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Simultaneous Chemical Stimulation of the Hypothalamus and Dorsal Hippocampus in the Waking Cat

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NAGY, J. AND L. DECSI. *Simultaneous chemical stimulation of the hypothalamus and dorsal hippocampus in the waking cat.* PHARMAC. BIOCHEM. BEHAV. 2(3) 285–292, 1974. – The spontaneous behaviour of waking cats was hardly, if at all, influenced by the injection of carbachol or noradrenaline into the dorsal hippocampus. On some occasions mild sedation and shift of the EEG pattern towards synchronization were observed. Carbachol injection into the anterior hypothalamus evoked a characteristic rage reaction with complete EEG arousal and theta-wave dominance in the hippocampal lead. Previous application of either carbachol or noradrenaline, but not nicotine, in the dorsal hippocampus inhibited or fully abolished the carbachol-induced hypothalamic emotional-behavioural reaction. The inhibitory effect of intrahippocampal carbachol on the hypothalamic rage reaction was easily counteracted by previous topical, intra-hippocampal application of a few μg of atropine. Intrahippocampal injection of dopamine or of the alpha-receptor stimulatory drug phenylephrine did not inhibit the carbachol-induced hypothalamic emotional reaction, while the beta-receptor stimulatory agent isoprenaline did. It is concluded that the dorsal hippocampus of the cat contains both a cholinergic and adrenergic receptor system. The cholinergic system presumably consists of muscarinic receptors and the adrenergic of beta-type adrenergic receptors.

Intracerebral microinjections Rage Hypothalamus Hippocampus Simultaneous chemical stimulation Cholinergic-adrenergic interaction

THE EFFECTS of chemical stimulation of the hippocampus have been analyzed by several authors. However, not many of them have used putative transmitter substances. MacLean [9] was the first to describe characteristic EEG and behavioural changes after intrahippocampal deposition of crystalline carbamylcholine (CCH) in the waking cat. In the experiments of Green and Lomax [4] the injection of CCH into the dorsal hippocampus resulted in a decreased ability of the animals to learn an alternation task. Low doses (0.5–4 μg) of CCH were found by Hull *et al.* [6] to have no effect on the bar-pressing activity of cats, while higher doses (4–10 μg) caused seizures in the two animals investigated. Spike-discharges after intrahippocampal administration of CCH and DFP were also observed by Baker and Benedict [1]. Activation of primary and secondary hippocampal epileptogenic foci was described by Guerrero-Figueroa *et al.* [5] after local application of ACH. Increased drinking in the rat was elicited by CCH stimulation of the dorsal hippocampus in the experiments of Lewitt *et al.* [7]. All these data unequivocally point to the presence in the dorsal hippocampus of a cholinergic system whose direct chemical stimulation elicits not only alterations in the local electrical activity but also complex behavioural effects.

It is also known that chemical stimulation of certain subcortical structures in the waking cat evokes a characteristic emotional-behavioural reaction, best called a rage reaction. This reaction can be elicited, *inter alia*, by CCH stimulation of the hypothalamus [2, 3, 8, 10, 15].

The limbic system plays an important part in the behavioural and affective-emotional reaction. This was also shown by the previous finding [11] that the CCH induced hypothalamic rage reaction was subject to amygdaloid influences, i.e. it was inhibited by simultaneous chemical stimulation of certain regions of the amygdala. In the present experiments the effect of the chemical stimulation of another part of the limbic system, the dorsal hippocampus, on the rage reaction evoked by CCH stimulation of the anterior hypothalamus has been investigated in the waking cat.

