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On the Neurochemical Basis of Self-Stimulation with Midbrain Raphe Electrode Placements

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DEAKIN, J. F. W. *On the neurochemical basis of self-stimulation with midbrain raphe electrode placements*. PHARMAC. BIOCHEM. BEHAV. 13(4) 525–530, 1980.—Bar-pressing for electrical stimulation of the median raphe nucleus in rats was not attenuated by 5HT receptor blockade with metergoline or cyproheptadine, by 5HT depletion induced with parachloroamphetamine or by prior destruction of ascending 5HT pathways with intracerebral microinjections of 5,7-dihydroxytryptamine. Furthermore, in a shuttle box paradigm in which rats could both initiate and terminate stimulation, parachlorophenylalanine did not antagonize initiation of stimulation. It is concluded that the rewarding effects of raphe stimulation are not mediated by serotonergic mechanisms. In contrast to these results, alpha-methyl-paratyrosine induced catecholamine depletion exerted an inhibitory effect on initiation behaviour without impairing termination of stimulation. It is concluded that the rewarding component of raphe stimulation is mediated by catecholamines. Termination of (escape from) stimulation was not materially affected by catecholamine or 5HT depletion suggesting the aversive component of raphe stimulation may not be mediated by these monoamines.

Neurochemical basis   Self-stimulation   Midbrain raphe   Electrode placement

IT has been suggested that the behavioural effects of rewards and punishments may be mediated respectively by central catecholamine and serotonin containing neurones. These suggestions were based on evidence that catecholamines but not serotonin may be involved in brain stimulation reward (reviewed in [3]) whereas serotonin rather than catecholamines mediate the suppressive effects of punishment on operant and other behaviours [10, 11, 24, 27].

This orderly scheme for reciprocal reward and punishment mediating functions of catecholamine and serotonin containing neurones has, however, been called into question by the finding that self-stimulation behaviour can be obtained with electrodes implanted in the serotonergic cell bodies of the raphe nuclei which give rise to forebrain 5HT projections [1, 15, 20, 22, 23]. Furthermore, there are some reports that raphe self-stimulation is antagonised by drugs which block serotonergic neurotransmission [18,26]. Because such findings pose considerable problems for an exclusively catecholaminergic mediation of reward and for the 5HT theory of punishment, the phenomenon of self-stimulation from the raphe has been examined.

The effects of 5HT receptor blocking agents and of 5HT depletion upon self-stimulation rates from the raphe nuclei were investigated to assess the possibility of a serotonergic substrate for the behaviour as suggested by the studies of Milliaresis, Bouchard and Jacobowitz [18] and Van der Kooy, Fibiger and Phillips [26]. However, earlier studies had suggested a catecholamine mediation for raphe self-stimulation since the behaviour was attenuated by catecholamine synthesis inhibition with alpha-methylparatyrosine [23] and by chlorpromazine [17,18]. Raphe self-stimulation could for example, be mediated by direct activation of the prominent system of catecholamine cell bodies and fibres passing through the raphe and known as the periventricular catecholamine system [16]. These pharmacological and anatomical considerations suggesting a catecholaminergic basis for raphe self-stimulation, if corroborated, could go some way to reconciling raphe self-stimulation with the catecholamine theory of reward and the 5HT theory of punishment. However, this reconciliation would necessarily involve the assumption that stimulation of the raphe is at once reinforcing and aversive, with catecholamines subserving the reinforcing component and serotonin the aversive effects. Therefore, an attempt was made to separately quantify reinforcing and possible aversive components of raphe stimulation by allowing animals to initiate and to terminate (escape) electrical stimulation of the raphe in a shuttle-box; a strategy described for other self-stimulation sites by Hunt *et al.* [14]. The effects of pharmacological manipulation of catecholamine and serotonin neurotransmission on initiation and escape behaviour were investigated.

METHOD

Implantation of Electrodes

Bipolar twisted stainless-steel electrodes (Plastic Products 0.27 mm external diameter) were used for all experiments. Electrodes were insulated except for the cross-sectional area of the tip. Electrodes were attached to the electrode holder of a Kopf stereotaxic frame and the electrode wires bent into the vertical position using a set-square. The tip of the electrode was then lowered to interaural zero using the A-P and vertical micromanipulators and the interaural zero coordinates recorded.

