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Suppression of Motor Conditioning by the Injection of 3 M KCL in the Caudate Nuclei of Cats

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PRADO-ALCALA, R. A., J. GRINBERG-ZYLBERBAUM, J. ALVAREZ-LEEFMANS AND H. BRUST-CARMONA, *Suppression of motor conditioning by the injection of 3 M KCl in the caudate nuclei of cats.* PHYSIOL. BEHAV. **10**(1) 59–64, 1973. –The performance of three motor conditioned responses: a pavlovian type and two instrumental responses were severely impaired in cats, after the application of 5 μl of 3 M KCl in the caudate nuclei (CN). Furthermore, during the first 2–3 min the cats kept moving from one place to the other exploring the box as if they were unable to recognize the conditioning environment and to restrain their movements. These findings are consistent with the view that the CN activity is necessary for the motor regulation which subserves learned responses, and for the analysis and/or storage mechanisms of afferent information.

Motor conditioning Caudate nucleus 3 M KCl Recent memory

THE PARTICIPATION of the caudate nucleus (CN) in learning processes has been shown by different authors [17, 19]. Many reports have indicated that the CN activity is necessary for the performance [20] or for the inhibition [3] of learned motor responses. It has been postulated, however, that the CN could participate not only in those mechanisms but in the analysis and integration of the afferent information [21], as well as in the storage mechanisms [7, 16].

In the present study we wanted to extend the examination of the CN participation in learning processes by investigating the modification of a Pavlovian conditioned response, an instrumental motor conditioned response and lever pressing behavior, during blockade of the CN's electrical activity elicited by means of topical application of 3 M KCl. This method was chosen since KCl 3 M produces depolarization of the affected neurons followed by a functional recovery [11] and permitted us to study the effects of the temporal impairment of the function of the CN in cats, that could be used as their own controls when recovered.

METHOD

The first two types of conditional responses (CR) were investigated in a soundproof box, measuring 96.0x68.0x52.5 cm illuminated by a 50 W lamp. In the middle of its floor an alley 30 cm wide and 96.0 cm long limited by two walls 20 cm high was located. A door at the frontal end of the box was provided with a one-way vision glass. A 19.0x21.0x2 cm platform could be moved along the alley towards or away from the reinforcing cup of 4 ml capacity, placed on the alley 7 cm away from the door and 10.5 cm above the floor. The cup could be filled with milk from the exterior of the box. The box was provided with constant air flow and a temperature of 18–23°C, as well as a white noice. The conditioned stimuli (CS) consisted in 4 flashes 1/sec, delivered by means of a photostimulator Grass PS2, with the intensity control set at 2; its lamp was placed in the midline of the roof, above the reinforcing cup.

Seven cats were trained to sit on the platform, which for 3 of them was placed near the reinforcing cup and for the other four it was at 75 cm. Thus the first group learned that after each CS they obtained a constant amount of milk (2.0 ml) by only lowering their heads. This was considered as a simple conditioned response or a Pavlovian conditioned response (PCR). The other 4 cats had to walk to the cup after the delivery of the CS to obtain the milk and return to the starting point. Only after they returned to the platform another CS was delivered. This response was called instru-

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mental conditioned response (ICR). The movements of the cats, were detected by two photocells placed beneath two holes on the floor, one at 16 cm and the other at 41.5 cm from the door and recorded in a Physiograph (Narco Bio-Systems). The artifact of the CS was also recorded. The interval between trials ranged between 10–30 sec. A more detailed description of the procedure of conditioning has been published elsewhere [2]. The cats of the ICR group were given daily (except Sundays) 3 series of trials consisting of 15 trials. For the PCR the daily session consisted of 25 trials. The criterion for conditioning was set at 90% correct responses during three consecutive days. After the animals had reached the criterion level, under general anaesthesia (40 mg/kg nembutal I.P.) double walled cannulae (0.5 mm external dia.) were stereotaxically implanted bilaterally in the head of the CN (A 16.0, L 4.5, H + 4.5 after Jasper and Ajmone Marsan Atlas, [14]).

The animals of the second group (8 animals) were trained in a Lehigh Valley environmental box with a lever pressing intelligence panel on a free operant schedule: each time the animal pressed the lever (LP) 0.5 ml of milk were delivered. The daily session lasted 24 min. The number of pressings was automatically recorded and later on, the mean of LP/min was calculated.

