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Lesions in the septal nuclei of the rat raise mean systemic arterial pressure and prevent the development of sound-withdrawal hypertension

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Midline lesions in the hippocampal commisure involving the bed nucleus (I) in the forebrains of rats, and lesions which destroyed the medial septal nuclei (III), caused mean systemic arterial pressure to rise by 30–40 mm Hg in female rats of an inbred hypertensive Wistar strain. Lesions involving the anteromedial portions of both lateral septal nuclei (II) raised mean arterial pressure by 10–20 mm Hg. After pentolinium, mean arterial pressure remained raised, by approximately 20 mm Hg, in the 7th postoperative week after lesions I, II and III. The mean systemic arterial pressures of intact and sham-lesioned rats rose by 30–40 mm Hg when exposed to reduction of sound level from 65–85 to 35–45 db for 5–6 weeks: this treatment did not influence the mean arterial pressures of rats with lesions I, II or III. The pressor effects of (−)-noradrenaline (0·25 μg), tyramine (25 μg), angiotensin (10 ng) and vasopressin (0·2 mU) did not differ in ganglion blocked sham-lesioned and lesioned animals. Organ/body weight ratios did not increase in the lesioned animals; overall, both the pituitary and adrenal weights were just significantly ($P > 0{\cdot}05$) reduced. Lesions II and III caused a significant reduction in kidney weight and lesion II a significant rise in the index of thyroid activity.

Female rats of an inbred Wistar strain rapidly developed hypertension when transferred from the normal rat room (NRR) to a semi-anechoic sound-proofed room (SPR) in the tenth or eleventh week of life. This hypertension was evident after two weeks in the SPR, reached peak value at four weeks and then stabilized by the sixth week approximately 10 mm Hg below the peak value and 30 mm Hg above the mean value found for control animals which had remained in the NRR. No hypertension developed in the SPR when sounds from the NRR were relayed, unaltered, into the SPR (Marwood & Lockett, 1973).

Adrenalectomized animals developed this sound-withdrawal hypertension (SWH) only when supplied with glucocorticoid. Hypophysectomized animals, in receipt of glucocorticoid did not develop hypertension. It was, therefore, considered probable that glucocorticoid played a permissive and the hypophysis an essential role in the genesis of SWH (Marwood, Ilett & Lockett, 1973).

The generation of hypertension by withdrawal of sound must be initiated in the brain through connections of the auditory pathway. One end result of the changed input to these connections is most probably a change in hypophysial secretion since one or more hypophysial hormones have been shown to play an essential role in the development of SWH. Since interplay between the reticular activating system, the

limbic system and the hypothalamus can so greatly influence hypophysial secretion (Mason, 1958, 1959) an attempt has been made to discover whether interruption of connections between the limbic system and the hypothalamus influence mean arterial pressure and/or prevent the development of SWH. Fibres travelling from the limbic system to the hypothalamus arise in the prosubiculum of the hippocampus; most of these fibres travel in the ventral portion of the fimbria to reach the arcuate nucleus of the hypothalamus via the medial corticohypothalamic tracts. Some fibres do, however, project from the hippocampus to the septal nuclei (including the bed nucleus of the hippocampal commissure) via the pre- and post commissural fornix (Raisman, 1970; Stumpf, 1970).

The purpose of the present work has, therefore, been to discover the effect of lesions placed in the septal nuclei of the rat forebrain on mean systemic arterial pressure and/or on ability to develop SWH. Effects of these lesions on cardiovascular reactivity to certain pressor agents have also been tested.

METHODS

Female rats (136), initially weighing 165–180 g, were used. All were of the same inbred Wistar strain used by Marwood & Lockett (1973), and Marwood & others (1973). This strain has gradually developed spontaneous hypertension in the course of the last nine years (Marwood & Lockett, 1973b).

