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Ambulation, Rearing and Cholinergic Systems in the Septum

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Septal lesions and systemic anticholinergic treatment have similar effects on a range of behaviours including open field ambulation and rearing. The present experiments investigated the possibility that this similarity stems from an action of the drugs on cholinergic systems in the septum (Carlton, 1969).
Systemic injections of 1.2 mg/kg of scopolamine blocked rearing and the habituation of ambulation as has been previously reported. Injections of 5 μg of scopolamine into the medial septal nucleus had no effect. Biochemical assays in uninjected animals showed that high medial septal acetylcholinesterase activty was correlated to high levels of ambulation but only in animals with low dorsolateral septal acetylcholinesterase activity. There was no relationship between septal acetylcholinesterase activity and rearing behaviour.
It was concluded that ambulation and rearing are controlled by separate systems in the septum and that neither system employs muscarinic cholinergic synapses in the medial septal nucleus.

Introduction

It has been suggested (Carlton, 1969) that some of the effects of anticholinergic drugs may be mediated via cholinergic systems in the septum and hippocampus. This idea receives support from experiments which employ intraseptal injections of anticholinergic drugs (Levitt & Buerger, 1970; Krikstone & Levitt, 1970; Hamilton & Grossman, 1969) or which investigate the interaction of systemic injections with septal lesions (Ellen, Aitken, Sims & Stahl, 1975; see also review by Bignami, 1976). However behavioural effects common to septal lesions and to systemic injections of anticholinergic drugs are not always reproduced by intraseptal injection of the drugs (Hamilton, McCleary & Grossman, 1968).

Rearing and habituation of ambulation in an open field are blocked by septal lesions (Kemble & Nagel, 1975) and by systemic administration of scopolamine (Hughes, Blampied & Stewart, 1975). Injection of procaine into the medial septal nucleus reproduces the effect of large septal lesions on ambulation but does not change rearing (Note 1). This suggests that two different systems, both passing through the septum, control ambulation and rearing respectively.

The hippocampal theta rhythm is controlled by neurones in the medial septal nucleus (Stumpf, 1965), occurs consistently during movement (Whishaw & Vanderwolf, 1973), and is sensitive to anticholinergic drugs (Vanderwolf, Kramis, Gillespie & Bland, 1975). Loss of theta rhythm in different parts of the hippocampus due to lesions in the medial septum, fornix and fimbria is accompanied by loss of acetylcholinesterase in the same parts (Rawlins, Feldon & Gray, 1979). It seems possible therefore that habituation of ambulation is controlled by ascending cholinergic systems passing through the medial septum (Lewis & Shute, 1967) and influencing hippocampal theta rhythm. However, while acetylcholinesterase would be expected to be present at cholinergic synapses, its presence is no guarantee that the systems with which it is associated are necessarily cholinergic (Silver, 1974, p. 353-4).

The first two experiments investigated the extent to which intraseptal injections of scopolamine reproduce the effects on ambulation of systemic scopolamine and of intraseptal injections of procaine. Since the effects of anticholinergics on habituation are to some extent test-dependent (File, 1976; Feigley & Hamilton, 1971) the effects of systemic Scopolamine reported in the literature were first replicated in the current apparatus. scopolamine has been reported to reduce defaecation in an exploratory situation (Mulas, Crabai & Pepeu, 1970) so this was also measured.

Experiments 1 and 2

Methods

Animals. Subjects were naive male Wistar rats weighing between 200-300gm, caged in squads of four in plastic boxes. The boxes measured 22 × 35× 16 cm

and water and food was available ad lib in the wire cage top. Lighting was via an external window. Each animal received only one session in the experimental apparatus.

Behavioural Testing. The open field consisted of a white, plastic-covered table (125 × 75 cm) marked off in 25 cm squares. It was placed in the centre of a blacked-out testing room and illuminated with a 15W lamp placed 150 cm above its centre. The experimenter sat approximately 1 metre from one corner of the table and recorded responses by pressing push buttons connected to recording equipment outside the room. Animals were brought in their home cages into the testing room in squads of four. They were placed, one at a time, on the marked off square nearest the experimenter's seat and the number of lines crossed by the animal's body, excluding the tail, and the number of times the animal reared were recorded in one minute bins for 10 minutes. The total number of faecal boli was counted at the end of the 10 minute period.

Data Analysis. The observations of ambulation and rearing were submitted to analysis of variance after application of square root and reciprocal transforms respectively to normalise the data (Zar, 1974). Effects which depended on minutes were assessed for the presence of orthogonal polyomial components (Snedecor & Cochran, 1967).

Surgery and Histology. Subjects were anaesthetised with an i.p. injection of a nembutal-chloral hydrate mixture and implanted with a 21g stainless steel outer cannula with a 27g inner obturator (Grossman, 1962). This was then attached to the skull with dental cement and four stainless steel screws. Subjects were allowed to recover for one week after the operation before testing commenced. Coordinates, with skull level between lambda and bregma were 1.00mm A to bregma, 5.5mm below the skull and on the midline.

