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...taneous alternation and middle ear disease[1]

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CUES FOR SPONTANEOUS ALTERNATION[1]

ROBERT J. DOUGLAS[2]
University of Michigan

Large numbers of possible cues for spontaneous alternation by rats in T mazes were tested both in isolation and in combination in an attempt to discover which stimuli determine the response. Free-trial spontaneous alternation represents the addition of a relatively weak odor-trail avoidance and a much more powerful tendency to turn in opposite directions at a choice point. No other effective alternation cues could be found, and the magnitude of these 2 tendencies was sufficiently high to account for all observed alternation. It was suggested that rats, at least, have a sense of relative direction or position in space, and that the receptors are located in the inner ear.

...n the second of two consecutive un...arded trials in the T maze, a rat typ...ly enters the alley which was not ...ited on the first trial. This phenomenon, ...ned "spontaneous alternation," has been ... subject of a large number of studies ...e Dember & Fowler, 1958), but the ...ecise cues used by the rat in alternating ...ve never been determined. Many insights ...o the nature of spontaneous alternation ...d its relation to other factors such as ...ention and maze learning could be ...ed through the answers to such stim...-based questions as: does alternation ...ur to any attendable stimulus or only to ...estricted set of cues? Is there a relation ...ween attention to a stimulus and sub...uent alternation to that stimulus? Is ... rate of alternation a direct function ...the number of cues present, or is it ...tively constant despite variation in the ...mber of available cues? Do cues which ... ineffective by themselves summate to ...duce alternation when they are com...ed?

...n order to investigate the types of ...uli which were important in the deter...ation of spontaneous alternation, a method had to be devised which would make possible the measurement of alternation rates in response to single types of stimuli. In a previous study, Walker, Dember, Earl, and Karoly (1955) used a rotating ± maze to subdivide possible alternation cues into broad classes such as intramaze, extramaze, and response-induced stimuli. The relative importance of any one class was determined by allowing the rat to alternate either to that class or to a combination of the other two. Unfortunately, this method has been exploited almost to its limit, and a further subdivision of stimuli into classes such as visual or auditory would be extremely involved. In addition, the ± maze has the inherent weakness of failing to make a differentiation between alternation of one cue class and repetition of responses to the other opposed classes. For these reasons the method chosen for this series of experiments consisted basically of varying all stimuli on the two trials except for those deliberately held constant. Since alternation of a stimulus is impossible unless *S* is exposed twice to that stimulus, all observed alternation could then be attributed to the constant cues. For this purpose two different mazes of identical size were used in conjunction with two different testing rooms. In a typical experiment the rat was given a first trial in Maze 1 located in Room 1, and a following trial in Maze 2 in Room 2, with both situations containing a pair of stimuli differing along some dimension such as brightness. For example, the right alleys of both mazes might contain white inserts

[1] This study represents a major part of the ...or's doctoral dissertation, University of Michi..., 1964. Members of the committee were E. L. ...ker, W. L. Hays, and R. D. Alexander, with ...L. Isaacson serving as chairman. The manu...t was prepared while the author was the re...nt of United States Public Health Postdoc...l Fellowship MH 23, 382. Partial support for ... study was also provided by Grant DA-MD-...-63-G76 from the Office of the Surgeon ...eral, United States Army, to R. L. Isaacson.

[2] Now at Department of Psychiatry, Stanford ...dical Center, Palo Alto, California.

and the left alleys black. This method was used first to test alternation to the broad stimulus classes investigated by Walker, Dember, Earl, and Karoly (1955), then to study alternation to stimuli within single modalities, and finally to investigate the effects of stimulus combinations once again. Important criticisms of this procedure are that other types of stimuli are also inadvertently being held constant, such as those common to the two nearly identical mazes, and that alternation of body turns are confounded with alternation to the constant stimuli. Fortunately, both objections could be largely dismissed after the first experiment.

Method

Subjects and Housing

Three groups of *Ss* were used. Group 1 consisted of 48 male hooded rats, supplied by the Windsor Biology Gardens, Bloomington, Indiana, roughly 120 days old at the start of the experiment. Groups 2 and 3 consisted of 40 and 24 6-mo.-old male hooded rats, respectively, drawn from a population bred at the University of Michigan. These three groups were individually housed, placed on ad-lib food and water schedules, and gentled prior to testing; several hyperemotional animals were discarded and replaced by others from the same population.

Equipment

Two T mazes of identical size and shape were constructed of ½-in. plywood. Main alleys and cross arms were 16 in. long. Alleys were 4 in. wide and 6 in. high, and were covered with wire mesh. The first 6 in. of the main alley constituted a start box, separated from the rest of the maze by a sliding door. Sliding doors were also located at the entrances to the side alleys. Each maze had a hooded 7½-w. light bulb wired in place directly over the choice point, which provided the only illumination used during testing. Neither of the mazes was provided with a floor, and both were placed over sheets of heavy opaque brown paper which was used to cover the tops of the tables on which the mazes rested during testing. Two adjacent rooms were used for testing, with the maze usually pointing north in one of the rooms and west in the other. Mazes were placed on tables which were placed within 6 in. of the walls.

General Procedure

The *Ss* were carried 8 at a time to the experimental area in separate compartments of a felt-covered carrying box, which was placed in the hall outside the testing rooms. As needed, *Ss* were removed one at a time; all 8 were usually finished within ½ hr., with *Ss* in a given group always tested in the same order, all being given a particular test on the same day, and none tested more than once a day. Testing was done between A.M. and 3 P.M. Most tests were repeated one or more times at a later date.

On a typical test, *S* was given a first trial in one of the mazes in one of the rooms and a second trial in the other maze in the other room, with the constant stimuli added to both situations. On the first trial *S* was placed in the start box and after a 10-sec. wait, the door to the main alley was raised. When *S*'s whole body was in one of the side alleys, the door to that alley was lowered and the response scored. After a further 10-sec. interval *S* was removed and carried to the second maze for a second, identical, trial. On some tests it was not possible to carry *S* directly from one maze to the other, as stimulus manipulations were carried out between trials. In these cases *S* was gently placed in a pressed-fiber wastebasket covered with a thin felt pad. The time necessary for intertrial manipulations was calculated during practice "dry runs" and in all cases refers to the time elapsed between mazes, not between successive choices. The results were probably not affected by the differing intertrial intervals, as Walker (1956) found that lengthening the interval even up to several hours had no effect on the alternation rate. In addition several of the tests were given with differing intertrial intervals and the same results were obtained in each case.

Treatment of Data

The presence of alternation was judged on the basis of a χ^2 test comparing the observed distribution of alternation and nonalternation responses with a distribution based on chance expectancies. Differences between rates obtained under differing conditions were tested by means of a χ^2 comparison of the two distributions. Since these distributions often included the results of the same *Ss* tested on more than one occasion, the validity of this test depended on the observations being independent. An analysis of literally thousands of observations showed that this assumption was for all practical purposes true when a homogeneous group of *Ss* was used. This analysis showed that the probability of an individual in such a group alternating was best estimated by the group mean on a previous test, and not by the previous history of the individual (Douglas, 1964). Thus, the results of several individuals each tested once are comparable to results obtained from one individual run several times.

Determination of chance rates of alternation was based on an intensive investigation of initial response tendencies. Since the best estimate of alternation occurring by chance is $1 - [(p_R)^2 + (p_L)^2]$ (Douglas & Isaacson, 1965), it is important that stimulus or response biases be known. Group 1 was found to have no detectable turn bias, the percentage of right turns on initial trials was 51.3% in 768 total observations. Groups 2 and 3, however, had the usual 2:1 right-turn bias repeatedly found in animals from this population, and, in addition, Group 1 *Ss* were found to have marked stimulus "preferences" for one of the paired cues used on several of the tests. Biases such as these have been found to act independently on the 2 alternation trials, and to spuriously detract from alternation (Douglas & Isaacson, 1965), because alternation under biased and unbiased conditions is differentially affected by chance responses. This problem can be solved by either mathematically extracting chance or random factors from the observed alternation rate or by "adjusting" scores obtained under a bias so that they are comparable to scores obtained in the absence of a bias. The latter method was used here, as it results in scores which are comparable to those reported in the literature without the necessity of a mental transformation of the figures. The rationale for this method is presented in detail in Douglas (1964), but in simpler form is as follows.

Suppose that there were a true underlying tendency for a rat to alternate spontaneously, but that this tendency were only operative, say, 60% of the time. Then one might reasonably expect that of 100 rats given 2 consecutive trials in the T maze, 60 would alternate because of this underlying tendency. The remaining 40 would not all repeat, however, as their second response would be independent of the first, and whether these animals would alternate by chance or not would depend on the magnitude of the stimulus or response bias. If no bias existed, then one would expect half, or 20 *Ss*, to alternate spuriously while the other half would fail to do so. Thus, 80 rats out of 100 would be observed to alternate, even though they had only a 60% true alternation tendency.

