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Choline Uptake and Permanent Memory Storage[1]

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RAUCA, CH., E. KAMMERER AND H. MATTHIES. *Choline uptake and permanent memory storage.* PHARMAC. BIOCHEM. BEHAV. 13(1) 21–25, 1980.—The uptake of ^{3}H-choline and its incorporation into ^{3}H-acetylcholine was studied in vitro on hippocampus slices obtained from animals showing a good or poor long-term memory. The animals were selected on the basis of their retention performance when tested by a brightness discrimination model. The ^{3}H-choline uptake and the incorporation of ^{3}H-choline into ^{3}H-acetylcholine was higher in hippocampus slices from animals showing good retention compared to those from animals with poor retention. The level of high affinity uptake of choline into hippocampus slices may serve as an indicator of the cholinergic activity in this structure under in vivo conditions. The present findings suggest that individual differences in the activity level of the hippocampal cholinergic system do exist and are capable of influencing the retention of the individual animals to a variable degree.

Acetylcholine Choline Uptake Incorporation Long-term memory Brightness discrimination Hippocampus

RECENTLY many papers reported on the participation of hippocampal cholinergic systems in acquisition and consolidation of conditioned reactions [4, 6, 19].

The pharmacological influence of cholinomimetics and cholinolytics on cholinergic synapses in the hippocampus led to, in part, contradictory findings concerning the effects of these drugs on acquisition and consolidation of long-term memory [8, 33, 35]. These contradictory findings have been individual and species differences in the activity of cholinergic neurons in the hippocampus [9, 14, 15]. In experiments attempting to characterize the activity status of hippocampal cholinergic systems and the influence of the latter on the process of memory consolidation, the activities of acetylcholine-synthesizing and acetylcholine-degradating enzymes were determined [14,15]. The results of that study revealed a correlative relationship between activity of choline acetylase and retention performance of the animals. The activity of choline acetylase is an essential factor in the regulation of acetylcholine synthesis. However, it is not clear if under physiological conditions the enzyme functions in the saturation range and if it can be considered as a limiting factor for acetylcholine synthesis [12, 17, 18].

In the brain the availability of the precursor of acetylcholine synthesis is limited and determined by the activity of the Na^+-dependent high affinity uptake system for choline [5,29].

On the one hand, acetylcholine synthesis and/or acetylcholine turnover can be controlled by coupling the functional activity of cholinergic neurons with the Na^+-dependent high affinity uptake mechanisms for choline [16, 30, 31, 32] and, on the other hand, the release of the cholinergic transmitter evoked by depolarisation can exert an influence on choline uptake [3, 7, 21, 26]. Therefore, the Na^+-dependent high affinity choline uptake can be considered as a limiting step for the rate of acetylcholine synthesis and consequently that link in the cholinergic transmitter mechanisms which most likely should reflect different functional activities of populations of cholinergic neurons.

In view of the above mentioned relationship which exists between the behaviour of animals in the learning experiment and/or their ability to consolidate an acquired experience and the functional activity of cholinergic neurons in the hippocampus, the Na^+-dependent high affinity choline uptake should be considered to represent the neurochemical correlate of this relationship.

In the present study, the uptake of tritium-labelled choline and its incorporation into ^{3}H-acetylcholine was studied on hippocampus slices obtained from animals showing a good or poor long-term memory. The animals were selected on the basis of their retention performance when tested by a brightness discrimination model. It was the aim of this study to find neurochemical correlates in the cholinergic system of the hippocampus on animals showing nearly identical acquisition behaviour but different ability concerning the consolidation tested by a 24-hour retention procedure.

METHOD

Training Procedure

Thirty male Wistar rats weighing 150–180 g were trained in a semiautomaic y-c chamber to learn a footshock-

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motivated brightness discrimination reaction [23, 24, 25]. The training session involved 31 runs. After every three runs the side of illumination in the alleys was changed so as to avoid position training. Running into the dark alley of the chamber was punished by application of a 1 mA footshock.

In one of experiments, twenty-four hours after training, retention was tested during a relearning session using the same procedure as described for training. In order to measure the retention performance, the following parameters were calculated: (a) Difference in the number of positive responses (ΔR): a positive response means, that the last run before and the first run after changing the side of illumination are correct. ΔR was calculated by subtraction of positive responses observed in the training session from the number of positive responses in the relearning session. (b) Difference between the number of errors during training and relearning multiplied by 100 and divided by the number of errors during training (% savings). The ΔR-value served as a criterion for classification of animals into two groups. Animals showing "good retention" (group A) achieved a ΔR of 3 or>3, while animals exhibiting "poor retention" (group B) showed a ΔR of 2 or<2. In a second series the uptake of ^{3}H-choline was estimated in naive animals in comparison with trained rats, using the same training procedure as described before.