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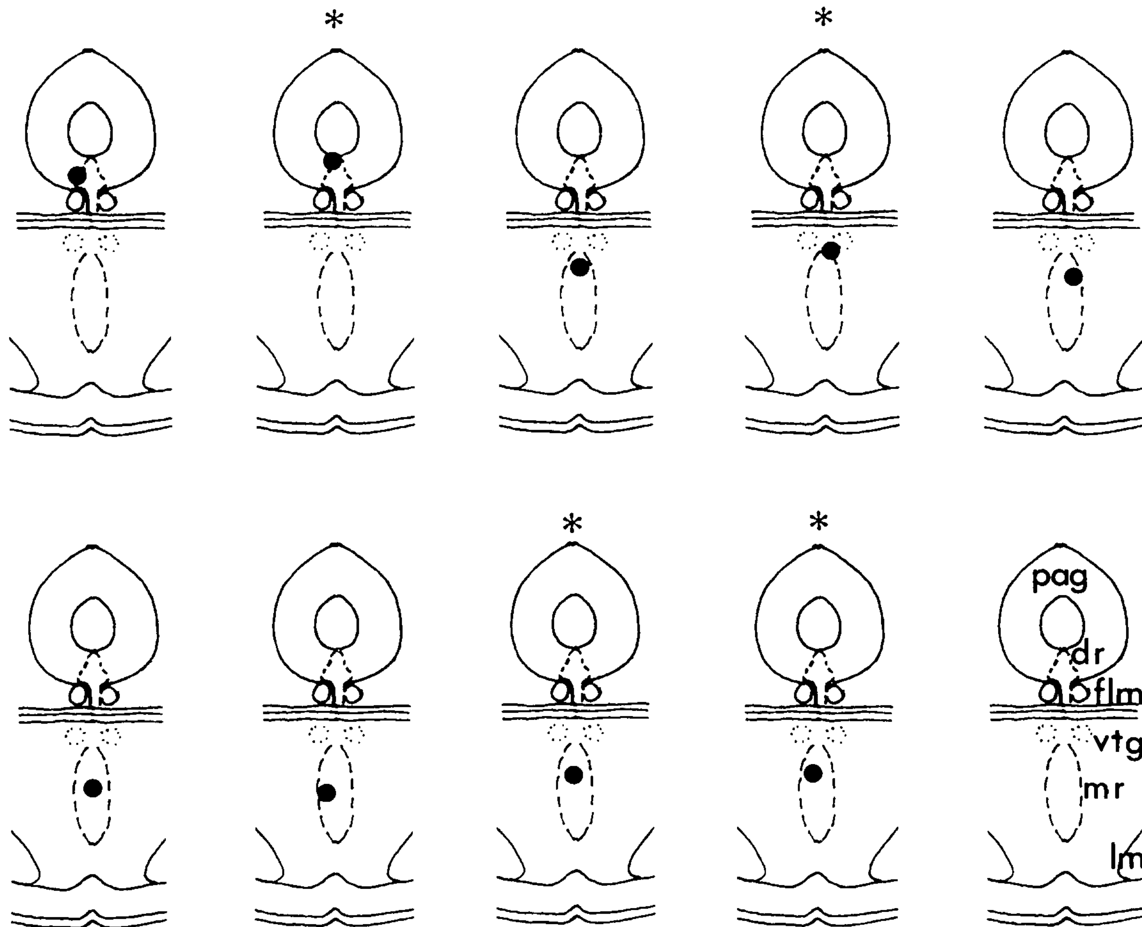

FIG. 1. Location of electrode tips in metergoline experiment. Dots indicate location of electrode tips on coronal sections of the brain stem at the level of the raphe nuclei. The same A-P coordinates were used throughout. pag=periaqueductal grey matter; dr=dorsal raphe nucleus; flm=medial longitudinal fasiculus; vtg=ventral tegmental nucleus of Gudden; mr=median raphe nucleus; lm=medial lemniscus. Sections marked with an asterisk indicate location of electrodes in animals which also received cyproheptadine. At least 7 days elapsed between cyproheptadine and metergoline administration.

Animals were anaesthetised with Avertin (tribromoethanol 1% in a 2.5% v/v iso-butyl alcohol-saline vehicle) and placed in the head holder with the tooth-bar 1 mm above the interaural plane to produce the orientation required by the stereotaxic atlas of Fyfkova and Marsala [8]. Coordinates were used which reliably placed the electrode tips in the dorsal or median raphe nuclei.

