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Hippocampal Corticosterone Receptors and Novelty-Induced Behavioral Activity: Effect of Kainic Acid Lesion in the Hippocampus

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Rats were injected bilaterally in the dorsal and ventral hippocampus with kainic acid (KA) or with artificial CSF and their behavior and brain corticosterone (B) receptor systems were studied. The hippocampal KA injection destroyed part of the pyramidal neurons and of the dentate gyrus neurons. These neurons contain a receptor system for B. At 2 weeks after the KA lesion this B receptor system displays an increase in apparent maximal binding capacity (B_{max}) of approximately 25%. The compensatory increase in B receptor concentration is reflected in an increased uptake of [^{3}H]B in cell nuclei of hippocampal slices incubated in vitro with saturating concentrations of the steroid. Administration of a tracer dose of [^{3}H]B shows that labelled steroid can enter in vivo the cell nuclear compartment of the KA-lesioned lobe.

The role of B was investigated on novelty-induced behavioral activities of KA-lesioned and sham-lesioned animals in a large open and a small closed field at 10 days after bilateral adrenalectomy (ADX) or sham-ADX which is 14 days after the (sham) lesion. B (300 μg/kg rat) was administered s.c. 1 h prior to the test. KA lesion resulted in an increase in exploratory activity and a reduction in grooming and immobility. After ADX the effect of KA on exploration was reduced in the 5 min open field and abolished in the 30 min closed field. ADX animals displayed more grooming behavior (closed-field). B replacement of ADX rats reinstated the exploratory hyperactivity of KA-lesioned rats. On some components of the behavior such as ambulation in open-field and locomotion in closed field, there was even a larger responsiveness to B in the KA-lesioned rats than in the control animals.

It is concluded that (1) after KA lesion of receptor containing neurons, the remaining tissue displays a compensatory increase in number of B receptor sites; (2) B is required for full expression of exploratory activity of rats with or without KA lesions; (3) the KA-lesioned rats display a larger responsiveness to B; and (4) the increased number of B receptor sites may underlie the larger responsiveness to B.

INTRODUCTION

Corticosterone (B), the principal glucocorticoid of the rat, restores certain behavioral disturbances that occur after bilateral adrenalectomy (ADX). ADX disturbs forced extinction of a passive avoidance response[2,3] and extinction of appetitive motivated behavior[28]. Moreover, novelty-induced exploratory behavior is reduced in rats adrenalectomized 10 days previously[4,42]. These behaviors are normalized with replacement of low doses of B, but not with dexamethasone (DEX), aldosterone (ALDO), deoxycorticosterone or progesterone[3,4,28,42,43].

The dose of B necessary to restore behavior of ADX rats and the remarkable specificity of B in this respect meet the properties of the B receptor system in rat brain. The receptor system specifically binds B, but not synthetic glucocorticoids such as dexamethasone and is predominantly retained in cell nuclei of neurons of limbic brain regions, in particular the hippocampus[11,13,22,25,37,38,41]. Moreover, the B-receptor system has a low binding capacity and 40–80% of the binding sites is occupied by endogenous hormone at plasma B levels in the physiological range[3]. These characteristics of localization, steroid specificity and capacity favored the hypothesis that the hippocampal B receptors mediate the modulatory action of B on adrenal dependent behavior[4,10].

Previously, it was found that unilateral removal of the dorsal hippocampus resulted in a compensatory

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increase in number of B-receptor sites in the remaining contralateral lobe[32]. In the present study it was attempted to modify the number of B receptor containing neurons in the hippocampus with an intrahippocampal injection of kainic acid (KA)[39]. Intraventricular or systemic injection of KA destroys, besides other neurons, the hippocampal pyramidal neurons, but spares the neurons of the dentate gyrus[31]. Intrahippocampal injection restricts neuronal loss to the hippocampus per se and causes a loss of dentate gyrus and pyramidal neurons[14,15]. However, this route of administration is also known to leave at larger distance from the injection site a number of hippocampal neurons undamaged[12,45].

A bilateral injection of KA in the dorsal and ventral hippocampus was used in this study. In the first series of experiments it was found that the number of soluble B-receptor sites was increased in the remaining hippocampal tissue after this lesion. In view of this finding the behavioral responsiveness to B replacement was investigated of rats that were adrenalectomized and KA lesioned.

METHODS

Animals and surgery

Male Wistar rats weighing 200–220 g at the time of operation were housed 4–5 per cage under a controlled light–dark cycle (light on from 05.00 to 19.00 h). Surgery and intracerebral injections were performed under Hypnorm anesthesia. Kainic acid (KA, Sigma, 3 nmol/μl artificial CSF)[5] or the vehicle were infused for 2 min per each injection into the dorsal and ventral hippocampi (3 and 1.5 nmol, respectively) with the aid of a guide cannula. The tip of the guide cannula was inserted stereotaxically according to the atlas of De Groot[8]: dorsal hippocampus A 3.4, L 2.3, D 2.4; and ventral hippocampus A 2.2, L 4.8, D 0.5. The needle protruded 100 μm into the brain. In order to prevent severe epileptic seizures diazepam (1 mg/rat) was injected i.p. at the end of the operation, and this injection was repeated at the appearance of tremor or convulsions. Before returning animals to their home cages, another 1 mg of diazepam was injected intramuscularly. After the antiepileptic treatment, the mortality was less than 10% in the KA-injected group. Bilateral ADX or sham-ADX occurred under ether anesthesia and was performed through the lumbar approach 4 days after the intrahippocampal injections. The ADX rats were supplied with saline solution for drinking.