Some weeks after testing they were anaesthetised with a lethal dose of nembutal and perfused through the heart with saline followed by formal saline. The brain was removed and kept in formal saline. Frozen sections were then taken at 50μ and all sections mounted and stained with sudan black. Any animal whose cannula tip was more than 0.5mm from the medial septal nucleus (Konig & Klippel, 1963) was excluded from the data analysis.

Injections. Systemic injections of 1.2 mg/kg scopolamine (BDH) as 1.2 mg/ml in saline were administered via 1 ml disposable syringes and 25g disposale needles. Control animals received an equivalent volume (1 ml/kg) of saline.

The systemic dose of scopolamine was chosen in relation to its effects on hippocampal theta rhythm. Below a dose of 0.9mg/kg scopolamine has only a small effect on theta rhythm. At doses of 1.0mg/kg and above it produces a consistent blockade of non-movement theta (Note 2).

Intraseptal injections of 1μl of 5 μg/μl scopolamine were administered via a Hamilton microsyringe and injection cannula. The tip of the injection cannula after insertion was level with the tip of the previously implanted outer guide cannula. For controls, the injection cannula was inserted but no fluid ejected.

The dose of intraseptal scopolamine was chosen in relation to its lack of effect on hippocampal theta rhythm and in relation to the effects of intraseptal amlobarbitone in the same task.

0.5μl of 20mg/l amylobarbitone (i.e. 0.01 μg) produces effects on ambulation equivalent to a systemic injection of 20 mg/kg of this drug. If absorption and transport of scopolamine were similar then an equivalent dose to 1.0 mg/kg i.p. (see above) would be 0.5μl of 1.0 mg/l. However, in a septal mapping experiment it was found that doses larger by a factor of 10^6 did not affect theta rhythm (Note 3). Systemic injections of barbiturate result in even distribtion through all tissues (Richards & Taylor, 1956). It seems unlikely that scopolamine would undergo concentration by a factor of 10^6 in passing from the periphery to its active site. This dose (i.e. 1μl of 5μg/μl) was therefore used as being one which should (by a factor of 10^6) affect ambulation if appropriate cholinergic synapses were present, but also being one which did not affect theta rhythm and hence might be expected *not* to affect ambulation.

Testing commenced 10 minutes after injection.

Results and Discussion

As is shown in Figure 1 injection of 1.2 mg/kg scopolamine i.p. effectively blocked habituation of ambulation (Drug × minutes linear deviation: $F_{1,306}=25.0$, $p<0.0005$; quadratic deviation: $F_{1,306}=21.5$, $p<0.0005$). It also produced a substantial reduction in rearing (Drug = 0.189 rears/minute, Control = 0.822 rears/minute, $t=4.74$, $df=34$, $p<0.0005$). These results replicate in the present apparatus effects of scopolamine previously reported in the literature. The drug also produced a significant reduction in open field defaecation (Drug = 1.12 boli/min, Control = 4.35 boli/min, $t=3.88$, $df=34$, $p<0.0005$).

Figure 1 also shows the result of injecting 1μl of 5μg/μl scopolamine into the medial septal nucleus. All animals showed substantial habituation of ambulation (minutes linear component: $F_{1,189}=51.5$, $p<0.0005$) but there was no sign of any difference between the treated and control groups (Drug × Minutes linear deviation: $F<0.0$) The treatment produced an apparent increase in rearing (Drug < 0.76 rears/minute, Control = 0.33 rears/minute) but there was considerable variability and this difference did not approach significance ($t=0.24$, $df=23$). An apparent modest reduction in defaecation (Drug