The animals were considered as being conditioned when they pressed the lever at a constant rate (8±2 pressings/min) during three consecutive days. Subsequently, cannulae were implanted in 3 cats in the head of the CN, in other 2 cats in the amydgaloid nuclei, (A 10, L 10, H - 4.5) and in 3 more in cortical areas (A 16, L 4.5, H 1 mm below the dura mater).

Conditioning sessions were reassumed 24 hr after surgery. When the animals had reacquired the criterion level the following procedures were performed; the first day 5 μl of KCl 3 M were injected bilaterally, the second day the same volume of isotonic saline solution or sham injection, or a control session without any injection was performed. This pattern was repeated until 2 microinjections of KCl were performed in each cat of the PCR group, one in each cat of the ICR group, 5 in the CN-LP group, 3 in the amygdala LP and 3 in the cortex LP groups. All injections were made 4 min before the second series in the ICR group. In the LP and PCR groups, injections were made 4 min before the daily training session. Injections were always delivered in 18–22 sec.

At the end of the experiments, under deep nembutal anaesthesia, the cat's brain was perfused first with saline solution and then with formaline solution 10%. The brains were taken out and left in formaline solution for 8–15 days. Then, they were cut in sections of 50 μ thick by the freezing technique. The cannulae placement was identified through the method described by Guzman-Flores, *et al.* [12].

In one of the cats of the ICR group a motivation test was performed, for this purpose 30 ml of milk were offered to the cat before the injection of KCl in the CN, and after the injection the 30 ml were presented every 10 sec during 40 min. The statistical value of the results was tested using a variance analysis test (random blocks test described by Edwards, [9].

RESULTS

In the first session following the cannulae implantation the number of positive responses in all types of conditioning PCR, ICR and LP decreased, but after 3–5 days all the animals regained the prior levels of conditioning.

All animals, immediately after the application of KCl in the CN, performed circling movements ipsilateral to the CN in which the injection was first performed. In 2–3 min more, they walked normally, and the righting and jumping reflexes were normal. In this condition, the cats of the PCR group did not sit on the platform of the conditioning box but moved continuously, explored the box and finally sat anywhere. The CS produced neither the orienting response to the reinforcing cup nor lowering of the head. This effect persisted for 20–30 min. The next day, the animals sat on the platform and showed the PCR as prior to the KCl application. Figure 1 illustrates the clear cut effects obtained in two cats. The difference obtained between the control series and experimental ones showed a $p<0.001$ (Table 1). In contrast, the injection of saline solution (0.8%) or the sham injection did not produce a significative change of either the spontaneous or the learned behavior (Fig. 1).

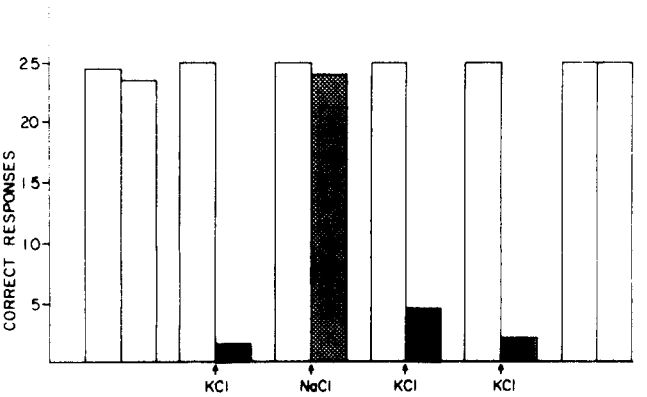

FIG. 1. The columns represent the daily number of correct Pavlovian conditioned response (PCR), out of 25 trials in two animals. The topical application of 3 M KCl in the caudate nuclei produced a significative decrease of the PCR. The same volume of NaCl (0.9%) did not modify the responses.

In the ICR group the bilateral microinjection of 3 M KCl in the CN caused the same spontaneous behavior previously described. In the conditioning box the animals kept moving back and forth in the aisle, going to and from the reinforcement cup.

After 30–45 min the animals sat on the platform and performed few ICRs (Fig. 2). During the third series the cats showed the same number of correct responses as prior to the KCl application. The next day the animals were completely recovered and the ICR reappeared at the same level as before the injection. The differences in the number of correct responses in the 4 control sessions compared with those obtained after the microinjections, performed once in the 4 cats, were found significant at the level of $p<0.01$ (Table 1). Once again, the NaCl or the sham injection did not change the ICR.