Corticosterone treatment

Corticosterone (Organon, Oss, The Netherlands) was dissolved in 2% ethanol/saline and was injected s.c. in a dose of 30 μg/100 g body wt. and in a volume of 0.2 ml/100 g body wt. The vehicle was given to the control animals.

Behavioral procedures

Behavioral tests were performed 10 days after ADX and sham-ADX, that is 14 days after the bilateral intrahippocampal KA injections. This time interval was selected in view of the time course of degeneration and regeneration of hippocampal nerve terminals following KA lesion[26]. The 14 days after the KA-lesion is presumably at the end of a period of intense synaptic regeneration. The 10 day period after ADX was selected in view of previous observations that showed this interval to be necessary for demonstration of the effect of B on exploratory behavior[4,42]. Open-field activity was measured 1 h after B or vehicle injections in a circular open field. The open-field apparatus consisted of a walled circular arena (radius: 40 cm, height 31 cm). The floor was divided into oblong blocks with an 8 cm radius circle in the center for scoring ambulatory activity. The room remained dark during testing and a 60 W lamp 40 cm from the floor served as light source. The number of floor units crossed by the rats served as the measure of ambulation and the number of rearings and the occurrence of grooming episodes were also recorded during a 5 min observation period.

The novelty-induced behavioral activity was also tested in a small closed field that consisted of mesh-covered separate chambers (30 × 30 × 30 cm) with non-transparent walls from all sides except the front wall, through which they were dimly illuminated. The floor of chambers was covered with wooden shavings like that of the rats home cages. Simultaneously 6 rats were observed during a 30-min observation period. The observation started 45 min following the B or vehicle injections. The technique of behavioral sampling of 15 s each was used to describe behavior. The behavioral events were grouped into 4 categories: (1) locomotion, all horizontal movements

such as ambulation, sniffing with head turning etc; (2) rearing, all vertical movements; (3) grooming, including face washing, licking or scratching different parts of the body; and (4) immobility, which covered all episodes without apparent voluntary and involuntary movements in the standing, rearing, sitting, and lying position. All behavioral data were transformed to percentage of the control, which allows a better comparison.

Corticosterone receptor assay

In all experiments the rats were sacrificed 15 days after bilateral hippocampal KA lesions, i.e. 11 days after ADX. For the in vitro binding studies with hippocampal cytosol, the rats were anesthetized with Nembutal and perfused with saline through the heart. Hippocampal lobes were removed from the brain on ice, weighed, homogenized, centrifuged and assayed for [^{3}H]B binding as previously described[11]. [^{3}H]B (spec. act. 50 Ci/mmol, New England Nuclear) was added to aliquots of the cytosol in a concentration range of 1×10^{-10} M to 2×10^{-8} M. The incubation medium was 5 mM Tris buffer (Tris/hydroxymethyl/aminomethane) containing 1 mM EDTA (ethylene-diamine-tetra-acetate, disodium salt), 1 mM 2-mercaptoethanol and 5% glycerol adjusted to pH = 7.4 with hydrochloric acid. Non-specific binding was determined in parallel incubations containing a 500-fold excess of unlabeled B. The data were analyzed according to Scatchard[33].

The competition by unlabeled steroids for the binding sites labeled with [^{3}H]B under the various experimental conditions was measured as described previously[41].A fixed amount of [^{3}H]B (5 nM) was used and various concentrations of unlabeled steroids were added (5, 15, 50, 150 and 500 nM unlabeled steroid).

For study of the uptake of [^{3}H]B in cell nuclei, rats were lesioned unilaterally in the dorsal and ventral hippocampus with KA. The contralateral lobe served as control for the ipsilateral KA-injected lobe in the same animal. Sacrifice was 2 weeks after the lesion and 10 days after ADX. Cell nuclear uptake in vivo of [^{3}H]B was performed as described previously[11,22]. Briefly, [^{3}H]B was injected intravenously in the tail vein in a dose of 1 nmol dissolved in 200 μl of 2% ethanol/saline. Injected animals were sacrificed after 1 h by decapitation. The hippocampus was dissected, weighed and a cell nuclear fraction was purified by sucrose density centrifugation[25]. The radioactive steroid was extracted from this fraction and from an aliquot of the tissue homogenate with scintillation fluid (Baker Chemicals, Deventer, The Netherlands).

Cell nuclear uptake in vitro of [^{3}H]B in tissue slices was performed as described previously[11,23]. Briefly, tissue slices of 0.3 mm thickness were prepared with a tissue chopper. The slices were incubated with 2×10^{-8} M [^{3}H]B with or without 10^{-5} M unlabeled hormone for 60 min at 25 °C in a Krebs–Ringer bicarbonate buffer, pH = 7.4 equilibrated with 95% O_2–5% CO_2. Slices were recovered by centrifugation and cell nuclei were isolated from slices with the standard method. All results of receptor assays and cell nuclear uptake experiments were expressed in fmol B/mg cytosol protein or cell nuclear protein. Protein determination was performed by the method of Lowry et al.[20]. The DNA content of the cell nuclear fraction was measured by the method of Burton[6].

Unlabeled steroids, except Ru 26988, were a gift from Organon International (Oss, The Netherlands). Ru 26988 was kindly donated by the Roussel-Uclaf Research Center (Romainville, France).

