Adult, male, albino rats were trained to discriminate between two patterned stimuli in a T-maze. Performance on this task was assessed following intravitreal injection of 1 μmol glutamate. Discrimination performance declined to nearly random levels by 1 day postinjection and remained significantly depressed for 2 weeks. However, by 2 months after injection, there was evidence of behavioral recovery to preinjection levels despite significant loss of inner retinal neurons. Task-related experience immediately following or 2 months after injection proved both necessary and facilitative for recovery.

Lucas and Newhouse\(^1\) were the first to show that monosodium glutamate (GLU) causes irreversible, degenerative changes in the inner retina when injected subcutaneously into rodents before eye opening. Degeneration is characterized by damage and loss of ganglion and amacrine cells with subsequent thinning of inner retinal layers.\(^2\) Other CNS involvement has been noted with neural death occurring in the periventricular arcuate region of the hypothalamus.\(^3,4\) GLU treatment also affects visual behavior,\(^5,6\) but it is unclear whether behavioral effects were due to retinal damage, damage to other parts of the CNS, or both. We have utilized intravitreal injections of micromolar amounts of GLU to induce degeneration of inner retinal neurons in adult rats while avoiding brain involvement. Preliminary results\(^7\) indicated that intravitreal GLU injections adversely affect performance of albino rats on a visual discrimination task, but that recovery to control response levels was possible despite severe degenerative changes in the retina. The research reported here confirms and extends these preliminary findings in a series of three experiments.

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Materials and Methods

Experiment 1

Time-course assessment of behavioral performance after intravitreal glutamate injection: Ten, male, Sprague-Dawley (SD) rats (250-300 g) had their left eyes surgically removed and after recuperating for 1 week were trained to perform a visual pattern discrimination task in a T-maze. Two patterned stimuli consisting of either horizontally or vertically oriented white bars measuring 2.54 × 1.27 cm were painted on panels in which doors were cut (Fig. 1). Rats were trained to discriminate the vertically oriented white bars (S+) by opening the stimulus panel door and thereby gain access to a goal box containing powdered rat chow for reinforcement. The goal box behind the stimulus panel with horizontally oriented white bars (S−) also contained powdered rat chow. However, the S− panel door remained locked throughout all training and testing.

Distance between the stimulus panels and the “choice area” was 30.5 cm. Thus, the height and width of the stimulus bars corresponded to 4.8° and 2.4° visual angle subtended, respectively, values well above the acuity threshold for rats.\(^8,9\)

All training and testing were carried out in a darkened room in which the T-maze was positioned on a table centrally located with one lamp illuminating the maze. This lamp contained a 60 watt incandescent bulb and was placed 80 cm directly over the “choice area.” Direct illuminance intensity on the floor of the “choice area” was 35.4 lux and 24 lux in the approach areas to the stimulus panels. The value for reflected illuminance of both stimulus panels as measured from the “choice area” was 3.2 lux.

Rats were run 20 trials/day until at least 80% correct responses were attained on 3 consecutive days.
(criterion performance level [CPL]) using three, different, random stimulus presentation sequences after Gellerman.\textsuperscript{10} Stimulus panels were alternated manually. If the next trial required that the panels remain in the same positions, the panels were still handled as if being switched so as to avoid auditory cues. Next, rats were assigned randomly to either a normal saline (NS)-injection group (\(N = 5\)) or a GLU-injection group (\(N = 5\)). Subjects in the NS group were intravitreally injected with 5 \(\mu\)l sterile NS and subjects in the GLU group were injected similarly with 1 \(\mu\)mol GLU (monosodium L-glutamate, Sigma) in 5 \(\mu\)l volume (pH 7.0). All rats were tested on 1, 3, 7, 14, 28, and 56 days postinjection (DPI).

Subjects were housed under a 12 hr/12 hr lighting schedule when not being trained or tested. Also, rats in this study were handled in accordance with the ARVO Resolution on the Use of Research Animals.

