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BRIEF COMMUNICATION

The Role of Hippocampal Cholinergic Mechanisms in the Aquisition of a Barpress Response

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JAFFARD, R., A. EBEL, C. DESTRADE, G. AYAD, P. MANDEL AND B. CARDO. *The role of hippocampal cholinergic mechanisms in the acquisition of a barpress response.* PHARMAC. BIOCHEM. BEHAV. 5(3) 371–374, 1976. – The role of hippocampal cholinergic mechanisms in learning a bar-press response reinforced with food was investigated. Firstly, an interstrain comparison showed that mice having a low choline acetyltransferase activity in the dorsal hippocampus were quicker to associate the barpress with reinforcement. Secondly, when the activity of this enzyme was reduced by a subseizure electrical stimulation of the hippocampus learning was accelerated. It is suggested that acetylcholine availability at the hippocampal synapses slowed the apparition of these learned responses.

CRF lever press conditioning Strain differences Electrical stimulation of the hippocampus
Choline acetyltransferase Acetylcholinesterase

NUMEROUS experiments have been concerned with the involvement of cholinergic hippocampal mechanisms in behavioural inhibition [4,23]. Some results further support the view that internal inhibition related to these cholinergic systems [11] constitute a negative factor in learning active responses [4] such as the shuttle box problem [1,7] or a barpress response [17].

The present experiment was designed to evaluate the hippocampal cholinergic regulation of the acquisition of a barpress response on CRF (continuous reinforcement). For this purpose the neurochemical correlates of learning ability [14] were investigated in 3 inbred strains of mice, 1 of them being submitted to an electrical stimulation of the hippocampus [2]. The cholinergic mechanisms were studied by testing the 2 main enzymes involved in acetylcholine (Ach) synthesis (Choline acetyltransferase: ChAc; Ec 2.3.1.6) and degradation (Acetylcholinesterase: AChE; Ec 3.1.1.7).

METHOD

Animals

Animals were male BALB/c Orl (n = 114), C57BL/6 Orl (n = 47) and C57BR Orl (n = 47) mice approximately 12 weeks old at the end of experiments. At 8 weeks of age they were housed individually in cages in a constant temperature room (21°C) with ad lib access to food and water.

Procedure

According to previously described procedures [2,21] 57 animals of the BALB/c strain were implanted bilaterally under general anesthesia (sodium penthiobarbital:100 mg/kg) with bipolar electrode in the CA1 field of dorsal hippocampus. Eight days later the threshold intensity values (S) producing hippocampal after-discharges were individually determined. Animals of the 3 strains, including implanted BALB/c mice were then assigned to different groups (see Table 1) and were used for behavioral or neurochemical studies.

Behaviour. Operant behaviour with food reward was studied in a Skinner box in which the lever and and food cup are separated by a small partition [2,9]. A progressive food deprivation schedule was intitiated for all animals 3 days before the learning session began.

TABLE 1

INITIAL LEVEL OF RESPONSES, RESPONSES TO ACHIEVE THE CRITERION IN CRF LEVER PRESS CONDITIONING (LEFT) AND ENZYMATIC ACTIVITIES IN THE DORSAL HIPPOCAMPUS (RIGHT) OF NON IMPLANTED (NI) INBRED STRAINS OF MICE (UPPER PART OF THE TABLE). TIME-DEPENDENT EFFECTS OF HIPPOCAMPAL STIMULATION (ST) ON THESE PARAMETERS IN BALB/c MICE (LOWER PART). RESULTS ARE EXPRESSED AS MEANS ± SEM

		BEHAVIORAL DATA		BIOCHEMICAL DATA	
	GROUPS	RESPONSES DURING THE FIRST 5 MIN	RESPONSES TO CRITERION	ChAc (1)	AChE (2)
INTERSTRAIN COMPARISON	BALB/c NI	6.1 ± 0.3	25.8 ± 1.1	15.32 ± 0.35	4.15 ± 0.09
	C57BL/6 NI	5.6 ± 0.3	19.9 ± 1.2	11.11 ± 0.51	3.88 ± 0.05
	C57BR NI	5.3 ± 0.3	11.6 ± 1.2	10.22 ± 0.46	3.73 ± 0.07
EFFECT OF HIPPOCAMPAL STIMULATION	BALB/c NST	6.1 ± 0.2	26.4 ± 1.0	15.06 ± 0.64	3.98 ± 0.08
	BALB/c ST - 5 min	6.2 ± 0.5	26.5 ± 1.2	14.51 ± 0.70	4.28 ± 0.08
	BALB/c ST - 24 hr	6.0 ± 0.3	16.9 ± 0.6	10.65 ± 0.61	3.99 ± 0.08

(1) μmol/g proteins $^{-1}$ H $^{-1}$
(2) mmol/g proteins $^{-1}$ H $^{-1}$

At the start of this session, BALB/c mice were at 17% under their free-feeding weight; for C57BL/6 and C57BR weight losses were respectively 19 and 16% [3,9]. Each animal was introduced in the apparatus for a 30 min acquisition session on CRF schedule. Responses (lever press followed by eating) were registered on a pen-recorder. Before training, implanted mice of the BALB/c strain were assigned to 3 groups (see Table 1): the animals of 2 groups were stimulated during 80 sec at 50% of the intensity threshold (S/2) already individually determined (S/2 = 9.6 ± 0.8 μA). This hippocampal stimulation was initiated 5 min (ST-5 min group) or 24 hr (ST-24 hr group) before the start of the learning session. Mice of the remaining third group were not stimulated (NST group).