METHOD

The experiments were performed on a total of 40 cats of both sexes weighing 2–4 kg. Prior to surgery a 3-day antibiotic pretreatment was performed with 1 daily dose of 200,000 I.U. of depot penicillin (Retardillin®, United Works of Pharmaceutical and Dietetic Products, Budapest) given intramuscularly. Under pentobarbital anesthesia (40 mg/kg) cannulae and electrodes were implanted stereotax-

ically in the dorsal hippocampus and in the anteromedial hypothalamus, generally on the left side. In addition, silver ball electrodes were placed on the acoustic and/or motor cortex. The electrodes were made of Nicrothal 80 TE-40 wire, 0,3 mm in dia., insulated except for 1 mm at the tips. EEG recordings were made from both monopolar and bipolar leads. In the case of a monopolar lead the reference electrode was fixed to the frontal bone in the midline of the skull. The chronically implanted cannula had a diameter of 0.8 mm and served as a guide cannula for a thinner one (0.2 mm in diameter), which was used for the injection. The guide cannulae were insulated with enamel except for 1 mm at the tips; cannula and electrode were 1 mm apart. The thinner, inner cannula used for injecting the drug and the tubing connecting it to the microinjector were previously filled with the solution to be injected. The microinjector ensured an accuracy of ±0.1 μl. The volume administered was 5 or 10 μl; the substance to be injected was dissolved in 0.85 per cent sodium chloride containing 0.003 M NaH_2PO_4-Na_2HPO_4 buffer, adjusting the pH of the solution to 7.3. The injected solutions were sterile and had a temperature of 37°C. After the operation the animals received the antibiotic treatment for 3 more days and were not used for experiments for at least 8 days.

The stereotaxic coordinates used in the experiments were as follows (Snider and Niemer [14]):

Anteromedial hypothalamus Fr: 13.5; L: 2.0; H: −3.0.

Dorsal hippocampal formation Fr: 4.5; L: 4.5; H: +8.0.

The injection of the drugs into the hippocampus was performed 15 min prior to the intrahypothalamic carbachol administration evoking the rage reaction. The dose of carbachol varied between 2.5 and 10 μg according to the response of the animal, but was always the same in a particular animal.

Quantitative measurement of the Carbachol-induced hypothalamic rage reaction was performed as follows. The animal was placed in a sound-proof cage fitted with a microphone which was connected to a tape recorder. The animal's growling and hissing, characteristic signs of the rage reaction, were recorded on magnetic tape. Then, by playing the tape back, the periods of growling and hissing were measured and the ratio of their sum total to the whole experimental period was established.

At the end of the experiments the cats were anesthetized and their brains perfused through the carotid arteries first with saline and then with 80–100 ml of 10% Formol. The exact localizations of the electrodes and cannulae were determined in 50 μ frozen sections or in 30 μ paraffin-embedded sections.

The drugs used were as follows: carbachol (choline chloride carbamate, Pharmaceutical Works Chinoin, Budapest), *l*-noradrenaline bitartarate (Rhone-Poulenc), nicotine bitartarate (Fluka), phenylephrine-HCl (Koch-Light), dopamine-HCl (Fluka), isoprenaline-HCl (United Works of Pharmaceutical and Dietic Products, Budapest). All the doses given refer to the corresponding salt.

RESULTS

Effect of Cholinergic Stimulation of the Hippocampus

General behavioural effects. The spontaneous behaviour of the animals was left practically unaffected by the CCH doses given. The cats behaved as they generally do in a more or less habituated environment, were engaged in orientation and grooming, occasionally they sat down, etc. The animals appeared somewhat sedated; seizures were never seen within the dose range used.

Effect on the hypothalamic rage reaction. Table 1 shows the general arrangement of the experiments as well as the effect of three doses of CCH. This series of experiments was performed on one and the same animal. As seen from Table 1, stimulation of the dorsal hippocampus with CCH inhibited the hypothalamic rage reaction. In this animal a dose of 2.5 μg of CCH, injected into the hippocampus, completely abolished the hypothalamic rage reaction.

Inhibition in per cent, as given in the tables, means the percentage inhibition of vocalization under the actual drug treatment, as compared with the mean of the two control experiments (Carbachol administration in the hypothalamus only), one performed some days prior to, and the other some days after, the intrahippocampal drug administration.

Table 2 shows the average values of all experiments. Injection of 0.62 μg CCH into the dorsal hippocampus caused a 21% inhibition of the hypothalamic rage reaction. The inhibition was 42% after 1.25 μg CCH, and an average of 62% after 2.5 μg. The inhibitory effect proved to be statistically highly significant ($p<0.001$). The ED_{50} of CCH calculated from the data in Table 2 is 1.9 μg.