Burr-holes were made with a dental drill and 10*B*A screws screwed into place around a central burr-hole through which the electrode was lowered to the appropriate coordinates. Dental cement was then poured round the screws and the plastic base of the electrode. When the cement was dry the electrode holder was removed, the electrode being held in place by the dental cement. Skin was closed with silk sutures.

Self-Stimulation

Lever pressing. Animals were trained to self-stimulate in sound attenuated Technical Services operant boxes equipped with a single lever. Continuous reinforcement was used throughout such that every lever press made by the rat produced a single 200 msec train of electrical stimulation delivered to the animal's electrode through a connecting lead.

Animals were shaped for self-stimulation at least seven days post-operatively. Stimulation parameters were controlled by Neurolog logic to produce 200 msec trains of 1 msec bipolar pulses with a frequency of 100 Hz. Current was continuously monitored on an oscilloscope.

Shuttle box. A Technical Services shuttle box with a pivoted floor and no barrier between the two sides was used. When animals moved to the left-hand side of the shuttle box continuous brain stimulation was delivered; animals could terminate stimulation by moving to the right-hand side. Initiation of brain stimulation switched on a digital timer and termination of stimulation switched the timer off thus recording the time taken for the animal to terminate (escape in the nomenclature of Hunt *et al.*, [14]) the stimulation. A second digital timer recorded the time taken for the animal to re-initiate stimulation. As each initiation or escape occurred the initiation or escape latency was printed out using a Venner printer.

Animals were given daily test sessions; in the experiments involving alpha-methylparatyrosine (AMPT) rats were run at a stimulation intensity of 30 μA for 15 min then at 70

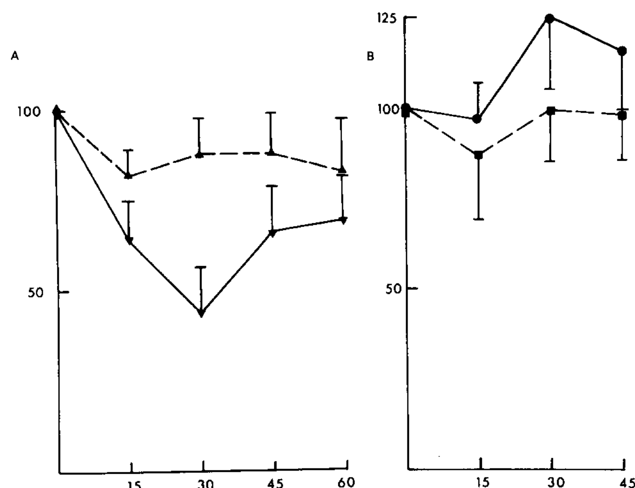

FIG. 2. Effects of 5HT blockers on raphe self-stimulation (a) ▼——▼ Metergoline (5 mg/kg; IP N=9) treated animals. ▲-----▲ The same animals injected with vehicle the previous day. (b) ●———● Cyproheptadine (5 mg/kg; IP N=4) treated animals. ■------■ The same animals injected with vehicle the previous day. Bar pressing rates expressed as per cent pre-injected rates, ordinates. The reduction in self-stimulation rates in the metergoline treated animals at 30 min is significantly different from rates on the vehicle injection day ($p<0.01$; t-test). Ordinate shows lever presses expressed as per cent of first 15 min (pre-drug rates). Abscissa: time after drug injection.

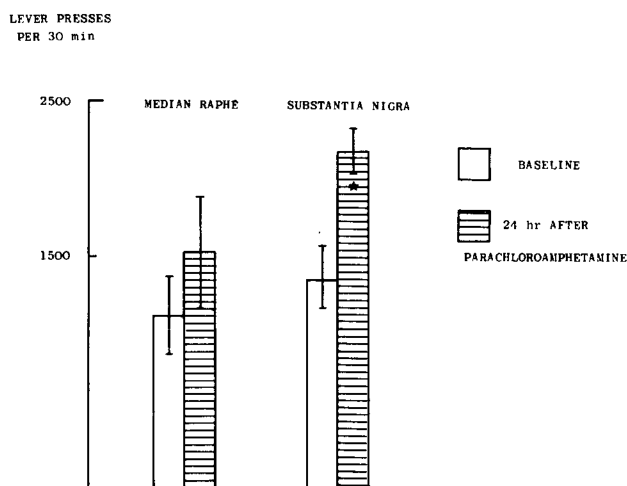

FIG. 3. Effects of PCA on median raphe self-stimulation.