Uptake and Incorporation of ^{3}H-choline in Hippocampus Slices

The in vitro test using the hippocampus slice model was performed one week after the check of retention (series 1) or training (series 2), respectively.

The tissue slices were prepared as described in detail elsewhere [34]. From the hippocampus of each animal two parallel samples of 12 slices were used for duplicate in vitro measurement of choline uptake and incorporation. The samples were preincubated in Krebs-Henseleit-solution [20] with aeration (95% O_2 and 5% CO_2) at 37°C for 20 min. After addition of 5 μCi ^{3}H-choline (purchased from the Radiochemical Centre, Amersham, Great Britain, specific activity 16.5 Ci/mmole in series 1 or 13.0 Ci/mmole in series 2) the samples were incubated under identical conditions for 5 min. Thereafter the slices were thoroughly washed with cold Krebs-Henseleit-solution and frozen on dry ice.

Extraction of ^{3}H-choline and ^{3}H-acetycholine from the Tissue and Measurement of Radioactivity

The extraction of labelled choline and acetylcholine and subsequent thin layer chromatographic separation of the substances have already been described [2,28]. In order to calculate the individual recovery of ^{3}H-choline and contamination of ^{3}H-acetylcholine due to ^{3}H-choline an internal standard of ^{14}C-choline was applied to each sample during extraction and thin layer chromatographic separation. Radioactivity of labelled choline and acetylcholine was measured in a toluene scintillator (6.0 g PPO, 0.25 g POPOP, 100 g naphthalene, 140 ml methanol, toluene ad 1000 ml) on an Intertechnique scintillation spectrometer (Plaisir, France) and computed as disintegrations per minutes. The uptake of choline was expressed either as absolute values

$$\frac{\text{d.p.m. } ^3\text{H-choline}_{(\text{tissue})} \times 100}{\text{mg protein} \times \%\text{ recovery of } ^{14}\text{C-choline}} \quad (1)$$

or as corrected values related to the radioactivity in the incubation medium.

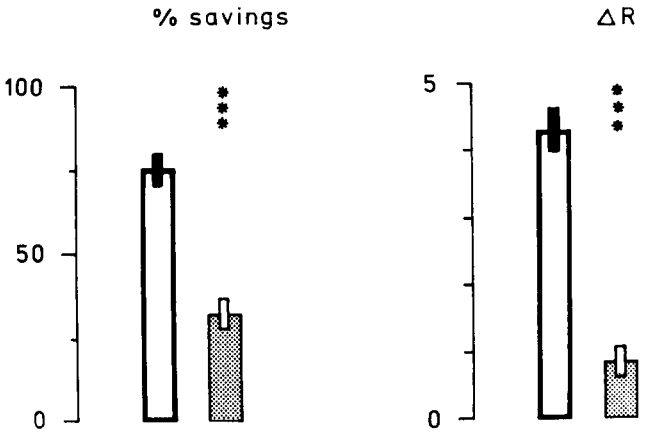

FIG. 1. The % savings and the ΔR values for A (empty bars; n=18) and B (dotted bars; n=12) groups are represented. The statistical evaluation was performed using the Mann-Whitney U-test; denotes ***$p<0.001$.

$$\frac{\text{d.p.m. } ^3\text{H-choline}_{(\text{tissue})} \times 100}{\text{mg protein} \times \%\text{ recovery of } ^{14}\text{C-choline}} \times \frac{1}{^3\text{H-choline/0.01 } \mu\text{l incubation medium}} \quad (2)$$

The incorporation of ^{3}H-choline into acetylcholine was expressed in a similar manner using the d.p.m. ^{3}H-acetylcholine of tissue. The protein content in the hippocampus slices was determined by an amido black technique [27].

Statistical Methods

Statistical evaluation of the findings was performed using the U-test. Calculation of regression and correlation functions between ΔR and choline uptake was carried out by a Hewlett-Packard calculator 9100 A.