EEG effects. The alterations in spontaneous electrical activity of the brain after the intrahypothalamic injection of CCH are shown in Fig. 1. Between Parts A and B, 5 μg of CCH were injected into the left anterior hypothalamus, in response to which 2 changes were easily observed: (1) A tendency to desynchronization which is strong in the hypothalamus on both sides, and also in the acoustic cortex. (2) Appearance of a strong dominance of theta-waves in the dorsal hippocampus.

Very interestingly, the above EEG alterations can also be observed when the behavioural effect, i.e. the rage reaction, is fully inhibited by the intrahippocampal injection of CCH. This is demonstrated in Fig. 2.

Part A of Fig. 2 shows the control leads, and Part B the electrical activity of the regions implicated 7 min after the monolateral intrahippocampal injection of 1.25 μg of CCH. In response to this dose of CCH a tendency to synchronization appeared in all leads, and became still stronger 14 min after the CCH injection (Part C of the figure). The high voltage slow activity was immediately desynchronized by a click stimulus, with return to the original pattern within a few seconds. Between Parts C and D of Fig. 2, 5 μg of CCH were injected into the left anterior hypothalamus. Part D shows the electrical activity recorded 11 min after this intervention. The animal became fully awake, the electrical records showed low voltage fast activity in all leads (especially pronounced in the acoustic cortex): at the same time, the theta-waves markedly increased in number in the hippocampal lead.

Thus the EEG changes are practically identical with those seen in Fig. 1, where CCH injected only into the hypothalamus elicited a strong rage reaction, while in the experiment shown in Fig. 2 the previous intrahippocampal CCH injection fully inhibited the hypothalamic rage reaction. However, the spontaneous electrical activity of the brain changed in the very same manner as it generally did during a rage reaction. A previous injection of 5 μg of atropine sulfate into the hippocampus completely abol-

TABLE 1

AN EXAMPLE OF THE GENERAL EXPERIMENTAL ARRANGEMENT

Day After Operation	Treatment	Vocalization in Sec 5 min	10 min	15 min	20 min	Inhibition in Per Cent
20	2.5 μg Carbachol into the left hypoth. ant.	127.3	269.6	365.2	432.9	
23	2.5 μg Carbachol into the left form. hipp. dors. + 2.5 μg Carbachol into the left hypoth. ant.	0.0	0.0	0.0	0.0	−100.0
30	2.5 μg Carbachol into the left hypoth. ant.	114.7	265.8	360.4	441.5	
33	1.25 μg Carbachol into the left form. hipp. dors. + 2.5 μg Carbachol into the left hypoth. ant.	53.8	144.5	182.7	197.5	− 55.4
38	2.5 μg Carbachol into the left hypoth. ant.	79.0	220.7	339.1	415.0	
40	0.62 μg Carbachol into the left form. hipp. dors. + 2.5 μg Carbachol into the left hypoth. ant.	66.6	167.2	225.5	282.2	− 32.0
43	2.5 μg Carbachol into the left hypoth. ant.	131.2	274.3	359.4	429.6	

ished the inhibitory effect of intrahippocampal CCH administration. In these experiments the atropine was given through the same permanent cannula 5 min prior to the CCH administration (n=3).

Nicotine bitartarate, in a dose of 50 μg injected into the dorsal hippocampus, failed to elicit any essential behavioural change in the animals, nor did it inhibit the CCH-induced hypothalamic rage reaction (n=3). This observation means that muscarinic receptors might be responsible for the inhibitory effect of CCH. This assumption is supported by the ability of atropine completely to antagonize the inhibitory effect of intrahippocampal CCH on the hypothalamus.

Effect of Adrenergic Stimulation of the Hippocampus

Noradrenaline:

General behavioural effects. After monolateral injection of 20–50 μg of noradrenaline into the hippocampus the cats became calm with little, if any, spontaneous movement and gave the impression of being somewhat sedated.

