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Impairment of Instrumental Learning in Rats by Glutamic Acid Diethyl Ester

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FREED, W. J. AND R. J. WYATT. *Impairment of instrumental learning in rats by glutamic acid diethyl ester.* PHARMAC. BIOCHEM. BEHAV. **14**(2) 223–226, 1981.—Glutamic acid diethyl ester (GDEE), a putative antagonist of glutamate-induced neuronal excitations, was administered prior to an instrumental conditioning task motivated by food reinforcement. A profound impairment of learning was produced in animals receiving 240 or 480 mg/kg of GDEE. Performance was not impaired by GDEE in rats that had previously learned the task. These findings support suggestions that central excitatory processes play an important role in learning phenomena, in particular when these learning phenomena involve acquisition of new behavioral patterns.

Glutamic acid diethyl ester Instrumental learning Central excitatory processes

THERE is some evidence that glutamate plays an important role in the mammalian brain as an excitatory neurotransmitter [9,24]. Glutamate is found in high concentrations in the hippocampus and cortex [24, 25, 27] and may be the transmitter for excitatory hippocampal afferents [8, 18, 30, 35] and the corticostriatal pathway [28, 33, 36]. Glutamate is capable of exciting most, if not all, neurons [9,24] and is released both from the brain and peripheral nerves in association with activity [23,28]. Van Harreveld and Fifkova [19,21] have suggested that patterned release of glutamate occurs in localized areas of the brain during learning, and that this phenomenon produces a patterned facilitation of synaptic transmission that facilitates, or forms the basis for, learning. Experimental evidence supporting this hypothesis includes the finding that proline, a putative glutamate antagonist [20, 34, 40] and related compounds inhibit passive avoidance learning in chicks [5, 6, 21]. Intracerebral administration of glutamic acid, which might be expected to disrupt any such patterned facilitation, impairs instrumental learning in rats [15], which is also consistent with this hypothesis.

Glutamic acid diethyl ester (GDEE) is a partially effective antagonist of glutamate-induced neuronal excitations [7, 17, 34, 36, 37]. GDEE has been found to be particularly effective in the hippocampus, where it inhibits the action of glutamate and dampens evoked field potentials [34,37]. GDEE has also been found to competitively inhibit the binding of glutamate to its putative membrane receptor [29]. The relative effectiveness of GDEE in the hippocampus, a brain area that has an important role in some forms of learning [3, 22, 31] suggested to us that GDEE might be capable of interfering with learning. The purpose of the present study, therefore, was to determine whether GDEE would impair instrumental learning in rats.

METHOD

Animals

Thirty-two male Sprague-Dawley albino rats, initially weighing 200–350 g, were deprived of food and maintained at approximately 85% of their initial free-feeding weights. Animals had free access to water except during testing.

Apparatus

Testing was conducted in four similar chambers constructed within modified camping coolers, with three walls consisting of white plastic and the floor and fourth wall consisting of clear Plexiglas. Masking noise was provided by exhaust fans, and the chambers were dimly illuminated by light entering through the ventillation outlets. Two bars, two pilot lamps (one above each bar), and a food cup were mounted on the Plexiglas wall. The size and layout of the chambers were essentially the same as that described previously [15]. Reinforcement contingencies were programmed electronically.

Drugs and Injections

L-glutamic acid diethyl ester HCl (Sigma Chemical Co.) was administered IP in a volume of 10 ml/kg. The highest concentration of GDEE was dissolved in distilled water, and this solution was diluted with normal saline. Control animals received normal saline. Solutions were prepared freshly before use.

