Physiology and Behavior. Vol. 4, pp. 365–370. Pergamon Press, 1969. Printed in Great Britain

Effects of Intracerebral Implantation of Corticosteroids on Extinction of an Avoidance Response in Rats

Tj. B. VAN WIMERSMA GREIDANUS AND D. DE WIED

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands

(Received 7 January 1969)

VAN WIMERSMA GREIDANUS, Tj. B. AND D. DE WIED. *Effects of intracerebral implantation of corticosteroids on extinction of an avoidance response in rats.* PHYSIOL. BEHAV. 4 (3) 365–370, 1969.—Intracerebral implantation of dexamethasone phosphate facilitated the rate of extinction of a pole jumping avoidance response when implanted into various areas in the median and posterior thalamus and in the ventriculus lateralis. Corticosterone, the rat's natural corticosteroid, also facilitated extinction of the avoidance response, but mainly if implanted in or near the nucleus parafascicularis. Implantations of either dexamethasone phosphate or corticosterone in other areas like hippocampus, nucleus septi lateralis, nucleus caudatus putamen, nucleus interstitialis striae terminalis and nucleus ventralis thalami did not result in a modification of the extinction rate.

Avoidance conditioning Extinction Intracerebral implantation Dexamethasone phosphate Corticosterone

SUBCUTANEOUSLY injected corticosteroids have been shown to facilitate extinction of a conditioned avoidance response (CAR) in rats [2, 21]. In order to determine the site of action of corticosteroids in facilitating the rate of extinction of an avoidance response a study was designed in which these steroids were placed into several areas of the CNS of the conscious rat during extinction sessions of a pole jumping avoidance response.

MATERIAL AND METHODS

Male rats of an inbred Wistar strain were used, weighing 110–130 gram. Avoidance conditioning was studied in a pole jumping test as described earlier [20]. The conditioned stimulus (CS) was a light produced by a 40 W bulb placed on top of the box. The CS was presented 5 sec prior to the unconditioned stimulus (US) of shock, delivered through the grid floor of the box if the rat had not avoided by jumping into the pole. The CS was terminated as soon as the animal jumped into the pole.

Ten acquisition trials were given each day for 4 consecutive days, with a variable intertrial onset interval, averaging 60 sec. The following day extinction trials were run; the CS was terminated at the end of the 5 sec period if no response had occurred and shock was not applied anymore. Rats which made 8 or more positive responses out of ten extinction trials were used for further experimentations.

To direct implantation of a steroid into various parts of the brain a stainless steel plate equipped with 12 holes was used. Tubes, with a dia. of 0.80 mm, of the same material, protruding 2.50 mm from the lower side of the plate, were attached to the holes in the plate. This plate was fixed to the rat skull with dental cement [see for details 13]. Through the tubes needles of different length containing crystalline steroid at the tip could be directed into the brain. The operated animals were allowed to recover from the operation while placed in individual cages. Rats in which the plate was not well fixed, were discarded from further participation in the experiment.

After reaching their pre-operative body weight, which usually took 3–8 days, animals were reconditioned. The intertrial onset interval was reduced from 60 to an average of 40 sec. After reconditioning, which generally took 2–4 sessions of ten trials, extinction trials were run.

When at least 8 positive responses out of ten extinction trials were scored, an empty needle was implanted into the brain of the rat through one of the twelve holes in the plate. Four hours after insertion, the empty needle was removed and the effect of this sham implantation was studied in a second extinction session.

The difference between the scores of these two extinction sessions was expressed as a percentage of the score of the first extinction session. This value was used as an index of the rate of extinction. The next day acquisition trials were performed again. Reconditioning was continued for 2–4 days. Thereafter 10 extinction trials were run. If the animal made 8 or more positive responses out of these extinction trials, the same needle was placed into the same hole as in sham implantation, but this time the needle was filled with a crystalline steroid. Four hr later ten extinction trials were run again. The difference between the rate of extinction

