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Low-Frequency Septal Stimulation Increases Tyrosine Hydroxylase Activity in the Hippocampus

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GRAHAM-JONES, S., L. HOLT, J. A. GRAY AND M. FILLENZ. *Low-frequency septal stimulation increases tyrosine hydroxylase activity in the hippocampus.* PHARMACOL BIOCHEM BEHAV **23**(4) 489–493, 1985.—Rats were chronically implanted with recording electrodes in the dorsomedial subiculum and stimulating electrodes in the septal area. Low-frequency septal stimulation (regular inter-pulse interval = 130 msec) was used over a period of 10 days to drive the hippocampal theta rhythm at 7.7 Hz (a régime shown previously to proactively facilitate acquisition of barpressing and retard its extinction), and high frequency (77 Hz) stimulation was used to block theta rhythm (a régime shown previously to proactively retard acquisition of barpressing and facilitate its extinction). The activity of soluble tyrosine hydroxylase from hippocampus increased in animals killed 15–33 days after the end of the period of 7.7 Hz septal stimulation but not after high-frequency stimulation. Irregular low-frequency stimulation with a mean inter-pulse of 130 msec produced the same effect on tyrosine hydroxylase activity as regular 7.7 Hz theta-driving. The possible relations between the effects of low-frequency septal stimulation on hippocampal tyrosine hydroxylase and on behavior are discussed.

Tyrosine hydroxylase Hippocampus Septal stimulation Hippocampal theta rhythm Stimulation frequency

UNDER appropriate conditions repeated exposure over a period of days to stressors (e.g., footshock, or a swim in cold water) gives rise to increased behavioral tolerance to both the pre-exposed and other stressors [8, 12, 23, 25]. This increased behavioral tolerance is sometimes accompanied by evidence of increased activity in the brain of the rate-limiting enzyme in catecholamine synthesis, tyrosine hydroxylase (TH) [24,25], and may even be a consequence of this neurochemical change.

Recently we have shown that increased behavioral tolerance to stressors may also be produced by low-frequency stimulation of the septal area [13] where the pacemaker cells for the hippocampal theta rhythm are located [19,20]. Septal stimulation for a period of 10 days (90 sec total stimulation per day) at a frequency of 7.7 Hz, driving hippocampal theta at the same frequency, proactively increased the resistance to extinction of a response acquired for food reward after the period of stimulation was over [13], an effect that can also be proactively produced by repeated footshock [8] and which can be regarded as one manifestation of increased tolerance for stress [12]. These behavioral effects of septal stimulation were present 3–4 weeks after the termination of the period of stimulation. The design of these behavioral experiments [13,14] precluded investigation of extinction earlier than about 3 weeks after the termination of stimulation, so the time to onset of the increased resistance to extinction is unknown. However, the time at which this effect was in fact observed is of the same order of magnitude as the time it takes for increased TH levels to appear in the hippocampus after the enzyme is induced in the locus coeruleus (the nucleus of origin of the noradrenergic innervation of the hippocampal formation) by reserpine [26]. In the present experiments, therefore, we measured TH activity in the hippocampus after rats were exposed to the same combination of septal stimulation and behavioral testing that has been shown to give rise to increased resistance to extinction [13].

Besides 7.7 Hz theta-driving stimulation (square-wave pulses at regular intervals of 130 msec), two other types of stimulation were delivered (in different animals) to the septal area. First, a high-frequency pulse train (77 Hz) was used to block the hippocampal theta rhythm [2]. This type of stimulation has been shown to have the opposite effect on behavior to that of 7.7 Hz theta-driving: whereas theta-driving proactively facilitated acquisition of a food-rewarded barpressing response and increased its resistance to extinction [13], theta-blocking proactively retarded acquisition and facilitated extinction of this response [14]. Second, in order

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to determine whether the effects of 7.7 Hz theta-driving depended on the average density of pulses delivered or upon their regularity (necessary to elicit hippocampal theta), some rats were given an irregular pulse train with an average frequency of 7.7 Hz.

TH activity was measured in synaptosomes and after solubilisation. The synaptosomal assay measures the activity of the enzyme in its physiological environment, i.e., the nerve terminal; the assay of soluble TH under saturating conditions for substrate and cofactor is a test of changes in the enzyme itself.