Now if a bias had been present, a different rate of alternation would have been observed, even though the underlying tendency were identical in each case. Suppose that one of the two side alleys was black and the other white, and that *Ss* had an 80% black preference and a 60% true alternation tendency. Then of the original 100 *Ss*, 80 would have gone to the right on the first trial and 20 to the left. On the second trial, (.6)(80) + (.6)(20), or 60, would truly alternate, while of the remaining 40 *Ss*, (.8)(32) + (1 − .8)(8), or 27.2, would fail to alternate because of the bias, while only (1 − .8)(32) + (.8)(8), or 12.8, would spuriously alternate. In this case the observed alternation rate would be 60 + 12.8, or 72.8%, as compared to an unbiased rate of 80%, even though in both cases the true alternation tendency has been assumed to be 60%. Reasoning such as this has been used for the derivation of formulas which allow biased alternation rates to be converted into equivalent unbiased rates, and one of the possible forms of this equation is shown below.

Adjusted rate (% alt.)

$$= 50\left\{1 + \left[\frac{n - \left(\frac{n_R}{(p_R)^2 + (p_L)^2}\right)}{n}\right]\right\}$$

Where: n = the number of observations
n_R = the number of nonalternation responses
p_R = initial probability of a right turn (or approach to one of the stimuli)
p_L = initial probability of a left turn (or approach to the other stimulus)

This formula was used throughout this study whenever evidence of a stimulus or response bias was found, although a slightly different version (Douglas, 1964) must be used when the observed rate is below chance.

Experiment 1

First, the tendency to alternate in response to all cues combined was tested by giving *Ss* consecutive trials in the same maze in the same room with the same paper floor. In this manner all possible cues were held constant. Next, the stimuli were broken down into the three classes used by Walker, Dember, Earl, and Karoly (1955): intramaze, extramaze, and response-induced cues. Alternation to each class was tested separately, with the following definitions used for each class. Intramaze cues are those with which *S* could conceivably come into contact, including the inner surface of the maze and paper floor. Extramaze stimuli are those originating outside the confines of the maze, including the subfloor under the immediate paper floor and the visual, olfactory, and auditory stimuli of the environment. Response stimuli are those generated by the actual muscle and joint movements involved in making a turning response.

Method

Alternation to all cues simultaneously was tested on seven different occasions scattered throughout the series of experiments in order to insure that the basic rate was not undergoing a change. On three occasions the intertrial interval was 30 sec., and in four sessions it was approximately 10 sec.

Alternation to response-induced stimuli was tested in two different ways. On the first test, each Group 1 *S* was tested in four separate sessions with each of the 2 trials given in a different maze and room, with a different paper floor, for a total of 192 observations. For the first two sessions the intertrial interval was 30 sec., with S being confined to the wastebasket during the interval, and for the last two sessions the intertrial interval was about 10 sec., as S was carried directly from one maze to the next.

The second test of response alternation involved the use of a maze in which S could make two consecutive turns without being handled between responses. This maze was like the T maze used in the other experiments with the exception that another cross alley was added at the end of each side alley, so that after the first turn S would once again come to a choice point where a second turn was made. Sliding doors were used to prevent retracing. Group 1 *Ss* were tested on four separate occasions in this maze. Fluorescent room lights were used for illumination, and the maze was centered under a fixture.

Intramaze cues were tested by giving S 2 consecutive trials in the same maze, with the same paper floor, but with the maze transported from room to room between trials. Before the first trial the position of the maze on the paper was outlined with grease pencil so that it could be replaced in a correct position. Group 1 *Ss* were given 1 trial in this maze, and were then removed and placed in the wastebasket while the maze and paper floor were carried to their positions in the next room. This procedure required an intertrial interval of 40 sec. Then *Ss* were given their second trial in the second room, with the order of rooms balanced. The *Ss* were given this test twice, for a total of 96 observations.

Alternation of extramaze cues was tested by giving Group 1 *Ss* consecutive trials in two different mazes using two different paper floors, but with both mazes placed over the same spot on the same table in the same room. This procedure held constant the cues provided by the environment, but varied all others. Changing the mazes and papers required an intertrial interval of 30 sec. Each S was tested on two different daily sessions, once in each room, for a total of 96 observations.

Results

The test for all-cue alternation yielded a mean rate for all seven sessions of 80.4%. No significant difference was found between rates when different intertrial intervals were used (82% at 10 sec., 79.2% at 30 sec.), and variations between sessions were very slight, with the range being less than 13%. The group was remarkably homogeneous, and the probability of an individual alternating on a given session could not be predicted by its previous history as accurately as it could by the group mean.

Alternation to response-induced stimuli was found to occur at a rate very near to chance expectancies. On the first test, with some handling between trials, *Ss* alternated 51% of the time, as compared to a chance 50%. On the second test, where S was allowed to make two consecutive turns without handling, alternation occurred at a 49% rate. When the results of the two tests are combined, the mean alternation rate to response-induced stimuli was found to be exactly 50%, for a total of 394 observations.

Alternation to intramaze cues was found to occur 61.5% of the time. This rate was significantly higher than a chance 50% ($\chi^2 = 5.0$, $p < .05$), but was significantly below the all-cue rate ($\chi^2 = 16.8$, $p < .01$).

Alternation to extramaze cues occurred at a 75% rate, which was significantly higher than the intramaze rate ($\chi^2 = 4.1$, $p < .05$). In fact, this rate was so high that it did not reliably differ from that found for all cues combined ($\chi^2 = 1.3$).

Discussion

The presently observed rate of all-cue alternation (80.4%) suggests that the present Group 1 *Ss* were rather typical in this respect, as this figure is neither especially high or low in comparison to the literature.

The failure to find evidence for the alternation of body turns per se in the present experiment should come as no surprise. Both Montgomery (1952) and Estes and Schoeffler (1955) presented evidence against the existence of response alternation, and it has never been demonstrated that alternation of body turns occurs in a normal T-maze situation. Walker, Dember, Earl, Fawl, and Karoly (1955) did report that making the turning response more figural through the use of a special three-dimensional maze appeared to result in an increase in response alternation. However, this maze was a modified version of the ± maze, and interpretation of the results must be guarded. Since that maze had two different approaches to the same goal box, their *Ss* may have been repeating approaches to that goal box, which in that case would be scored as alternation. In any event, the present failure to find any evidence of response-induced alternation, even in a rather ideal situation in which no handling occurred between responses, indicates that response alternation is not a measurable factor in normal T-maze alternation tests, and that special experimental controls to rule out this tendency are not necessary.

The present finding that extramaze cues were more potent elicitors of alternation than were intramaze cues is at variance with the report by Walker, Dember, Earl, Fawl, and Karoly (1955) in which intramaze cues were found to be relatively more important. As was discussed earlier, however, their test involved the use of the ± maze. It will be shown later that alternation of extramaze cues might be expected to be reduced in such a situation, and that the results are not necessarily in conflict.

As was stated earlier, a possible objection to the present procedures could be that some stimuli are being inadvertently held constant, e.g., those of the response itself and those common to the two nearly identical mazes. The results of the first response stimuli experiment, however, showed that even when *both* factors were present no alternation could be detected. The results of some of the later experiments will also add evidence that these possible complications can safely be dismissed.

At first glance it might seem odd that when intramaze cues (61.5% alternation) were added to extramaze cues (75% alternation), as in the all-cue experiment, the resulting alternation rate was only 80.4%. If the first two figures are directly manipulated according to the rules of probability, as if independent, one would predict that alternation to the combined classes would be 90%, which is reliably higher than the observed rate ($\chi^2 = 33.5$, $p < .01$). This could lead to the conclusion that the cues do not act independently or that there is some tendency for the absence of one cue class to be compensated for by an increased use of the remaining class. However, this method is erroneous, as both figures contain a great deal of spurious alternation due to the presence of false positives, and while the true underlying alternation tendencies might be expected to interact additively, the error component should not. In the present case, where no bias was found, the extraction of error and the estimation of "true alternation" rates is relatively easy, as for every *S* that for some spurious reason did not alternate, there was probably one which did alternate, and a good estimate for true alternation is: (% alternation) − (% nonalternation). The complete rationale for the use of this, and the expanded form of the formula, can be found in Douglas (1964). Thus, the figures quoted above can be reduced to 23% for intramaze cues and 50% for extramaze. Now if these figures are combined additively, as if independent, the result is an expected true alternation rate to the two cue classes combined of 11.5 + 38.5 + 11.5, or 61.5% true alternation. In order to convert this figure into a "raw" rate the expected error must be added back in. Since of the 38.5% of the *Ss* which would not truly alternate, half would be expected to alternate spuriously while half would not, we should add 19.23% to our earlier true 61.5%, for an expected raw alternation rate of 80.75%, which is remarkably close to the actual observed figure of 80.4%. This result suggests that the reaction of the rat to these different cue classes is additive and independent. Additional evidence on this point will be presented later.

Experiment 2

Visual, tactile, and olfactory stimuli were considered as possible bases of the 61.5% alternation rate found for intramaze cues.