□ Preinjection baseline rates.

▤ Lever pressing rates 24 hr post-PCA (10 mg/kg; IP)

Substantia nigra/ventral tegmental area N=4; Median raphe N=8. Ordinate shows total number of lever pressing in 30 min test sessions before (open columns) and 24 hr after PCA injection (shaded columns).

μA for a further 15 min, and, in the PCPA experiment, for 20 min at 70 μA. Stimulation was delivered from a 50 Hz sinusoidal stimulator.

Animals acquired shuttling behaviour in a single session. Baseline values for initiation and escape latencies were obtained over the following six days. AMPT (150 mg/kg, IP) was administered four hours before the eighth session. One week after the AMPT experiment, baseline was re-established over seven days. Animals were injected with PCPA (parachlorophenylalanine 300 mg/kg IP) after the seventh session and shuttle box self-stimulation followed for 3 days after drug administration.

It was not uncommon for 100–200 crossings to be made in a single test session each producing an initiation or termination latency data point. To reduce this data the first 20 and last 20 initiation or termination latencies were averaged to give a daily mean value.

5,7-Dihydroxytryptamine Lesions

To destroy ascending 5HT projections, 8 μg of 5,7-dihydroxytryptamine creatinine sulphate were microinjected into both the dorsal and median raphe nuclei in 2 μl 0.2 mg/ml ascorbic acid in saline vehicle. The rate of injection was 1 μl/min. After one week, for post-operative recovery and to allow for degeneration of 5HT neurones, animals were implanted with median raphe nucleus stimulating electrodes. After a further 5 days, animals were shaped for self-stimulation behaviour and, having acquired the behaviour, the animals were allowed to self-stimulate for a further 30 min. The animals were killed by cervical dislocation and the brains rapidly removed and the cortex dissected off. Cortical 5HT concentrations were assayed by the method of Curzon and Green [5]. A group of six control animals were treated in the same way at the same time except that they did not receive microinjections.

Histology

At the completion of experiments brain stems were removed and fixed in a formalin-saline solution. Alternate 30 μ sections were stained with cresyl fast violet and the sections examined under a microscope to confirm location of electrode tips in the raphe nuclei.

Drugs

The putative 5HT receptor blocking agents Metergoline (Farmitalia) and Cyproheptadine (Merck, Sharpe and Dohme) were dissolved in 0.7% ascorbic acid/saline vehicle and were injected at a dose of 5 mg/kg, IP. On control days vehicle alone was injected. The 5HT depleting agents parachloroamphetamine (PCA) and parachlorophenylalanine methyl ester (PCPA) were obtained from Sigma and injected in saline.

RESULTS

5HT blockers and raphe self-stimulation. Figure 1 shows the location of electrode tips used in the metergoline study. Figure 2 shows successive 15 min lever pressing rates expressed as a percentage of 15 min pre-injection rate on the metergoline injection day and the rate on the preceding vehicle injection day. The figure shows that metergoline caused a significant slowing of self-stimulation rates at 30 min post drug which had largely recovered by 45 min at which time lever pressing rate was not significantly different from that 45 min following vehicle injection.

In four animals no reduction in self-stimulation rates was

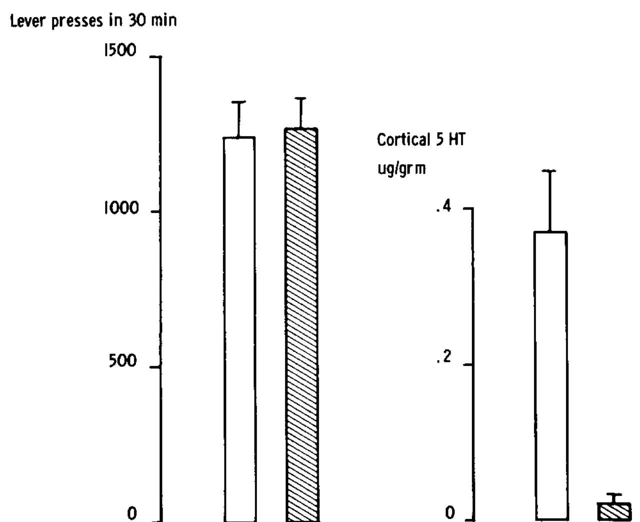

FIG. 4. Lack of effect of 5,7 DHT lesions of median raphe nucleus on median raphe nucleus self-stimulation.

non-injected control animals N=6

5,7DHT lesioned animals N=12

Left hand ordinate shows total number of lever presses in 30 min following acquisition of lever pressing behaviour in control (open columns) and 5HT depleted animals (shaded column). Right hand ordinate shows cortical 5HT concentrations in control (open column) and 5,7 DHT lesioned animals (shaded column).