METHOD

The experiments had five phases: surgery, stimulation, acquisition of the barpressing response, extinction of the barpressing response, and killing plus TH assay in the hippocampus. All methods other than biochemical have been fully described, as have the behavioral results [13,14]; these aspects of the experiments will therefore only be summarised here.

General

Subjects were male Sprague-Dawley rats 90–120 days old at surgery, caged singly, maintained on food deprivation (10 g/day), and implanted with a chronic bipolar recording electrode in the dorsomedial subiculum (stereotaxic coordinates: 6 mm posterior to bregma; 2 mm left; 5 mm deep from surface of skull; skull flat between bregma and lambda) and two stimulation electrodes bilaterally in the lateral septal area (coordinates: 0.5 mm anterior; 0.7 mm lateral; 5.5 mm deep) [15]. Square-wave, constant-current stimulation (pulse-width = 0.5 msec) was delivered across the tips of the two septal electrodes [14]. Three stimulation schedules were used [13,14] as follows. *Theta-driving* consisted of 6-sec trains of pulses at 7.7 Hz (regular inter-pulse interval = 130 msec) occurring on a random time (RT) schedule with an average probability of 1 train/30 sec. Current intensity was set at 20% above each animal's threshold for driving the hippocampal theta rhythm. *Irregular stimulation* was identical to theta-driving except that, within each train, pulses occurred at random inter-pulse intervals with a mean of 130 msec and a minimum interval of 20 msec. Current intensity was set at 20% above each animals's threshold for the reliable occurrence of evoked hippocampal potentials. *Theta-blocking* consisted of 2-sec trains of pulses at 77 Hz (regular inter-pulse interval = 13 msec) at a fixed interval of 20 sec between trains. These changes in train duration and scheduling were necessary to avoid seizures in the theta-blocking condition. Current intensity was set at 20% above each animal's threshold for blocking the hippocampal theta rhythm, i.e., replacing theta with desynchronised, low-voltage, fast activity. Controls were treated identically to stimulated animals in every respect except that no stimulation was delivered. Average total stimulation time was kept constant at 90 sec/session by varying session length between schedules (about 8.5 min for theta-driving and irregular stimulation, about 16.5 min for theta-blocking).

Immediately after the stimulation phase behavioral training commenced [13,14]. All rats acquired a discrete trial fixed-ratio 5 barpress response in a Skinner box equipped with a single retractable lever and a pellet dispenser. Reward consisted of a single 45-mg pellet. There were 4 trials/day at a 1-min inter-trial interval during which the lever was retracted. The duration of acquisition varied between experiments from 15 to 21 consecutive days. Extinction followed immediately after the end of acquisition and lasted 12 consecutive days. The only difference from acquisition was that food pellets were no longer delivered.

The animals were killed the day after extinction testing ended by stunning and immediate decapitation. The brains were removed within 2 min and the hippocampus dissected out on ice [4].

Biochemical

Hippocampi were homogenised in 0.32 M sucrose and a crude synaptosomal fraction (P_2 pellet) prepared by two successive centrifugations at $1000 \times g$ for 10 min and $15000 \times g$ for 20 min.

Synaptosomal TH assay. The P_2 pellet was resuspended in incubation medium, pH 7.4, containing NaCl 125 mM, KCl 5 mM, CaCl 1 mM, MgCl 1 mM, Tris·HCl 50 mM, glucose 10 mM and ascorbate 1 mM. ^{3}H-3,5,tyrosine (Amersham Radiochemicals) was added to a final concentration of 20 μM, which was saturating. Synaptosomes were incubated at 37°C for 20 min; the reaction was stopped by cooling to 0°C and adding 5% trichloracetic acid. ^3H_2O was eluted on a double Dowex 50/Dowex 1 column as described previously [18].

Soluble TH assay. Synaptosomes were disrupted by mixing resuspended P_2 pellets with an equal volume of NaPO buffer 5 mM, pH 7.2, containing 0.4% Triton X100. Aliquots were incubated with catalase, tetrahydrobiopterin 1.27 mM and ^{3}H-tyrosine 10 μM. Samples were incubated at 37° for 10 min and ^3H_2O measured as described previously. The rate of tyrosine hydroxylation was expressed as fmol ^3H_2O/min/mg protein.