Brain lesions. Lesions were placed in the brains of anaesthetized rats (sodium methohexital, 45 mg kg^{-1}, i.p.) by the method of Krieg (1946) using stereotaxic co-ordinates adapted from those of de Groot (1959) to our strain of rats used. The Krieg stereotaxic instrument (model 51200, C. H. Stoetling & Co., U.S.A.) zeroed on the interaural line, was used to mark the position on the skull through which a 0·5mm diameter hole was subsequently drilled to allow passage of the electrode. This electrode was positioned in the brain at an angle of 15° from the vertical to avoid injury to the superior sagittal sinus. A current of 2 mA was passed for 10 s before the electrode was removed and the skin incisions were sutured. The actual position of each lesion was ascertained histologically, post mortem. Rats with lesions in the hippocampal commissure (Fig. 1) with involvement of the midline bed nucleus con-

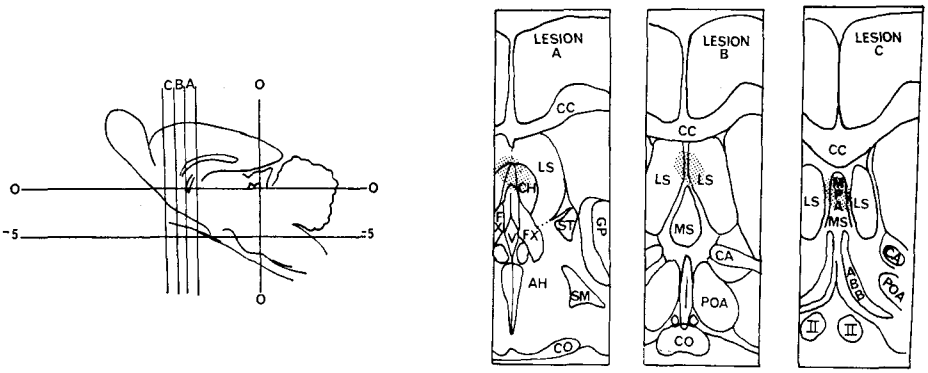

FIG. 1. The sites of lesions made in the forebrains of rats are indicated by diagrams adapted from de Groot, J. (1959), *Transcripts of the Royal Netherlands Academy of Science*, **52**, 1. The shaded areas show the positions of lesions A, B and C. Key:—ABB, Diagonal Band of Broca: AH, Ante hypothalamus: CA, Ante commissure: CC, Corpus callosum: CH, Hippocampal commissure: CO, Optic chiasma: FX, Fornix: GP, Globus pallidum: LS, Lateral septal nucleus: MPA, Area parolfactoria medialis: MS, Media septal nucleus: POA, Preoptic area: SM, Stria medullaris: ST, stria terminalis: V, Ventricle.

stituted group I. Rats with lesions involving the anteromedial portions of the lateral septal nuclei, and those with lesions in the medial septal nucleus constituted groups II and III, respectively. Rats with lesions just lateral to these targets constituted the sham-operated animals.

Mean systemic arterial pressure was recorded under pentobarbitone sodium anaesthesia (45 mg kg^{-1}, i.p.) from a cannulated carotid artery by means of an E &M force displacement transducer coupled to a Nesco pen recorder. Drugs were administered intravenously in 0·1 ml 0·9% NaCl through a polythene cannula in an external jugular vein. Block to transmission in autonomic ganglia was induced with pentolinium ions (1 mg, i.v., 5 mg, s.c.) before the pressor responses to (−)-noradrenaline, 0·25 μg; tyramine, 25 μg; angiotensin II-val^5-amide 10 ng and vasopressin 0·2 mU were recorded. These selected doses had been shown to produce submaximal effects in the weight range of the female rats used.

Tests for ability to develop sound-withdrawal hypertension were made by transference of animals from the stock room for female rats (NRR) to the semi-anechoic sound-proofed room (SPR) on or after the 8th post-lesion day for a period of 6 weeks. The sound levels in the NRR ranged from 65–85 db and in the SPR from 35–45 db (Marwood & Lockett, 1973a).

Postmortem examinations were made at the termination of each experiment. The adrenal glands, spleen, kidneys, thymus gland and heart were excised, cleaned of adherent fat and connective tissue, and weighed. Buffered formol saline was used to fix the brain and thyroid glands for histological examination.