RESULTS

Effect of kainic acid lesion on histology, tissue weight, protein and DNA content of the hippocampus

A massive loss of pyramidal and also of granular cells was found in the vicinity of the KA lesions in the dorsal and ventral hippocampus. At larger distance from the injection site a number of pyramidal and granular cells escaped the neurotoxic lesioning. Fig. 1 is representative for such a lesion. The KA injection is applied unilaterally to make a comparison possible with the undamaged contralateral hippocampus lobe. The figure shows that large amounts of the CAIII, CAIV and dentate gyrus neurons are removed. However, the figure also shows that some neuron containing parts of the ipsilateral lobe are still present.

The weight loss of the ipsilateral KA-injected lobe (—27%) was not so high as the decrease in protein content (—56%). A larger proportion of protein was soluble in the KA-lesioned animals; cytosol protein decreased by 40%, while the concentration of cyto-

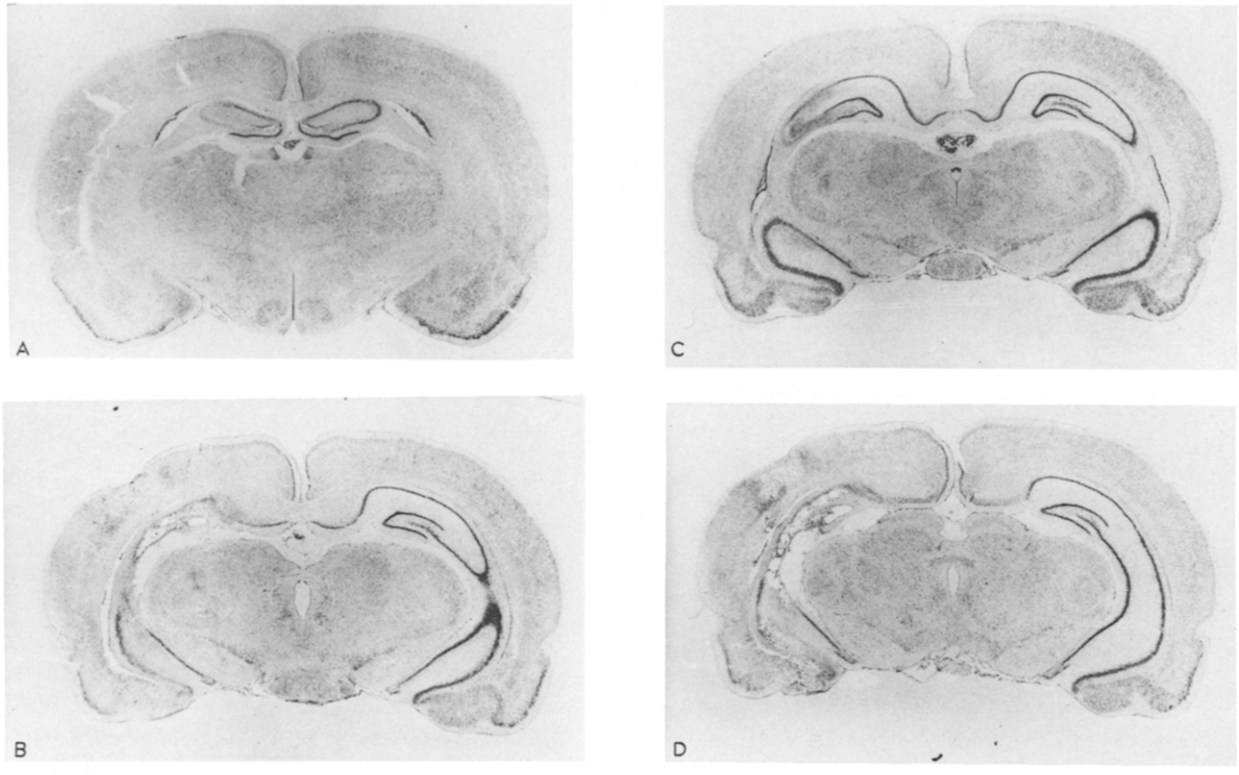

Fig. 1. Histology of unilateral kainic acid lesion in dorsal and ventral hippocampus. Note that in the two sections A and C mainly CAIII and CAIV neurons are removed and that gyrus dentatus and CAI neurons escaped the lesion. B and D show almost complete lesioning of pyramidal and dentate gyrus neurons.

TABLE I

Unilateral lesion of the hippocampus with kainic acid: effect on tissue weight, protein and DNA content

Data are mean of n = 10 animals ± S.E.M.

	KA-ipsilateral	*contralateral*	*% change*	*KA-ipsilateral (μg/mg tissue)*	*Contralateral (μg/mg tissue)*
Tissue weight (mg)	43.5 ± 1.6	59.6 ± 1.4	—27		
Tissue protein (mg)	2.2 ± 0.1	5.0 ± 0.1	—56	50.7 ± 3.0	83.3 ± 1.6
Cytosol protein (mg)	1.4 ± 0.2	2.3 ± 0.1	—40	32.7 ± 3.0	38.4 ± 1.6
Tissue DNA (μg)	37.7 ± 7.3	43.6 ± 3.4	—14	0.9 ± 0.2	0.7 ± 0.1

solic protein per mg tissue slightly increased. The DNA content of the KA injected lobe was slightly, but not significantly, lower than that of the contralateral lobe (—14%). The DNA concentration per mg tissue which may be considered a measure of cell density, was not altered significantly in the ipsilateral KA-lesioned side (Table I).