Experiments

The data reported were gathered in four separate experiments, indicated here by the letters A–D. Experiments A and B are Experiments I and II, respectively, in Holt and Gray's [13] report; Experiment D is reported by Holt and Gray [14]; and Experiment C, in which there was no behavioral training, is reported only here.

Experiment A. There were 3 groups of 8 rats each: Food Control, Correlated Theta-driving, and Uncorrelated Theta-driving. During the stimulation phase all rats received, besides any programmed stimulation, response-independent delivery of single 45-mg food pellets on a RT-30 sec schedule. The two stimulated groups received the theta-driving stimulation described above. In the Correlated Theta-driving group food-pellets were always delivered during the last second of the 6-sec pulse-trains, stimulation and food being delivered by the same RT-30 schedule. In the Uncorrelated Theta-driving group food delivery and septal stimulation were randomly related to each other. There were 15 days of barpress acquisition and 12 days of extinction between the end of stimulation and the day of killing.

Experiment B. There were 4 groups of 6 rats each: Uncorrelated Theta-driving and Food Control groups were treated as in Experiment A; the Theta-driving group received the theta-driving stimulation as described above, but no food delivery; and the Control group received neither stimulation nor food delivery during the stimulation phase. There were 18 days of barpress acquisition and 12 of extinction between stimulation and killing.

Experiment C. There were 3 groups of 8 rats each: Theta-

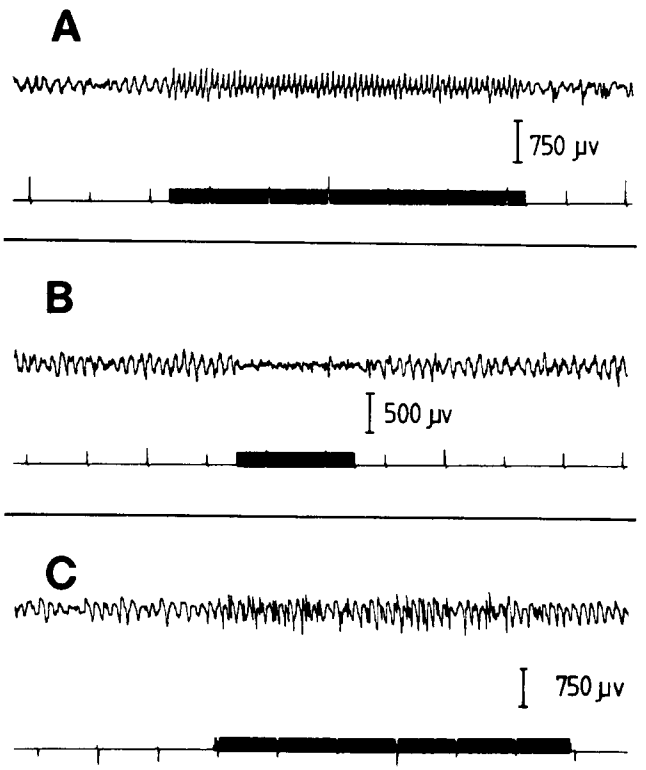

FIG. 1. Examples of hippocampal electrical activity elicited by theta-driving (A), theta-blocking (B) and irregular (C) septal stimulation. (For details of stimulation parameters, see the Method section.) The bottom trace in each panel shows time (in seconds) and periods of stimulation (solid black).

TABLE 1

SEPTAL STIMULATION AND HIPPOCAMPAL TH

Experiment	Group	N	Mean Stimulating Current (μA ± SEM)	Soluble TH*
A	Food Control	6	—	1230 ± 104
	Correlated Theta-driving	6	84.9 ± 2.5	1156 ± 173
	Uncorrelated Theta-driving	6	79.9 ± 2.0	1866 ± 184
B	Control	6	—	1324 ± 172
	Food Control	6	—	1329 ± 119
	Uncorrelated Theta-driving	6	58.8 ± 1.9	1646 ± 205
	Theta-driving	6	65.5 ± 1.9	1554 ± 173
C	Control	7	—	974 ± 234
	Theta-driving	7	114.6 ± 3.9	1305 ± 221
	Irregular Stimulation	6	114.3 ± 4.5	1331 ± 197
D	Control	8	—	426 ± 27
	Blocking	8	65.0 ± 2.3	413 ± 91

*Percentage of control (Experiments B, C) or food control (Experiment A) mean (fmol/min/mg P_2 protein) ± the standard error of that mean.

driving, Irregular Stimulation and Control, each receiving the treatment during the stimulation phase described above. No food was delivered during the stimulation phase and there was no behavioral training of any kind. Animals remained undisturbed in their home cages for 15 days after the end of the stimulation phase and were then killed.