TABLE 1

EFFECT OF TWO SUBSEQUENT INSERTIONS OF AN EMPTY NEEDLE ON THE RATE OF EXTINCTION OF A POLE JUMPING AVOIDANCE RESPONSE

Number of pos. responses in 1st extinction session	Number of pos. responses in 2nd extinction session 4 hr after 1st insertion	First extinction index	Number of pos. responses in 1st extinction session after reconditioning	Number of pos. responses in 2nd extinction session 4 hr after 2nd insertion	Second extinction index	Difference between 1st and 2nd extinction index	Localization of needle tip in the brain
10	7	30%	10	6	40%	10%	nucleus parafascicularis
8	7	12,5%	10	9	10%	2,5%	,,
10	6	40%	9	6	33%	7%	,,
10	10	0	8	7	12,5%	12,5%	,,
10	9	10%	10	10	0	10%	,,
8	9	−12,5%	10	10	0	12,5%	nucleus medialis thalami pars medialis
8	7	12,5%	9	7	22%	9,5%	fasciculus medialis prosencephali
10	10	0	10	8	20%	20%	tractus septo-hypothalamicus
10	9	10%	10	10	0	10%	nucleus caudatus putamen
10	7	30%	9	8	11%	19%	nucleus lateralis thalami
9	6	33%	8	6	25%	8%	nucleus reticularis thalami

following implantation of the empty needle and that following implantation of the needle with steroid at the tip was taken as an index of the effect of the steroid on the rate of extinction of the CAR.

To investigate the possibility of an effect on extinction of a second insertion of the needle at the same place, control experiments were done. Both insertions were carried out with an empty needle. Results of the control experiments (Table 1) indicated that the difference between the rate of extinction following two subsequent implantations of an empty needle never exceeded the 20 per cent level. Accordingly it was decided that steroid implantation in the brain had a facilitating effect on extinction of the CAR if the rate of extinction following steroid implantation minus the extinction rate following insertion of the empty needle was 30 per cent or more.

After the experiment was completed the places which had been reached by the needle tip were located by staining with Evans Blue. The animals were then sacrificed and the brains were removed. After fixation, the places of the Evans Blue stains were identified, using an atlas for the rat brain as a reference [14].

The steroids used were dexamethasone phosphate and corticosterone. Approximately 10 μg of dexamethasone phosphate was applicated via the tip of the needle. Almost all of it disappeared during the 4 hr of implantation. The needle tip could contain approximately 20 μg of corticosterone of which about 7 μg disappeared during the time of implantation.

RESULTS

(*a*). *Control Experiments* (7 *Animals*)

The control experiments showed that the second insertion itself on the same place in the brain had no effect on extinction. The control experiments concerned the nucleus medialis thalami, nucleus lateralis thalami, nucleus reticularis thalami, nucleus caudatus putamen, tractus septohypothalamicus and nucleus parafascicularis (5x). The effect of two subsequent insertions of an empty needle is shown in Table 1.

(*b*). *Implantation of Dexamethasone Phosphate* (31 *Animals*)

Results of 39 implantations with this steroid are depicted in three sagittal diagrams of the ratbrain (Figs. 1, 2 and 3). It can be seen that the application of dexamethasone around the 580 μ sagittal plane facilitated extinction of the CAR at various places. Of the total of 13 areas 8 were found to facilitate extinction of the CAR (Fig. 1). These were the cerebrospinal fluid (2x), nucleus parafascicularis, nucleus subparafascicularis, nucleus periventricularis, nucleus anterior medialis thalami, nucleus medialis thalami pars medialis and the nucleus posteromedianus thalami. The fasciculus retroflexus, lemniscus medialis and decussatio supramamillaris appeared ineffective.

Around the 1160 μ sagittal plane 3 out of 15 implantations turned out to be effective: the liquor of the ventriculus lateralis (2x) and the nucleus parafascicularis. No effect on the extinction was found after implantation of dexamethasone in the nucleus anterior ventralis thalami, nucleus