Effect of kainic acid lesion on the hippocampal corticosterone receptor system

The maximal binding capacity (B_{max}) and affinity (K_d) of the receptor sites for B after bilateral KA injections are summarized in Table II. The B_{max} after KA lesion showed an increase of 29 and 20% in the two experiments. However, when the total amount of receptor sites in the whole hippocampus was calculated, practically no difference in B_{max} between KA- and sham-injected animals was found. There was no sign of curve linearity in the Scatchard plots.

Fig. 2 shows the competition by unlabeled B, the mineralocorticoid aldosterone (ALDO) and the 'pure' glucocorticoid Ru 26988[30] for the binding sites

TABLE II

Binding constants of hippocampal corticosterone receptor system after bilateral kainic acid lesion in the hippocampus

		Expt. 1		*Expt. 2*	
		KA	*Sham*	*KA*	*Sham*
B_{max}	fmol/mg cytosol protein	414	320	393	328
K_d	nM	4.9	3.1	5.1	4.3
B_{max}	fmol/hippocampus lobe	1176	1082	1116	1108

TABLE III

Uptake in tissue and cell nuclei of hippocampus after unilateral kainic acid lesion

Data are expressed as fmol [^{3}H]B/mg protein. In vitro are data from uptake in tissue slices incubated for 1 h at 25 °C in a concentration of 2×10^{-8} M labeled steroid subtracted with uptake data in the presence of a 500-fold excess of unlabeled B to correct for non-specific uptake. In vivo are data of uptake in hippocampus 1 h after s.c. administration of 50 μCi (0.2 μg) [^{3}H]B. N, cell nuclear fraction; WH, tissue; N/WH, ratio of cell nuclear vs tissue uptake; n, number of animals ± S.E.M.; KA, kainic acid.

	In vitro				*In vivo*			
	N	*WH*	*N/WH*	*n*	*N*	*WH*	*N/WH*	*n*
KA-ipsilateral	426 ± 55	3570 ± 737	0.12	(8)	76 ± 9	38.5 ± 4.9	2.0 ± 0.3	(4)
Contralateral	310 ± 37	1419 ± 174	0.22	(8)	168 ± 36	33.9 ± 5.3	4.8 ± 0.3	(4)

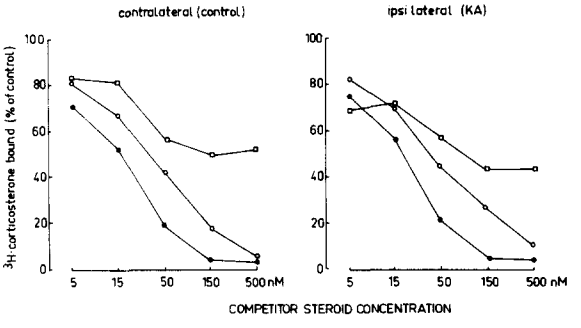

Fig. 2. Competition of B, ALDO and Ru 26988 for specific binding of [^{3}H]B in hippocampus cytosol. Labeled steroids were present in concentrations of approximately 5 nM. The values shown are the means of 3 separate binding experiments. The S.E. values were less than 10% for each point. The concentration of unlabeled radioligand (cL-IC_{50}) and competing steroid (cC-IC_{50}) required to decrease the binding measured in the absence of competitor by 50% was determined. The ratio (cL-IC_{50}/cC-IC_{50}) × 100 represents the relative binding affinity (RBA). RBA for KA-lesioned hippocampus: B, 100; ALDO, 64; Ru 26988, 46. For control hippocampus: B, 100; ALDO, 62; Ru 26988, 38. ●——●, B; ○——○, ALDO; □——□, Ru 26988.

labeled with B. Hippocampal cytosol was incubated with a fixed amount (5 nM) of [^{3}H]B in the presence of increasing concentrations of unlabeled steroids. The relative binding affinity (RBA) of the 3 steroids for [^{3}H]B binding was calculated from the displacement of the labeled steroid (see legend Fig. 2). The order of potency for [^{3}H]B labeled sites was: B > ALDO > Ru 26988 and did not differ significantly for KA lesioned and sham-injected animals (cf. RBA KA lesioned hippocampus: B = 100, ALDO = 64, Ru 26988 = 46; control hippocampus: B = 100, ALDO = 62, Ru 26988 = 38).

Effect of kainic acid lesion on cell nuclear uptake of [^{3}H] corticosterone in the hippocampus in vivo and in vitro in tissue slices

Since it is thought that B exerts its effect on hippocampus function via the receptor system at the genomic level, the cell nuclear uptake process of [^{3}H]B was investigated in the KA-lesioned animals. In these experiments, the KA lesion was applied unilaterally in the dorsal and ventral hippocampus. The uptake of [^{3}H]B in the cell nuclei of the ipsilateral KA lesioned hippocampus was measured and the uptake in the contralateral hippocampus lobe served as control in the same animal.