The microinjection of 3 M KCl in the CN of cats trained to lever press (LP) evoked circling movements, similar to those observed in the other two groups of animals, which disappeared in a few min. In the box, the animals explored

TABLE 1

	PCR		ICR		LP	
	Control	KCl	Control	KCl	Control	KCl
Number of sessions	6	6	4	4	15	15
Mean number of correct responses	25.0	2.7	15.0	4.5	8.7	2.1
n_1	1		1		1	
n_2	5		3		14	
F	236.3886		779.9151		57.0120	
p	< 0.001		< 0.01		< 0.001	

Statistical analysis of the results obtained in control (no injection) and experimental (KCl injection) sessions performed in cats with cannulae implanted in the caudate nucleus.

PCR, Pavlovian conditioned response; ICR, Instrumental conditioned response; LP, Lever pressing.

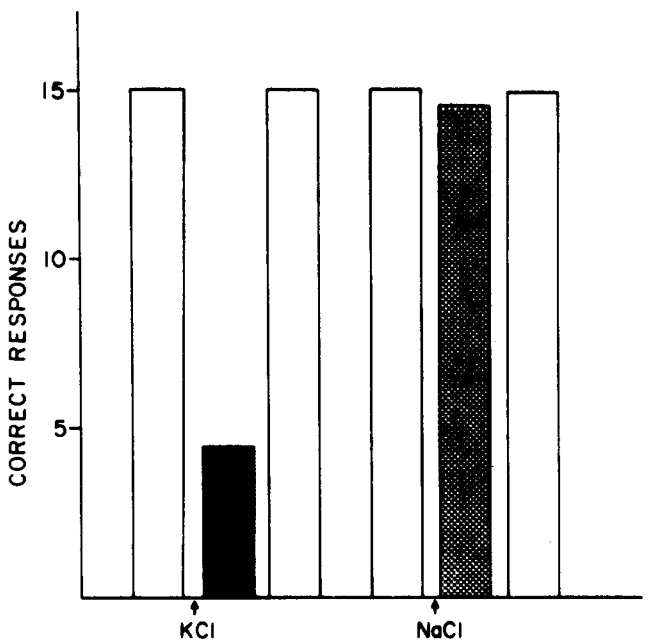

FIG. 2. The columns represent the mean number of correct instrumental conditioned responses (ICR) in each of the three series of the sessions performed in three cats. After the microinjection of KCl 3 M there was a significative impairment in the ICR which the application of NaCl did not alter.

it as they did in the first two or three initial sessions. If by accident they touched the lever, activating the automatic reinforcement system, the animals did not react to it. However, if the cats reached the reinforcement cup they drank the milk. This condition persisted for the whole session after the first microinjection. On the next day the animals pressed the lever at the same rate as the day previous to the injection (Fig. 3). The effect was found statistically significant at the level of $p<0.001$ (Table 1). Consecutively to the 9 microinjections of KCl in cortical areas of the 3 cats the LP rate slightly decreased (Fig. 4). The same result was observed after the microinjection into the amygdaloid complex (Fig. 5). The differences observed were not statistically significant (Table 2).

The animal which was repeatedly confronted with milk during the time of KCl effects in the CN, eagerly drank it.

Histological studies indicated that the cannulae were placed in the dorsomedial part of the caudate nucleus' head; in the parietal cortical areas and in the dorsolateral nucleus or dorsomedial nucleus of the amygdala (Fig. 6).

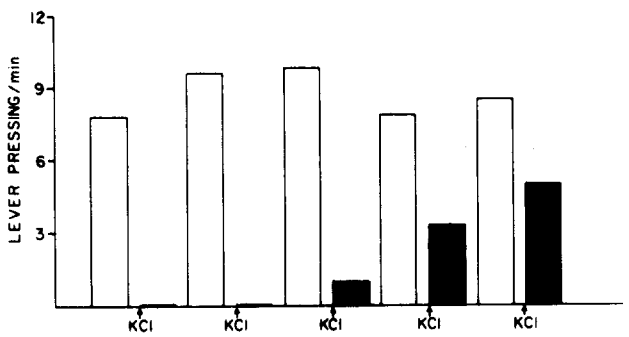

FIG. 3. The bilateral topical application of 3 M KCl in the caudate nuclei abolished the lever pressing (LP). The columns represent the average of LP rate/min in three cats in which five applications were performed.

