Of the total number of neurones recorded in this region of the prefrontal cortex 42 % were sensitive to the injections of apomorphine. At the dose of 0.1 mg/kg, apomorphine produced a large decrease (by approximately 70 %) of the spontaneous firing rate of these cells in the medial prefrontal cortex (n = 14, n = the number of cells). The activity of the control cells recorded simultaneously in the somatosensory

Fig. 1. Examples of the recording sites. The outlines were taken from the atlas of König and Klippel.

As shown in this figure most of the recordings were made in the medial wall of the anterior telencephalon, in the region of the prefrontal cortex.
Fig. 2. The mean firing rate of two neurones recorded simultaneously in the prefrontal cortex and hippocampus after single injections of isotonic saline and apomorphine (APM, 0.1 mg/kg). This represents a typical example of the time course of the effect of apomorphine on the spontaneous firing rate of cells in the prefrontal cortex.

cortex or hippocampus was not altered by apomorphine. The decrease of firing rate of different prefrontal neurones produced by apomorphine lasted for 10–30 min after which time the units recovered their initial baseline firing rate. The effect of apomorphine was gradual and reached a maximum 2 min after injection. A typical example of the time-course of the effect of a single injection of apomorphine is shown in Fig. 2. In general, the lowest effective dose of apomorphine was 0.06 mg/kg \( (n = 6) \), but in some cases effects were observed with a dose of 0.016 mg/kg \( (n = 2) \). Previous blocking of dopamine receptors by spiroperidol (0.1 mg/kg), administered at least 15 min previously through the catheter, blocked the effects produced by apomorphine \( (n = 5) \) (see Fig. 4).

These findings were investigated further in preliminary experiments carried out with amphetamine and L-DOPA (see Fig. 3B). Amphetamine produced a complete inhibition of spontaneous firing rate in the cells of the medial prefrontal cortex at the dose of 2 mg/kg \( (n = 2) \). A total dose of 4 mg/kg produced a protracted depression of neuronal activity, lasting for more than 1 h \( (n = 2) \). The majority of the control units was also affected by amphetamine injections, which produced an increase in their spontaneous firing rate for a short period (see e.g., Fig. 3B). Pretreatment with injections of spiroperidol (0.1 mg/kg) prevented the cells in the medial prefrontal cortex, but not in the control cells, from being affected by a single injection of amphetamine \( (2 \text{ mg/kg}, n = 2) \). A second injection of amphetamine \( (1–2 \text{ mg/kg}) \) then decreased the firing rates of cells, perhaps by overcoming the competitive receptor blockade produced by the spiroperidol.

Injections of L-DOPA administered as a single dose of 100 mg/kg did not produce
Fig. 3. Effects of saline, apomorphine (APM, 0.06 mg/kg), and amphetamine (AMP, 2 mg/kg) on the firing rate of single neurones in the prefrontal cortex and the somatosensory cortex recorded simultaneously. In A, the firing rate before and after saline injections and the effects of a single injection of APM are shown. The firing rate of the prefrontal cell returned to its initial value in 14–15 min. B shows the firing rate of the same cell more than 1 h after the apomorphine injection and shows the effects of a single injection of amphetamine (AMP), 2 mg/kg. As shown in the figure, the firing rate of the prefrontal cell was completely inhibited after injections of both APM and AMP. Amphetamine had a small effect on the control cell.

any effect on the spontaneous firing rate of the cells in the medial prefrontal cortex. When the L-DOPA concentration in the central nervous system was increased by inhibiting its peripheral decarboxylation with R04-4602 (50 mg/kg, i.p.), the injections of L-DOPA produced a decrease of the firing rate of the prefrontal cells for approximately 30 min (n = 2).
BASELINE
A PREFRONTAL CORTEX
B CORTICAL CONTROL

5 min. after AMP injection

25 min. after AMP injection

35 min. after AMP injection

2 min. after APM injection

15 min. after APM injection

35 min. after APM injection

15 min. after SPIROPERIDOL injection

1 sec
These results show that the spontaneous firing rate of neurones in the medial prefrontal cortex is decreased by injections of drugs which act on dopamine receptors either directly (apomorphine) or through the release of dopamine (amphetamine, L-DOPA). The finding that the effect produced by apomorphine was seen in the neurones in the prefrontal cortex but not in the control neurones recorded simultaneously in another cortical area or in the hippocampus, and that the effect can be blocked by the prior injection of spiroperidol, suggests that the inhibitory effect is due to the stimulation of dopamine receptors. These results, on the other hand, do not rule out the possibility that these cells can also receive noradrenergic inhibitory inputs. The fact that the other drugs used in this study (amphetamine, L-DOPA) act through the release and synthesis of dopamine as well as noradrenaline and produced a similar inhibitory effect on cell firing (although the effects of 2 mg/kg of amphetamine were blocked with spiroperidol) would, certainly, leave that possibility open to further research. It is clear, however, that the discussion of this point seems premature on the basis of our preliminary experiments with amphetamine and L-DOPA. Nevertheless, our findings are consistent with the hypothesis that dopaminergic terminals in the medial prefrontal cortex have an inhibitory function, and is in agreement with the hypothesised role of dopamine as an inhibitory neurotransmitter (see references cited by Groves et al.11). This hypothesis could be investigated further by applying apomorphine systemically and dopamine iontophoretically through a multi-barrelled recording electrode assembly to cells activated by electrical stimulation of the A10 dopamine cell group. It will be of interest to determine the possible behavioural functions of this dopaminergic inhibition of the prefrontal cortex. One recent finding from our laboratory is that apomorphine has a specific inhibitory effect on self-stimulation of the prefrontal cortex16. Thus, dopaminergic inhibition in the prefrontal cortex may have an important role in motivated and rewarded behaviour.

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Fig. 4. Photographs of spikes of single neurones recorded simultaneously in the prefrontal cortex and in the somatosensory cortex are shown. The baseline firing rate of the cells was measured for 15 min during which a saline control injection was performed to ensure stability. After that time a single dose of 2 mg/kg of amphetamine (AMP) was injected intravenously. The figure shows the effects 5, 25 and 35 min after the AMP injection. After the firing rate of the prefrontal cell had returned to its initial value, the rate was measured for 10 min more, and then apomorphine (APM, 0.1 mg/kg) was injected in a single dose. The figure shows the effects 2, 15 and 35 min after the injection. After recovery of the firing rate spiroperidol (0.1 mg/kg) (which blocks dopamine receptors) was injected in a single dose. Twenty minutes after the injection of spiroperidol, apomorphine (0.1 mg/kg) was injected again. The figure shows the effects of spiroperidol 15 min after the spiroperidol injection, and the effects of apomorphine 15 min after the apomorphine injection was performed. It can be seen that APM as well as AMP produce an inhibition of the spontaneous firing rate of the neurone in the medial prefrontal cortex but not in the control unit. Spiroperidol prevented the cell in the medial prefrontal cortex from being affected by the second AMP injection.