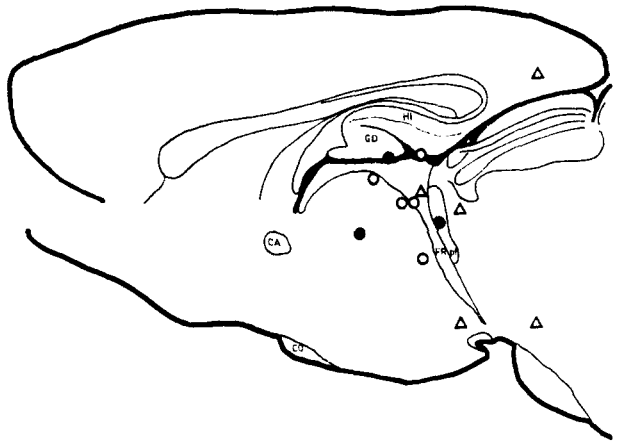

FIG. 1. Reconstruction of 13 dexamethasone phosphate implantations between the sagittal zero plane and the 740 μ lateral section superimposed on a drawing representing section L 580 μ from König and Klippel.
CA: commissura anterior. CO: chiasma opticum. FR: fasciculus retroflexus. GD: gyrus dentatus. pf: nucleus parafascicularis. ●: difference between extinction rate after sham implantation and after steroid implantation > 75%. O: id. < 75% and > 30%. △: id. < 30%.

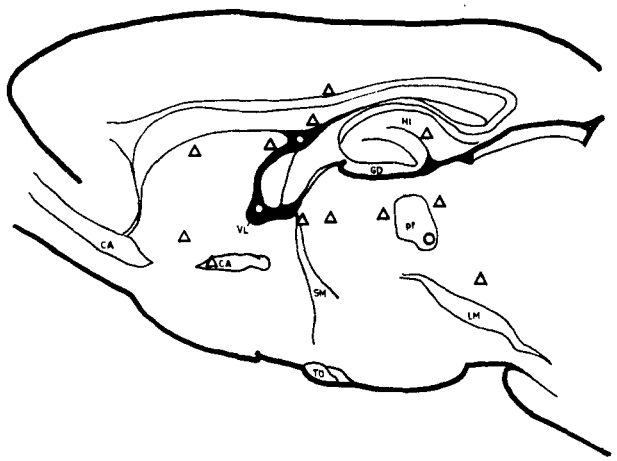

FIG. 2. Reconstruction of 15 dexamethasone phosphate implantations between the 940 μ and 1490 μ lateral section superimposed on a drawing representing section L 1160 μ from König and Klippel
CA: commissura anterior. GD: gyrus dentatus. HI: hippocampus. LM: lemniscus medialis. pf: nucleus parafascicularis. SM: stria medullaris thalami. TO: tractus opticus. VL: ventriculus lateralis. O: difference between extinction rate after sham implantation and after steroid implantation < 75% and > 30%. △: id. < 30%.

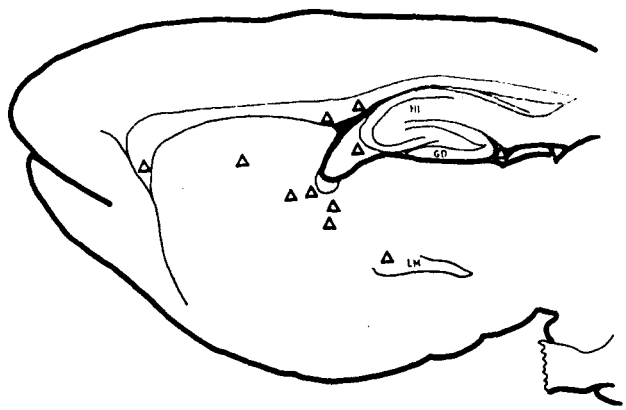

FIG. 3. Reconstruction of 11 dexamethasone phosphate implantations more lateral than the 1490 μ section superimposed on a drawing representing section L 2250 μ from König and Klippel. For explanation of symbols and abbreviations see Figs. 1 and 2.

medialis thalami pars lateralis, nucleus septi lateralis, nucleus accumbens, nucleus interstitialis striae terminalis, nucleus caudatus putamen, hippocampus and formatio reticularis.

No sensitive structures were encountered in the more laterally located 2250 μ plane: i.a. nucleus ventralis thalami, nucleus caudatus putamen, globus pallidus and nucleus reticularis thalami. The facilitating effect of dexamethasone implantation on the extinction of CAR is shown in Table 2.