Method

Visual cues. Three types of visual intramaze stimuli were investigated: brightness differences, pattern differences, and stimuli specific to the maze. The 40 Group 2 *Ss*, used for the brightness test, were run in two mazes which differed in dimensions from those used in most of the other experiments; both T mazes had side alleys about 2 ft. long, and one had a main alley 1 ft. long, while the other had a 2-ft. main alley. One maze had a wooden floor while the other had a grid floor. These were placed against different walls of the same room, and were aligned at right angles to each other. The procedures were similar to those of Experiment 1, with an intertrial interval of less than 10 sec. Brightness differences were produced by lining the right alleys of both mazes with black cardboard inserts and the left alleys with white. Each *S* was then given 1 trial in each maze. Several days later the experiment was repeated with the white inserts in the right alleys and the black in the left, for a total of 80 observations.

Visual pattern cues were tested in almost the same way as the brightness cues, except that the 24 Group 3 *Ss* were given 1 trial in each maze, with the right alleys containing cardboard inserts having a double row of black five-pointed stars against a white background, while the left alleys had black circles in place of the stars. Circles and stars were of equal area, so the inserts did not differ in brightness. Several days later the positions of the stars and circles were reversed and the test repeated, for a total of 48 observations.

Visual and other stimuli specific to a given maze (excluding the floor) were tested by giving the 48 Group 1 *Ss* a single session with 2 consecutive trials in one of the two mazes used in Experiment 1, but with that maze transported from room to room between trials, and with a different paper floor used on each trial. The intertrial interval was 30 sec., during which *S* was confined to the wastebasket. While this method held constant the visual stimuli of the maze walls and ceiling, other types of stimuli, such as tactile, were not ruled out, of course.

Tactile stimuli. Tactile cues were provided by two pairs of floor inserts constructed of ¼-in. wood. Two sets, rather than one, were used in order to rule out specific odor or visual cues. Each pair consisted of one smooth board and one covered with ¼-in. wire mesh. Both were painted black. These were placed in the side alleys so that they met at the center of the choice point, and a white board was placed in the main alley so that *Ss* did not have to step up at the choice point. Group 1 *Ss* were given this test on two sessions: for the first session, the wire-covered inserts were in the right alleys of both mazes and the smooth boards in the left, and for the second, the positions of the inserts were reversed. On a given test *S* was given 1 trial in one of the mazes in one of the rooms, and then carried directly to the next maze in the next room for the second trial, with an intertrial interval of less than 10 sec. The order in which mazes and rooms were used was balanced, and 96 observations were made with tactile cues. As the inserts met in the center of the choice point, *S* could sample floor textures before making a response.

Olfactory cues. As the maze-specific stimulus test included possible odor differences within a maze, no further tests were made in the intramaze category. Deliberately produced odor differences were later tested as extramaze cues, although the odors were actually within the maze.

The effectiveness of *S*'s odor trail from the previous trial as an alternation cue was tested by giving the Group 1 *Ss* consecutive trials in two different mazes in two different rooms, but with the same paper floor used in both cases. Each *S* received a fresh paper floor for its 2 trials. Before the start of the first trial the position of the maze on the paper was outlined in grease pencil so that the second maze could be placed over the same area as the first. The *Ss* were confined to the wastebasket between trials while the paper was being shifted, with an intertrial interval of 30 sec. The test was given on two occasions, for a total of 96 observations.

The possibility that *S* might react to its own odor in a form other than an odor trail was tested by placing a 2 × 2 in. gauze pad in its cage 24 hr. before testing. Before *S*'s first trial this pad was placed in one of the side alleys very close to the choice point, and a clean pad placed in an equivalent position in the opposite side alley. After *S* had responded, these pads were removed and placed in the corresponding locations in the next maze and *S* given a second trial. The interval between trials was about 10 sec., and *Ss* were tested on two sessions, for a total of 96 observations.

Results

Visual cues. Alternation with the black and white brightness stimuli occurred only 37.5% of the time, which on the surface appears to be considerably less than chance. However, *Ss* from the population from which Groups 2 and 3 were drawn have been repeatedly demonstrated to have a right-turn tendency of about .67. In this particular case, this tendency was not quite high enough to reach significance (60%), although it also did not differ reliably from the estimated true value for this bias. In addition, a significant "preference" for black vs. white was found (67.5%, $\chi^2 = 9.8$, $p < .01$). Both biases acted to spuriously reduce the observed rate of alternation (Douglas & Isaacson, 1965), and when the present rate of 37.5% was corrected for these tendencies, the adjusted rate of 47.3% did not reliably differ from 50% ($\chi^2 = 0.2$). Thus, it can safely be concluded that these *Ss* were neither alternating nor repeating in response to brightness differences.

Alternation to the visual pattern cues was found at a rate of exactly 50%. When this figure was corrected for the turn bias, it corresponded to a nonbiased rate of 54.5%, which did not reliably exceed chance ($\chi^2 = 0.4$). Thus, *Ss* appear not to have alternated either brightness or pattern visual differences. The present visual stimuli were probably discriminably different to these *Ss*, as the present author had earlier trained two rats to make this discrimination in fewer than 50 trials each.

Maze-specific cues. Alternation to maze-specific cues, whether visual or otherwise, occurred at a rate of only 47.9%. As the unbiased Group 1 *Ss* were used, the chance rate was 50%, and the observed rate did not reliably differ from chance ($\chi^2 = 0.1$). Although the number of observations in this case was only 48, the results did not appear to warrant further testing. The evidence at this point was clearly against the hypothesis that visual cues of any type were the basis for alternation. A total of 176 observations in the three "visual" tests had failed to pick up even a hint of alternation, despite the fact that the brightness and pattern cues differed far more than the visual stimuli

[wh]ich might normally be present in an [un]differentiated T maze.

[T]actile cues. Alternation to tactile cues [wa]s found to occur at the low rate of 33.3%. [The]re was, however, a significant 76% tend[enc]y to respond to the mesh-covered floor [ins]ert in preference to the smooth one ($\chi^2 =$ [illegible].0, $p < .01$). When the alternation rate [wa]s corrected for this bias, the adjusted [ra]te of 45.6% did not differ reliably from [illegible]% ($\chi^2 = 0.7$). Thus, there was no observa[bl]e tendency for alternation of tactile floor [ins]erts.

[O]lfactory cues. An effective alternation [cu]e was finally found in *S*'s own odor trail. There was a 65.6% tendency for these [Ss] to enter the alley which had the paper [flo]or which had not been walked upon [on] the preceding trial. This rate was [si]gnificantly higher than a chance 50% ($\chi^2 = 11.5$, $p < .01$), but did not come [cl]ose to differing reliably from the 61.5% [ra]te for alternation to all intramaze cues combined. It appears very likely that all intramaze cue alternation was due to avoidance of odor trail, as no other effective stimuli were found. It is assumed that the present results can be considered to represent odor-trail avoidance, and not a reaction to visual or tactile floor stimuli, as these types of cues had previously been found to be ineffective.

When alternation to *S*'s own odor on a gauze pad was tested, it was found to occur at a nonsignificant rate of 55.2% ($\chi^2 = 1.0$). There was also no detectable tendency for this pad to be approached or avoided on the first trial. Thus, the odor by itself did not appear to elicit alternation unless it was in the form of a trail made by *S* on the previous trial.

Discussion

The only effective alternation cue in the intramaze class appears to be the individual's odor trail. When this was absent, no "compensatory" alternation to the remaining cues was found, and when it was present, alternation occurred at a rate not reliably different from that observed when all intramaze stimuli were combined. The data indicate, however, that the failure of *S* to alternate to visual and tactile stimuli is not due to *S*'s failure to "notice" these cues. There were clear and significant tendencies for *Ss* to respond differentially to black vs. white and wire-mesh vs. smooth floor. Such tendencies, however, act independently on the 2 trials, and an analysis of the data indicated that the probability of making a response to black or wire mesh on the second trial was constant, whatever the first response had been. Alternation, on the other hand, implies that the second response is determined by the first. If "attention" is objectively defined in terms of these differential responses, then it can be concluded that attention to a stimulus does not imply that that stimulus will be alternated.

The failure to find alternation to visual stimuli agrees with the report by Dember (1958) that blinded rats alternate at a normal rate. In addition, the present results indicate that Dember's animals were not merely "compensating" for the loss of vision through the use of cues not normally used in alternation. These results, however, cast considerable doubt on the hypothesis that alternation is a response to changed stimuli (Dember, 1956). In that study rats were found to approach the alley which had been changed in brightness between trials, even though on the test trials both alleys were of equal brightness. While there can be little doubt that rats do make such responses, this does not imply that the phenomenon is identical to spontaneous alternation. While rats approach stimuli which are changed in brightness, the present results show that they do not alternate in response to unchanged visual stimuli. In the usual T-maze alternation situation, stimuli remain unchanged between trials by *E*, and if differences do exist from trial to trial these are due to the presence of an odor trail (or possibly urine or fecal boli) in the just visited alley. An approach to this change would result in spontaneous repetition, rather than alternation.