Procedure

Magazine training. The bars were disconnected and food

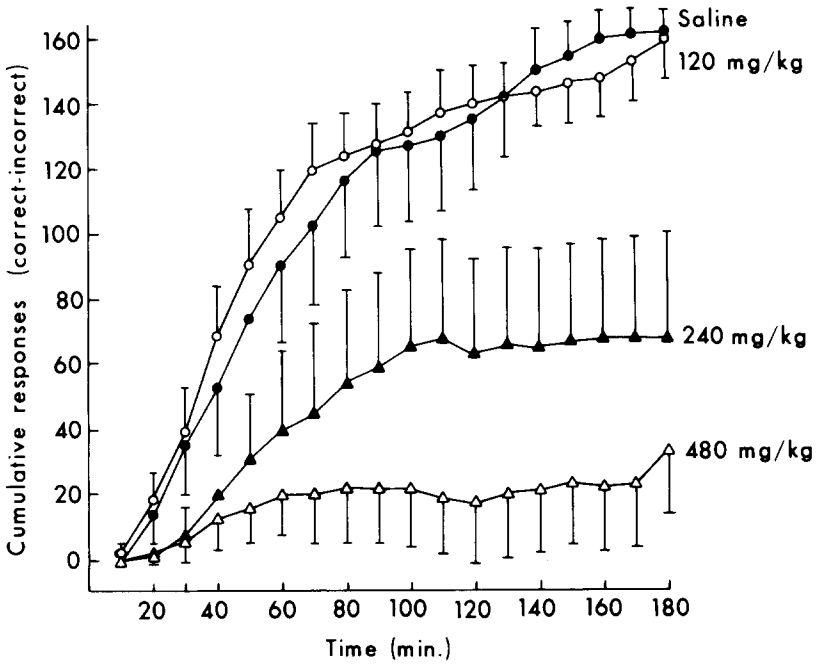

FIG. 1. Cumulative differential responses (responses on the active bar minus responses on the inactive bars) as a function of time for animals receiving saline or one of three doses of glutamic acid diethyl ester HCl (IP, 10 ml/kg). Points shown are means; vertical bars indicate SEM (some of these have been omitted for clarity). There are eight animals per group.

pellets were delivered noncontingently at a rate of one per minute for 60 minutes.

Learning. The next day one of the bars, either the right or the left, was activated so that each bar press resulted in the delivery of one 45-mg Noyes food pellet (correct bar). The other bar remained inactive (incorrect bar). The position of the correct and incorrect bars remained the same for each particular animal for all phases of testing.

The maximum duration of the learning test was 180 min, but the test was terminated if and when any animal completed 200 correct responses. For the first 60 min, the animals received food pellets noncontingently at a rate of one per 5 min. After 60 min, the noncontingent food pellets were discontinued and the pilot lamp above the active bar was illuminated. Numbers of bar presses were recorded every 10 min.

Drugs were administered 5 min prior to the start of the learning tests. For additional animals, when noted, drugs were administered 30 min prior to the start of the learning test.

Recall. Two days after the learning test, the animals were again placed in the chambers and the pilot lamp above the active bar was illuminated. No noncontingent food pellets were delivered. Animals were tested for 30 min. No drugs were administered.

Performance. The animals were satiated and allowed to recuperate for approximately two weeks, and again deprived of food for 24 hr. Each animal was tested for one 30 min session using the procedure described under recall. Sixteen animals that bar pressed at rates of 100 or more correct responses per 30 min were divided into two equivalent groups on the basis of prior drug treatment. On the following day, one group received 480 mg/kg GDEE IP, and the other received saline; after 30 min the animals were tested for 40 min. Time to the first correct response, time to complete 10 correct responses, and total numbers of correct and incorrect responses were recorded.

RESULTS

Learning

GDEE was found to impair acquisition of the bar-press response in that two highest doses of GDEE essentially prevented learning (Fig. 1). Total numbers of correct (reinforced) responses were decreased, while the number of incorrect (unreinforced) responses was unchanged (Table 1). Although there was a tendency for numbers of incorrect responses to be increased, this was not significant (Table 1). In addition, the time required for the animals to reach a criterion of 90% correct responses was greatly increased by GDEE, and the mean percentage of responses that were incorrect was increased to about 40% by the two highest doses of GDEE (Table 1). Seven of the eight animals that received 480 mg/kg GDEE never reached a criterion of 90% correct responses, while 6 of 8 animals given 240 mg/kg did not reach this criterion, and only 2 of 8 animals that received 120 mg/kg or saline did not reach this criterion.