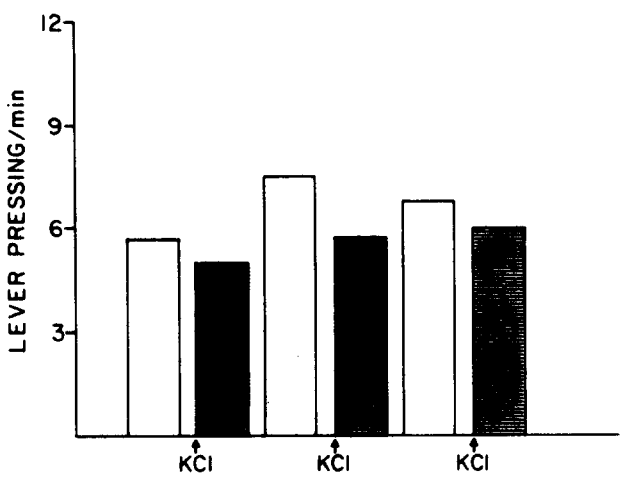

FIG. 4. The bilateral topical application of 3 M KCl in the cortical areas did not impair the lever pressing rate of three cats in which three injections were performed. Columns represent the same as in Fig. 3.

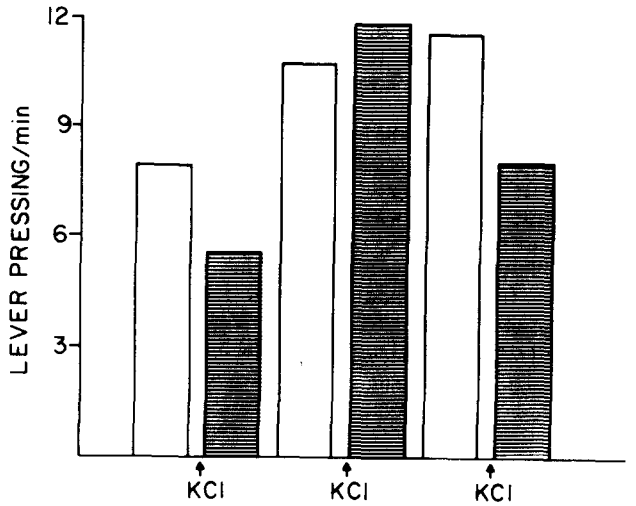

FIG. 5. No statistical difference was found in the LP performance in the 3 cats injected with KCl into the amygdala as compared with control sessions. Columns represent the same as in Fig. 3.

DISCUSSION

It was clearly observed that after the blockade of the CN electrical activity the cats kept moving from one place to the other exploring the box and did not react to the conditioning stimuli; in the Skinner box, they did not press the lever nor reacted to the noise produced by the reinforcement mechanisms. This behavior was very similar to that showed in the first training session.

These results support the idea that the motor regulation which subserved learned responses depends in a very important way on the CN activity, and indicate that this is a basic function since, simple learned motor responses Pavlovian type or instrumental are equally affected by the CN inhibition. Therefore, the present results seem to agree with the idea already expressed by other authors [4, 13, 17] that the CN could be part of the efferent pathway of the neuronal circuitry responsible for the CR.

However, it is possible that the conditioning information or its integration is stored (engram) in the CN. Thus, during the CN blockade there might be a derangement in the retrieval of information previously stored, as has been proposed by Dean and Davis [7], or in the instantaneous analysis of the information reaching the brain [13], since the animals seem to be unable to recognize the conditioning situation or the CS. These suppositions have further support by the results described in this paper as well as by the existence of caudate influences upon the hippocampus which has been involved in memory processes [6, 9] and by the modifications of the afferent evoked potentials by CN electrical stimulation [7].

During the imprinting of the engram seems to be very important the cerebral cortex participation, since the inhibition of this structure affected the CRs, but when the RC is very well consolidated the cortical lesion did not affect the RC [2]. Furthermore, Buresova and Bures [5] had recently described that visual discrimination responses very well consolidated were not changed by cortical spreading depression.

In conclusion the CN plays an important role in the maintenance and performance of motor conditioned responses and could be one of the subcortical analyzer structures suggested in Anokhin model of conditioning [1] as well as the storage place for the engram of motor conditioned responses.