The finding of an apparent tendency of the rat to avoid its odor trail suggested that a rat might also react to the trail of the previous animal. No formal test of this possibility was made, but the data from several experiments in which the same paper floor had been used for different animals were analyzed. In the records from four daily sessions there were found to be 187 possibilities for *Ss* to respond to the odor trail of the immediately preceding rat. Avoidance of this trail was found to occur only 50.8% of the time, so *Ss* did not appear to be responding differentially to the trail of the preceding *S*. This finding must be limited, however, to rats which are individually housed, as nothing is known about a possible avoidance tendency in animals housed in group cages where dominance hierarchies might emerge.

Experiment 3

Experiment 1 showed that alternation of extramaze cues occurred at a high (75%) rate. In the following experiment an attempt was made to discover the specific types of stimuli which might have contributed to this alternation. The effectiveness of visual and auditory cues, odor differences, and deep-floor stimuli were tested both individually and in combination.

Method

Visual extramaze cues. Group 1 *Ss* were used in this and all subsequent experiments. Visual extramaze stimuli were provided by stripes which were attached to the inside top surface of a cardboard box which covered the maze. On the right half of the inside ceiling were placed ½-in. red and white stripes running parallel to the cross alleys. On the left side similar stripes were arranged vertically to the cross alleys. Reflected light from the maze made these stimuli clearly visible from underneath. Two conditions were used for this test. In the first, *S* was given 1 trial in one maze in one room and the next trial, 30 sec. later, in the other maze in the other room, with the box placed over each maze during the test. In the second test the same maze was used for both trials, which added maze-specific cues to the situation. Each *S* was tested twice, for a total of 96 observations, and the intertrial interval was 40 sec., as both the maze and the covering box had to be transported from room to room.

Deep-floor cues. The possibility of deep-floor cues being important in alternation was suggested by the work of Shepard (1959), in which these cues were suspected of being important in the learning of complex mazes by the rat. The effectiveness of floor resonance differences as alternation cues was tested through the use of a special subfloor, constructed for this purpose, which was large enough (23 × 42 in.) to form a floor for the entire maze. The right half of this floor was made of three layers of ¼-in. fiberboard, while the left half was a solid piece of ¾-in. plywood. The dividing line between the two halves was placed under the centerline of the main alley so that the right half of the main alley and the entire right side alley were over the fiberboard layers. The subfloor was placed on the previously used tables, and raised from the surface by ¾-in. wood pedestals. A finger tap on either side produced noticeably different sounds.

The *Ss* were tested by giving them 1 trial in each maze and room, with the subfloor used on both trials, but covered with a new paper floor for each trial in order to rule out odor-trail cues. The intertrial interval was 40 sec., and two sessions were run, for a total of 96 observations.

Odor differences. Even though the possible odor differences within a maze had been dismissed as alternation cues, it was still not known whether strong odor differences might produce alternation. This possibility was tested through the use of different smelling substances which were actually placed within the maze, although it was suspected that if odor differences were normally used by rats they might well originate outside the maze. The olfactory stimuli were a liquid soap with a strong peppermint smell and a decidedly aromatic pipe tobacco, placed in open vials and taped to the insides of the ends of the cross alleys. Tobacco was at the left in both mazes and the soap at the right; positions were not switched because of a fear that the effects might be lingering. The *Ss* were given 1 trial in each maze and room, with an intertrial interval of 10 sec. or less. Each *S* was run on two different occasions for a total of 96 observations.

Auditory cues. The auditory stimulus consisted of a photographic timer which emitted a buzzing sound of a mild but clearly audible intensity. Each *S* was given 1 trial in each maze and room with this buzzer either to the left or right on both trials. The intertrial interval was 10 sec., with *S* and the turned-off buzzer being transported directly from one maze to the next, and each *S* was tested once, for a total of 48 2-trial observations.

Cue combination. Because of the negative results which will be discussed later, it was decided that an attempt would be made to reconstruct an extramaze alternation cue combination by using simultaneously most of the stimuli which had previously been used separately. In this experiment a single maze was used for both trials, although these trials were given in different rooms. To make the maze portable, the subfloor used in the deep-floor cue experiment was attached by a hinge to this maze so that different paper floors could be inserted easily between trials, the auditory cue buzzer was attached to the outside of the butt of the right side alley, the two odor vials were attached inside the butt ends of the side alleys, and the extramaze box was attached to the maze. It was possible to switch rooms and start the next trial within about 50 sec. Each *S* was given 1 trial with these combined cues in one room, and was confined to the wastebasket until the maze was placed in the next room for the next trial. Each *S* was tested on two sessions for a total of 96 2-trial observations. The constant or alternatable cues were a combination of visual maze-specific and extramaze cues, auditory, deep-floor, and odor difference cues. Odor-trail cues were eliminated by using different paper floors on the two trials.

Results

Visual extramaze cues. Alternation to extramaze cues did not differ significantly from chance, either when these cues were isolated (45.8%) or when they were used in combination with maze-specific cues (51.0%). Since no stimulus biases were found, the chance rate was 50%. The experiment added further evidence against the possibility of spontaneous alternation occurring to visual cues of any type.

Deep-floor or vibratory cues. Alternation to subfloor cues was found at a rate of 52.1%, with no stimulus bias present. This did not, of course, come close to being significantly different from chance, and it can be concluded that deep-floor cues probably did not affect alternation, as these cues were far more intense than would be found in the usual subfloor underlying a maze.

Odor differences. Alternation to the odor stimuli occurred at a rate of only 41.7%. There was, however, a significant tendency to approach the peppermint smell rather than the tobacco (66.7%, $\chi^2 = 10.6$, $p < .01$). When the alternation rate was adjusted for this bias, the resulting rate of 47.9% did not differ significantly from chance ($\chi^2 = 0.3$). Since these odor differences were more marked than would normally occur in a alternation situation, and since the bias of these *S*s indicates that they "noticed" the odors, it is unlikely that alternation of odor differences occurs in the T maze.

Auditory cues. Alternation to auditory cues occurred at a rate of 47.9%, which did not differ reliably from 50%. No tendency was found to either approach or avoid the side closer to the buzzer.

Cue combination. When *S*s were given the opportunity to alternate simultaneously to maze-specific plus visual extramaze plus auditory plus odor-difference plus deep-floor cues, the astounding result was that they failed to alternate at greater than a chance rate. When the observed raw rate of 47.9% was corrected for the previously mentioned odor preference the result was an adjusted rate of 52.1%. This was significantly different from the 75% rate measured earlier when two different mazes had been used in the same position in the same room (with odor trail, of course, ruled out), and the difference was reliable at far beyond the 1% level ($\chi^2 = 21.8$).

Discussion

At this point the search for the cues which had been responsible for the high rate of "extramaze" alternation in Experiment 1 could be considered to be a total failure, despite the fact that every type of stimulus of which the author (and many others) *could* conceive had been tested both alone and in combination with all others. The results did, however, show that there was no simple relationship between the ease with which stimulus differences can be learned and their use as effective alternation cues. Obviously, the failure to find an effective extramaze alternation cue must have been due to a failure to include it in the series of tests. The extramaze alternation test in Experiment 1 must have included some factor missing from this experiment. The discovery of the important difference came about entirely by accident when the author reran the auditory cue test using a different stimulus (a music box). On that test the mazes were realigned in their separate rooms so that both were now pointing in the same direction, while in all previous experiments (except for the extramaze cue test in Experiment 1) the mazes had pointed in directions differing by 90°. Surprisingly enough, *S*s began to alternate at a very high rate, in contrast to the earlier negative results. At this point the mazes were once again pointed in perpendicular directions, whereupon alternation fell back to a chance 50%. These results suggested that the rat was somehow able to use spatial direction cues in alternating, and that these were the important cues missing from this experiment but present in Experiment 1.

Experiment 4

Although in the music box experiment discussed above *S*s alternated spatial direction at almost the same rate as they had alternated to the extramaze cues in Experiment 1 (74% vs. 75%), it was still not known whether the results were an artifact due to some stimulus present in both rooms and *detectable* in both cases only when the mazes were in the precise locations used. For this reason the experiment was repeated, but with the parallel-alley mazes pointed in a common direction 90° to the left of the earlier positions. In addition, the experiment was repeated with the maze alleys aligned in parallel, but with each maze pointing in the opposite direction. In this way an alternation of spatial direction would require that S make the same turn twice in a row. The music box was, of course, discarded.

Method

Mazes parallel, pointed same direction. Mazes were placed so that they pointed in the same direction, with alleys parallel, but with each placed against the west wall rather than the north. The S was given 1 trial in one maze in one room and carried directly to the next maze and room for the second trial, with an intertrial interval of 10 sec. or less. Each S was tested on two sessions for a total of 96 observations.

Mazes parallel, opposite directions. This test was done both using two rooms and with both mazes in the same room. In the two-room test, the mazes pointed (*a*) north and south, and (*b*) east and west. The *Ss* were given two sessions in each condition, for a total of 192 observations. Procedure was otherwise identical to that above. This experiment was repeated using both mazes in the larger of the two testing rooms. In this test the mazes were pointed toward each other and placed on the same large table in the center of the room. Both north-south and east-west alignments were used, and a total of 192 observations made, using test procedures outlined above.