RESULTS

The retention check in series 1, 24 hr after training, using the brightness discrimination reaction showed that 60% of the animals (18 of a total of 30) achieved a ΔR value of 3 or>3 and 40% of the animals (12 of a total of 30) a ΔR value of 2 (n=1) or<2 (n=11). The ΔR values and the % savings for the A and B groups are represented in Fig. 1.

The training behaviour of the animals of both groups showed differences as to the number of positive responses but only small deviations in the number of negative runs (reinforcements). The relearning test showed that in group A the number of positive responses (from 3.17 ± 0.35 to 7.39 ± 0.35; $p<0.001$) was considerably more increased than for group B animals (from 4.83 ± 0.51 to 5.25 ± 0.41; $p<0.05$). The number of negative runs in the relearning test was found to be substantially decreased in group A (from 11.28 ± 0.79 to 3.22 ± 0.60; $p<0.01$) to an amount more pronounced than in the B group (from 9.17 ± 0.90 to 6.00 ± 0.74; $p<0.05$).

Uptake and incorporation of ^{3}H-choline were studied in vitro on hippocampal slices from the animals of groups A and B. The hippocampus slices from group A animals revealed a significantly higher uptake of ^{3}H-choline than slices from group B animals. Fig. 2 depicts the ^{3}H-choline uptake expressed in d.p.m./mg protein (absolute values, formula 1 in METHOD).

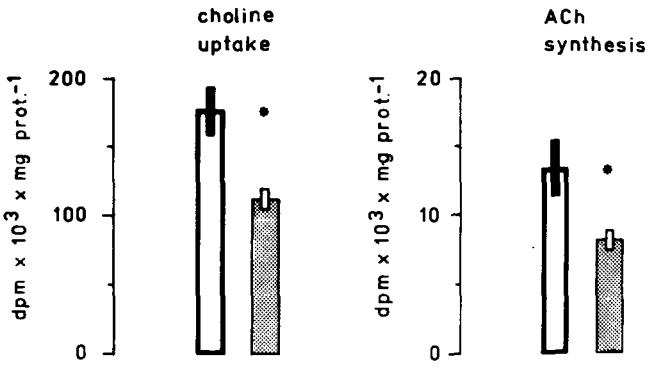

FIG. 2. The uptake and incorporation of ^{3}H-choline on hippocampus slices from animals of A (empty bars; n=18) and B (dotted bars; n=12) groups expressed as absolute values (see METHOD, formula 1). The statistical evaluation was performed using the Mann-Whitney U-test; denotes $*p<0.05$.

$$Q = \frac{\text{dpm ACh}}{\text{dpm choline}} \times 100$$

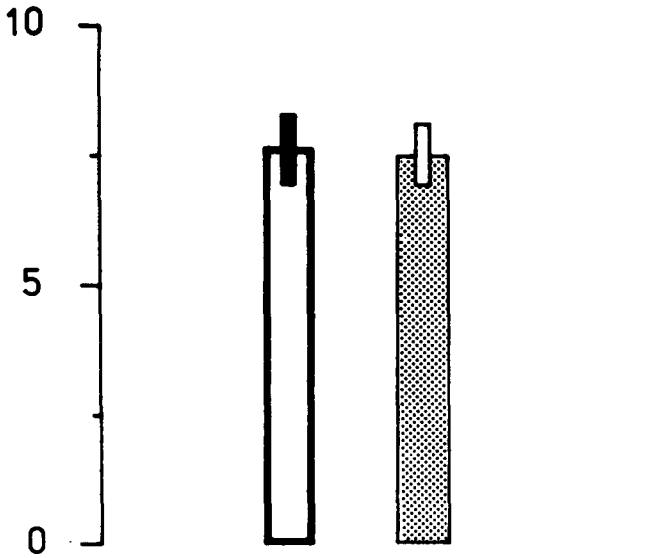

FIG. 4. The calculated Q values, i.e. relationship of absolute values for ^{3}H-acetylcholine×100, divided by the absolute values for ^{3}H-choline, of A (empty bars; n=18) and B (dotted bars; n=12) groups are represented.

The relation of corrected values (formula 2 in METHOD) for the uptake of ^{3}H-choline to the retention performance of the animals, expressed as ΔR values, was calculated to be a linear correlation, as demonstrated in Fig. 3.

The classification of all animals in groups based exclusively on the percentage savings and the corresponding values for the ^{3}H-choline uptake are represented in Table 1. The stepwise decrease in the ^{3}H-choline uptake paralleling the % savings was to be expected, due to correlative relationships between ΔR values and ^{3}H-choline uptake.