Effect on the hypothalamic rage reaction. Table 3 shows the effect of adrenergic stimulation of the dorsal hippocampus on the rage reaction evoked by intrahypothalamic injection of CCH. As can be seen, 20 μg of noradrenaline caused a 33%, and 50 μg of noradrenaline a 67% inhibition. The effect is statistically highly significant ($p<0.001$). The ED_{50} value of noradrenaline is 35 μg, i.e. about 20-times as high as that of CCH.

EEG effects. Figure 3 shows the EEG alterations evoked by intrahippocampal injection of noradrenaline. Part A of the figure represents the control record. Part B shows the spontaneous electrical activity 4 min after intrahippocampal injection of 50 μg of noradrenaline. Between Parts B and C 5 μg of CCH were injected into the left anterior hypothalamus. As a consequence, the

TABLE 2

EFFECT OF INTRAHIPPOCAMPAL CARBACHOL ON THE HYPOTHALAMIC RAGE REACTION

N†	Treatment	Vocalization in Sec*	Inhibition in Per Cent
10	Carbachol into the ant. hypoth.	471 ± 81.4	
5	0.62 μg Carbachol into the dorsal hipp. form. + Carbachol into the ant. hypoth.	372 ± 104.2	−21
14	Carbachol into the ant. hypoth.	438 ± 58.6	
7	1.25 μg Carbachol into the dorsal hipp. form. + Carbachol into the ant. hypoth.	255 ± 93.1	−42
18	Carbachol into the ant. hypoth.	435 ± 57.6	
9	2.5 μg Carbachol into the dorsal hipp. form. + Carbachol into the ant. hypoth.	166 ± 71.4	−62 $p<0.001$

*Mean ± S.E. during 20 min after the intrahypothalamic Carbachol injection
†Number of experiments

tendency to synchronization elicited by the intrahippocampal noradrenaline injection ceased, and low voltage fast activity appeared in all leads.

Other Adrenergic Stimulants:

Intrahippocampal injection of 50 μg of dopamine had no influence on the hypothalamic rage reaction (n=3), nor did it cause any essential alteration in either the spontaneous behaviour of the animals or in the EEG patterns.

Also ineffective was in these respects a dose of 50 μg of phenylephrine, an adrenergic alpha-receptor stimulatory drug (n=3).

The CCH-induced hypothalamic rage reaction was inhibited by the intrahippocampal injection of 50 μg of isoprenaline, an adrenergic beta-receptor stimulatory agent (n=4). The efficiency of this drug was somewhat below that of noradrenaline.

DISCUSSION

From the above results it seems that the dorsal hippocampus of the cat contains at least two functional systems, probably of differing chemical mediation. Chemical stimulation of these systems elicits slight behavioural and EEG alterations and markedly inhibits the rage reaction evoked by chemical (carbachol) stimulation of the anterior hypothalamus.

Cholinergic System

The behavioural effect of carbachol injected into the dorsal hippocampus is, by itself, rather poor. The animal becomes a little sedated, and the EEG pattern is shifted towards synchronization. However, the alimentary conditioned reflexes are not influenced by monolateral carbachol administration, although a bilateral injection may have an inhibitory effect [12]. No autonomic signs were seen after intrahippocampal application of carbachol at the doses used.

On the other hand, a rage reaction evoked by intrahypothalamic injection of carbachol was greatly inhibited by intrahippocampal administration of carbachol.

The inhibitory effect of intrahippocampally applied carbachol on the hypothalamic rage reaction is probably due to the influence of muscarinic receptors. For the implication of muscarinic receptors speaks the finding that local pretreatment with small amounts of atropine completely abolished the inhibitory action of intrahippocampal carbachol on the hypothalamic rage reaction. This assumption is corroborated also by the finding that even high doses of nicotine fail to exert any such effect. (Similarly, the hypothalamic rage reaction cannot be inhibited by intrahippocampal application of Diazepam although this drug is fairly effective in inhibiting the rage reaction if injected into the amygdala [13]).