Results

Parallel mazes, same direction. A very high rate of alternation was once again found in this situation (81.3%). When this figure was combined with the earlier results from these same conditions, a mean of 77.6% alternation was found for what will tentatively be termed spatial-direction alternation. This rate is well above chance ($\chi^2 = 58.5$, $p < .01$), and more than sufficient in magnitude to account for all of what was thought to be extramaze cue alternation. These results indicate that alternation when two mazes are used is dependent upon alley orientation rather than on the location of the maze, and that the extramaze cue is actually spatial direction, or at least relative direction.

Mazes parallel, opposite directions. On the first part of this test, where the parallel-alley mazes were pointed in opposite directions and placed in two different rooms, alternation of spatial direction dropped to 61.5%, which was significantly lower than in the same-direction condition above ($\chi^2 = 12.5$, $p < .01$). This rate was, however, well above chance ($\chi^2 = 10.1$, $p < .01$).

On the second part of this test, in which both mazes were in the same room, a rate of 63.5% was found. This rate is nearly identical to that found when two rooms were used, and is also both reliably higher than chance ($\chi^2 = 14.1$, $p < .01$) and lower than that found in the same-direction experiment ($\chi^2 = 9.8$, $p < .01$). Once again the results indicate that alignment and direction are the important factors, and not the number of rooms used.

Discussion

These results leave little room for doubt that the results of the extramaze cue test in Experiment 1 were attributable to a tendency of S to turn in opposite spatial directions, and probably not due to environmental stimuli, as had originally been supposed. An extensive logical analysis showing that the present results could not possibly have been due to *Ss'* reactions to room cues can be found in Douglas (1964). For present purposes it can be stated that almost identical results were obtained with the mazes in the same or in different rooms, or in different positions within those rooms. In addition, the results of the second response cue test in Experiment 1 showed that if S had made its first response as a reaction to some room cue, then its second response was not in the opposite direction to that cue, but was instead independent of the first.

Although alternation of spatial direction was definitely lower when the mazes were pointed in opposite, rather than in the same, directions, the reason for this effect can only be guessed. It was probably not due to any opposition from a tendency to make alternate body turns, as the results of Experiment 1 showed that such a tendency probably did not exist. In any event, the back-to-back configuration greatly resembles that of a ± maze, and may explain why Walker, Dember, Earl, Fawl, and Karoly (1955) arrived at a much lower estimate for the strength of extramaze cue alternation than was found here in Experiment 1. One reason for the reduction alternation rate in the opposite-direction situation may have been that the rats were slightly disoriented by the extra turning involved between trials. If this were so, then deliberate turning between trials should have an even greater effect in reducing spatial alternation.

Experiment 5

At this point the evidence clearly indicated that the greater part of the rats' spontaneous alternation in the T maze was based on a tendency to turn in opposite directions at a choice point. It was suspected that this tendency might well be based on information supplied by the semicircular canals which could be interfered with by spatial disorientation produced by turning between trials. If this were true, then it should be possible to disrupt alternation even in "normal" T-maze situations, as well as in the parallel-alley, two-maze situations.

Method

The mazes were once again placed in separate rooms with their alleys parallel, and with both mazes pointing in the same direction. The *Ss* were again given 1 trial in each maze, with *E* carrying *S* directly from one room to the next between trials. This time, however, *E* made a 360° pirouette while carrying *S* from one maze to the next. Each *S* was tested once, for a total of 48 observations.

In an attempt to disorient *S* more completely after the first trial, *S* was carried into a darkened room across the hall and subjected to eight rapid turns (of varying degrees) in alternating directions in a horizontal plane. The *S* was placed in *E*'s hand during this procedure, with the body and head held parallel to the floor; the turns were from left to right and vice versa. Each *S* was tested on two sessions for a total of 96 observations.

As the procedure above appeared to produce some emotionality, two control experiments were run. In the first, the procedure above was repeated except that 12 turns in rapidly alternating directions were made in an up-and-down plane, rather than horizontally, with *S* still held parallel to the floor. This procedure appeared to produce an even greater emotional reaction in the rats than the one above. The *Ss* were tested on two sessions for a total of 96 observations.

In the second control experiment an attempt was made to rule out the possibility that these procedures might merely disrupt behavior in general through fear or other side effects. The *Ss* were tested in a normal all-cue T-maze situation, with horizontal turns between trials. If this procedure disrupted behavior in general, then alternation should fall to the chance level, but if it only affected spatial alternation, then *Ss* should alternate at a rate characteristic of odor-trail avoidance. Each *S* was given 2 consecutive trials in the same maze in the same room with the same paper floor on both trials. In short, *Ss* were tested in an orthodox spontaneous-alternation situation, except that the eight turns in a horizontal plane were given between trials. The intertrial interval in these turning tests was roughly 30 sec. This test was given in two sessions, for a total of 96 observations.

Results

When *S* was given only one 360° horizontal turn between trials, alternation was found to occur at the rate of 64.6%, which was very close to the rate observed when the mazes were pointing in opposite directions (62.5%). Thus, it would appear that turning between trials might account for the lower rate found with opposite-direction mazes. The present 64.6% rate just missed being significantly lower than the rate obtained under the same conditions, but without a turn between trials ($\chi^2 = 3.5$, $p < .07$). This failure was probably due to the fact that only 48 observations were made, but the results did not appear to warrant further testing.

When *S* was turned eight times in a horizontal plane between trials, alternation occurred at a rate very close to chance (53.1%, $\chi^2 = 0.4$). This rate was, of course, significantly lower than the near 75% for *Ss* that had not been turned between trials ($\chi^2 = 24.5, p < .01$).

When *S* was turned 12 times in a vertical plane between trials, alternation occurred at the very high rate of 79.2%, a figure very close to that expected in this situation if no disruption were produced by the vertical turning. Since the emotionality produced by this procedure appeared to at least equal, if not exceed, that of the horizontal turning, it is very unlikely that the reduction of alternation to a near-chance rate in the latter situation was due to emotional factors.

When *Ss* were tested in a normal all-cue alternation situation, but with eight horizontal turns between trials, the alternation rate (66.7%) was found to be significantly lower than the normal all-cue rate of near 80% ($\chi^2 = 7.6, p < .01$). Since this rate was very close to the 65.6% found for odor-trail avoidance, the amount of alternation observed in the present situation was very likely due to the odor-trail avoidance tendency, which was apparently not affected by the turning procedure. These results suggest that the effects of horizontal turning were due to a selective disorientation of the direction cues normally used by the rat.

Discussion

The evidence now appears to clearly favor the hypothesis that alternation is based on a relatively weak odor-trail avoidance tendency interacting additively with a much more powerful tendency to turn in opposite spatial directions at a choice point. Evidence for the first factor consists of the findings that rats alternated at a moderate rate when the only constant cues available on the two trials were those provided by the paper floor. In Experiment 2 it was shown that this tendency is probably not due to the possible visual or tactile cues associated with this floor.

Evidence for spatial alternation consists of the observations that alternation of direction in space occurred at about the same rate when the rat was run in the same location and when the two trials were given in separate rooms, as long as the mazes were parallel and pointing in the same direction. Alternation was reduced either when the mazes were pointed in opposite directions in the same or different rooms, or when *S* was given one 360° turn between trials. Further, alternation was found at a normal rate when *S* was turned in a vertical plane (up and down) between trials but reduced to a rate very near chance when *Ss* were turned in a horizontal plane between trials. When the latter conditions were repeated with the addition of odor-trail cues, *Ss* alternated at a rate characteristic of odor-trail avoidance. This strongly suggests that the rat is capable of knowing its position in space relative to a previous position, and uses this information in alternation. The most obvious candidate for the relevant sensory organs is the vestibular system, more specifically the semicircular canals, which are sensitive to acceleration of turning movements in three planes, only one of which, the horizontal one, would be particularly relevant here. This information could conceivably be the basis for a spatial position sense. As a further check on this "vestibular" hypothesis the present author investigated spontaneous alternation in rats with middle ear disease, a disease which results in progressive destruction of the inner ear organs, usually unilaterally. While such unilateral destruction of the cochlea probably does not greatly interfere with audition, a unilateral insult profoundly disrupts the paired and balanced vestibular system. It was found that these animals appeared to completely lack spatial alternation, while still retaining normal odor-trail avoidance tendencies (Douglas, 1966). If such a direction or position sense exists, then the possibility of cortical representation must be considered. Unfortunately, there is no general agreement among anatomists as to the location or even the existence of a vestibular projection area. It has been reported, however, that ablation of parietal cortex in monkeys apparently results in a loss of spatial position sense in monkeys (Ettlinger & Kalsbeck, 1962). This suggests that equivalent lesions in rats should eliminate spatial alternation through the elimination of the sensory system involved.