Furthermore, a higher incorporation of ^{3}H-choline into ^{3}H-acetylcholine was observed for group A animals over group B animals (Fig. 2).

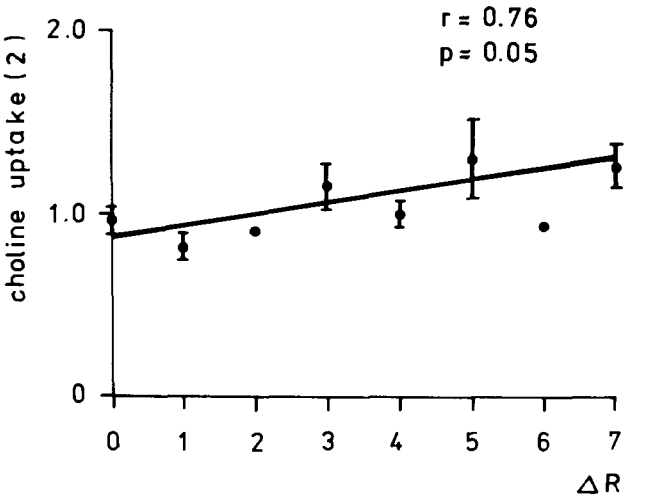

FIG. 3. The ^{3}H-choline uptake, expressed as corrected values (see METHOD, formula 2), is plotted against retention performance of all animals (n=30).

TABLE 1

THE CLASSIFICATION OF ALL ANIMALS INTO GROUPS BASED ON THE PERCENTAGE SAVINGS AND THE CORRESPONDING VALUES FOR THE ^{3}H-CHOLINE UPTAKE

Group	% Saving $\bar{X}$ ± SEM)	^{3}H-choline uptake (corrected values, $\bar{X}$ ± SEM)
1 (60–100% savings) (n=14)	82.4 ± 3.3	1161 ± 79
2 (40–50% savings) (n= 9)	47.4 ± 1.3	1007 ± 103
3 (0–25% savings) (n= 7)	18.0 ± 3.1	948 ± 35*

Significant difference between group 1 and 3; $*p<0.05$.

However, including the animals of both groups, a highly significant ($p<0.0027$) linear correlation was found to exist between ^{3}H-choline uptake and ^{3}H-acetylcholine synthesis.

The calculated Q values, i.e. relationship of the d.p.m. for ^{3}H-acetylcholine×100, divided by the d.p.m. for ^{3}H-choline, showed no differences between groups (Fig. 4).

In a second series of experiments, the uptake of ^{3}H-choline and its incorporation into acetylcholine was estimated one week after training session. Naive rats of the same stock and age served as controls. The number of negative runs (9.50 ± 0.43) and positive responses (4.83 ± 0.48) in this series of trained animals was in essential identical with these of group A and B in series 1. The mean number of negative runs was considerably higher than that observed in the relearning test. The uptake and incorporation of ^{3}H-choline, expressed as absolute values, in hippocampus slices of trained animals showed no differences compared to naive animals (Table 2).

DISCUSSION

In the present experiments, the classification of the

TABLE 2

UPTAKE AND INCORPORATION OF ^{3}H-CHOLINE IN HIPPOCAMPUS SLICES OF TRAINED AND NAIVE ANIMALS (ABSOLUTE VALUES)

	Trained animals n=6			Naive animals n=6		
Uptake of ^{3}H-choline	84	635 ± 7	821	100	917 ± 6	030
Incorporation of ^{3}H-acetylcholine	4	337 ±	649	3	980 ±	390

Differences between trained and naive animals are not significant.

animals into two groups, i.e. of either good or poor retention, was based on the values ΔR and % savings which were achieved in the relearning test of a brightness discrimination. One week after testing the retention of the trained animals, the uptake and incorporation of ^{3}H-choline were measured in hippocampus slices obtained from these animals. This period was chosen because, most likely, the activity changes of cholinergic systems in the hippocampus, elicited by the training or relearning procedures, would have decayed after one week.

This assumption is supported by the fact, that in series 2 no differences in the uptake and incorporation of choline between trained and naive animals could be observed. To eliminate the differences in the specific activities of the choline isotopes used in both series, the absolute values for the uptake can be expressed as p moles/mg protein/5 min. Using these calculations the values for the uptake of choline were 49.0 ± 4.8 p moles in group A, 30.8 ± 1.9 p moles in group B of series 1, 35.3 ± 2.1 p moles in naive animals and 29.6 ± 2.7 p moles in trained animals of series 2. Animals with good retention only showed a higher activity of the uptake system.