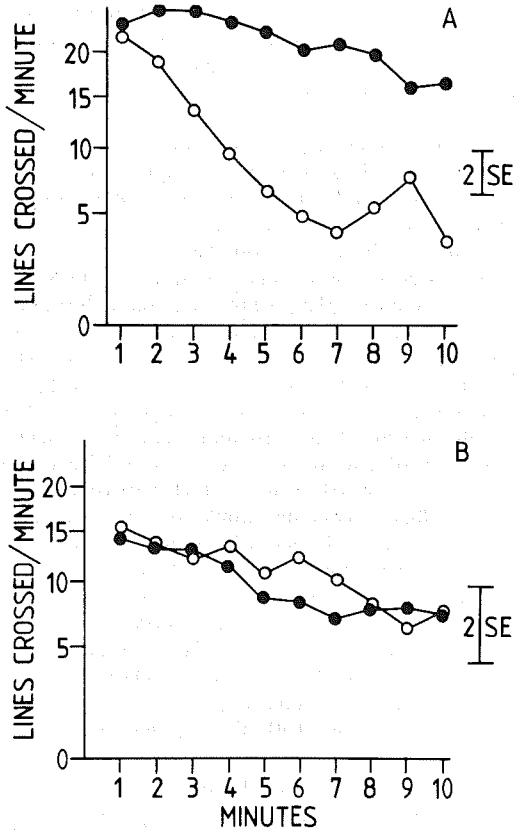

Figure 1A. Open field ambulation in rats treated with 1.2 mg/Kg i.p. Scopolamine (•) compared with saline injected controls (o). Ordinate is ambulation with a nonlinear scale resulting from a square root transformation used to normalise variance. Data were collected in one minute bins during the 10 minute session as represented by the abscissa. The bar marked 2SE represents two standard errors for a comparison *between* groups.

Figure 1. B. Open field ambulaltion in rats injected in the medial septum with 5μg of scopolamine (•) compared with sham injected controls (o).

= 0.24 boli/minute, Control = 0.34 boli/minute) also did not approach significance (t = 0.28, df = 23). Intraseptal injection of scopolamine, therefore, produced no effects on any measure.

The first experiment demonstrated that in the present testing apparatus intraperitoneal injection of scopolamine produces a large and highly significant reduction in the habituation of open field ambulation and significantly reduces rearing and defaecation. By contrast injection of 5μg of scopolamine into the dorsal aspect of the medial septal nucleus had no significant effects. Such a lack of effect has been reported previously (Kelsey & Grossman, 1969). The failure to affect rearing is not surprising as total blockade of the medial septum does not change this, however both intraseptal procaine and intraseptal amylobarbitone (0.01μg) do affect ambulation (Note 1). This suggests that the habituation of ambulation depends on non-cholinergic (or at least non-muscarinic) synapses in the medial septum.

This conclusion should be treated with some caution. The present amount and location of injection of scopolamine in the septum is without effect on hippocampal theta rhythm (Note 3). However Bennett (1975) reports that in the cat injections of scopolamine into the septum can block theta rhythm. While this could be due to diffusion to, for example, the hippocampus, an extensive mapping study of the effects of intraseptal scopolamine in the rat is required before we can conclude that no part of the critical circuitry in the medial septum is cholinergic.

As noted earlier, acetylcholinesterase containing systems run through the septum to the hippocampus. This provides us with a second, independent method of assessing the involvement in open field behavioural of septal systems which are likely to be cholinergic (whether nicotinic or muscarinic). Since there are relatively high levels of acetylcholinesterase in both the medial and lateral septum, it is possible that open field behaviour depends on an interaction between cholinergic systems in both areas. Experiment 3, therefore, investigates the correlational relationships between medial and lateral septal acetylcholinesterase levels and open field behaviour.

Experiment 3

Method

Open field testing was as for Experiments 1 and 2 but no injections were given. Immediately after its individual test, each animal was killed, and the brain was removed and placed on a petri dish embedded in carbon dioxide ice. Samples were taken from the brain by cutting it in half vertically through the midline and then scooping out medial and dorso-lateral septal samples. The medial sample was taken by scooping out from an area the maximum anterior and dorsal extent of which was bounded by the genu of the corpus callosum and the maximum ventral and posterior extent of which was bounded by the anterior commissure. The depth of the scoop was limited, as far as possible, to 1mm. The dorsal-lateral septal sample was then taken, scooping within the same anterior-posterior limits, more dorsally and to greater depth. The samples were homogenised and assayed for acetylcholinesterase activity (AChE) by the method of Ellman, Courtney, Adey & Featherstone (1961) as modified by Durkin (Note 3).

The animals were divided for analysis into approximately equal sized groups on the basis of the AChE scores. The critical value dividing the high and low medial AChE groups was 17.0 μmoles of acetylthiocholine hydrolysed per hour per mg of protein. The groups were further subdivided into high and low dorsolateral AChE groups, the critical value being 11.0 μmoles of acetylthiocholine hydrolysed per hour per mg of protein.

Results and Discussion

The relationship between ambulation and septal acetylcholinesterase activity is demonstrated in Figure 2. The only effect of acetylcholinesterase which achieved significance was the medial × dorsolateral × minutes interaction ($F9,81=2.26$, $p<0.05$) which involved a highly significant linear deviation ($F1,81=10.7$, $p<0.0025$). Examination of the slopes of the regression lines for this interaction showed that the linear deviation arose from similar habituation of ambulation in the high medial-high dorsolateral group (slope = -0.130, square root data) and the low medial-low dorsolateral group (slope = -0.150) and similar habituation in the low medial-high dorsolateral group (slope =-0.330) and high medial-low dorsolateral group (slope =-0.335); while all other slope comarisons exceed 2 standard errors (0.11).

