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Influence of Post-Training Intrahippocampally Applied Oxotremorine on the Consolidation of a Brightness Discrimination[1]

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GRECKSCH, G., T. OTT AND H. MATTHIES. *Influence of posttraining intrahippocampally applied oxotremorine on the consolidation of a brightness discrimination.* PHARMAC. BIOCHEM. BEHAV. 8(3) 215–218, 1978. – The posttraining intrahippocampal injection of oxotremorine revealed an improvement of the retention performance in a brightness discrimination task. The oxotremorine effect seemed to be dependent on distinct variables of training and was restricted to rats exhibiting a good acquisition performance. Scopolamine impaired the retention performance of animals with few training errors. The role of hippocampal cholinergic synapses for consolidation was discussed.

Oxotremorine Scopolamine Cholinergic synapses Memory consolidation Brightness discrimination Hippocampus Interindividual variability

SINCE the investigations of Buresova *et al.* [2] with atropine, many neuropharmacological studies revealed the functional activity of cholinergic synapses of the Central Nervous System to be a critical variable of acquisition and consolidation. However, the heterogenous results scarcely permit general conclusions to be drawn with regard to the role of cholinergic synapses during memory formation. The existence of contradictory findings (for reviews see [1, 7, 11, 14]) may be explained by (1) quite limited possibilities of a selective influence of potentially involved different cholinergic systems, (2) lack of specific behavioral parameters required for quantitative evaluation and (3) use of different learning models.

The posttrial application of drugs enables consolidation processes to be influenced in a relatively selective manner [13]. However, studies employing a posttrial administration of cholinolytic drugs showed improvement in retention [5,6], impairment of retention [20,25] or no influences on retention [8].

In studies with physostigmine, Stratton and Petrinovich [22] obtained qualitatively different effects, depending on the learning ability of the animals. Whereas good learners showed impaired retention after the cholinergic activation, the retention of poor learners was improved.

In our previous experiments, using an intrahippocampal injection of atropine, the selective cholinolytic influence on consolidation revealed an improvement in retention [18]. Subsequent studies using scopolamine at various doses showed that the mean effect obtained from all animals was a positive influence of this drug on consolidation, but a detailed analysis of the results exhibited positive as well as negative effects depending on the dose applied and the number of training errors [15].

The experiments presented in this paper were designed to examine the influence of cholinergic activation of dorsal hippocampus on consolidation by an intrahippocampal posttrial application of oxotremorine using the same training model, namely, a footshock motivated brightness discrimination as described by Ott *et al.* [16]. A total of 221 male Wistar rats from our own breeding stock, weighing 200–250 g at the time of implantation, were used. One week before the experiments, the animals were provided with chronically implanted microcannula into the dorsal hippocampus using the coordintes AP = 3.1 mm; lateral = 3.1 mm and 3.1 mm deep according to Skinner [19]. The microinjections of substances were accomplished using a Hamilton microsyringe, connected to the inner cannula through a teflon hose. One µl solution per hippocampus was infused within approximately 30 sec. Immediately before the experiment, all animals received an infusion of one µl of artificial cerebrospinal fluid (ACF) bilaterally into the hippocampus.

METHOD

Trainings Procedure

Initially, the animals were allowed to stay for 10 min in the semiautomatic Y-chamber for habituation. Thereafter by application of 1 mA current to the grid floor the animals escaped from the starting compartment and had to run in the illuminated alley of the chamber, since entering the non-illuminated alley was punished by an electric foot-

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shock. A run was evaluated as correct when the animals ran directly into the illuminated alley of the chamber. After every three runs the direction of the illuminated alley was changed so as to avoid position training. The mean time between two runs was 60 sec (39–90 sec). The training sessions involved 22, 31 or 40 runs.

Retention Test

The retention was tested 24 hr after training using a relearning procedure performed in the same way as the training procedure.