Histological techniques. Fixed tissues were dehydrated and then infiltrated with paraplast in a Shandon Elliott automatic tissue processor and were embedded in paraplast. Sections, cut at 7 μm on a Reichert rotary microtome, were stained either with haematoxylin and chromatrope 2R (thyroid) or with Weil's stain (brain) and were mounted in Depex medium. Serial sections of each brain were scanned to determine the site of the lesion.

Index of thyroid activity. Two hundred consecutive alveoli were inspected by high powered visual microscopy in a section across each gland, using a moving stage, to determine the ratio of the number of alveoli surrounded by cubical cells to the number surrounded by flattened cells. Each gland afforded a mean of three estimates.

RESULTS

The effect of lesions placed in the septal nuclei of the rat forebrain on mean systemic arterial pressure, and on cardiovascular responses to selected pressor agents

Data shown in Table 1 demonstrate that the mean systemic arterial pressures of sham-operated animals stored for 7 weeks in the NRR did not differ significantly from those of intact animals. In contrast, the mean pressures found for rats with midline lesions in the hippocampal commissure involving the bed nucleus (group I), and for those with lesions in the medial septal nucleus (group III), were highly significantly raised above control levels. The hypertensions induced by these two lesions did not differ significantly and amounted to a rise in pressure of 30 to 40 mm Hg. Lesions involving the anteromedial portions of the two lateral septal nuclei (group II) produced a lesser degree of hypertension ($P < 0·05$) which was also highly significant ($P < 0·01$). This difference in the mean arterial pressures of groups I and III and group II rats disappeared after induction of blockade of transmission in the autonomic ganglia: the mean pressures recorded for lesioned animals were, however, very

Table 1. *Effect of forebrain lesions on mean systemic arterial pressure in rats of an inbred Wistar strain stored for 5 weeks post-operatively in the normal rat room (70–80 db) or in the sound-proofed room (35–45 db) for 6 weeks.*

Site of lesion (if any)	Body weight g	Mean systemic pressure in mm Hg: Before block	After ganglion block
Sound level 65–85 db for 7 weeks post-operatively			
Sham-operated	215 ± 7·3(8)	150·9 ± 1·75(8)	73·6 ± 1·34(8)
Unoperated	216 ± 4·7(8)	148·8 ± 2·14(8)	75·2 ± 2·26(8)
Lesioned in:—			
I Hippocampal commissure	208 ± 5·2(5)	189·3 ± 2·29(5)**	94·0 ± 3·40(5)**
II Anteromedial portions of lateral septal nuclei ..	210 ± 5·0(11)	171·2 ± 1·74(11)**	91·6 ± 3·32(11)**
III Medial septal nucleus ..	212 ± 6·1(7)	183·1 ± 2·23(7)**	93·6 ± 2·97(7)**
Sound level 35–45 db for 6 weeks			
Sham-operated	212 ± 5·2(8)	186·2 ± 4·10(8)	98·1 ± 2·36(8)
Unoperated	116 ± 4·4(12)	180·6 ± 3·31(12)	94·2 ± 2·74(12)
Lesioned in:—			
I Hippocampal commissure	221 ± 10·2(5)	189·6 ± 3·4(5)	—
II Anteromedial portions of lateral septal nuclei ..	213 ± 4·9(16)	159·2 ± 4·3(16)**	89·2 ± 2·79(8)
III Medial septal nucleus ..	213 ± 3·8(18)	186·5 ± 2·4(18)	95·2 ± 3·15(8)

The values shown are means ± their standard errors followed by the numbers of animals contributing to each mean in parentheses. The significance of differences between the mean values for lesioned and for sham operated animals has been examined by *t*-test and is indicated by asterisks: one, $P < 0.05$; two, $P < 0.01$.