Table III shows the uptake in the cell nuclear fraction of hippocampus slices incubated with a saturating amount of [^{3}H]B (2×10^{-8} M)[11]. The in vitro cell nuclear uptake in slices of the KA lesioned lobe is under these conditions significantly higher than in the contralateral lobe. Moreover, the cell nuclear uptake is in the same order as the B_{max} of the soluble receptor system (Table II). Table III also shows the cell

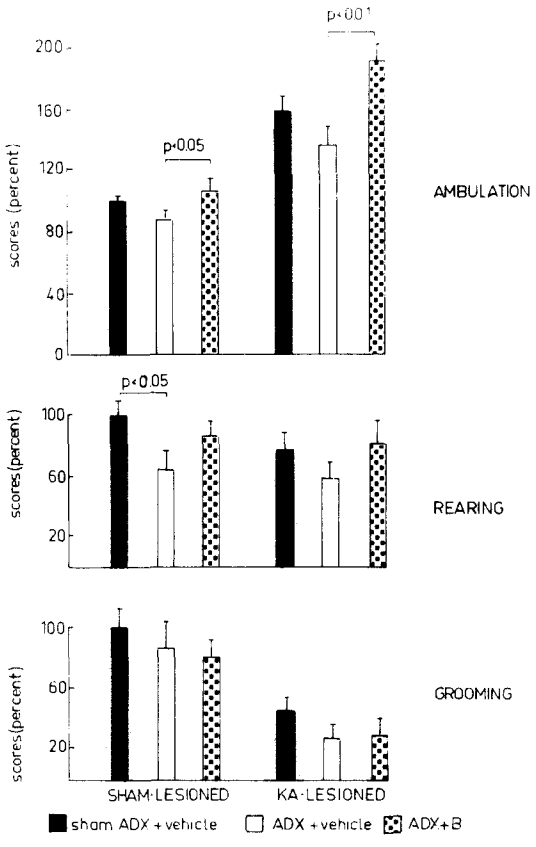

Fig. 3. Open-field activity (ambulation, rearing and grooming) of CSF injected (sham-lesioned) and bilaterally kainic acid injected (KA-lesioned) groups of animals 14 days after lesioning. B (300 μg/kg rat) or vehicle (2% ethanol/saline) were injected s.c. 1 h prior to the test. Groups consist of 12–16 animals. Data are given as percent scores of sham-ADX animals (100%). Student's *t*-test was used to evaluate the data.

nuclear uptake in vivo at 1 h after administration of [^{3}H]B. In this experiment it was found that the tracer amount of [^{3}H]B distributed very differently between the two hippocampus lobes. The amount of radioactivity extracted from the cell nuclei of the ipsilateral KA-lesioned side is about half of the amount in the contralateral lobe, when expressed in fmol/mg protein. In contrast, the uptake of the tracer in the tissue as measured in the homogenate was higher at the lesioned ipsilateral side. However, in one aspect the in vivo and in vitro cell nuclear uptake data are similar. When the uptake data are expressed as ratio of the amount localized in the tissue and in the cell nuclear compartment (N/WH), this ratio is in vivo and in vitro reduced in the ipsilateral KA-lesioned side. This implies that less ^{3}H-steroid is retained in cell nuclei relative to a larger uptake in the whole tissue.

Effect of B on novelty-induced behavior of kainic lesioned rats

Fig. 3 shows ambulation, rearing and grooming of bilateral KA- or sham-lesioned rats, that were adrenalectomized 10 days previously and received B (300 μg/kg rat) or saline s.c. 1 h prior to the open field test. These data were compared with the results obtained in sham-ADX rats. The bilateral KA lesion resulted in a substantial increase in ambulation of the 3 groups (ADX, ADX + B, sham-ADX) of animals as compared to the CSF controls ($P < 0.001$). The number of rearings was slightly, but not significantly decreased in lesioned rats, while KA-lesioned rats groomed less than the sham-lesioned controls ($t_{30} = 3.66$, $P < 0.01$). ADX caused a slight decrease in ambulation and rearing of KA- and sham-lesioned animals, that reached significance in the number of rearings of the sham-lesioned animals only. B replacement of ADX rats increased significantly ($P < 0.01$) ambulation after KA- as well as sham-lesion. However, the increase of ambulation after B was twice as much in the KA-lesioned group than in the non-lesioned animals.

The data obtained by time sampling of the behavior in a closed field of KA-lesioned and sham-lesioned control animals are shown in Table IV. Bilateral KA lesion did increase the frequency of rearing and locomotion and decrease the amount of grooming of the sham-ADX rats. These behavioral parameters were unaffected by KA in the ADX animals. B-replacement (300 μg/kg) 1 h prior to the behavioral test increased the frequency of locomotion, while the amount of grooming decreased. Locomotion and grooming were restored to the level of the sham-ADX rats after B replacement. The effect of B was more pronounced in the KA-lesioned animals than in the sham-lesioned animals ($n = 15$, $P < 0.05$). As compared to the ADX controls the increase in locomotion was 92% in the KA-lesioned group and 39% in the sham-lesioned groups after B. KA-lesion did not alter the responsiveness to B in the other 3 behavioral parameters scored.

DISCUSSION

The present study has disclosed novel aspects of

TABLE IV

Effects of adrenalectomy and corticosterone administration on novelty induced behavioral activities and immobility of rats injected with artificial CSF or kainic acid bilaterally into hippocampal lobes

Median frequencies of a total of 100 time-sampled scores are shown. ADX and sham-ADX occurred 10 days before the test on spontaneous activity by placing the animals in a closed field. Locomotion, rearing, grooming and immobility were scored during 30 min at 15 s intervals as described in the Methods section. 300 μg B/kg body wt. or vehicle were injected 45 min prior to test. Groups consist of 9 animals.