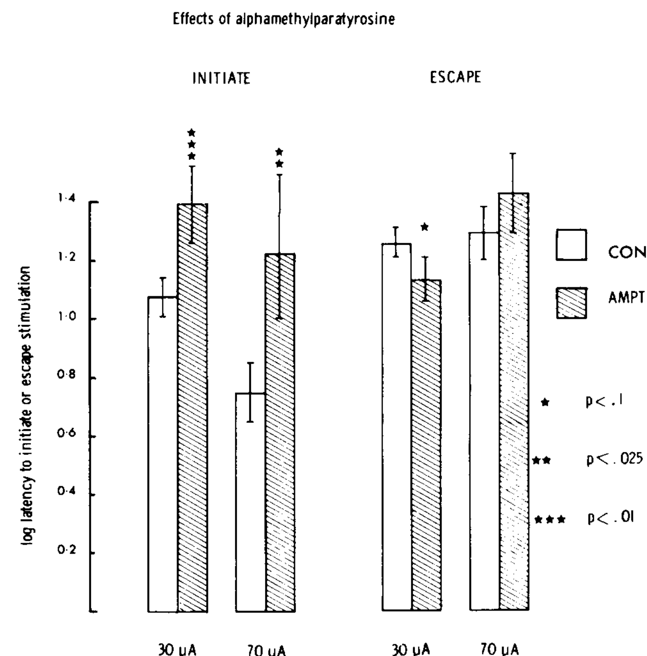

FIG. 5. Effects of AMPT on initiation and escape latencies at two intensities of median raphe stimulation. Left hand columns show log initiation latencies (ordinate) at 30 and 70 μA stimulus intensity. Open columns show latencies on the day before AMPT, shaded columns 4 hrs after AMPT the next day. Right hand columns show log escape latencies (ordinate) at 30 and 70 μA stimulus intensity. Open columns show latencies on the day before AMPT and shaded columns 4 hrs after AMPT. N=11. Statistical comparisons pre and post-drug by t-test. Vertical bars=SEM.

observed following cyproheptadine (5 mg/kg IP, Figure 2).

Effects of 5HT depletion, induced by parachloroamphetamine (PCA) pretreatment, on raphe self-stimultion. Twenty-four hours after PCA (10 mg/kg, IP) ventral tegmental area self-stimulators (N=4) showed a significant increase in lever pressing rates, (Fig. 3). No significant effect on median raphe (N=9) self-stimulation was observed although the trend was also towards an increase. All electrode tips were found to be located in the raphe on histological examination.

Effects of destruction of ascending 5HT neurones with 5,7-dihydroxytryptamine (5,7 DHT) microinjections. 5,7 DHT lesioned animals (N=12) showed a 90% decrease in cortical 5HT concentrations (Fig. 4) and in most cases fluorimetric excitation scans showed no peak at 360 nM. Despite this severe reduction in cortical 5HT concentrations all lesioned animals were rapidly shaped to self-stimulate and the subsequent 30 min lever pressing total was identical to that of non-lesioned control animals (N=6).

Effects of AMPT and PCPA on initiation and escape from raphe stimulation. To maintain homogeneity of variance, initiation and termination latencies were transformed to logarithms and latencies discussed below refer to the transformed data.

To investigate stability of baseline pre-drug initiation and termination latencies, data from the six daily sessions before AMPT were subjected to analysis of variance for repeated measures. No significant main effect of days was observed for initiation latencies, $F(5,90)=0.81$; p=N.S., indicating stable baseline performance.

A significant main effect of stimulus intensity was observed for baseline initiation latencies, $F(1,18)=6.62$; $p<0.05$, indicating that initiation was significantly more rapid at 70 μA than at 30 μA throughout the baseline period. In contrast stimulation intensity did not affect termination latencies, $F(1,18)=0.13$; p=N.S.

The four left hand histograms of Fig. 5 compare initiation latencies on the day before and 4 hours after AMPT (150 mg/kg, IP) administration. It can be seen there is a significant slowing of initiation (increase in initiation latencies) after AMPT. This was confirmed by including initiation latencies under AMPT in the previously mentioned analysis of variance which then revealed a significant main effect of days, $F(6,10)=7.00$; $p<0.01$.

