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BRIEF COMMUNICATION

Effects of Electrical Stimulation of the Reticular Formation and Chlorpromazine on Performance of Trace Conditioned Avoidance Response in the Rat[1,2,3]

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ELIASSON, M. AND C. KORNETSKY. *Effects of electrical stimulation of the reticular formation and chlorpromazine on performance of trace conditioned avoidance response in the rat.* PHARMAC. BIOCHEM. BEHAV. 1(6) 731-734, 1973.–The effects of electrical stimulation to the mesencephalic reticular formation and chlorpromazine on the performance of a trace conditioned avoidance response by rats were studied. Either treatment alone impaired the performance; this impairment was a function of level of stimulation or dose of the drug, respectively. The performance deficit was not present when a high intensity of stimulation of the reticular formation was combined with a moderate dose of chlorpromazine. However, the combination of a high dose of the drug with a low stimulation intensity interfered with the avoidance responding more than any other condition tested. These effects appeared to be independent of neutral or negative reinforcement effects of the stimulation, as tested in an independent situation.

Brain stimulation Reticular formation Chlorpromazine Trace conditioned avoidance response Rats

THE EFFECTS of electrical stimulation of the reticular formation on rats' performance of behavioral tasks have been reported to be a function of the nature of the task as well as of the properties of the stimulation [7,8]. Wilson and Radloff [8] found that an operant schedule requiring a higher rate of barpressing for food reinforcement was impaired significantly by a stimulation intensity that had no effects on a low rate of responding. Vierck [7] reported other types of behavior such as continuous avoidance performance to be impaired by electrical stimulation to the reticular formation and that exploration and gross activity in rats were decreased. Also attention behavior is inhibited by electrical stimulation to reticular placements. This impairment can be reversed by chlorpromazine, which by itself produced the same kind of performance deficit [2,5].

The purpose of the present study was to investigate further the relationship of reticular stimulation and chlorpromazine by testing several levels of stimulation intensities and drug doses on a task that was maintained in a manner different from food deprivation, and retained the essential feature of alertness for a proper performance. For these reasons a trace conditioned avoidance response was chosen. The effects of chlorpromazine on avoidance responding are well documented [1,3].

METHOD

Animals and Implantation Procedure

Four mature male albino rats of the Holtzman strain were implanted stereotaxically with bipolar stainless steel electrodes (0.25 mm dia.) that were insulated except for the cross section at the tips. Each animal received two electrodes placed bilaterally. The surgery was performed under Equi-Thesin anesthesia (Jensen-Salsbery Laboratories) at a dose of 0.3 cc per 100 g of body weight IP. Atropine sulphate was also administered at 3 mg/kg SC. The coordinates were: A.P. 6 mm, L. 2 mm, V. 5.5 mm, with the area between bregma and lambda kept in a horizontal position. The animals were housed individually in stainless steel cages

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and provided with food and water ad lib.

At the completion of testing the animals were sacrificed and perfused. The brains were saved for verification of the electrode placements.

Apparatus

Electrical stimulation to the reticular formation delivered from a constant current stimulator (Nuclear Chicago) consisted of biphasic rectangular pulses. The duration of each pulse was 0.2 msec with a delay of 0.2 msec. Intensities ranged from 0.01 ma to 0.15 ma at a frequency of 100 Hz, and a train duration of 500 msec.

Avoidance training and testing took place in a Plexiglas chamber inside a sound-attenuating box and with a wheel manipuladum on one of the walls and other details as described by Latz *et al.* [6]. A house buzzer served as the conditioned stimulus (CS); while the unconditioned stimulus (US) was a crambled foot shock through the grid floor at 0.5 ma.

Reinforcing properties of the electrical stimulation were tested in a box 43 cm long, 30 cm high, and 23 cm wide. A steel bar placed across the middle of the box 2.5 cm above the floor divided the box into two equally large areas. Timers were connected to the apparatus, automatically recording time spent in either compartment plus the total testing time.

Procedure

In training of the avoidance responding the CS duration was started at 10 sec duration and decreased stepwise until the final condition of 2 sec, followed by an 8 sec long silent period - the trace – before the US. A response (A one quarter turn of the wheel) caused the shock to be avoided and the CS terminated. A wheel turn after onset of the US, which had a duration of 5 sec was an escape response and terminated the shock. The CS was presented at irregular intervals, on the average every 30 sec.