Taking into considerations findings obtained on synaptosomes [10, 11, 13] and our results on hippocampus slices [28] it should be assessed that the capacity of the Na^+-dependent high affinity transport system, was by far not exhausted and the measured amount of intracellular ^{3}H-choline was exclusively taken up in the cell by the Na^+-dependent high affinity transport system.

The choline content of the hippocampus slices was not determined. Therefore, it must be reservedly noted that the differences observed in ^{3}H-choline uptake between animals with good and poor retention performance can occur only if the choline pool size is identical for both groups.

The ^{3}H-choline uptake into hippocampus slices from animals with different ability for consolidation of long-term memory of a brightness discrimination reaction showed statistically significant deviations.

Likewise, the incorporation of ^{3}H-choline into ^{3}H-acetylcholine was higher in hippocampus slices from animals showing good retention compared to those from animals with poor retention.

As described in METHOD, runnings into the dark alley of the Y-maze were punished by application of footshocks in the training as well as in the relearning session. Animals in group B made more errors during the retention test and therefore have received more footshocks than animals in group A. The difference of punishments in the relearning session between group A (3.22 ± 0.60) and B (6.00 ± 0.74) was smaller than that observed in trained rats of series 2. One week after training, the uptake of choline was the same as observed in naive animals. Therefore it can be assumed that differences in the uptake of choline, measured 7 days after training or relearning, are not due to the number of footshocks but may be considered as a reflection of the ability for consolidation of long-term memory.

Furthermore, a linear correlation was found between ^{3}H-choline uptake and incorporation into ^{3}H-acetylcholine. Animals with good and poor retention (7.52% ± 0.66 and 7.38% ± 0.51, respectively) showed an almost identical percentage relationship of ^{3}H-choline incorporation to ^{3}H-choline uptake. This finding can be interpreted to mean that in our experiments the choline uptake may be the rate-limiting step for the acetylcholine synthesis [22,26]. An enhanced neuronal activity of the hippocampus in vivo elicited by electrical stimulation of the septo-hippocampal tract or by application of convulsive drugs leads to an increase in the V_{max}-values for the Na^+-dependent high affinity choline transport and a rise in the acetylcholine turnover in vitro [1, 16, 31, 32]. In view of these considerations, the conclusion can be drawn differences existing in activity level of cholinergic neurons of the hippocampus in vivo are reflected in vitro by the magnitude of the Na^+-dependent high affinity choline uptake in hippocampus slices [1, 30, 31].

The level of the Na^+-dependent high affinity uptake of ^{3}H-choline into hippocampus slices may serve as an indicator of the cholinergic activity in this structure under in vitro conditions.

A linear correlation was found between the corrected d.p.m. for the ^{3}H-choline uptake and the retention performance expressed as ΔR values. This relationship suggests that a definite activity level of hippocampal cholinergic systems is required for the formation of a 24-hr retention of a brightness discrimination reaction. However, the level of the cholinergic activity in the hippocampus shows a great individual variability and is related to the extent of consolidation of a long-term memory. In experiments on different mice strains, Jaffard [15] found, on the basis of determination of activity of the acetylcholine-synthesizing enzyme and its relation to the formation of long-term memory, a correlation between activity of the hippocampal cholinergic systems and memory consolidation. Izquierdo [14] observed that the consolidation of a positive conditioned reaction was accompanied by an increase in choline acetylase activity in hippocampus and frontal cortex. Grecksch [9] in experiments on rats characterized the individual activity of hippocampal cholinergic systems by the psychomotoric activity and the effect of cholinominetics and cholinolytics on the latter, and found correlation between single parameters and the retention capability of the animals.

The stimulation of the medial septum (eliciting slow-wave rhythmic activity in the hippocampus) immediately after training led to an improvement of the 24-hr retention of a brightness discrimination reaction [36].

The cholinergic septo-hippocampal system is decisively involved in the consolidation of the brightness discrimination reaction as well as in the formation of long-term memory of many conditioned reactions.

The present findings suggest that individual differences in the activity level of the hippocampal cholinergic system do exist and are capable of influencing the retention performance of the individual animals to a variable degree. Thus, functional differences in neuronal activity in the hippocampus in vivo can be proved in studies on hippocampus slices in vitro.

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