There was a trend to greater ambulation in animals with high medial acetylcholinesterase ($F1,9=3.9$, $p<0.1$), but this appears to be due largely to animals with low dorsolateral acetylcholinesterase. In the low dorsolateral groups ambulation is greater in high-medial than low-medial animals during minutes 2-7 by amounts close to 2 standard errors. By contrast in the high dorsolateral groups high-medial animals show greater ambulation than low-medial animals by this amount only on minute 9 and possibly 10.

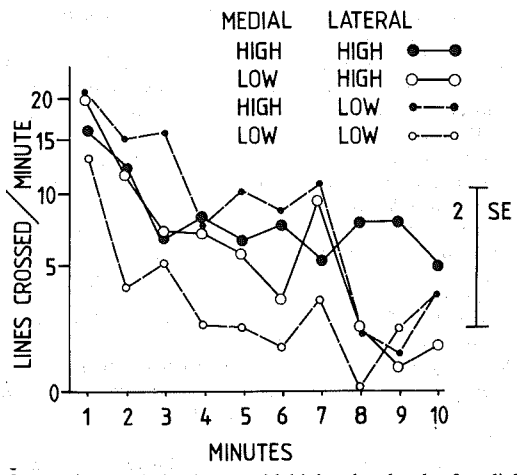

Figure 2. Ambulation in rats with high or low levels of medial septal acetylcholinesterase and high or low levels of lateral septal acetylcholinesterase.

The data on rearing were very much more variable and yielded no F ratios which approached the 10% level of significance.

Analysis of defaecation scores showed no effect of medial acetylcholinesterase and no interaction of medial and lateral acetylcholinesterase. However animals in the low lateral acetylcholinesterase groups showed no defaecation at all. This effect was significant (High medial-High lateral = 0..40 boli/minute, Low medial-High lateral = 0.23 boli/minute, Both Low lateral = 0.0 boli/minute; main effect of lateral acetylcholinesterase $F1,8=7.0$, $p<0.05$).

These findings, in apparent contrast to those with intracranial injections implicate the medial septum in the control of ambulation. Three points need to be noted here: firstly, the activity measured could be due to fibres of passage rather than a synaptic region; secondly, the results are correlational so the order of cause and effect cannot be determined; thirdly, a high level of the breakdown enzyme acetylcholinesterase could be associated, in a cholinergic pathway, either with high or with low levels of transmission. With respect to this last point the high level of ambulation associated with a high level of medial septal acetylcholinesterase can usefully be compared with the high level of defaecation associated with a high level of lateral septal acetylcholinesterase. In the former case high acetylcholinesterase is equivalent to a systemic injection of scopolamine, in the latter it is the opposite.

Inspection of Figure 2 suggests that ambulation shows a relationship with medial septal acetylcholinesterase only when lateral septal cholinesterase activity is low. As obtained here the medial sample would include both the medial septal nucleus and the dorsal part of the diagonal band nucleus however these nuclei appear to be functionally homogenous with respect to acetylcholinesterase (Srebro, Mellgren & Hallmark, 1976). The lateral sample should contain the intermediate and internal parts of the dorsal septal nucleus (Srebro et al, 1976). It might be argued that the observed result could be obtained by variation in the anterio-posterior siting of the

samples taken. Relatively anterior samples could be from the external part of the dorsal septal nucleus (which has low cholinesterase activity) in the lateral case and the anterior part of the medial septal nucleus in the medial case. Relatively posterior samples could be from the intermediate and internal parts of the dorsal septal nucleus (which has high cholinesterase activity) in the lateral case and the dorsal part of the diagonal band nucleus in the medial case (Srebro et al, 1976). The observed interaction of medial and dorsolateral activity in relation to ambulation would then be attributable to a functional differentiation between the medial septal nucleus and the diagonal band nucleus. Such an explantion totally fails to account for the very strong relationship observed between dorsolateral activity and defaecation and can therefore be discounted.

General Discussion

The present experiments suggest that there are three separate systems controlling open field ambulation, rearing and defaecation respectively. The effects of systemic injections of scopolamine imply that all three depend on muscarinic cholinergic transmission at some point. The intraseptal injections of scopolamine indicate that such transmission is not occurring in the dorsal part of the medial septal nucleus although they do not entirely rule out other parts of the medial septum. The acetylcholinesterase data point to separate systems, in the medial septum controlling ambulation and in the dorsolateral septum controlling defaecation. The septal control of rearing (Kemble & Nagel, 1975) does not appear to depend on acetylcholinesterase levels in either the medial or dorsolateral septum. These data provide only partial support for Carlton's (1969) suggestion that the effects of anticholinergic drugs may be mediated via cholinergic systems in the septum and hippocampus. Although they provide no counterinstances to the hypothesis they do show that a number of separate systems are involved and they suggest that the hippocampus rather than the septum may be the site of the muscarinic synapses involved in ambulation, rearing and defaecation.

Reference Notes

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