Evaluation

The number of incorrect runs during training and relearning was determined for the calculation of percentage of savings:

$$\text{Savings percent} = \frac{\text{training errors} - \text{relearning errors}}{\text{training errors}} \times 100.$$

Treatment

Five and twenty-five hundredths μg oxotremorine, dissolved in ACF were bilaterally applied to the animals immediately after the training procedure whereas the control animals received 1 μl of ACF per hippocampus. In comparison two further groups were treated posttraining with 0.1 mg/kg scopolamine and saline solution.

Statistics

The statistical evaluation was accomplished using the Mann-Whitney U test or by the two-tailed *t*-test, when the number of animals under investigation was equal to 40.

RESULTS

In all training groups, i.e., training sessions involving 22, 31 or 40 runs, the posttrial application of oxotremorine revealed a statistically significant retention improvement over the corresponding controls (Table 1). This effect was most pronounced for the group with the shortest training (22 runs). This result suggest that the oxotremorine effect seems to be dependent on distinct variables of training. To examine this question the population of trained animals performing 31 runs was analysed in more detailed terms.

Figure 1 represents the mean values of training and relearning errors. As demonstrated in this figure, the retention values of oxotremorine-treated rats were significantly higher than those of controls, whereas both groups exhibited identical training performance.

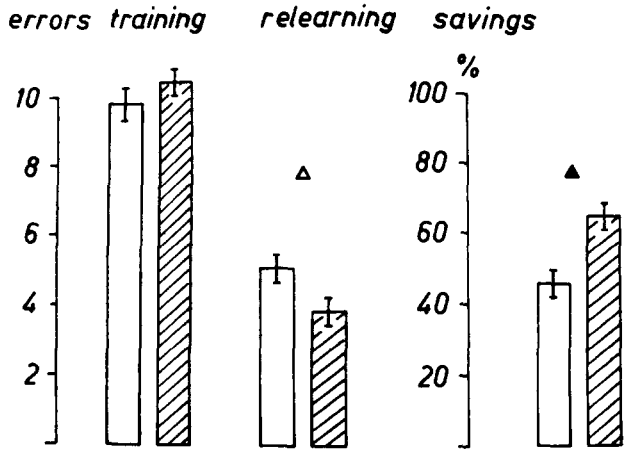

FIG. 1. Effect of a posttraining injection of 5.25 μg oxotremorine on the retention after a training session involving 31 runs (shaded bars = oxotremorine group; open bars = control group).

However, when arranging the oxotremorine-treated and control animals in several groups according to the number of training errors, a dependence of oxotremorine effect on the number of training errors become evident (Fig. 2). Thus, posttraining application of oxotremorine facilitated retention to a considerable extent in animals with less training errors, i.e., the oxotremorine effect respresented in the average value was actually confined to rats exhibiting a good acquisition performance. However, this interpretation presumed that the relative frequency of animals belonging to the two groups was based on statistical distribution in symmetric terms. As is shown in the upper part of Fig. 2, this statistical requirement is fulfilled.

These findings seemed to differ from previous results showing a statistically significant improvement of the retention of the brightness discrimination reaction after posttraining application of cholinolytic drugs [15,18]. Even in further experiments, the posttraining injection of 0.1 mg/kg scopolamine had no influence on retention calculated on the basis of mean values involving all animals. However, if the animals were arranged in groups according to the number of training errors, a clear cut effect of scopolamine on retention became evident.

As demonstrated by the straight line regression curves,

TABLE 1

INFLUENCE ON RETENTION INDICES ± SEM BY APPLICATION OF 5.25 μg OXOTREMORINE PER HIPPOCAMPUS

Number of Runs During a Training Session	% of Retention Indices		No. of Animals	*p*
	Control Group	Oxotremorine Group		
22	10 ± 6.84	46 ± 8.80	9	<0.03
31	46 ± 3.44	65 ± 3.26	117	<0.001
40	48 ± 6.94	67 ± 4.24	27	<0.05