TABLE 1

EFFECTS OF GLUTAMIC ACID DIETHYL ESTER (GDEE) ON SEVERAL MEASURES (MEAN ± SEM) OF LEARNING

Measure	Dose (mg/kg) 0	120	240	480	F(df)	p
Time to obtain 100 food pellets (min)	71 ± 16	56 ± 6.8	125 ± 22	135 ± 22	4.85 (3,28)	0.008
Time to achieve 90% correct responses overall (min)	90 ± 23	68 ± 25	145 ± 23	170 ± 10	5.03 (3,28)	0.007
Time to the first 10-min interval with 90% correct responses (min)	50 ± 13	25 ± 3	120 ± 27	124 ± 27	6.19 (3,28)	0.003
Total number of correct responses	182 ± 9.0	181 ± 14.0	96 ± 35.2	77 ± 27.4	5.39 (3,28)	0.005
Total number of incorrect responses	21 ± 9.1	21 ± 7.5	28 ± 10.8	50 ± 25	0.65 (3,28)	0.519
Percentage of total responses that were incorrect	8.6 ± 3.1	9.4 ± 3.0	41.9 ± 11.5	40.2 ± 7.0	6.88 (3,28)	0.002
Recall: Latency to first correct response (sec)	67 ± 20	76 ± 21	138 ± 65	586 ± 344	2.90 (3,23)	0.056
Recall: Latency of 10 correct responses (sec)	174 ± 33	180 ± 28	616 ± 201	879 ± 377	3.21 (3,23)	0.041
Recall: Correct responses in the first 10 min	43 ± 3.6	36 ± 2.7	24 ± 8.2	18 ± 5.1	4.32 (3,27)	0.013
Recall: Correct responses from 10 to 30 min	66 ± 5.5	52 ± 5.9	52 ± 122	42 ± 12.2	1.13 (3,27)	0.355

In the recall testing, the animals that had received the higher dosages of GDEE made fewer correct responses initially (for the first 10 min), but similar numbers of responses during the final 20 min of the 30-min testing session (Table 1).

None of four animals given 480 mg/kg GDEE 30 min prior to the start of the learning session achieved 90% correct responses.

Performance

When the animals had previously learned to respond, their performance was not affected by GDEE. There was no difference between the animals in terms of : (i) Latency to the first correct response (62 ± 14 sec for GDEE vs 96 ± 25 sec for saline), (ii) Time to complete 10 correct responses (141 ± 16 sec for GDEE vs 180 ± 32 sec for saline), (iii) Total number of correct responses (186 ± 23 for GDEE vs 204 ± 7.1 for saline), and (iv) Total number of incorrect responses (15 ± 5.4 for GDEE vs 13 ± 4.5 for saline) (means ± SEM). None of these differences approached statistical significance ($p>0.2$, one-tailed t-tests).

DISCUSSION

The present data demonstrate that GDEE impairs learning, but does not alter performance, of an instrumental response motivated by food reinforcement. This might be interpreted as a reflection of a primary role of glutamate in the process of learning; however, the present finding should be qualified on several grounds. First, it may be that instrumental learning can simply be disrupted more easily than performance of the learned response. Secondly, these effects might be due to some secondary effect of GDEE. There is, in fact, some question about the specificity of GDEE as a glutamate antagonist, particularly in brain areas other than the hippocampus. In particular, spontaneous neuronal firing rates and aspartate responses are frequently altered by GDEE [7, 17, 34]. But this may not be a serious difficulty, as aspartate and glutamate receptors do not seem to be entirely distinct [10,29], and spontaneous levels of an neuronal activity could be influenced by background glutamate levels. In addition, other sedatives, such as the barbiturates, do not have particularly profound effects on learning [14]. A final consideration is that these effects may be unique to this particular type of learning task. In the present study, acquisition of a reinforced behavioral pattern was inhibited by GDEE. Whether learning as studied in paradigms involving modulation or inhibition of established responses is sensitive to disruption by GDEE is unknown.

In certain model systems, excitation of neuronal pathways seems to facilitate subsequent transmission; these model systems include the kindling paradigm of Goddard [16], synaptic potentiation [4,12] and the development of epileptic foci and mirror foci following topical application of irritants to the cortical surface [26,39]. It has been suggested that a phenomenon somewhat akin to this occurs during the acquisition of new behaviors, comprising an initial event in the process of learning [3, 13, 19, 21, 32]. This notion is consistent with electrophysiological evidence that learning tends to be localized to the brain areas that participate in the perceptual and motor tasks that comprise the new experience [1].

Several considerations had led Van Harreveld [19,21] to suggest that glutamate plays an important role in these potentiative processes; for example, glutamate release accompanies cortical spreading depression, during which dendritic spine swelling is also observed [19]. In this connection, there is some evidence that the highly potentiable hippocampal perforant pathway releases glutamate as a transmit-

ter [8, 18, 30, 35]. An equally tenable hypothesis, however, would be that all forms of synaptic excitation participate in this kind of promotion of synaptic transmission, and that any apparent predominance of glutamate could merely be due to its greater ubiquity. This would be consistent with a significant literature that implicates acetylcholine, a second major excitatory transmitter in the mammalian brain, in learning phenomena [2,11]. The present findings, therefore, do not necessarily suggest a unique role of glutamate in learning phenomena, but they do lend support to suggestions that excitatory synaptic processes mediate some aspect of neuronal plasticity that contributes to learning.

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