While most of the results reported here were statistically significant and internally consistent, it is not intended that they be extended without reservation to alternation behavior observed under conditions other than the free-trial, unrewarded response procedures used here. There is some reason to believe that forced-trial alternation, with or without reward, differs in some respects from free-trial alternation. The forced-trial procedure typically involves blocking off one of the side alleys of the T maze so that *S* can visit only one of the alleys on the first trial (or first block of trials), with the second or test trial being a free choice, as the block has been removed before that trial. While alternation using this type of procedure should logically involve both odor-trail avoidance and spatial-direction alternation, it also would be expected to involve the added factor of an approach to change (Dember, 1956), as the now unblocked alley has obviously been changed between trials. If this tendency interacts additively with the other alternation tendencies, then one would expect that forced-trial alternation should generally be found to occur at a higher rate than free-trial alternation. In one study in which were observed under both conditions, it was found that forced-trial alternation was indeed higher than free-trial (Dember & Fowler, 1959). In fact, the magnitude of the difference was very close to that expected if spontaneous alternation and the approach to change were independent and additive factors.

This analysis helps to explain the apparently contradictory finding by Estes and Schoeffler (1955) that odor-trail avoidance does not occur. In that study a forced-trial procedure was used, and a possible odor-trail avoidance tendency was opposed by both spatial-direction alternation and the tendency to approach change. Considering that the former tendency is relatively weak, while the latter two are very powerful, it is not surprising that an odor-trail avoidance tendency was not detected. This argument is presented in mathematical detail in Douglas (1964). The negative findings of Wingfield and Dennis (1934) can be explained in a similar manner, as they tested odor-trail avoidance in opposition to spatial-direction alternation. In both cases their results would have been expected even if it were assumed that odor-trail avoidance did exist.

The present series of experiments has done much to clarify the relation between learning and alternation cues. Walker and Paradise (1958) reported that those cues which were alternated at the highest rate were those which were also learned the fastest. In that study, however, stimuli were not actually isolated, and alternation was attributed to a certain cue mainly by inference. The present study showed that a number of cues which are easily learned were simply not alternated to, with the best example being the visual brightness stimuli. Thus, the lack of alternation to a stimulus does not imply that it will be difficult for the rat to learn to respond differentially to that stimulus. On the other hand, there is some evidence that the main determiner of alternation, a spatial direction sense, may be very important in the learning of complex mazes. Watson (1907) found that if rats were first trained to perfection in a maze, and then the maze turned so that it faced in a different direction in the room, the animals began to make a great many errors. The greatest decrement was found when the rotation was 90°, with a milder effect found at 180°. If the maze was not rotated, but merely moved in one direction between trials, then his animals acted as if nothing were amiss and continued in their error-free performance. These and related findings led Watson to conclude at one point that the semicircular canals must be the most important receptor system in complex maze learning. Shepard (1959) also reported that two of his rats suddenly lost their ability to succeed in his complex maze at the onset of middle ear disease, a condition which, as was mentioned earlier, disrupts the vestibular system. It is probably no coincidence that this disease also eliminates alternation of spatial direction.

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umerical Estimates of Neurons and Glia in Lateral eniculate Body During Retrograde Degeneration [1]

KAO LIANG CHOW AND JAMES H. DEWSON, III
Division of Neurology and Department of Psychiatry, Stanford University School of Medicine, Palo Alto, California

ABSTRACT Studies were made of decrease of neurons and proliferation of glial cells in the dorsal nuclei of lateral geniculate body of 32 rabbits and 12 cats who had undergone unilateral ablation of visual cortex up to 60 weeks prior to sacrifice. Results show that degeneration occurred in 80% of neurons in rabbit lateral geniculate body within three days, and 95% disappeared within four weeks. In comparison, degeneration was present in only 70% of neurons in the cat nucleus at the seventh post-operative day, and about 26% remained normal up to the fiftieth week. Glial cell reactions were differential and complex: astrocytes showed rapid proliferation with gradual return to normal, while oligodendrocytes and microglia increased more slowly, maintaining a high level over 50 weeks.

Since Nissl first demonstrated that de- nerative changes of nerve cells resulted om destruction of the axons over half a ntury ago, numerous studies have de- cribed the process of retrograde cell de- generation in a wide variety of pathological nd experimental material. Einarson ('60) as reviewed the literature extensively, so his will not again be detailed here. Be- ause of the irreversible and localized na- ure of the cell-atrophied zone in the alamus, retrograde degeneration has en used by investigators to determine e projection of the thalamo-cortical con- ections of many different species. Results ave shown that almost all of the dorsal alamic nuclei sustain retrograde degen- ation following ablation of corresponding rtical areas (Clark, '49; Peacock and mbs, '65; Powell, '52; Rose and Woolsey, 3; Walker, '35, '38).

The completeness of cell loss in the ected regions varies from nucleus to cleus. The dorsal nucleus of lateral niculate body (for brevity, the term "lat- l geniculate" will be used hereafter) ows a sharply delineated zone of cell ophy as well as a severe loss of the urons within the degenerated area after al lesion of the visual cortex. Lashley 1) reported practically no intermediate nsition between a normal number of ls in the unaffected region and no neu- ns in the degenerated part of this nucleus rat. A similar, almost total depletion of neurons during retrograde degeneration has been found in the lateral geniculate of rabbit, monkey, and man (Polyak, '32; Powell, '52; Rose and Woolsey, '43; Walker, '35, '38). In the cat, however, many neurons persist in the lateral geniculate following destruction of visual cortex (Minkowski, '13; Waller and Barris, '37). Whether these remaining neurons represent the cells with short axons is not known (O'Leary, '40). No quantitative determinations on the time-course of the disintegration of the neurons in the lateral geniculate during retrograde cell-atrophy have come to our attention.

In common to many types of traumatic and degenerative lesions of the brain, proliferation of glial cells is present in the lateral geniculate during retrograde degeneration (Glees, '55; Penfield, '32; Rio Hortega, '32; Windle, '58). Several studies on the gliosis of the lateral geniculate during retrograde degeneration are relevant to the present experiment. Albers (in Windle, '58) failed to find significant differences of the amount of proteolytic enzymes between the degenerated and normal lateral geniculate. Koch, Ranck and Newman ('62) reported an increased concentration of

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was also used. All the numerical estimates were expressed in a unit volume of 0.0002 mm³ (0.01 mm² × 20 μ) for rabbit, and 0.00026 mm³ (0.01 mm² × 26 μ) for cat. For tissues prepared by the frozen technique, these represent cell-density values. Because of the uncertainty about the extent of tissue shrinkage in paraffin-embedded material, the values computed from the thionin-stained slides should not be compared directly with such estimates of other reports.

We chose to count only those neurons whose nucleoli were clearly visible. The criterion for inclusion of the glia cells in the celloidin sections was a well-outlined nucleus. All whole cells were counted in the silver-stained slides; cell fragments were not included. The estimates also included half of the cells that were situated on the boundary of the counting field. Since our measurements were relative ones (the cell ratios of the degenerated to the control lateral geniculates), we were not concerned with many of the sources of bias commonly recognized in numerical determinations of cell populations (Agduhr, '41; Bok and Van Erp Taalman Kip, '39; Haug, '56). We were aware, also, that the distinction between oligodendrocytes and microglia is often an uncertain one. We have arbitrarily made separate estimates for these two types of glia cells, and computed the data separately.

On every slide through the lateral geniculate of every animal used for cell-count, total areas of the normal and the degenerated geniculates were computed. Additionally, the area of degeneration within the affected nucleus was measured with a planimeter. Separate sums of areas of the control and degenerated nuclei as well as the degenerated zones were then computed. Percentages which represent both the ratio of the degenerated sub-area to the total area of the affected nucleus, and the ratio of the total areas of the degenerated to control nuclei are listed in tables 1 and 2. Area measurements were taken only from materials used for cell-counts. These tables also include the post-operative survival-times of the animals, the thickness of the sections, and the staining method utilized (thionin or Cajal and Rio Hortega).

RESULTS

The histological findings described below apply only to the lateral geniculate ipsilateral to the cortical ablation; the lateral geniculate ipsilateral to the intact visual area invariably remained normal.

Rabbit. Over 10% of the geniculate neurons underwent chromatolysis one day after the operation; by the third postoperative day, approximately 80% of the cells were degenerated. These neurons showed swollen nuclei surrounded by dusty Nissl substances, and rows of vacuoles crowded at the periphery of the perikaryon. In addition, widespread damage of the capillaries was also apparent. By the end of one week, the degenerated cells consisted of a large nucleus with a thin rim of cytoplasm; indeed, in many cases, only an isolated nucleus was left. All normal neurons disappeared between the fourth and ninth weeks. The time course of the depletion of neurons is shown in figure 1. Except for the estimates of the first day and the estimate of total neurons at the third day, all differences between the number of neurons in the degenerated and normal lateral geniculates are statistically significant at < 0.001 level.