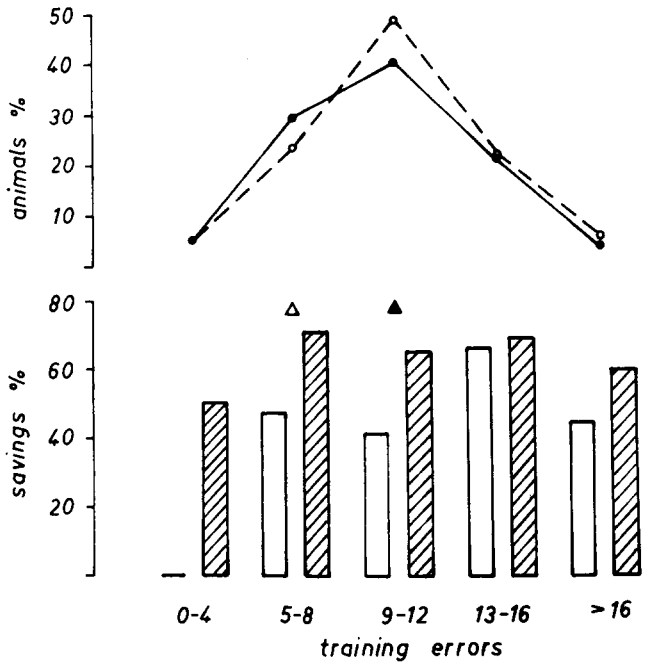

FIG. 2. Effect of oxotremorine on retention vs number of training errors after a training session involving 31 runs (shaded bars = oxotremorine group; open bars = control group). Top: Frequency distribution of the animals vs number of training errors (solid line = control group; broken line = oxotremorine group).

scopolamine impaired the retention performance of animals with few training errors and vice versa, the retention performance was improved in animals exhibiting many training errors (Fig. 3).

In addition to these results, the regression curves calculated for the findings represented in Fig. 2 reveal that scopolamine and oxotremorine influenced the retention performance in a qualitatively different manner, depending on the number of training errors.

DISCUSSION

Generally the present results permit the conclusion that the effectivity of consolidation processes, working after the training period are critically dependent on the functional activity of the cholinergic afferences of the hippocampus. Likewise, experiments with 7 Hz stimulation of the medial septum (instead of oxotremorine application) also suggest the importance of the cholinergic septo-hippocampal system for the consolidation process, since this kind of cholinergic activation [21], also revealed an improvement of retention [24]. Thus, both the stimulation of medial septum [23] and the intrahippocampal injection of oxotremorine [17] elicited a hippocampal theta rhythm. The findings obtained after electrical stimulation of the hippocampus also supported the assumption that cholinergic hippocampal mechanisms exerted effects on consolidation [4,10].

Furthermore, the present findings suggest that the functional activity of hippocampal cholinergic synapses is shifted by training towards a distinct optimum. A similar conclusion was drawn by Deutsch [3]. This interpretation is consistent with the hypothesis postulating that cholinergic synapses are altered by learning processes. The different functional states of cholinergic terminals at different times in the acquisition and consolidation of a learned behavior were also suggested by the fact that the hippocampal acetylcholine fractions show very strong changes in a relative short time [12]. The effectivity of the process, stimulated by training, is at least partly dependent on the genetically determined and individually different activity level of the septo-hippocampal cholinergic system. For instance, Izquierdo [9] found differences in the choline acetylase activity in the hippocampus in dependence on the discriminating performance. Taking this aspect into consideration it becomes clear that the effect of cholinergic