Treatments	*Locomotion*		*Rearing*		*Grooming*		*Immobility*	
	CSF	*KA*	*CSF*	*KA*	*CSF*	*KA*	*CSF*	*KA*
Sham-ADX vehicle	31	44[a]	9	21[a]	30	21[b]	30	13[b]
ADX vehicle	26	25++	7	10++	47+	46++	21	29
ADX B	36*	48**	10	13	28**	24*	24	18
ANOVA (Kruskal–Wallis)	0.05	0.01	n.s.	0.01	0.02	0.02	n.s.	0.05

+$p < 0.05$; ++$p < 0.01$ vs sham-ADX-vehicle groups (Mann–Whitney U-test)
*$p < 0.05$, **$p < 0.01$ vs ADX-vehicle groups (Mann–Whitney U-test)
[a]$p < 0.02$, [b]$p < 0.05$ vs sham-ADX CSF injected groups (Mann-Whitney U-test)

the interaction of B with its receptor system in the brain in relation to behavior. Firstly, partial removal of the B receptor-containing neurons in the hippocampus by KA lesion resulted in an increase of soluble receptor sites measured in vitro in the remaining tissue. These sites may be confined to neurons or be present in glial cells that have proliferated in the damaged hippocampus lobe. Secondly, ADX virtually abolished the increase in exploratory activities as induced by novelty in rats with hippocampal KA lesion. B administration to ADX rats reinstated the exploratory hyperactivity of KA lesioned rats. On some components of this behavior even an enlarged responsiveness to B was observed.

Our former observations suggested that exploratory behavior of the rat in novel environments is affected by the level of circulating B[4,42]. In accordance with those findings reduced exploratory activities were observed in this study following ADX of sham-lesioned rats in both the open- and the closed-field test situations and these behaviors were reinstated by B. The two test situations should be viewed as complementary ones. While the behavior of the rats is directed by novelty in both situations, the immediate response to novelty, in particular that of ambulation, is reflected in the open-field, while the short-term habituation to the novel environment (shift from locomotor activity to immobility through grooming periods) could be seen in the closed-field. ADX-induced changes were certainly not dramatic, but were reflected in reduced exploratory (ambulatory and locomotion) activities with a shift to increased grooming in the closed-field situation.

The KA-lesions in the hippocampi resulted in substantially increased exploratory activities, in particular the horizontal ones in the open field and both the horizontal and vertical ones in the closed-field, and in diminished grooming activity. These changes evoked by KA lesion in exploratory behavior resemble those found in rats with hippocampal damage[14–19]. It has also been shown that hippocampectomy reduces novelty-induced grooming[1,7]. Removal of the adrenals in the KA-lesioned rats slightly diminished (open-field) or virtually abolished (closed-field) the increases in exploratory activities and caused a shift towards increased grooming behavior (closed-field). The former findings resemble those of Iuvone and Van Hartesveldt[16] who showed that ADX prevents the increase in exploratory behavior of rats with hippocampal damage.

That administration of corticosterone to ADX rats 'restores' the exploratory activities towards the control levels in KA-lesioned rats suggests that the behavioral deficits in ADX rats were due to the absence of adrenocortical hormone. This study also showed that some components of the exploratory behavior, in particular ambulation, in the open-field exhibited larger responsiveness to B in KA-lesioned rats than

in the sham-lesioned rats. The increase in ambulation following B injection was twice as high in lesioned ADX rats. Accordingly, the effect of B on novelty-induced behavior becomes more potent, when the hippocampus is lesioned. In this respect B and the impaired hippocampal function act in the same direction, when the animal is exposed to a novel environment. In other words B seems to exert an inhibitory infuence on the expression of hippocampal function. A similar conclusion was reached by McEwen[21] using as criteria the B effects on appetitive conditioned behavior[28], neuroendocrine responses and hippocampus electrophysiology.

In view of the synergistic action of B and the KA-lesion in the hippocampus on novelty-induced behavior it is important to know where this action of B takes place. There are a number of arguments that point to the involvement of the hippocampal B receptor system in this respect. These arguments are the specificity of B action on behavior and the low replacement dose of the steroid that correlates very well with the properties of the B-receptor system in hippocampal neurons[4,10,21]. The effect of B was specific in novelty-induced behavior[4,42], as well as in aversively[2,3] or appetitive[28] motivated behaviors. Potent synthetic glucocorticoids such as dexamethasone or mineralocorticoids were ineffective[42,43]. The dose of B administered to ADX animals led to a physiological level of receptor occupation[3] and in all these parameters B replacement restored the behavior to that observed in sham-ADX animals. On the basis of this line of reasoning it seems reasonable to assume that the B-receptor system in the remaining hippocampal cells is implicated in mediating the increased responsiveness to B after KA lesion. The hippocampus has an influence on locomotor activity probably via modulation of meso-limbic or nigrostriatal dopamine systems, but these latter structures do not contain steroid-sensitive receptor containing cells. Interestingly, autoradiographical data have demonstrated that motor neurons in the spinal cord can retain labeled B[37].

The present study demonstrates that 2 weeks after the KA-lesion the number of receptor sites per unit of protein actually is increased. The increase in receptor capacity (B_{max}) proceeded to such an extent that for the whole hippocampus it compensated the considerable loss of receptor due to lesioning of receptor containing neurons. The B-receptor sites in KA-lesioned tissue represent a pool that is indistinguishable of the receptor sites in the hippocampus of control animals in terms of its relative binding affinity towards B, Ru 26988 and ALDO. It is conceivable that the larger number of B receptors in the hippocampus underlies the increased responsiveness to B after KA lesion. Alternatively, it cannot be excluded that the larger number of receptor sites stems from glial cells proliferating after tissue damage.