Neurochemical analysis. For the neurochemical investigations, animals of the 3 lines were maintained under the same conditions as for the behavioural studies (see Method above). Two sets of experiments were performed: ChAc and AChE activities were measured in the dorsal hippocampus of BALB/c, C57BL/6 and C57BR strains (non implanted). In the BALB/c strain enzyme activities were investigated 5 min or 24 hr after hippocampal bilateral stimulation (80 sec at S/2). A group of non stimulated mice was used as control.

Dissection. The animals were killed by cervical dislocation, the brain rapidly removed and placed on dry ice. The 2 dorsal hippocampus were dissected and immediately frozen. The whole operation requires less than 90 sec.

Analytical. For biochemical determinations the samples were homogenized at 0–2°C in 0.5% Triton X-100 (100 μg tissue/1 μl) using Potter-Elvehjem glass homogenisers. Samples were diluted with 0.05% bovine serum albumin. Choline acetyltransferase was measured according to the micro-technique of McCaman and Hunt [15] as modified by Goldberg *et al.* [6] by following the incorporation of (1^{-14}C) acetate from acetyl-CoA into acetylcholine. The (1^{-14}C) acetylcholine formed was precipitated with potassium periodide and the amount of radioactivity present was counted in a Packard scintillation counter. The enzyme activity was calculated from the known specific activity of the (1^{-14}C) acetyl-CoA (NEN Chemicals, 59.2 mCi/mmole).

Acetylcholinesterase was determined by the method of McCaman *et al.* [16], based on the hydrolysis of (1^{-14}C) acetylcholine (NEN Chemicals, 2.43 mCi/mmole). Unhydrolyzed substrate was precipitated as a Reinecke salt. Nonspecific cholinesterases were inhibited by addition of iso-octamethyl-pyro-phosphoramide (10^{-6}M). Proteins were measured by the method of Lowry *et al.* [13].

RESULTS

Behavioural and biochemical data are summarized in the table.

Behavioural data

The speed of acquisition was individually determined by the number of responses registered before the animal had reached a stable criterion of 10 responses or more during 5 consecutive min.

A two-way analysis of variance showed a statistically significant effect of strain (upper part of the table) on responses to criterion ($F(2,78) = 37.5$, $p<0.001$) with C57BR>C57BL/6>BALB/c ($p<0.001$ in each case).

Hippocampal stimulation on BALB/c (lower part of the table) lowered the mean number of responses to criterion when this treatment was applied 24 hr before the learning

session began ($t(15) = 7.63$, $p<0.001$) but not when the delay interval was only 5 min ($t = 0.06$).

Implantation by itself had no effect. In addition, there were no differences between performances of the 6 groups during the 1st 5 min of the session ($F(5,96) = 1.1$).

Biochemical Data

As can be seen in the upper right part of the table activity of ChAc was much greater in the dorsal hippocampus of BALB/c than C57BL/6 ($t(22) = 5.61$, $p<0.001$) and C57BR ($t(22) = 6.55$, $p<0.001$). Electrical stimulation of the hippocampus in BALB/c reduced ChAc activity in the ST 24 hr group ($t(22) = 6.01$, $p<0.001$) but was ineffective in the ST 5 min group.

Slight differences were also observed between AChE activity of the 6 groups but hippocampal stimulation had no effect on this enzyme. Finally, ChAc activity was positively correlated with responses to criterion ($r = +0.94$; $p<0.02$).

DISCUSSION

Our results show that the speed of acquisition of a simple barpress response associated with food reward is inversely correlated with choline acetyltransferase activity in the dorsal hippocampus. This was suggested both by interstrain differences and by the neurochemical and behavioural effects of hippocampal electrical stimulation on BALB/c [5]. They further support the view that acetylcholine availability at the synaptic level constitute a negative factor in learning such an active response.

This conclusion is in accord with several experimental findings: First, it has been demonstrated that lesions of the septo-hippocampal pathway which mediate response inhibition [20] enhance active avoidance in rats [7] and mice [18] and have similar effects on a CRF lever press conditioning [17]; in addition, anticholinergic drugs generally lead to similar results [1,10]. Furthermore, interstrain comparisons in different behavioural patterns strongly suggest that C57 mice exhibits behavioural inhibitory defects. Thus, C57 mice were shown to be poor problem solvers in learning tasks involving response inhibition (unpublished observations). In contrast higher exploratory behaviour has been observed in these mice when compared to BALB/c and DBA strains [11, 19, 22].

Such interstrains differences in exploratory behaviour might explain the apparently elevated speed of acquisition shown by C57 mice during the 1st 5 min of the learning session, i.e. an elevated exploratory behaviour may tend to accelerate acquisition by increasing the number of fortuitous responses and consequently the opportunity for learning [1]. However, this non-specific hypothesis only partly explains these observations, since the initial level of responding (1st 5 min of the session) is the same in the 6 groups.

It seems that these differences are not cognitive; in fact it has been shown that for BALB/c mice, the number of responses necessary to reach the criterion is greatly reduced when the learning sessions are spaced over longer intervals [9] while the inverse phenomenon is observed in C57 mice [3,8].

Finally it seems reasonable to postulate that a defect in cholinergic hippocampal mechanisms facilitates earlier occurrence of learned responses when subjects are submitted to a single acquisition session.

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