The animals were trained until their performances reached an asymptote and were then habituated to having the stimulation cable attached to the electrodes.

Animals received one train of stimulation in the middle of each intertrial interval, i.e. on the average every 30 sec. The testing of the effects of stimulation started with the lowest intensity 0.01 ma, and increased from session to session in steps of 0.02 ma up to an intensity where overt motor reactions could be observed e.g. contralateral turning. The highest intensity combined with chlorpromazine was one without interfering motor components. The effect of electrical stimulation to the reticular formation was then tested in a descending order of intensity.

Three intensities were chosen for testing together with chlorpromazine. The first two levels were 0.01 ma and 0.05 ma. The third one was just below the threshold for a strong motor response in each individual animal. For one animal this intensity was 0.10 ma and for the others 0.15 ma.

Chlorpromazine or saline was injected IP 15 min before the session. Each electrode point was tested with the three current intensities and the three doses of the drug. Testing was alternating from session to session between the two electrode points in each animal. For two of the animals the first testing condition was a combination of the high dose of chlorpromazine with the low current intensity. The other two animals were tested the first time with the smallest dose of the drug and the high stimulation intensity. The conditions were reversed in the animal pairs for the start of testing of the second electrode point. No animal was ever tested with the same treatment combination on two consecutive testing occasions. The conditions were then altered one step for each session.

A session lasted 45 min and occurred 5 6 times a week. Chlorpromazine and/or stimulation was usually administered twice a week with control runs (with saline and no stimulation) in between. The performance was always allowed to return to the pretreatment level for each animal before the next treatment was applied.

Percentage avoidance, percentage escape, number of responses, and mean response time/avoidance were recorded.

Reinforcing effects of the electrical stimulation were tested prior to the avoidance training. The animal was placed in the testing box and the total time spent in either compartment was recorded. Testing was performed in four blocks, each consisting of six sessions lasting 10 min. In the first and third blocks no stimulation was delivered to control for any spontaneous preferences of box-compartments. During the remaining sessions the animal received one train of stimulation, whenever it entered a predetermined side of the experimental chamber. This was repeated every 30 sec if the animal remained. The starting point alternated from session to session between compartments, but stimulation of one electrode was always received in the same compartment. When the second electrode placement was tested the compartment in which stimulation occurred was reversed. Time spent in either half of the box was calculated under both conditions for each animal and electrode and the differences tested for statistical significance by means of a double-tail t-test for correlated observations [9].

RESULTS

Three of the animals had their electrodes placed, as intended in the reticular formation, while the fourth animal had one electrode on the borderline of the reticular formation and the posterior thalamic nucleus and one in the lateral geniculate.

Only one electrode placement yielded significant reinforcement effects, here stimulation was negatively reinforcing ($t = 2.76$, df 5; $p<0.05$).

Effects of electrical stimulation on the CAR performance are shown in Fig. 1 for each animal. Only the highest intensity of stimulation had any effect on the performance and caused a considerable decrease of avoidance responding. This was observed at all placements, including the one in the lateral geniculate. The effects of brain stimulation for each reticular placement compared to the immediately preceding control performance for each animal yielded statistically significant effects on a correlated t-test ($t = 3.96$, df 2 and $t = 4.52$, df 3 respectively; $p<0.05$).

Effects of chlorpromazine on the CAR behavior (fig. 1) were significant only at the 2 mg/kg dose ($t = 4.34$, df 3; $p<0.05$).

Effects of stimulation and chlorpromazine combined were not identical for all animals and placements, as is evident from Fig. 2. The 1 mg/kg dose, which by itself did not affect the performance, counteracted the stimulation in all animals and at all placements, except those not in the intended area, bringing the performance back to within one standard deviation of the mean for the control condition.