The astrocytes in the normal lateral geniculates stained with Cajal's gold sublimate were round cells showing a few slender processes radiating outward from the perikaryon. We did not attempt to distinguish between protoplasmic and fibrous astrocytes in the present study. In the degenerated nuclei, the cell bodies showed signs of enlargement as early as the third post-operative day. The development of astrocytes reached a peak between the second and ninth post-operative weeks. During this period, the number of astrocytes in the degenerated nucleus tripled compared with its control nucleus. The individual cell bodies and cell processes grew in size and also increased in number and length to form an interwoven network. By the fifteenth week, some cell processes were broken and separated from the cell body. Further regression of the hypertrophied astrocytes continued until the fiftieth week, when the number of cells returned to control levels. The post-operative development of astrocytes is graphically illustrated in figure 2. The differences

TABLE 1

Data from rabbit experiments

Rabbit	Survival	Stain	Thickness	Total areas: affected of non-affected	Sub-areas: degenerated zones
	weeks		µ	%	%
CP 18	no op.	C.H.	20	—	—
CP 30	no op.	C.H.	20	—	—
CP 31	no op.	Thionin	20	—	—
CP 26	1 day	C.H.	20	98	65
CP 104	1 day	Thionin	40	—	—
CP 12	1 day	Thionin	20	100	85
CP 16	3 day	C.H.	20	107	75
CP 19	3 day	C.H.	20	—	—
CP 17	3 day	Thionin	20	103	74
CP 2	1	C.H.	20	103	81
CP 20	1	C.H.	20	103	60
CP 14	1	Thionin	40	101	67
CP 3	2	C.H.	20	—	—
CP 4	2	C.H.	20	96	78
CP 27	2	C.H.	20	107	66
CP 15	2	Thionin	40	110	72
CP 6	3	C.H.	20	—	—
CP 5	4	C.H.	20	101	89
CP 29	4	C.H.	20	100	70
CP 101	4	C.H.	20	—	—
CP 103	4	C.H.	20	—	—
CP 105	7	C.H.	20	—	—
CP 7	9	C.H.	20	102	78
CP 28	9	C.H.	20	94	70
CP 8	9	Thionin	40	103	88
CP 107	15	C.H.	20	—	—
CP 24	15	C.H.	20	92	73
CP 25	15	Thionin	20	107	54
CP 22	25	C.H.	20	84	55
CP 10	25	Thionin	20	89	73
CP 11	50	C.H.	20	88	53
CP 23	50	Thionin	20	85	42

TABLE 2

Data from cat experiments

Cat	Survival	Stain	Thickness	Total areas: affected of non-affected	Sub-areas: degenerated zones
	weeks		µ	%	%
G 18	0.5	C.H.	26	—	—
G 21	0.5	Thionin	26	—	—
G 16	1	C.H.	26	94	78
G 22	1	Thionin	26	—	—
G 17	6	C.H.	26	100	72
G 13	6	Thionin	52	—	—
G 10	10	Thionin	52	96	63
G 11	10	Thionin	52	104	54
G 15 R	14	C.H.	26	—	—
G 19	14	Thionin	26	99	58
G 15 L	27	C.H.	26	—	—
G 12	27	Thionin	26	93	62
G 23	50	Thionin	26	87	68

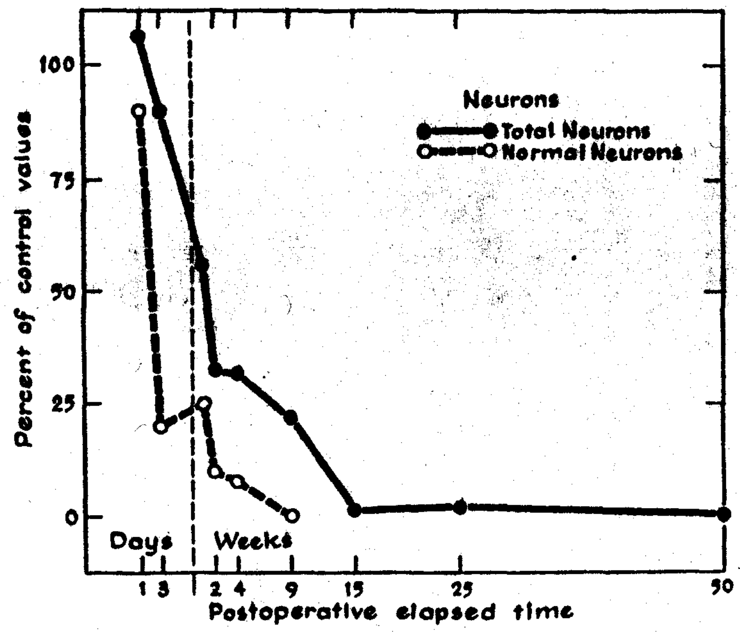


Fig. 1 Number of neurons in degenerated nucleus of the rabbit expressed as per cent of control (normal) lateral geniculate during post-operative survival.

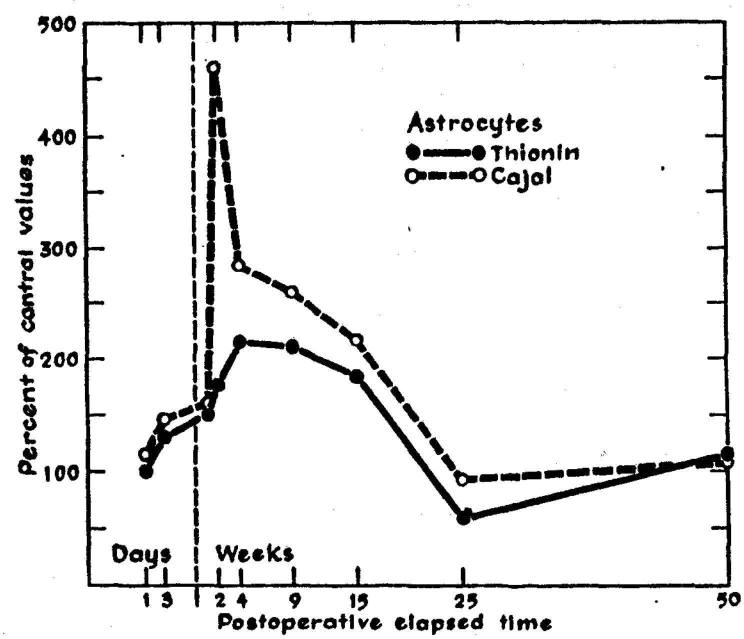


Fig. 2 Number of astrocytes in degenerated nucleus of the rabbit expressed as per cent of control (normal) lateral geniculate during post-operative survival.

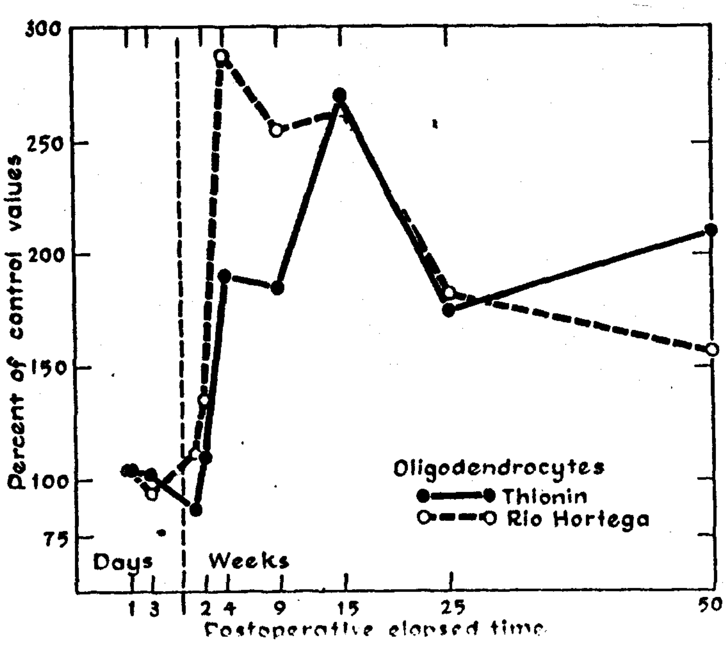
between the number of astrocytes in the degenerated and the control nuclei between three days and 15 weeks are significant at < 0.001 level; differences between the cell-counts of three other time-intervals (one day, 25 weeks, and 50 weeks) are not significant.

The oligodendrocytes constituted the most numerous elements in both the normal and degenerated lateral geniculates. Numerical estimates of these glial cells were similar to control values during the first post-operative week. The cells then began to increase during the second week and reached a maximum at the fifteenth week. Their number then declined but was maintained at between 150 and 200% of the control value up to the fiftieth week. With Rio Hortega silver carbonate stain, oligodendrocytes showed an increase in cell volume and in the number of cell processes during the height of the period of hypertrophy. The differences between the numerical estimates of the normal and degenerated nuclei at the time-intervals of four through 50 weeks are significant at < 0.001 level (fig. 3). The difference at the second post-operative week is significant at < 0.01 level.

The nuclei of microglia in the material stained with thionin were sometimes indistinguishable from those of small oligodendrocytes. In the Rio Hortega stained sections, most of the microglia could be easily identified by the shape of the perikaryon and distribution of the cell processes. Graphic representations of the development of microglia are illustrated by figure 4. In the silver-stained material, proliferation of microglia reached its maximum at the fourth week and subsequently declined to about 150% of control at the fiftieth week. All the differences between the number of cells in the normal and degenerated nuclei from the second week onward are significant at the 0.001 level.