Experiment D. There were 2 groups of 8 rats each: Theta-blocking (given the stimulation régime described above) and Control. No food was delivered during the stimulation phase. There were 21 days of acquisition and 12 of extinction between stimulation and killing.

In Experiments A, B and C, animals were allocated to the different stimulation conditions (other than control) after being matched for the threshold current (regular 7.7 Hz pulses) able to drive hippocampal theta. The group sizes given are for the start of each experiment. Data were lost for some animals at various stages of the experiments. Final group sizes are given in Table 1, together with the mean stimulating current intensities used in each group.

RESULTS

Figure 1 shows examples of the activity elicited in the hippocampal formation by theta-driving, theta-blocking and irregular stimulation of the septal area.

There were no effects of septal stimulation in any experiment on synaptosomal TH activity. The values for synaptosomal tyrosine hydroxylation rate in Experiment A in fmol ^3H_2O/min/mg synaptosomal protein were: control 207 ± 29, uncorrelated 223 ± 36 and correlated 229 ± 34, $n = 6$ for each group.

Table 1 shows the results of the soluble TH assay. Analysis of variance demonstrated in Experiment A a difference between groups, $F(2,15) = 6.13$, $p < 0.025$. Subsequent t-tests showed that this was due to a significant increase in TH activity in the Uncorrelated Theta-driving group relative to both the Food Controls, $t(15) = 2.85$, $p < 0.02$, and the Correlated Theta-driving group, $t(15) = 3.18$, $p < 0.02$; the latter two groups did not differ. The reliability of the effect of theta-driving in the Uncorrelated condition was demonstrated by a combined analysis of variance of the Uncorrelated Theta-driving and Food Control groups from Experiments A and B. This revealed a main effect of Stimulation, $F(1,20) = 9.08$, $p < 0.01$, with no effect of Replication and no interaction between these two factors. The delivery of food in Experiment B had no effect on TH activity: the means for the Control and Food Control groups were virtually identical, as were those for Uncorrelated Theta-driving and simple Theta-driving groups (Table 1). In Experiment C, the mean levels of TH activity were higher in both the Theta-driving and the Irregular Stimulation groups (which did not differ from each other) than among Controls (Table 1). A comparison between the combined stimulated groups and the Controls yielded $F(1,18) = 3.87$, which falls short of the 5% significance level ($p = 0.05$ at $F = 4.41$). However, a combined analysis of Experiments A–C again confirmed the reliability of the effect of low-frequency stimulation on hippocampal TH: there was a main effect of

Stimulation, $F(1,43)=8.44$, $p<0.01$, and no interaction between Stimulation and Replications.

In contrast to this effect of low-frequency septal stimulation, theta-blocking had no effect on TH activity in Experiment D ($F<1$).

DISCUSSION

Our results demonstrate a reliable increase in soluble, but not synaptosomal, TH activity in the hippocampus 15–30 days after low-frequency stimulation of the septal area. This effect appears not to depend on driving of the hippocampal theta rhythm, since levels of TH activity were similarly increased in the Theta-driving and Irregular Stimulation groups of Experiment C. It does depend on the use of low-frequency stimulation, however, since high-frequency theta-blocking stimulation failed to alter TH activity in Experiment D. Although the time which elapsed between stimulation and killing was longer in Experiment D than in the other experiments, the difference (33 days as compared to 30 days in Experiment B) is probably too small to account for the lack of effect of high-frequency septal stimulation on hippocampal TH. Thus, even if the increase in TH activity does not require the occurrence of regular trains of theta waves (Figure 1A), it may depend on the same low-frequency mechanisms as those which control theta.