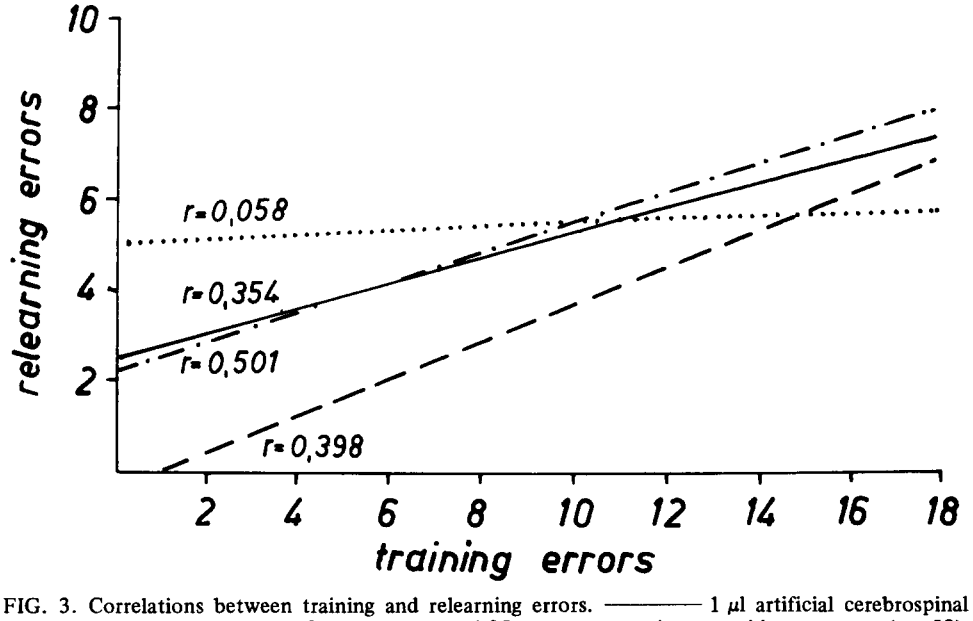

FIG. 3. Correlations between training and relearning errors. ———— 1 μl artificial cerebrospinal fluid per hippocampus (n = 59); — — — — 5.25 μg oxotremorine per hippocampus (n = 58); —·—·—·— 1 ml saline solution per 100 g of body weight (n = 44); ..... 0.1 mg/kg scopolamine IP (n = 24).

A week following surgery, the rats were screened for ICSS in an apparatus different from that used in the main experiment. The current producing asymptotic peak rates of responding was determined for each rat by a rate/intensity procedure [8]. These values varied from 20–40 μA for a 300 msec 50 Hz sine wave pulse, and were used in subsequent training.

The test apparatus was a standard operant chamber (Campden Instruments), 25 × 21 × 19 cm, housed in a sound-attenuating chest. On one wall was a Perspex panel, hinged at the top. To either side of the panel was a retractable lever. The discriminative stimuli were white noise (100 KHz, 66 ± 1 dB), and illumination of a lamp (2.8 W, 24 V) situated above and behind the panel. These stimuli were counterbalanced across rats as S+ or S−.

Procedure

Pretest training. Neither lever was present during this phase. On Session 1 each rat was shaped to panel-push according to a CRF schedule of brain stimulation. During Session 2, S+ and S− were introduced. S+ always preceded S− by 5 sec. S+ was initially 30 sec long, but was gradually reduced to 5 sec during Session 2. S− was always 5 sec long. This sequence of stimuli was presented at variable intertrial intervals averaging 4 sec in Session 2, gradually increasing to 12 sec in Session 3. Panel-pushing during S+ was reinforced on a CRF schedule. Responding during the intertrial interval lengthened it by 1 sec in Session 2, gradually increasing to 10 sec in Session 3. Responding during the interval between S+ and S−, or in S− itself also lengthened the intertrial interval by up to 10 sec. Session 3 ended when a stringent criterion had been met for performance during a single 5 min period: (1) the rat should average at least 4 responses per S+; (2) the proportion of responses during S+ to those during S− should average at least 95%; and (3) the proportion of responses during S+ to the total responses should average at least 85%. The final stage of training required each rat to attain the criterion for 3 consecutive 5 min periods on consecutive Sessions 4–6. Priming at the beginning of Sessions 4–6 was not generally necessary. Three rats of the 19 did not reach criterion performance and were used as untrained controls.