Table 3 includes the average numbers of neurons and glial cells calculated from all counts made on normal material. No corrections on tissue-shrinkage were made on the values obtained from the thionin-stained material.

Cat. The neurons in the cat lateral geniculate have been described as large,



work. They maintained this hypertrophied state from the sixth through the twenty-seventh week (fig. 5). The number of astrocytes subsided by the fiftieth week, but was still significantly higher than the normal level.

Figure 5 additionally depicts the progressive increase of oligodendrocytes and microglia throughout the various post-operative survival times. Both types of glia started to multiply one week after the ablation. They progressed more or less in a parallel fashion and continued without regression to reach a maximum at the fiftieth week.

With the exception of the estimates of the total neurons and the three types of glial cells at the third post-operative day, all the differences between the cell counts of experimental and control materials at each post-operative week were statistically significant at < 0.001 level. The averages of neurons and glial cells calculated from all the normal lateral geniculates of cats used in the cell count estimates are also included in table 3.

DISCUSSION

Neurons. The present results show that retrograde degeneration of the lateral geniculate progressed at a faster rate and reached a more complete stage in rabbit than in cat. Severe cell-atrophy appeared on the third post-operative day in rabbit but only at the seventh day in cat. Also, practically no normal neurons were left between the fourth and ninth weeks in rabbit, but about 30% still remained up to the fiftieth week in cat. Van Cravel and Verhart ('63) reported slow degeneration rates of the optic nerve in cat. Our findings add further evidence on the slow degeneration of fiber tracts in the central nervous system of this species. The large amount of residual, normal neurons in the degenerated nucleus probably will remain in cats with survival times even longer than 50 weeks. This is in contrast to the practically

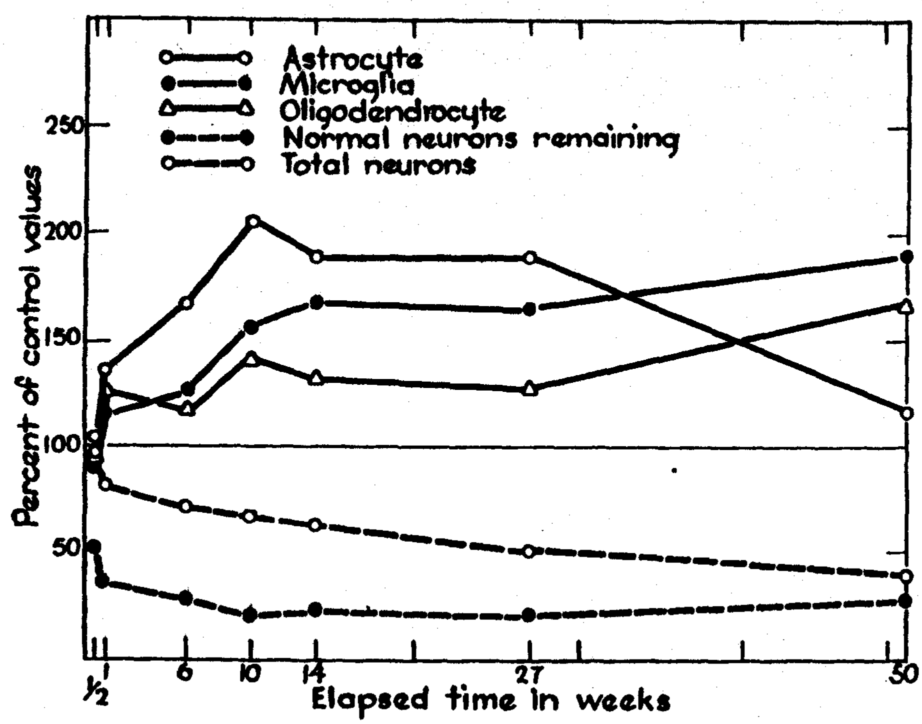


Fig. 5 Number of cells in degenerated nucleus expressed as per cent of control (normal) lateral geniculate of the cat during post-operative survival.

total absence of normal neurons in our rabbit material and in the degenerated lateral geniculate of rat, monkey, and chimpanzee (Lashley, '41; Polyak, '32; Walker, '35, '38). Both the above findings plus the fact that no retrograde degeneration of retinal ganglion cells could be demonstrated up to six months following unilateral optic tract section in cat (Chow, unpublished material) point to some species-specific peculiarities of the cat visual system.

Moreover, there is also a difference in the degree of sharpness of the boundaries between the degenerated and undegenerated zones of rabbit and cat. We have made serial cell-counts from the undegenerated to the degenerated areas of the lateral geniculates in cats and in rabbits that survived more than 14 weeks. The results showed that the boundaries in the rabbit material were sharp, consisting of only a narrow (less than 0.05 mm) transitional zone. In cat, this intermediate area extended up to 0.5 mm, within which neurons, especially the large ones, progressively decreased toward the center of degeneration. This finding was reminiscent of that reported for the opossum (Diamond and Utley, '63). Whether it suggests a similar overlapping projection of lateral geniculate and pulvinar to striate and its surrounding cortices remains to be determined. Finally, the question of the origin of the normal, small neurons which persisted in the center of the degenerated zone of cat may be raised. Are they the same ones that existed there before the process of degeneration, or could it be that some may migrate from the nearby intermediate, degenerated regions? Would these cells also degenerate if the cortical lesions had been larger, hence including areas other than the visual cortex?

Glial cells. The spread of gliosis during retrograde degeneration also appeared to be faster in rabbit than in cat. The development of astrocytes indicated a biphasic process. In rabbit, its initial proliferation reversed to the normal level by the twenty-fifth week. Even at the fiftieth week, the number of astrocytes in the degenerated nucleus of cat was still significantly higher than the control value, although had longer post-operative survival times been allowed, they probably also would return to the normal level. Furthermore, the cat material showed that both oligodendrocytes and microglia increased continuously throughout the post-operative period, reaching their peak at the fiftieth week. At the end of the present experiments, the degenerated zones of both cat and rabbit contained the same or higher numbers of astrocytes than the normal material, plus about 200% more oligodendrocytes and microglia than normal. This proportion may closely represent the final state of gliosis in a retrograde cell-atrophied zone.

The glia-counts made on the silver-stained rabbit sections gave lower values than those on the thionin sections. Since the latter were not corrected for tissue shrinkage, these counts were perhaps 20–30% higher than the actual values. Thus, our result indicates that the silver method is reliable and probably stained most of the glial cells. It should be noted that because of the small number of cells in each count, an occasionally large difference between the percentages based on the two different stains (such as the one between the percentage of astrocytes at the second operation week shown in fig. 2) may actually represent a difference of only one cell. The discrepancies between the two estimates of microglia at the last three post-operative periods (fig. 4) may reflect the difficulty in resolving the crowded nuclei of the increased number of capillary cells mixed with the clusters of microglia in the thionin sections.

Errors in numerical estimates. There are many possible sources of error inherent in any numerical studies. Some of the biasses, such as the staining quality, section thickness, subjective criteria, and so on, raised by many investigators are partially controlled in the present study by the use of relative measures.

Due to the incompleteness of the cortical lesion, the lateral geniculates showed only subtotal degeneration. Tables 1 and 2 show that the volume of the degenerated zone ranged from 42 to 88%. The question whether tissue shrinkage would increase the cell-density estimates may be raised in this regard. We are confident that this factor does not materially alter our results for the following reasons. First, the glial cells increased to a maximum between the second and fourth week in the rabbit and between the tenth and fourteenth week in cat. No decrease of the volume of the lateral geniculate was apparent during that early phase of degeneration. Second, at the end of the fiftieth week, both the oligodendrocytes and microglia in cat and rabbit maintained the high level of 150–200% of control. Although there is about 15% shrinkage of the degenerated zone, it could not alone account for such increased levels. Even allowing for the possibility of increasingly large areas of degeneration, the amount of shrinkage still could not reach more than 30%. Third, the neurons either disappeared completely (rabbit) or reached a steady state (cat) without showing any signs of being affected by continuous changes of volume. Fourth, changes in cell-counts were accompanied by morphological changes of glial cells such as increased size of cell body, thickening of cell processes, etc. They were highly correlated processes. Since the latter could be easily observed, the former could hardly be entirely produced by the shrinking of degenerated tissue. Although it might be desirable to use a larger number of animals at each time-interval for cell-count purposes to control for possible effects of individual variability, we nonetheless feel that the numerical estimates reported here represent the order of magnitude of relative changes of the number of neurons and glial cells during retrograde degeneration.

In summary, we have shown that (a) the time course of retrograde neuronal degeneration in the lateral geniculate body shows a species difference, (b) the proliferation of glial cells, in general, does not parallel the time course of neuronal degeneration, and (c) astrocytes, specifically, show a waxing and waning which does not parallel the development of oligodendrocytes and microglia.

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