TABLE 2

LEVER PRESSING RESPONSE

	AMIGDALA		CORTEX	
	Control	KCl	Control	KCl
Number of sessions	6	6	9	9
Mean number of correct responses	10.1	8.5	6.7	5.6
n_1	1		1	
n_2	5		5	
F	0.5903		0.2368	
P	No significative		No significative	

Statistical analysis of the results obtained in control (no injection) and experimental (KCl injection) sessions performed in cats with cannulae implanted in the amigdala or in the cerebral cortex.

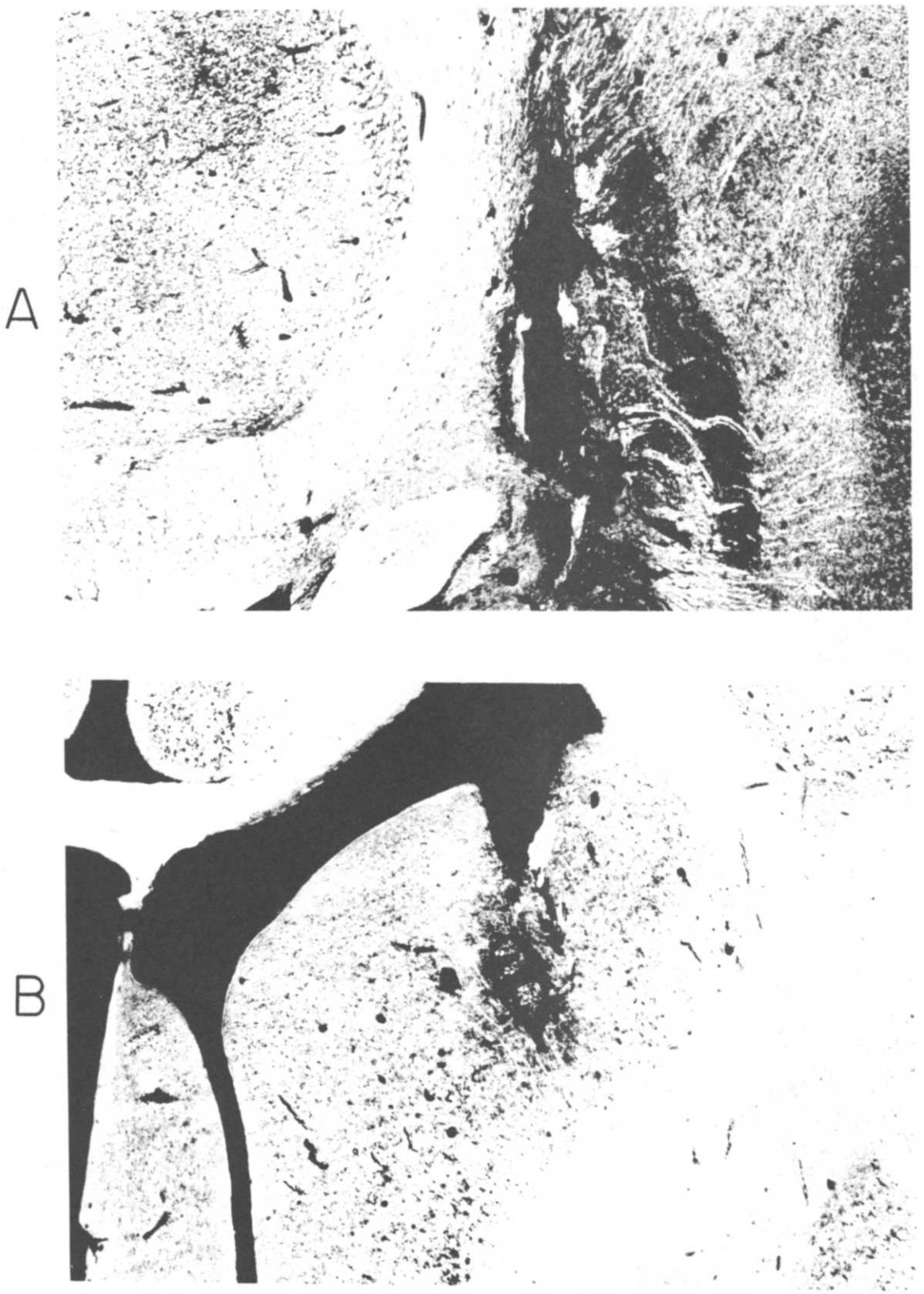

FIG. 6. Illustrates the cannula placement in the amygdala (A) and CN (B) of two cats.

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