Interestingly, the EEG effects of intrahypothalamically injected carbachol are hardly, if at all, influenced by carbachol injected into the hippocampus. The strong cortical and subcortical desynchronization as well as the appearance and dominance of hippocampal theta-waves observed during the rage reaction can also be seen when the rage reaction is completely inhibited by intrahippocampal carbachol application. Thus the emotional-behavioural reaction and the EEG alterations can be dissociated under certain conditions.

According to some data in the literature intrahippocampal carbachol application evokes spike potentials [1, 6, 9] and intrahippocampal acethylcholine activates a latent epileptogenic focus [5]. However, in our experience much higher doses were needed to evoke spike discharges than those necessary to inhibit the hypothalamic rage reaction completely. With the doses used and the localization employed in our experiments no spike activity was ever seen. Thus the inhibitory effect on the hypothalamus was certainly not the result of some non-physiological electric/functional alteration in hippocampal activity.

The inhibitory effect of intrahippocampal carbachol on the hypothalamic rage reaction is certainly not due to diffusion of the drug into the cerebral ventricle for carbachol elicits a rage reaction when injected into the cerebral ventricle (as shown previously, [3]).

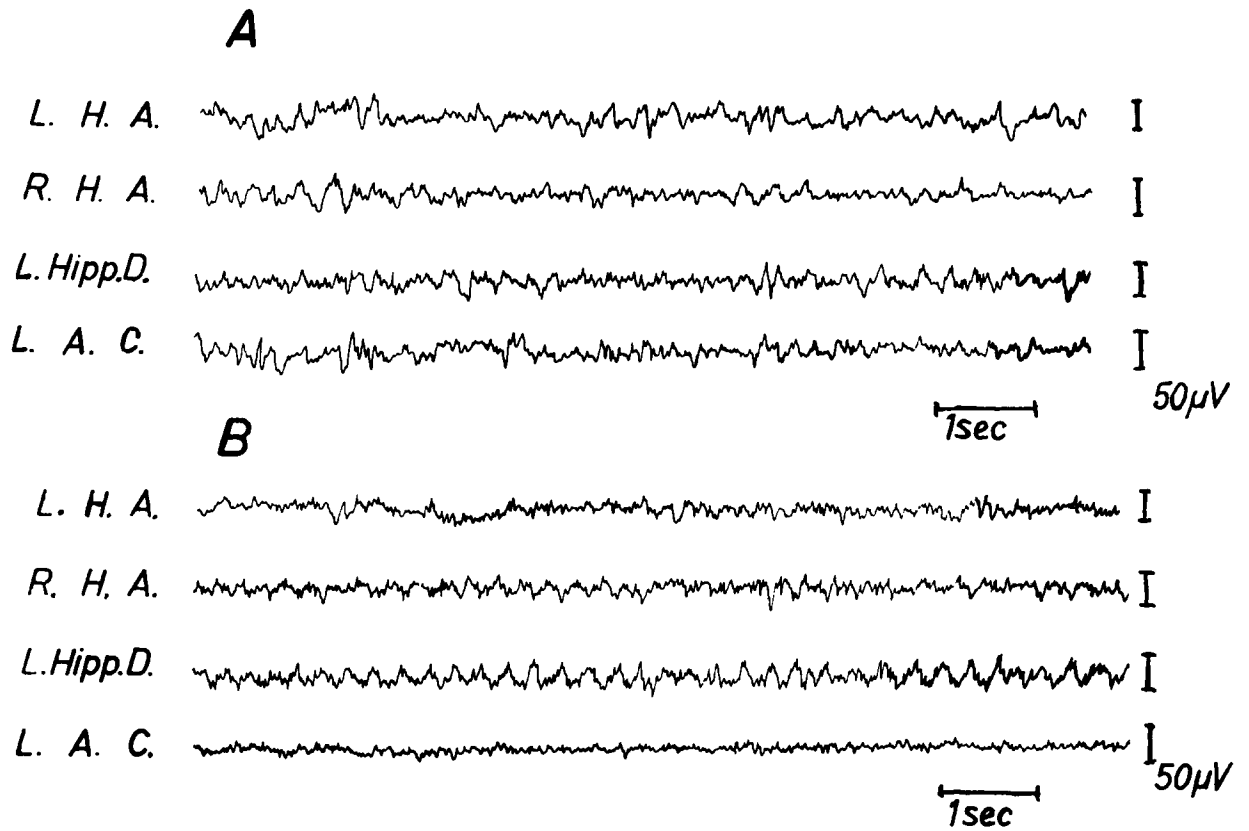

FIG. 1. Effect of 5 μg of carbachol injected into the left anterior hypothalamus on the spontaneous electrical activity of the brain in a waking cat. Part A: control record taken 5 min before carbachol. Part B: 5 min after carbachol. L.H.A. = left hypothalamus anterior. R.H.A. = right hypothalamus anterior. L.Hipp.D. = left hippocampus dorsalis. L.A.C. = left acoustic cortex.

Adrenergic System

The general behavioural effect of noradrenaline injected into the dorsal hippocampus is somewhat more pronounced than that of carbachol. The animal becomes sedated and the EEG pattern is shifted towards synchronization.