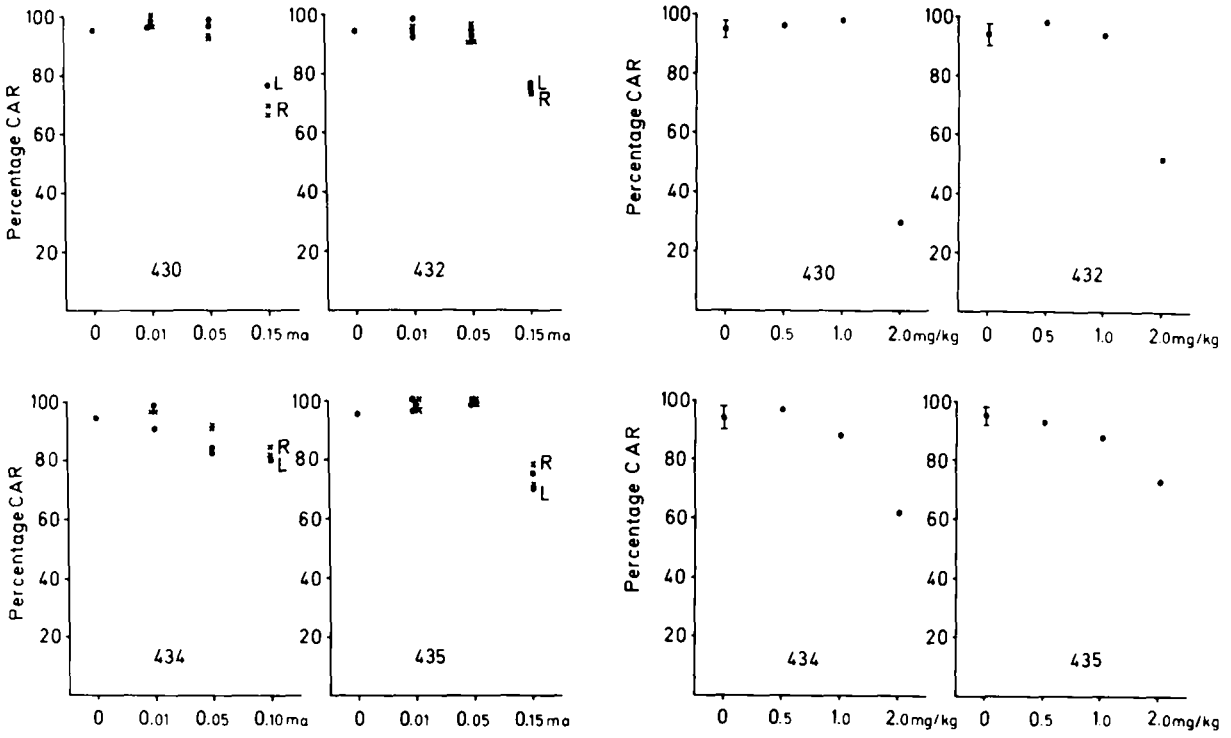

FIG. 1. Effects of electrical stimulation at different intensities on the CAR performance for individual animals and placements with repeated applications of stimulation (left hand figures) and effects of different doses of chlorpromazine for individual animals (right hand figures). Left side electrode placement is indicated by L, right side placement by R. Standard deviation for the saline performance is indicated.

When the dose of the drug was increased to 2 mg the performance was impaired beyond the control level. The exception was the placement with the negative reinforcement properties, where this dose was the most effective one in counteracting the stimulation effect. When this high dose of the drug was combined with a low or intermediate intensity of stimulation the result was a greater inhibition of the CAR performance than seen under any other condition.

The other measures of the performance: escape responding, number of responses, and mean response time did not attain any statistically significant effects as a consequence of the different treatments. Escape performance and mean response time were altered appreciably only by the treatment combination of 2 mg of the drug and 0.05 ma stimulation, but this was not true for every placement. The number of responses increased with impaired avoidance responding, except under the condition that also impaired escape, where the number of responses was not different from the control condition.

DISCUSSION

The trace conditioned avoidance response is suppressed by certain intensities of electrical stimulation to the reticular formation. Repeated applications of stimulation to the same placements show the effect to be reliable. This impairment is counteracted by a dose of chlorpromazine that by itself does not interfere with the performance.

The present data agree with previous findings with some modifications. The CAR is more resistant to disruptions by both treatments and the specific interactions between stimulation at reticular placements and the drug appear to have an optimum at which they cancel the effects of each other.