The four right hand histograms show that AMPT had minimal effects on termination latencies, the more rapid termination at 30 μA being significant at the borderline $p<0.1$ level.

Figure 6 shows initiation and escape latencies before and on the three days following PCPA administration. The reduction in initiation latencies at 3 days following PCPA is significant ($p<0.05$) but escape latencies were not influenced by PCPA.

DISCUSSION

In confirmation of many other studies (see introduction) it was observed that self-stimulation behaviour could be obtained with electrodes implanted into the raphe nuclei. Metergoline was found to produce a transient depression in lever pressing rates which had substantially recovered by 45 min post drug (Fig. 2). This time-course is in contrast to the ability of metergoline to completely abolish most of the ab-

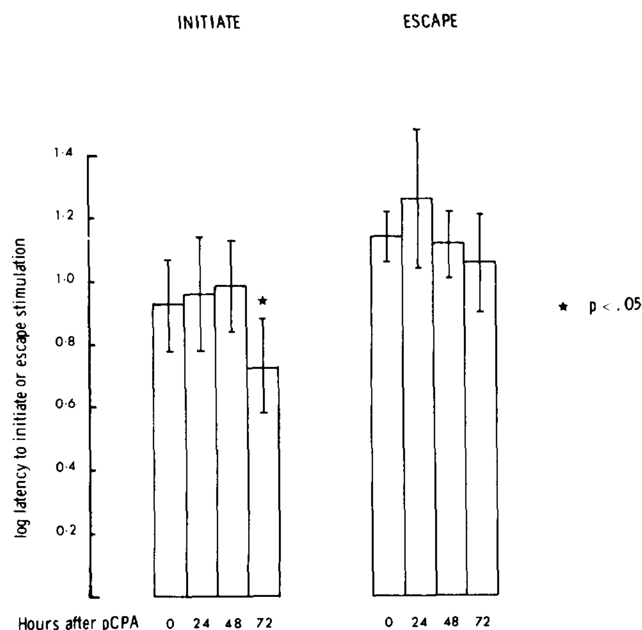

FIG. 6. Effects of PCPA on initiation and escape latencies at 70 μA stimulation of median raphe nucleus. Ordinate shows log initiation latencies (left hand columns) and log escape latencies (right hand columns) before PCPA (columns marked 0 hrs after PCPA) and on the following 3 days. Vertical bars=SEM. N=11. Statistical comparison pre and post drug by *t*-test.

normal behaviour induced by the combination of a monoamine oxidase inhibitor and the 5HT precursor tryptophan for at least one and a half hours after its administration [6]. That the effect of metergoline on raphe self-stimulation was not due to 5HT receptor blockade is further suggested by the lack of effect of another putative 5HT blocker, cyproheptadine, on raphe self-stimulation (Fig. 2).

Further evidence for a lack of involvement of 5HT in raphe self-stimulation is provided by the lack of effect of 5HT depletion caused by 24 hr pretreatment with parachloroamphetamine (PCA) (Fig. 3).

The degree of 5HT depletion 24 hr after 10 mg/kg PCA has been reported to be between 70 and 90% [13,21], however, there are regional variations in the efficacy of PCA [21] and a biochemical check on 5HT depletions was not made in the present study. Therefore, the effects of biochemically confirmed global forebrain 5HT depletion, produced by the neurotoxin 5,7 DHT, were investigated. In twelve animals 5,7 DHT microinjections were found at the end of the experiment to produce a 90% depletion in cortical 5HT concentrations and in most cases 5HT was undetectable using a fluorimetric assay method [5]. The same lesion in an earlier study was found to produce similar depletions in striatum and hippocampus [7]. Despite this severe destruction of ascending 5HT fibres all twelve lesioned animals were readily trained to self stimulate and maintained the behaviour at the same rate as control rats.

These results confirm three other reports that PCPA induced 5HT depletion did not reduce self-stimulation from the dorsal raphe nucleus [2, 17, 23]. However, Van der Kooy [26] and Milliaresis *et al.* [18] have reported reductions in lever pressing rates for raphe stimulation following PCPA and similar findings have been reported for hippocampal and caudate electrode placements [19,25]. These studies have utilised long daily test sessions of up to two hours and decrements in responding are progressive and most marked towards the end of the test sessions. This may explain why the earlier studies using shorter sessions failed to observe PCPA induced antagonism of raphe self-stimulation. It may be that, long sessions are required for electrical stimulation to deplete residual functional 5HT stores. However, there is no biochemical evidence for this suggestion and even at the end of long sessions the reductions in lever pressing rates are only of the order of 50%. Therefore, it seems unlikely that 5HT neurones are a vital link in the mediation of raphe self-stimulation.