(*c*). *Implantation of Corticosterone* (22 *Animals*)

Results of 26 implantations with corticosterone have been plotted on two sagittal brain diagrams (Figs. 4 and 5). Five effective sites for this steroid were found around the 580 μ sagittal plane (nucleus parafascicularis (3x), fasciculus retroflexus and cerebrospinal fluid) and only one at the more lateral level (just posterior of the nucleus parafascicularis). Ineffective implantations concerned the nucleus medialis thalami, nucleus ventralis thalami, nucleus septi lateralis, nucleus posterior hypothalami, nucleus interstitialis striae terminalis, hippocampus, fasciculus retroflexus and substantia nigra zona reticulata. The facilitating effect of corticosterone implantation on the extinction of CAR is shown in Table 3.

DISCUSSION

The present data show that implantation of dexamethasone phosphate or corticosterone in the thalamic parafascicular area facilitates extinction of a pole jumping avoidance response. The fact that dexamethasone phosphate implantations

TABLE 2

THE FACILITATING EFFECT OF DEXAMETHASONE PHOSPHATE IMPLANTATION ON THE RATE OF EXTINCTION OF A POLE JUMPING AVOIDANCE RESPONSE

Number of pos. responses in 1st extinction session	Number of pos. responses in 2nd extinction session 4 hr after insertion of empty needle	Number of pos. responses in 1st extinction session after reconditioning	Number of pos. responses in 2nd extinction session 4 hr after dexamethasone implantation	Extinction rate following steroid impl. minus extinction rate following sham implantation	Localization of needle tip in the brain
10	8	10	0	80%	nucleus anterior medialis thalami
9	7	10	3	48%	nucleus parafascicularis
10	9	10	0	90%	,, ,,
9	9	8	1	87%	cerebrospinal fluid
9	6	8	2	42%	nucleus posteromedianus thalami
10	7	10	1	60%	liquor ventriculus lateralis
10	7	9	2	48%	nucleus subparafascicularis
10	10	10	4	60%	nucleus periventricularis
9	9	10	3	70%	cerebrospinal fluid
9	9	9	4	55%	nucleus medialis thalami pars medialis
10	9	10	4	50%	liquor ventriculus lateralis

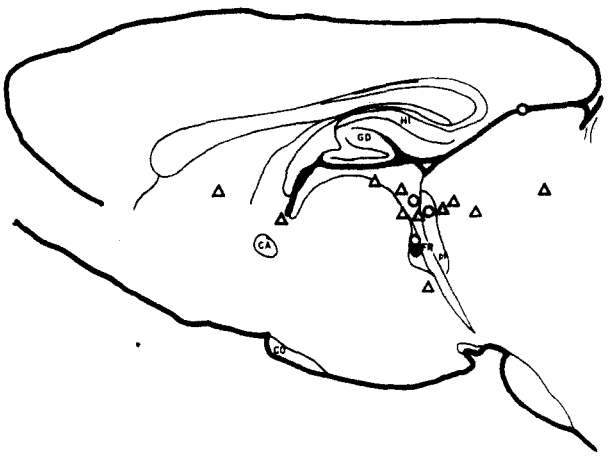

FIG. 4. Reconstruction of 16 corticosterone implantations between the sagittal zero plane and the 740 μ lateral section superimposed on a drawing representing section L 580 μ from König and Klippel. For explanation of symbols and abbreviations see Figs. 1 and 2.

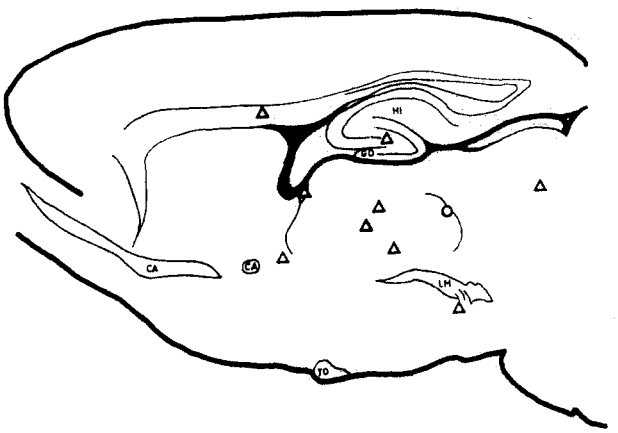

FIG. 5. Reconstruction of 10 corticosterone implantations more lateral than the 740 μ section superimposed on a drawing representing section L 1490 μ from König and Klippel. For explanation of symbols and abbreviations see Figs. 1 and 2.