The increase in hippocampal TH activity caused by low-frequency septal stimulation may depend upon quite subtle behavioral factors. In Experiment B this increase was unaffected by unsignalled food delivery, yet in Experiment A it occurred in the Uncorrelated but not in the Correlated Theta-driving group. These two groups correspond, respectively, to the 'truly random control' procedure [21] and a Pavlovian conditioning procedure (in which septal stimulation constitutes the conditioned stimulus and food delivery, the unconditioned stimulus). A difference between the behavioral effects of these two procedures is used to isolate changes which are due to the specific temporal relationship between conditioned and unconditioned stimuli in Pavlovian conditioning. Thus our results show that the effect of septal stimulation on hippocampal TH activity may be nullified if septal stimulation is made a conditioned stimulus for the delivery of food. In other unpublished experiments, moreover, we have consistently failed to observe any change in hippocampal TH activity when the behavioral training intervening between septal stimulation and killing involves footshock, although in other respects the experimental design was similar to that used here [9]. Such dependence of changes in brain catecholamine systems upon behavioral factors is not without precedent. For example, inescapable but not escapable footshock causes a short-term fall in brain noradrenaline levels and a long-term increase in TH activity [24,25].

There is a rough correlation between the behavioral effects of low-frequency septal stimulation and the neurochemical changes reported here. The uncorrelated Theta-driving and simple Theta-driving groups of Experiments A and B displayed facilitated acquisition and increased resistance to extinction of the barpressing response relative to unstimulated controls [13], while the Theta-blocking group of Experiment D [14] showed the reverse effects—retarded acquisition and decreased resistance to extinction (the behavioral effects of the Irregular Stimulation régime have not been studied yet in this task). Thus increased TH activity is generally accompanied by increased behavioral output. This is consistent with the analogy we drew (see Introduction) between theta-driving stimulation and stress, since repeated exposure to stressors can give rise to behavioral and neurochemical changes [8, 23, 24] similar to those observed in our experiments. However, the Correlated Theta-driving condition also retarded extinction of barpressing [13], yet it failed to alter TH activity (Experiment A). It is possible, therefore, that low-frequency septal stimulation can affect barpressing behavior in two ways: one associative (the Correlated Theta-driving condition) and not dependent upon changes in hippocampal TH, the other non-associative (the Uncorrelated and simple Theta-driving conditions) and dependent upon, or at least accompanied by, changes in hippocampal TH (for a discussion of the difference between associative and non-associative mechanisms in behavioral tolerance for stress, see [11,12]).

The exact nature of the changes in TH is not clear. The assay was carried out at the physiological pH of 7.2 in the presence of saturating cofactor and substrate concentration and the increase in activity therefore represents an increase in V_{max}; increases in V_{max} at this pH may be due either to enzyme induction or covalent modifications of the enzyme [1]. We have shown previously that synaptosomal and soluble TH activity do not necessarily show parallel changes. Thus reserpine, which causes enzyme induction [16], results in an increase in the V_{max} of the soluble enzyme but no increase in synaptosomal TH activity [5]. Conversely, repeated handling leads to a delayed, long-lasting increase in synaptosomal TH activity, but no change in V_{max} of soluble TH [10]. Normally less than 10% of the potential activity of the enzyme is expressed; the regulatory factors which limit the expression of the enzyme are not fully understood. The increase in the V_{max} may lead to an increased potential for tyrosine hydroxylation, which may require further regulatory mechanisms to be expressed [6].

The locus at which septal stimulation affects hippocampal TH activity is also unclear. The intra-hippocampal noradrenergic terminals belong to cell bodies which lie in the locus coeruleus and also innervate the septal nuclei [17]. However, antidromic stimulation does not lead to enzyme induction [7]. The septum sends cholinergic fibres to the hippocampus, and the noradrenergic terminals in the hippocampus have presynaptic muscarinic receptors [3]. Activation of these receptors depresses both basal and K^+-stimulated noradrenaline synthesis in hippocampal synaptosomes. Changes in the density of muscarinic receptors occur as a result of stress or the chronic administration of drugs [22]. Repeated electrical stimulation of the cholinergic septo-hippocampal pathway could lead to a decrease in the number of muscarinic receptors on noradrenergic terminals. This would lead to the removal of a tonic inhibition of synthesis and would appear as activation of the soluble enzyme, when compared with enzyme from control rats. An axo-axonal interaction between cholinergic septo-hippocampal fibres and the noradrenergic terminals could therefore underlie the change found in the enzyme isolated from the rats subjected to theta driving.

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