The increase in B_{max} of soluble receptor sites after KA-lesion is reflected in the increased capacity of the cell nuclear uptake in vitro in tissue slices. However, administration in vivo of a tracer dose of [^{3}H]B to unilateral-lesioned animals shows a reduced uptake in cell nuclei isolated from the lesioned hippocampus lobe. At present we cannot offer an unequivocal explanation of this apparent discrepancy. It could be related to the tracer dose or to an altered uptake and distribution kinetics of the ^{3}H-steroid in vivo in the tissue and cell nuclear compartment after the lesion. It also cannot be excluded that in vitro the increase in B_{max} is due to 'cryptic' receptor sites[40], e.g. receptor sites that are available for [^{3}H]B binding in vitro, but are not occupied by B in vivo[40]. At any rate this study shows that after KA lesion B receptor sites remain physiologically active in the cell nuclear uptake process.

In a previous study involving unilateral removal of the dorsal hippocampus, the contralateral lobe displayed a compensatory increase in receptor number as well[32]. It may well be that the increase in B receptor number after lesioning is part of the ability of the hippocampus to develop a morphological[26,34,35] and functional recovery[14,15,29]. There is a maximal behavioral activity change 1 week after the lesion, and locomotor activity subsedes during the second post-operative week[17,19]. At this time the increase in receptor number has developed[32] which persists for more than 3 months. It would, therefore, be of interest to investigate whether the larger magnitude of the B effect on behavior persists at a time hippocampal plasticity has permitted functional recovery.

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REFERENCES

1 Bär, P. R., Gispen, W. H. and Isaacson, R. L., Behavioral and regional neurochemical sequelae of hippocampal destruction in the rat, *Pharmacol. Biochem. Behav.*, 14 (1981) 305–312.
2 Bohus, B. and De Kloet, E. R., Behavioral effects of corticosterone related to putative glucocorticoid properties in the rat brain, *J. Endocrinol.*, 72 (1977) 64P–65P.
3 Bohus, B. and De Kloet, E. R., Adrenal steroids and extinction behavior: antagonism by progesterone, desoxycorticosterone and dexamethasone of a specific effect of corticosterone, *Life Sci.*, 28 (1981) 433–440.
4 Bohus, B., De Kloet, E. R. and Veldhuis, H. D., Adrenal steroids and behavioral adaptation: relationship to brain corticoid receptors. In D. W. Pfaff and D. Ganten (Eds.), *Current Topics in Neuroendocrinology, Vol. 2: Adrenal Action on the Brain*, Springer Verlag, Berlin, 1982, pp. 107–148.
5 Bradbury, M. W. B. and Davson, H., Transport of urea and certain monosaccharides between blood and fluid perfusing the cerebral ventricular system of rabbits, *J. Physiol. (Lond.)*, 170 (1964) 195–211.
6 Burton, K., A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, *Biochem. J.*, 62 (1956) 315–323.
7 Colbern, D., Isaacson, R. L., Bohus, B. and Gispen, W. H., Limbic-midbrain lesions and ACTH-induced excessive grooming, *Life Sci.*, 21 (1977) 393–402.
8 De Groot, J., *The Rat Forebrain in Stereotaxic Coordinates*, North Holland, Amsterdam, 1967.
9 De Kloet, E. R. and McEwen, B. S., Glucocorticoid interaction with brain and pituitary. In W. H. Gispen (Ed.), *Molecular and Functional Neurobiology*, Elsevier Publ. Co., Amsterdam, 1976, pp. 258–307.
10 De Kloet, E. R. and Veldhuis, H. D., Adrenocortical hormone action on the brain. In A. Lajtha (Ed.), *Handbook of Neurochemistry, Vol. 8*, Plenum Press, New York, in press.
11 De Kloet, E. R., Wallach, G. and McEwen, B. S., Differences in corticosterone and dexamethasone binding to rat brain and pituitary, *Endocrinology*, 96 (1975) 615–623.
12 Fonnum, F. and Walaas, I., The effect of intrahippocampal kainic acid injections and surgical lesions on neurotransmitters in hippocampus and septum, *J. Neurochem.*, 31 (1978) 1173–1181.
13 Gerlach, J. L. and McEwen, B. S., Rat brain binds adrenal steroid hormone: radioautography of hippocampus with corticosterone, *Science*, 175 (1972) 1133–1136.
14 Handelman, G. E. and Olton, D. S., Spatial memory following damage to hippocampal CA_3 pyramidal cells with kainic acid: impairment and recovery with preoperative training, *Brain Research*, 217 (1981) 41–58.
15 Handelman, G. E. and Olton, D. S., Recovery of function after neurotoxic damage to the hippocampal CA_3 region: importance of post-operative recovery interval and task experience, *Behav. Neural Biol.*, 33 (1981) 453–464.
16 Iuvone, P. M. and Van Hartesveldt, C., Diurnal locomotor activity in rats: effects of hippocampal ablation and adrenalectomy, *Behav. Biol.*, 19 (1977) 228–237.
17 Isaacson, R. L., *The Limbic System*, Plenum Press, New York, 1974.
18 Kim, C., Choi, H., Kim, J. K., Chang, H. K., Park, R. S. and Kang, I. Y., General behavioral activity and its component patterns in hippocampectomized rats, *Brain Research*, 19 (1970) 379–394.
19 Kimble, D. P., Changes in behavior of hippocampal lesioned rats across a 6-week postoperative period, *Physiol. Psychol.*, 4 (1976) 289–293.
20 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measured with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.
21 McEwen, B. S., Glucocorticoids and hippocampus: receptors in search of a function. In D. Ganten and D. W. Pfaff (Eds.), *Adrenal Actions on the Brain*, Springer Verlag, Berlin, 1982, pp. 1–22.
22 McEwen, B. S., De Kloet, E. R. and Wallach, G., Interactions in vivo and in vitro of corticoids and progesterone with cell nuclei and soluble macromolecules from rat brain regions and pituitary, *Brain Research*, 105 (1976) 129–136.
23 McEwen, B. S. and Wallach, G., Corticosterone binding to hippocampus: nuclear and cytosol binding in vitro, *Brain Research*, 57 (1973) 373–386.
24 McEwen, B. S. and Zigmond, R. E., Isolation of brain cell nuclei. In N. Marks and R. Rodnight (Eds.), *Research Methods in Neurochemistry, Vol. I*, Plenum Press, New York, pp. 140–161.
25 McEwen, B. S., Weiss, J. M. and Schwartz, L. S., Retention of corticosterone by cell nuclei from brain regions of adrenalectomized rats, *Brain Research*, 17 (1970) 471–482.
26 McWilliams, R. and Lynch, G., Terminal proliferation in the partially deafferented dentate gyrus: time courses for the appearance and removal of degeneration and the replacement of lost terminals, *J. comp. Neurol.*, 187 (1979) 191–198.
27 Meyer, J. S., Luine, V. N., Khylchevskaya, R. I. and McEwen, B. S., Glucocorticoids and hippocampal enzyme activity, *Brain Research*, 166 (1979) 172–175.
28 Micco, D. J. and McEwen, B. S., Glucocorticoids, the hippocampus, and behavior: interactive relation between task activation and steroid hormone binding specificity, *J. comp. physiol. Psychol.*, 94 (1980) 624–633.
29 Misslin, R., Haberkorn, E. and Ropartz, P., Responses to novelty and changes in behavior across a 3 week postoperative period in hippocampal-lesioned mice, *Physiol. Behav.*, 27 (1981) 413–418.
30 Moguilevski, M. and Raynaud, J. P., Evidence for a specific mineralocorticoid receptor in rat pituitary and brain, *J. Ster. Biochem.*, 12 (1980) 309–314.
31 Nadler, J. V., Perry, B. W. and Cotman, C. W., Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells, *Nature (Lond.)*, 271 (1978) 676–677.
32 Nyakas, C., De Kloet, E. R., Veldhuis, H. D. and Bohus, B., Corticosterone binding capacity increases in contralateral hippocampus after partial unilateral hippo-