Differences in baseline rates of responding have been shown to be of great importance for effects of drugs and brain stimulation [1, 8, 9,] and may account for at least some of the differences between the present and previous findings. The CAR with a slower pace and being performed very efficiently on a stable level seems less susceptive to the experimental manipulations.

The impairment seen after the high dose of the drug and low intensity stimulation had a different profile from that resulting from other treatments. The animals often did not escape the foot shock or had a very long latency. In addition, there was no increase of the number of wheel turns as a consequence of the many shocks received, as was the case at e.g. 2 mg of chlorpromazine, or this dose of the drug and high intensity stimulation, suggesting a more severely disrupted performance. In many instances there was a wheel turning response to the brain stimulation and no response to the CS that followed, which may indicate that the high dose of the drug could have reduced reactions to the peripheral signal, while the central stimulation at this intensity still was having some effect [4]. With high intensity stimulation, the arousal-attenuating effects of the drug was decreased leaving the animals more capable of an adequate reaction. The high dose of chlorpromazine together with the high intensity stimulation in most cases yielded an

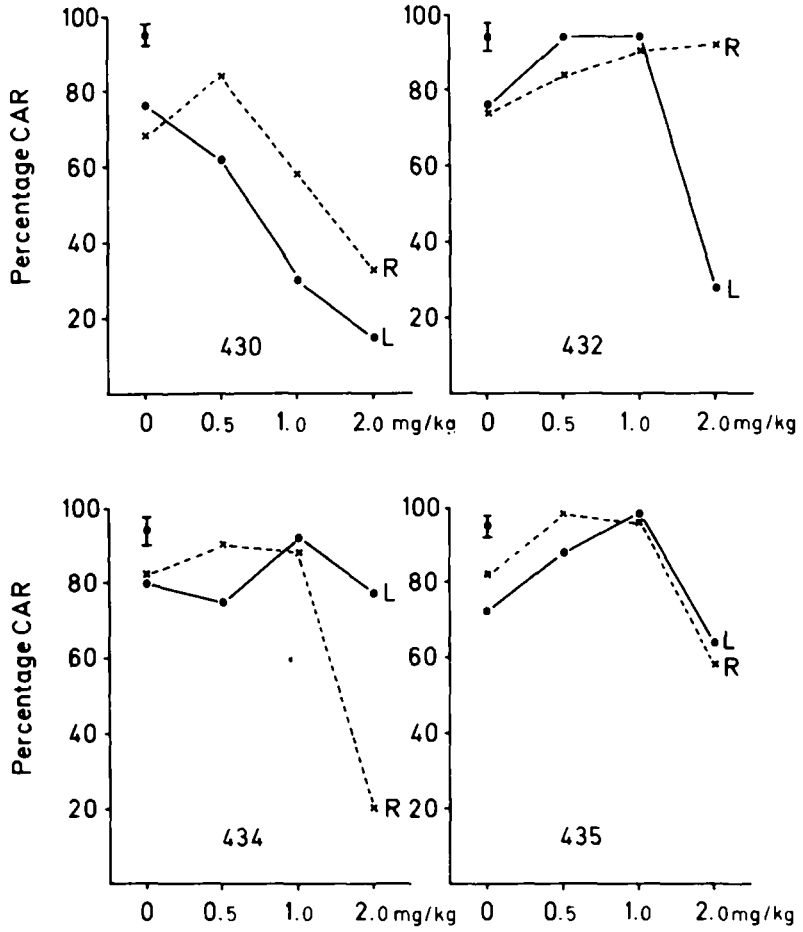

FIG. 2. Effects of saline or different doses of chlorpromazine and 0.10 0.15 ma stimulation for individual animals and placements. Animal 432 right electrode placement showed negative reinforcement properties. Animal 430 had its left placement outside the reticular formation and the right one on the borderline.

impaired avoidance performance, however, the escape responding was not impaired and number of responses or mean response time were not altered from control.

The chlorpromazine reversal effects on impairment of performance as a result of reticular formation stimulation do not appear to be limited to the food-reinforced attention tests. Nor can they be said to be a function of reinforcing effects of the stimulation, as the reversal occurred at placements lacking these properties. However, the specific interaction effects of drug dosage and stimulation intensity seem to be dependent on the parameters of the individual test.

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