Test phase; preference test. The 16 remaining rats were divided into 4 groups of 4, balanced according to training performance and whether light or noise had been S+. The 4 groups received 0, 5, 10 or 15 mg/kg of pipradrol fifteen min prior to each of three 1 hr sessions, each session being separated by 48 hr. In the test phase no ICS was given and panel-pushing had no consequence. The two levers were now present, each requiring 12.5 g for switch closure. Each response on one lever produced S+ (CR+), and on the other lever, S− (CR−). The stimuli were 1 sec long, and if additional responses were made during this time, the duration was reset to 1 sec. Contingent presentation of CR+ or CR− was counterbalanced over the levers for each group, and rats received CR+ at a lever position random with respect to the side of their electrode placement. The untrained rats were used as controls for possible effects of the drug on responding for stimulus change. One of these rats was given control injections prior to each of three 1 hr sessions and the other two received doses of 10 mg/kg of pipradrol. Lever press responses and panel-pushes were recorded at 5 min intervals. Data were analysed with a 3-factor repeated-measures ANOVAR, and post hoc comparisons were made using the Newman-Keuls test [22].

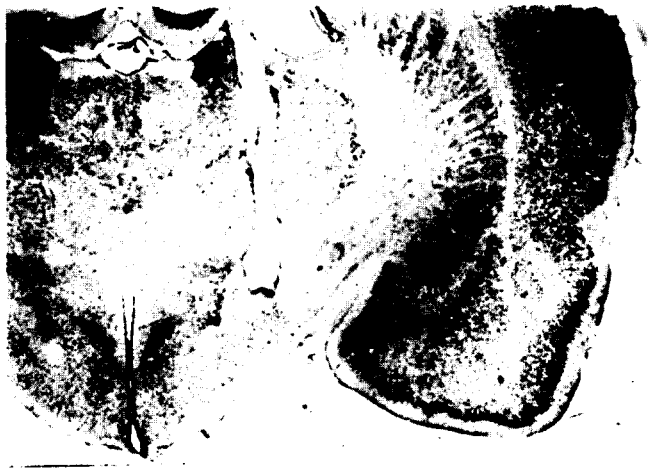

FIG. 1. Photomicrograph depicting a representative electrode placement in one of the rats.

Lever-press data were subjected to a square-root transformation to achieve homogeneity of variance [22], but panel-push data remained untransformed.

Histology

At the end of the experiment, all rats were sacrificed to verify electrode placement. The brains were perfused with Formalin, and every third 30 μ section was stained with cresylechtviolet. The histological examination verified that the tip of each of the electrodes implanted was in the vicinity of the lateral hypothalamus (see Fig. 1).

Drug. The drug employed was pipradrol hydrochloride (Meratran) dissolved in a 1 : 2 mixture of polyethylene glycol (BDH) and distilled water. The four doses of pipradrol employed (0, 5, 10 and 15 mg/kg) were injected intraperitoneally, fifteen min before each session, in a volume of 1 ml/1 kg body weight.

RESULTS

Pretest Training

A high level of differential responding between S+ and S− was obtained in all rats (mean 99.5 ± 0.3%), and each animal made the majority of its total panel-pushes during S+ (mean 89.5 ± 1.0%). The mean panel-pushing rate was 114.7 ± 8.2 responses per 5 min period.

Preference Test

Pipradrol produced a clear dose-dependent increase in responding on the lever providing CR+, but no marked effect on the lever providing CR− (see Fig. 2). This stimulatory action of the drug was highly significant, as assessed by the statistical interaction of drug dose with responding on the CR+ or CR− lever, $F(1,12) = 10.41$, $p<0.01$. Subsequent comparisons revealed that the interaction was attributable to the large increase in responding on the CR+ lever after 10 mg/kg relative to the other doses (Newman-Keuls, $p<0.001$). The facilitatory effect of 5 mg/kg just failed to reach significance ($p<0.10$). There were no significant effects of the drug on responding on the CR− lever, although it is interesting to note that responding after 10 mg/kg was less than that after 0 mg/kg in 3/4 cases. An additional analysis was performed to assess whether