campectomy, *Neurosci. Lett.*, 21 (1981) 339–343.
33 Scatchard, G., The attraction of protein for small molecules and ions, *Ann. N.Y. Acad. Sci.*, 51 (1949) 660–672.
34 Scheff, S. W. and Cotman, G., Chronic glucocorticoid therapy alters axon sprouting in the hippocampal dentate gyrus, *Exp. Neurol.*, 76 (1982) 644–654.
35 Scheff, S. W., Bernardo, L. S. and Cotman, C. W., Hydrocortisone administration retards axon sprouting in the rat dentate gyrus. *Exp. Neurol.*, 68 (1980) 195–201.
36 Siegel, S., *Nonparametric Statistics for Behavioral Sciences*, McGraw-Hill Book Co. Inc., New York, 1956.
37 Stumpf, W. E., Anatomical distribution of steroid hormone target neurons and circuitry in the brain. In M. Motta (Ed.), *The Endocrine Function of the Brain*. Raven Press, New York, 1980, pp. 43–49.
38 Stumpf, W. E. and Sar, M., Anatomical distribution of corticosterone-concentrating neurons in rat brain. In *Anatomical Neuroendocrinology, Int. Conf. Neurobiology of CNS-Hormone Interactions*, Chapel Hill, 1974, Karger, Basel, 1975, pp. 254–261.
39 Takemoto, T., Isolation and structural identification of naturally occurring excitatory amino acids. In G. McGeer, J. W. Olney and P. L. McGeer (Eds.), *Kainic Acid as a Tool in Neurobiology*, Raven Press, New York, 1978, pp. 1–15.
40 Turner, B. B. and McEwen, B. S., Hippocampal cytosol binding capacity of corticosterone: no depletion with nuclear loading, *Brain Research*, 189 (1980) 169–182.
41 Veldhuis, H. D., Van Koppen, C., Van Ittersum, M. and De Kloet, E. R., Specificity of the adrenal steroid receptor system in rat hippocampus, *Endocrinology*, 110 (1982) 2044–2051.
42 Veldhuis, H. D., De Kloet, E. R., Van Zoest, I. and Bohus, B., Adrenalectomy reduces exploratory behavior activity in the rat: a specific role of corticosterone, *Horm. Behav.*, 16 (1982) 191–198.
43 Veldhuis, H. D. and De Kloet, E. R., Antagonistic effects of aldosterone on corticosterone-induced changes in exploratory behavior of adrenalectomized rats, *Horm. Behav.*, in press.
44 Weijnen, J. A. W. M. and Slangen, J. L., Effects of ACTH-analogues on extinction of conditioned behavior. In D. De Wied and J. A. W. M. Weijnen (Eds.), *Pituitary, Adrenal and the Brain. Progress in Brain Research, Vol. 32*, Elsevier Publ. Co., Amsterdam, 1970, pp. 221–235.
45 Wood, P. L., Peralta, E., Cheney, D. L. and Costa, E., The turnover rate of ACh in the hippocampus after lesion of hippocampal pyramidal cells with kainic acid, *Neuropharmacologia*, 18 (1979) 519–523.