A striking difference between carbachol and noradrenaline after intrahippocampal application can be seen in their effect on the alimentary conditioned reflexes. Unlike carbachol, even 2 × 50 μg of noradrenaline injected bilaterally remain completely ineffective in this respect [12]. Similarly, no noteworthy autonomic signs were seen after intrahippocampal noradrenaline application.

However, the CCH-induced hypothalamic rage reaction is inhibited by intrahippocampal injection of noradrenaline, just like by that of carbachol. This may seem somewhat paradoxical, since the very same effect is elicited both by a cholinergic and adrenergic stimulant from the same cerebral region. The phenomenon is, however, not really unusual. Several peripheral organs (e.g. the vas deferens of the guinea-pig, the nictitating membrane of the cat, etc.) are influenced in the same sense by cholinergic and also by adrenergic drugs. In the central nervous system qualitatively identical effects can be elicited from the lateral amygdaloid nucleus by either carbachol or noradrenaline [11].

The inhibitory action of intrahippocampal noradrenaline is presumably a specific receptor effect. Dopamine is completely ineffective and so is the alpha-receptor stimulatory drug phenylephrine. On the other hand, the hypothalamic rage reaction can be inhibited by the beta-receptor stimulatory agent isoprenaline when injected into the dorsal hippocampus.

From the above findings it has been concluded that the dorsal hippocampus of the cat contains both an adrenergic and a cholinergic receptor system, which may overlap anatomically. The cholinergic system probably consists of muscarinic receptors, and the adrenergic system of beta-type receptors. The effects of chemical stimulation of these cholinergic and adrenergic systems have much in common, but are not identical.