Table 2. *Effect of forebrain lesions on cardiovascular reactivity to pressor agents in rats of an inbred Wistar strain stored for 5 weeks postoperatively in the normal rat room (70–80 db) or in the sound-proofed room (35–45 db) for 6 weeks.*

Site of lesion (if any)	Body weight g	mm rise in systemic pressure caused by various pressor agents: 0·25 µg (−)-noradrenaline	tyramine 25 µg	angiotensin 10 ng	vasopressin 0·2 mU
Sound level 65–85 db for 7 weeks post-operatively					
Sham-operated ..	215 ± 7·3(8)	82·4 ± 2·80(7)	64·0 ± 3·25(7)	67·7 ± 4·18(7)	51·4 ± 5·78(7)
Unoperated	216 ± 4·7(8)	78·8 ± 3·31(8)	62·6 ± 5·24(8)	64·2 ± 3·97(8)	45·6 ± 4·26(12)
Lesioned in:—					
III Hippocampal commissure ..	208 ± 5·2(5)	84·0 ± 5·18(5)	71·4 ± 3·66(5)	67·3 ± 4·40(5)	50·2 ± 2·28(5)
II Anteromedial portions of lateral septal nuclei ..	210 ± 5·0(11)	83·3 ± 0·98(11)	68·3 ± 4·56(11)	67·8 ± 2·73(11)	49·1 ± 4·07(11)
III Medial septal nucleus	212 ± 6·1(7)	84·1 ± 3·26(7)	69·7 ± 3·74(7)	69·0 ± 3·36(7)	5·09 ± 3·92(7)
Sound level 35–45 db for 6 weeks					
Sham-operated ..	212 ± 5·2(8)	76·4 ± 6·1(7)	61·8 ± 3·44(7)	72·2 ± 3·78(7)	46·4 ± 4·31(7)
Unoperated	116 ± 4·4(12)	74·8 ± 5·7(12)	64·3 ± 2·89(12)	74·6 ± 3·13(12)	47·3 ± 4·09(12)
Lesioned in:—					
I Hippocampal commissure ..	—	—	—	—	—
II Anteromedial portions of lateral septal nuclei ..	213 ± 4·9(16)	81·6 ± 4·88(8)	66·4 ± 4·07(8)	66·8 ± 4·54(8)	42·7 ± 5·15(6)
III Medial septal nucleus	213 ± 3·8(18)	77·3 ± 5·29(8)	70·6 ± 3·98(8)	68·2 ± 5·05(8)	45·3 ± 4·78(8)

The values shown are means ± their standard errors followed by the numbers of animals contributing to each mean in parenthesis. The significance of differences between the mean values for lesioned and for sham operated animals has been examined by *t*-test and is indicated by asterisks: one, $P < 0.05$; two, $P < 0.01$.

significantly greater than those found in sham-operated ganglion-blocked animals.

No changes in the pressor effects of (—)-noradrenaline, tyramine, angiotensin II-val^5-amide or vasopressin were found to have resulted from sham-operations or from the production of lesions I, II and III in animals examined in the 7th post-operative week under ganglionic-blockade (Table 2).

The effect of lesions in the septal nucleus of the rat forebrain on ability to develop sound-withdrawal hypertension (SWH)

Whereas sham-operated animals housed for 6 weeks in the SPR developed a degree of SWH not differing from that produced in normal rats, none of the lesioned animals developed SWH. The hypertensive states of lesioned animals stored in the SPR did not differ from those of lesioned animals stored in the NRR before or after ganglionic blockade.

No significant changes in the pressor effects of (—)-noradrenaline, tyramine, angiotensin II-val^5-amide or vasopressin resulted from 6 weeks of sound-withdrawal in normal, sham-operated or lesioned animals.