While the use of half-hour sessions may explain why PCA was ineffective in antagonising median raphe self-stimulation in the present experiment, this would not account for the lack of effect of 5HT blockers or of 5,7 DHT induced destruction of 5HT neurones in which the disruption of 5HT neurotransmission is independent of the duration of test sessions. Another possible explanation of the present results is that the electrode tips were located in a different part of the raphe to those used in other studies. However, in all cases, histological verification of raphe placement was obtained and drug or lesion effects did not seem to be dependent on precise location of electrode tips within the raphe.

It was argued in the introduction that raphe stimulation might have a catecholamine mediated rewarding component and a 5HT mediated aversive or a behaviourally inhibiting component. In an attempt to separate these components a shuttle-box stimulation paradigm similar to that described by Hunt and colleagues [14] was used. Increasing stimulation intensity from 30 to 70 μA was found to cause more rapid initiation of stimulation (Fig. 5) supporting the view that this measure reflects the rewarding component of the stimulus. At both intensities AMPT induced catecholamine depletion delayed initiation of stimulation (Fig. 5) suggesting that the rewarding component of raphe stimulation is mediated by catecholamines. In contrast, PCPA induced 5HT depletion was without major effect on initiation latencies (Fig. 6), a finding compatible with the view that the rewarding component of raphe stimulation is not mediated by 5HT neurones.

It was anticipated that latency to terminate stimulation would be a measure of the aversiveness of raphe stimulation [14]; however, this measure was not intensity dependent and was not greatly modified by 5HT or catecholamine depletion (Figs. 5 and 6). The fact that this measure was not intensity dependent suggests either aversive effects were already maximal at 30 μA or that termination latency is not a measure of aversiveness; it might, for example, be suggested that there is a decay in reinforcement during a continuous stimulus train, animals might terminate stimulation in order to obtain further reinforcement at the beginning of the next stimulus train. If, on the other hand, the stimulus does have an aversive component which is maximal at 30 μA this might explain why termination latencies are not more rapid at 70 μA and why AMPT was ineffective at this intensity. However, if the aversive component is serotonergic, depletion of 5HT following PCPA should have slowed termination latencies; this was not observed. Thus two possible conclusions from the lack of effect of stimulus intensity and drugs on termination latencies seem possible; either termination latency in this situation is not a good measure of an aversive component of raphe stimulation, or, that the aversive component is not serotonergic.

The finding that AMPT increased initiation latencies while slightly decreasing escape latencies (Fig. 5) indicates a

specificity of action on the rewarding component of raphe stimulation and rules out the possibility that the drug merely depresses some aspect of motor activity. A similar selective effect of AMPT on initiation latencies was reported by Hunt *et al.* [14] using animals with electrodes implanted into the medial forebrain bundle.

Other studies have implicated catecholamines in raphe self-stimulation behaviour. Simon *et al.* [23] reported antagonism of dorsal raphe self-stimulation following AMPT and the decline in lever pressing rates was dissociated from the decrease in activity produced by the drug established for the same dose in an earlier study. The finding that chlorpromazine antagonises raphe self-stimulation [17,18] is not decisive evidence for catecholamine mediation since the drug has 5HT receptor blocking properties [12]. Amphetamine has been reported to increase raphe self-stimulation [17,18] suggesting a catecholamine mediation since amphetamine releases catecholamines at low doses but 5HT at high doses only [4,9].

In summary it was observed that the rewarding effects of raphe stimulation could not be antagonised by four different pharmacological methods of impairing serotonergic neurotransmission. It therefore seems unlikely that raphe self-stimulation is mediated by serotonergic neurones; rather, the shuttle-box experiment suggests the rewarding effects of raphe stimulation are directly or indirectly mediated by a catecholamine system. An obvious candidate is the periventricular catecholamine system passing through the raphe nuclei. These findings are compatible with catecholamine theories of reward. While these experiments allow the possibility that 5HT neurones may mediate some of the behavioural effects of punishment, experiments using the shuttle-box paradigm failed to provide any corroboration for this view.

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