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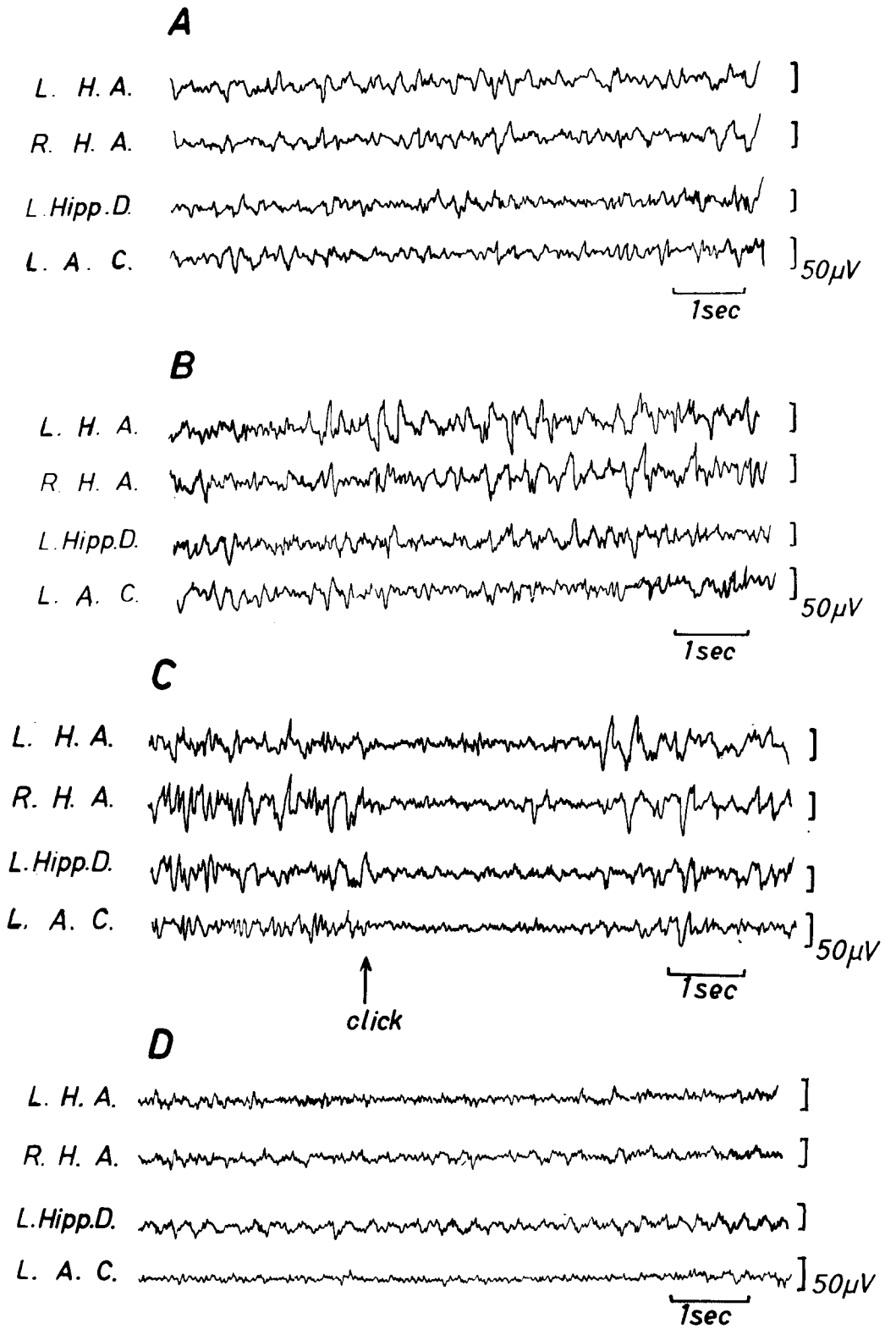

FIG. 2. Effect of intrahippocampal and intrahypothalamic carbachol, in combination, on the spontaneous electrical activity of the brain in a waking cat. Part A: control record taken 5 min before the first carbachol injection. Part B: 7 min after the injection of 1.25 μg of carbachol into the left dorsal hippocampus. Part C: the same after 14 min. Part D: 11 min after an additional injection of 5 μg of carbachol into the left anterior hypothalamus. L.H.A. = left hypothalamus anterior. R.H.A. = right hypothalamus anterior. L.Hipp.D. = left hippocampus dorsalis. L.A.C. = left acoustic cortex.

