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Intraventricular N-Acetyl-L-Glutamate Induces Retrograde Amnesia in Chicks[1]

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DAVIS, J. L., A. CHERKIN AND M. D. HEWITT. *Intraventricular N-acetyl-L-glutamate induces retrograde amnesia in chicks*. PHARMAC. BIOCHEM. BEHAV. 14(6)919–920, 1981.—Two-day-old chicks were injected intraventricularly with saline or an N-acetyl-L-glutamate solution. The amino acid analog produced retrograde amnesia without concomitant electrophysiological seizure activity. A mechanism involving the disruption of activity at memory-related glutamate synapses was suggested for the observed amnestic effect.

N-acetyl-L-glutamate Amnesia Chicks Glutamate Proline

VAN HARREVELD and Fifkova [6] hypothesized that neural activity associated with learning leads to a patterned release of glutamate into extracellular fluid, thereby increasing the permeability of dendritic membranes to sodium. Extracellular electrolytes then diffuse into the affected structures and water enters to maintain osmotic equilibrium, swelling the dendritic spines. The resultant decrease in their electrical resistance would increase the effectiveness of synaptic excitation.

Amino acids have previously been shown to cause amnesia, presumably by inhibiting effects of glutamate release. For example, L-proline and some of its analogs have been shown to impair memory processing without provoking toxic or electrophysiological disturbances. There is a very high correlation between the amnestic potency of these amino acids and their ability to inhibit spreading depression in the avian retina [5], a process known to involve the release of glutamate.

N-acetyl-L-glutamic acid (NAG), an amino acid analog, which also will be shown to have amnestic properties, affects primarily glutamate uptake in rat synaptosomes [2]. The effect is more specific than those produced by β-aminobutyric acid and α,γ-diaminobutyric acid which also interfere with uptake of other structurally similar amino acids in the same preparation. To our knowledge, this is the first suggestion that inhibition of glutamate reuptake also may produce a memory deficit. Disruption of the glutamate reuptake system could produce a "noisy" synaptic environment where an excess of glutamate present in non-patterned form would compete with new information-carrying glutamate release patterns.

The possibility that the amnestic effect might be due to covert brain seizures was ruled out by an electrophysiological examination of NAG at the dose used in the amnesia study.

METHOD

The training, injection, and testing paradigm has been described elsewhere [1]. Briefly, white Leghorn cockerels, 29 hr old are individually housed in a room controlled for temperature, relative humidity, light level, and absence of food and water. Chicks are aversively trained when they spontaneously peck at a 3 mm stainless steel bead previously dipped in methyl anthranilate, a strong gustatory aversant. At either 1 or 240 min after training, bilateral 5-μl intracranial injections of saline or NAG (300 or 150 mM) were given. Chicks were tested 24 hr later by presenting the dry training target for 10 sec, followed by a 10 sec presentation of a novel stimulus target (microminiature lamp) to control for general peck performance. An enhanced number of pecks elicited by presentation of the bead as compared with the controls indicate memory loss. All p values were calculated from normalized (square-root transformation) peck score data using student's t-test, one tailed. Injections and testing were "blind."

The implantation of the electrodes and recording procedures of the EEG have been described in detail elsewhere [3]. Each chick had active bilateral recording electrodes

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TABLE 1

Injection Interval (min)	Compound	Dose/chick (μmols)	N	Retention Test Peck Score Training Target ($\sqrt{\bar{p}} \pm$SD)		Novel Target ($\sqrt{\bar{p}} \pm$SD)	
1	NAG	3.0	62	1.69	1.64	1.75	1.23
240	NAG	3.0	48	1.03	1.16	1.31	1.21
1	NAG	1.5	22	0.31	0.53	1.70	1.48
1	saline	—	31	0.75	1.08	1.92	1.30

stereotaxically positioned in right and left ectostriata, an indifferent electrode under the comb and one in the dorsal neck muscles (ground). Under halothane anesthesia, the electrode assembly, with miniature amphenol recording connections, was embedded and fixed to the skull with Grip dental acrylic. The active recording electrodes were insulated to 0.5 mm of the tip. Recording was begun approximately 24 hr after surgery, always between 1300–1600 hr. Electrode locations were histologically verified.

Electrophysiological screening and amplifier adjustments to be used in the experiment were determined during the adaptation period. During this period recording leads were fastened and each chick was placed in a 8.5-cm dia. × 21.0-cm deep carton. The electrical activity was recorded on 6 channels of a Grass Model 7B polygraph. Two of the polygraph channels were used to monitor monopolar-EEG activity from each ectostriatum referenced to the comb, and a third channel recorded bipolar activity between hemispheres. The three EEG amplifiers were set at a band pass of 1 to 75 Hz (−3 dB points). The fourth channel recorded the same bipolar activity at a band pass of 10–75 Hz. With the last recording channel, multiple unit activity (MUA) recorded bipolarly from the ectostriatum was led to a Grass Model P511 H preamplifier (−3 dB band pass points at 300 Hz to 3 KHz); this activity was subsequently selected by high-pass filtering (−3 dB at 500 Hz: −2 dB at 100 Hz) with a David Kopf Model SFA 12 spike filter, and then was integrated with a David Kopf Model C10 DC converter. MUA was integrated above the threshold of system noise (10 μV) to a peak count of 1 K/sec. The integrated EEG and MUA were smoothed with low-pass filtering at 3 and 0.5 Hz, respectively.

Upon completion of a 15-min adaptation period, each chick was removed from the recording carton and manually restrained in a head holder precalibrated to guide the 27 ga needle of a Hamilton microliter injection syringe into the right and left lateral ventricles. A total of 5 μl/hemisphere of the 300 mM NAG was quickly injected into each ventricle through a hole in the acrylic recording assembly posterior to the ectostriatal electrodes. Within 5 sec after the injections, a 10-min post-injection recording period commenced.

RESULTS AND DISCUSSION

NAG at a dose of 3.0 μmols, given 1 min after training, is amnestic compared to saline ($p<0.01$), whereas 1.5 μmols at the same training-treatment interval does not differ statistically from saline (Table 1). The smaller dose of NAG significantly differs in amnestic effect from the larger dose ($p<0.001$). Delaying the injection for 240 min abolished the effect, confirming its retrograde nature, and suggesting that NAG is only capable of disrupting short-term memory. Memories that have been consolidated and are in long-term storage (and therefore no longer dependent upon glutamate activity) are not subject to disruption by NAG. An alternate explanation for the increased number of responses emitted during the retention test would be that NAG increased arousal or activity level with a concomitant increase in pecking. Statistical analyses of the novel lure data parallel to the aforementioned comparisons were all nonsignificant, indicating that NAG was not simply increasing nonspecific peck rates.

Neither seizure spiking nor isoelectricity was observed in ectostriata after NAG injection. In the chick, severe brain seizures induced by agents such as flurothyl [4], pentylenetetrazol and CO_2 (unpublished data) do not cause amnesia unless they also produce a large number of seizure spikes. Such severe seizures spread through the striatum [4]. Therefore, electrodes placed on the ectostriatum for reasons of both convenience and avoidance of damage to striatal tissue provide an adequate control against the possibility of amnesia induced by electrophysiological disturbances.

We conclude that the intraventricular injection of NAG produces retrograde amnesia for avoidance conditioning in the chick without concomitant seizure activity. A possible mechanism may involve the disruption of normal activity at memory-related glutamate synapses.

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