The influence of lesions in the septal nuclei of the rat forebrain on organ weights and on the index of thyroid activity

The weights of the hearts, spleens and thymus glands of the lesioned animals did *not* differ from those of sham-operated animals. The combined weights of the adrenals were similar in all lesion groups and tended throughout to be less than the adrenal weights found for sham-operated animals. This reduction in adrenal weight reached significance ($P < 0{\cdot}05$) only in group II of the lesioned animals. A small but just significant ($P < 0{\cdot}05$) reduction in kidney weight was observed in groups II and III. The weights of the pituitary glands of all groups of lesioned animals tended to be less than those of sham-operated animals; this difference, significant in no one group of lesioned animals, was significant ($P = 0{\cdot}05$) overall. A significant rise in the index of thyroid activity resulted from lesions in the hippocampal commissure: the smaller increase in this index observed in group II of the lesioned animals did not reach significance.

DISCUSSION

Lesions I and III, made in the tenth to eleventh week of the lives of these hypertensive female Wistar rats produced an hypertension (LH) in animals housed in the NRR (where the sound level ranged from 65–85 db), which did not differ in magnitude from the hypertension (SWH) developed by normal and by sham-lesioned rats exposed for 6 weeks to reduction in the sound level (to 35–45 db) by transfer to the SPR. Moreover, the existence of LH precluded the development of SWH.

In the sixth week of sound-withdrawal (in confirmation of Marwood & Lockett, 1973a) and in the seventh week post-lesion, the pressor effects of noradrenaline, tyramine, angiotensin and vasopressin were unchanged, despite the hypertensive state of the animals. This characteristic of LH and of SWH is in contrast with observations made on rats with other forms of experimentally induced hypertension. Increase in the pressor effects of noradrenaline (Hinke, 1965; Vacek, 1970; Beilin & Ziakas, 1972), angiotensin (Baum & Shropshire, 1967), tyramine (Finch, 1971) and vasopressin (Hinke, 1965) are found in ts made hypertensive with DOCA and salt.

Femoral arterial strips from rats with renal hypertension show a lowered contraction threshold to noradrenaline (Bandick & Sparks, 1970) and pressor responses shown by these rats have been variously reported as increased to tyramine alone (Ferrari, Maragno & others, 1968), to noradrenaline alone (McGregor & Smirk, 1968) or to both noradrenaline and tyramine (Finch, 1971). The patterns of the cardiovascular responses to pressor drugs found in rats with genetic hypertension still needs clarification. Increased pressor responses to noradrenaline (Laverty, 1961; Trajkov, Glavas & others, 1971; McGregor & Smirk, 1968) have been reported, but have not always been demonstrable (Clineschmidt, Geller & others, 1970; Hallback, Lundgren & Weiss, 1971). Increase in the pressor effect of angiotensin has also been observed in these animals (McGregor & Smirk, 1968; Shibayama, Mizogami & Sokabe, 1971; Trajkov & others, 1971).

The absence of cardiac hypertrophy in response to LH and SWH was unexpected. The overall tendency to reduction in adrenal weight seen in the lesioned animals was unaccompanied by any significant change in the weights of the spleens or thymus glands and hence does not indicate any marked increase or decrease in the rates of secretion of ACTH and/or of glucocorticoid (Guillemin, Clayton & others, 1957; Lipscomb & Nelson, 1960). The rise in the thyroid index of activity observed in rats with lesion I and a tendency to 'high normal' values for this index in rats with lesion II may relate to observations made by Lupulescu, Nicolescu & others (1962) and by Shizume, Matsuzaki & others (1962). The former workers reported an increase in thyroid activity after lesions in the septal area in rats, the latter an increase in the release of thyrotropic hormone following stimulation of the hippocampus in dogs.

The smaller degree of hypertension resulting from lesion II as compared to lesions I and II, accompanied, as in lesions I and III, by normal cardiovascular reactivity and inability to develop SWH, remains unexplained, as does the reduction in kidney weight which resulted from lesion II and lesion III.

Overall, it has been demonstrated that lesion I, II and III in septal nuclei of the rat forebrain prevent the development of SWH. SWH and LH resemble one another in magnitude and in their insignificant influence on cardiovascular reactivity to pressor agents. It is possible that these lesions interrupted inhibitory influences of the limbic system on the setting of mean systemic arterial pressure. Much more work is needed to establish or disprove the identity of LH and SWH.

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