TABLE 3

THE FACILITATING EFFECT OF CORTICOSTERONE IMPLANTATION ON THE RATE OF EXTINCTION OF A POLE JUMPING AVOIDANCE RESPONSE

Number of pos. responses in 1st extinction session	Number of pos. responses in 2nd extinction session 4 hr after insertion of empty needle	Number of pos. responses in 1st extinction session after reconditioning	Number of pos. responses in 2nd extinction session after corticosterone implantation	Extinction rate following steroid impl. minus extinction rate following sham implantation	Localization of needle tip in the brain
10	7	10	1	60%	nucleus parafascicularis
10	10	9	2	78%	,, ,,
10	10	9	6	33%	,, ,,
9	6	8	2	42%	just posterior of nucleus parafascicularis
10	10	10	6	40%	cerebrospinal fluid
10	10	10	7	30%	fasciculus retroflexus

had an effect in a larger area of the brain than corticosterone implantations, might be explained by the fact that the synthetic glucocorticoid is more potent than corticosterone and that it is more soluble than the rat's natural corticosteroid. Consequently a greater diffusion of dexamethasone out of the tip of the implanted needle can be expected and moreover a greater spreading through the brain within the 4 hr of implantation between the two extinction sessions. Besides, the fact that dexamethasone causes a rapid extinction of the CAR when implanted into the lateral ventricle suggests that it can reach the site of action via the cerebrospinal fluid. In support of this hypothesis is the observation that the more the sagittal zero plane was reached the more effective places were found. This observation indicates that most of the effective places were located around the third ventricle. It is however also possible that some of the steroid was diffused from the brain, entering the general circulation and in turn reaches the brain again, since small amounts of dexamethasone (2.5 μg) injected subcutaneously already facilitate extinction of the CAR in a similar situation (unpublished data).

The results presented here indicate that glucocorticosteroids exhibit their effect on conditioned avoidance behavior by a direct action on corticosteroid sensitive structures in the brain. Since ACTH and related peptides have an opposite effect i.e. inhibit the rate of extinction of a shuttlebox avoidance response [20, 21], the influence of glucocorticosteroids might be explained by the well known inhibiting action of these steroids on pituitary ACTH-release [15]. However, corticosteroid induced facilitation of extinction also occurs in hypophysectomized rats [21]. These results, together with those reported in the present study strongly suggest that glucocorticosteroids affect extinction of an avoidance response independent of pituitary ACTH.

The present experiments reveal that the nucleus parafascicularis is an important structure involved in the maintenance of an avoidance response, since implantation of this area with corticosteroids invariably led to extinction of the avoidance response. This area has been implicated before as related with extinction of avoidance behavior. Lesions destroying portions of the diffuse thalamic nuclei profoundly disturb the CAR. Thompson stated that the fact that the system of thalamic nuclei, which mediates the electrographic arousal response, also assists in mediating CAR is not coincidental [18]. Medial thalamic damage tends to destroy a pre-operatively established CAR [19]. Rapid extinction of a conditioned avoidance response took place in rats bearing cortisol implants in the mesencephalic reticular formation [1]. Other studies have demonstrated the role of the nucleus parafascicularis in maintaining avoidance responding [6, 8, 10, 11], and its possible role in the emotional reactions to painful stimuli [9]. Cardo reported a significant improvement in conditioning after stimulation of the parafascicular nucleus during acquisition of a CAR [7]. Experiments done in this laboratory have shown that bilateral lesions in the mid-caudal thalamus, destroying the parafascicular nuclei, facilitate the extinction of CAR [3, 4, 5]. This indicates that the corticosteroids inhibit the activity of these nuclei since their effect is similar to that of destruction of this area which leads to facilitation of extinction of the CAR.

In which way these steroids affect the function of the parafascicular nuclei remains, however, to be clarified. In this respect it is worth noting that related steroids have been shown to cause an increase of evoked potentials in the hypothalamus and the midbrain [12], to alter the firing rate of diencephalic units in cats [16] and the excitability of the CNS in rats [17, 22].