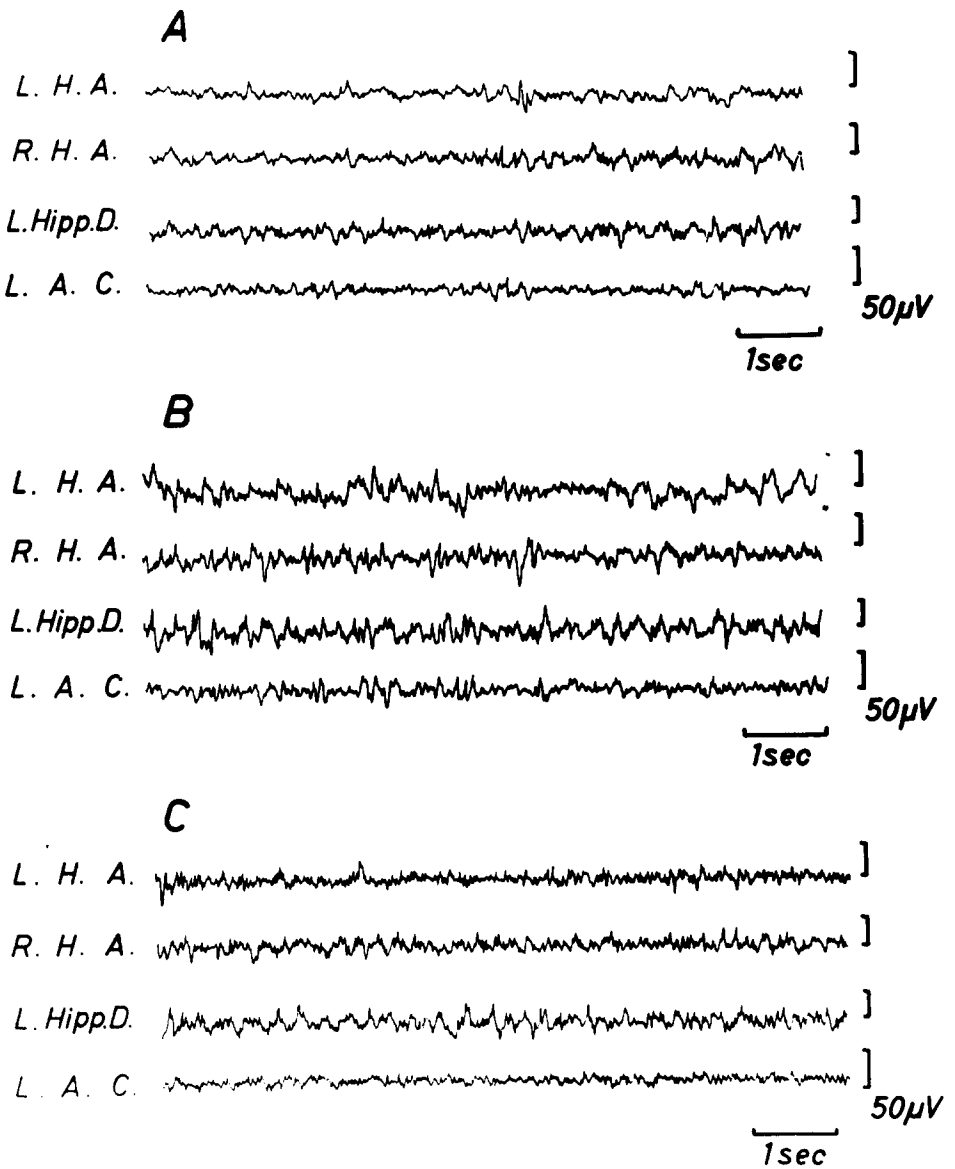

FIG. 3. Effects of intrahippocampal noradrenaline and intrahypothalamic carbachol, in combination, on the spontaneous electrical activity of the brain in a waking cat. Part A: control record taken 5 min before the first injection. Part B: 4 min after the injection of 50 μg of noradrenaline into the left dorsal hippocampus. Part C: 6 min after the additional injection of 5 μg of carbachol into the left anterior hypothalamus. L.H.A. = left hypothalamus anterior. R.H.A. = right hypothalamus anterior. L.Hipp.D. = left hippocampus dorsalis. L.A.C. = left acoustic cortex.

TABLE 3

EFFECT OF INTRAHIPPOCAMPAL NORADRENALINE ON THE HYPOTHALAMIC RAGE REACTION

N†	Treatment	Vocalization in Sec*	Inhibition in Per Cent
16	Carbachol into the ant. hypoth.	456 ± 56.2	
8	20 μg Noradrenaline into the dorsal hipp. form. + Carbachol into the ant. hypoth.	304 ± 91.8	−33
16	Carbachol into the ant. hypoth.	505 ± 59.0	
8	50 μg Noradrenaline into the dorsal hipp. form. + Carbachol into the ant. hypoth.	168 ± 67.0	−67 $p<0.001$

*Mean ± S.E. during 20 min after the intrahypothalamic Carbachol injection

†Number of experiments

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