**Experiment 2**

**Effects of task-related experience on behavioral recovery following intravitreal glutamate injection:** To investigate whether task-related experience was necessary to demonstrate recovery after GLU-induced retinal damage and if so, whether the rate of recovery would increase as the amount of experience was augmented, 11 male SD rats (250–300 g) had their left eyes surgically removed and were trained on the discrimination task as in experiment 1. They then were assigned randomly to one of the following groups. Group A (\(N = 3\)) rats were intravitreally injected with 5 \(\mu\)l sterile NS and tested on 1 and 56 DPI to control for any forgetting that may have occurred. Rats in groups B (\(N = 4\)) and C (\(N = 4\)) were similarly injected with 1 \(\mu\)mol GLU in 5 \(\mu\)l volume. Group B rats were tested on 1 and 56 DPI to determine if any task-related experience was necessary for recovery. Rats in group C were tested daily beginning 1 DPI until reaching CPL. If task-related experience facilitated recovery, then group C rats should demonstrate CPL prior to the 56 days required in experiment 1.

**Experiment 3**

**Histologic evaluation of retinal damage after intravitreal glutamate injection:** Both uninjected and injected eyes from all behavioral animals were retained for histologic comparison. Immediately after removal, whole eyes were immersed in fixative solution containing 1% paraformaldehyde and 1% glutaraldehyde in .1 M phosphate buffer (pH 7.2) for 2 or 3 min at room temperature. Next, eyes were dissected in a petri dish containing fixative solution. Cornea, iris, and lens were removed and the remaining eyecups fixed an additional 1–4 hr at room temperature. Eyecups were dehydrated through a graded series of ethyl alcohol and infiltrated and embedded in glycol methacrylate (Polysciences). Embedded tissue was sectioned along the superior-inferior axis through the optic disc at a thickness of 2 \(\mu\)m. Toluidine blue-stained sections were examined via the light microscope.

**Results**

**Experiment 1**

Results are illustrated graphically in Figure 2. NS rats maintained 91.6% correct responses over the testing period while GLU rats performed at a significantly lower overall level of 73.4% correct, \(F(1, 8) = 170.67, P < .001\). Although comparable with the NS group during the 3 days prior to injection (–3 to –1 DPI on the graph), GLU-injected animals showed a statistically significant decline in performance to an average of 60% correct by 1 DPI, \(F(1, 56) = 59.58, P < .001\). Response levels for these rats remained below 70% correct through day 14. By 28 DPI, correct responses increased to 76%, which by the Newman–Keuls procedure was significantly greater than the average performance at 1 DPI, \(P < .01\). Further recovery was evidenced by 56 DPI, when the average response level of GLU rats was 88% correct. This
Fig. 2. Graph illustrates performance of albino rats injected intravitreally with 1 \( \mu \)mol monosodium glutamate (GLUTAMATE) on the visual pattern discrimination task relative to the performance of saline-injected (NORMAL SALINE) control rats. Performance was assessed over a 56-day period after injection at the times indicated and the values represented are mean percent correct ± 1 SEM; \( n = 5 \) for each data point. CPL = Criterion performance level.

was not statistically different from the NS group and preinjection levels (−3 to −1 DPI). The reliability of this behavioral recovery was checked by running all

Table 1. Summary of behavioral data for groups B and C from experiment 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat no.</th>
<th>1 DPI*</th>
<th>56 DPI</th>
<th>Trials to recovery</th>
</tr>
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<tbody>
<tr>
<td>B</td>
<td>756</td>
<td>70</td>
<td>75</td>
<td>80</td>
</tr>
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<td>759</td>
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<td>65</td>
<td>140</td>
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<td></td>
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<tr>
<td></td>
<td>771</td>
<td>55</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>( \bar{x} )</td>
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<td>68.75</td>
<td>95</td>
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<tr>
<td></td>
<td>SEM</td>
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<td>3.75</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
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<td>—</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>743</td>
<td>55</td>
<td>—</td>
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<td></td>
<td>746</td>
<td>60</td>
<td>—</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>( \bar{x} )</td>
<td>55</td>
<td>—</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>2.04</td>
<td>—</td>
<td>22.54</td>
</tr>
</tbody>
</table>

DPI = Days postinjection.