REFERENCES

1. Bohus, B. Pituitary ACTH release and avoidance behavior of rats with cortisol inplants in mesencephalic reticular formation and median eminence. *Neuroendocrinology*, **3**: 355–365, 1968.
2. Bohus, B. and K. Lissak. Adrenocortical hormones and avoidance behavior of rats. *Int. J. Neuropharmac.* **7**: 301–306, 1968.
3. Bohus, B. and D. de Wied. Effect of α-MSH on extinction of a conditioned avoidance response in posterior lobectomized rats and in rats bearing lesions in the mid-caudal thalamus. *Acta physiol. pharmacol. neerl.* **14**: 336, 1967.
4. Bohus, B. and D. de Wied. Avoidance and escape behavior following medial thalamic lesions in rats. *J. comp. physiol. Psychol.* **64**: 26–29, 1967.
5. Bohus, B. and D. de Wied. Failure of α-MSH to delay extinction of conditioned avoidance behavior in rats with lesions in the parafascicular nuclei of the thalamus. *Physiol. Behav.* **2**: 221–223, 1967.
6. Cardo, B. Rôle de certains noyaux thalamiques dans l'élaboration et la conservation de divers conditionnements. *Psychol. Franç.* **10**: 344–351, 1965.
7. Cardo, B. Effets de la stimulation du noyau parafasciculaire thalamique sur l'acquisition d'un conditionnement d'évitement chez le rat. *Physiol. Behav.* **2**: 245–248, 1967.
8. Cardo, B. and F. Valade. Rôle du noyau thalamique parafasciculaire dans la conservation d'un conditionnement d'évitement chez le rat. *C.r. hebd. Séanc. Acad. Sci. (Paris)* **261**: 1399–1402, 1965.
9. Delacour, J., D. Albe-Fessard and S. Libouban. Rôle, chez le rat, de deux noyaux thalamiques dans le conditionnement instrumental. *Neuropsychologia* **4**: 101–112, 1966.
10. Delacour, J. and S. Libouban. Action différentielle de diverses lésions de noyaux relais thalamiques sur des conditionnement instrumentaux. *J. Physiol.* **56**: 555, 1964.
11. Delacour, J. and M. P. Santacana de Martinez. Rôle du thalamus median dans l'établissement et la rétention de conditionnement défensifs, classiques et instrumentaux. *Neuropsychologia* **5**: 237–252, 1967.
12. Feldman, S., J. C. Todt and R. W. Porter. Effect of adrenocortical hormones on evoked potentials in the brain stem. *Neurology* **11**: 109–115, 1961.
13. Hulst, S. G. T. and D. de Wied. Changes in body temperature and water intake following intracerebral implantation of carbachol in rats. *Physiol. Behav.* **2**: 367–371, 1967.
14. König, J. F. R. and R. A. Klippel. The Rat Brain. A stereotaxic atlas of the forebrain and lower parts of the brainstem. The Williams and Wilkins Company, Baltimore, 1963.
15. Mangili, G., M. Motta and L. Martini. Control of adrenocorticotropic hormone secretion. In: *Neuroendocrinology I*, edited by L. Martini and W. F. Ganong. New York–London: Acad. Press, 1966, pp. 298–344.
16. Slusher, M. A., J. E. Hyde and M. Laufer. Effect of intracerebral hydrocortisone on unit activity of diencephalon and midbrain in cats. *J. Neurophysiol.* **29**: 157–169, 1966.
17. Soulairac, A., Cl. Gottesmann and M.-J. Thangaprégassam. Variations de l'excitabilité nerveuse centrale chez le rat sous l'influence de diverses hormones stéroides. *J. Physiol.* **55**: 340–341, 1963.
18. Thompson, R. Thalamic structures critical for retention of an avoidance conditioned response in rats. *J. comp. physiol. Psychol.* **56**: 261–267, 1963.

19. Vanderwolf, C. H. The effect of medial thalamic lesions on previously established fear-motivated behavior. *Can. J. Psychol.* **17**: 183–187, 1963.
20. de Wied, D. Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. *Proc. Soc. exp. Biol.* **122**: 28–32, 1966.
21. de Wied, D. Opposite effects of ACTH and glucocorticosteroids on extinction of conditioned avoidance behavior. In: Proc. 2nd Intern. Congress of Hormonal Steroids, Exc. Med. Intern. Congress Series, 132, 1966, pp. 945–951.
22. Woodbury, D. M. Effect of hormones on brain excitability and electrolytes. *Rec. Progr. Horm. Res.* **10**: 65–104, 1954.