GLU-injected rats an additional two days. Every animal remained above CPL.

Experiment 2

Group A rats were above CPL both at 1 and 56 DPI, indicating good task retention. Results for groups B and C are summarized in Table 1. At 1 DPI, rats in group B showed expected behavioral deficits with an average correct response level of 63.75%; performance was similarly below CPL at 56 days with an average of 68.75% correct responses. When group B rats were run daily after day 56, an average of 95 trials was required before achieving preinjection response levels, ie, recovery. Thus some task-related experience was necessary for recovery.

Rats in group C all showed sharp declines in performance to an average of 55% correct 1 DPI. They recovered by 145 trials, which was not significantly different from group B. For purposes of comparison with GLU-treated rats in experiment 1, results from both GLU groups in this experiment were pooled. Rats run daily regardless of starting time recovered after an average of 120 trials over about six running days. The maximum running time was about ten days. Hence, GLU-injected rats in experiment 2 recovered approximately 1.5 months sooner than those in experiment 1.

To see if nonvisual cues could have mediated the recovery, the three NS rats from group A had their remaining right eyes surgically removed and after recuperation were run on the discrimination task for 7 more days. Average performance was 55% correct, a level which was not significantly above chance (ie, 50% correct) and which was similar to that for GLU-injected rats 1 DPI.

Experiment 3

An example of the irreversible degenerative changes after GLU injection is shown in Figure 3. The most striking feature of glutamate damage was a sharp reduction of inner retinal thickness, especially the inner plexiform layer, by 2 months postinjection. This thinning reflected a significant loss of ganglion and amacrine cells. Whereas exact quantification was not attempted in this experiment, ganglion cell loss was on the order of 50%. Reduction of inner nuclear layer cell numbers appeared somewhat less. Neuronal loss and thinning of the inner retina were evident, to a greater or lesser extent, in every GLU-injected rat. Examination of NS-injected eyes revealed no retinal cytopathology.
Discussion

In agreement with our preliminary work, intravitreal GLU injection caused deficits in pattern discrimination behavior during the first 1-2 weeks postinjection followed by a gradual recovery to control and preinjection levels by 56 days postinjection. Although it is impossible to guarantee that the rats used in this study learned the task solely on the basis of the orientational difference between S+ and S−, subjects did learn the discrimination, demonstrate significant deficits after glutamate injection, and recover by two months postinjection. Moreover, in experiment 2, when rats enucleated bilaterally and run on the task 7 additional days did not demonstrate above random response levels, this clearly showed that the behavioral response measured was mediated via visual input, the precise nature of which could be the basis of further work.

Our original findings have been extended by showing that similar to what has been demonstrated in studies of recovery of function after brain trauma, task-related experience proved both necessary and facilitative for recovery after GLU-induced retinal damage. Moreover, it was shown that intravitreal injection of micromolar amounts of GLU resulted in severe degenerative changes in the retina similar to those observed after neonatal rodents are injected subcutaneously, including significant reductions in ganglion and amacrine cell numbers. A precise quantification of cell loss after intravitreal GLU injection will be the topic of a later paper.

This is the first report of behavioral recovery following irreversible structural damage to neural retina. Although Wright and Harding have demonstrated recovery of olfactory behaviors in mice after section of primary olfactory nerves, this was shown to be a function of the growth of replacement neurons, a capacity the mammalian retina does not possess. Because the nature of the early retinal response to glutamate is one of massive edema, it is reasonable to assume that the initial precipitous drop in performance in GLU-injected rats was due to electrochemical disruption of the entire retina by influx of extracellular fluid. Subsequent recovery probably reflected resolution of the edema, reinstatement of functional integrity of surviving ganglion cells, and relearning of the task via these cells. There
was some indication from the histology of GLU-injected animals that rats with more residual ganglion cells recovered faster. However, additional work is needed to more precisely elucidate both the behavioral and anatomic parameters of this recovery phenomenon.

Key words: monosodium glutamate, retinal damage, visual behavior, pattern discrimination, T-maze, behavioral recovery, task-related experience, albino rat

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