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Partial Callosotomy and Left–Right Response Differentiation in the Rat: Separate Anterior and Posterior Facilitatory Effects

Michael Noonan
Departments of Psychology and Biology
Canisius College

Seymour Axelrod
Department of Psychiatry
State University of New York at Buffalo

Earlier research demonstrated that complete section of the corpus callosum in the rat reduced the number of trials required to acquire a left–right response differentiation (LRRD). This study was designed to investigate whether the facilitatory effect on LRRD could be produced by section of an anatomical subdivision of the callosum. Rats with sections of the anterior or posterior corpus callosum mastered the LRRD task faster than sham subjects, but more slowly than rats with total callosal section; section of the middle portion of the callosum had no such effect. The partial facilitatory effects of anterior and posterior callosotomy appear to be independent, and suggest that the callosal intermixing of lateral information, which contributes to left–right confusion, occurs at both the sensory and motor levels of processing.

Distinguishing between left and right proves particularly difficult for most animals (Corballis & Beale, 1976), and there is evidence from experiments testing the effects of commissurotomy on left–right mirror-image stimulus discrimination that suggests that the interhemispheric commissural connections, by allowing lateralized signals to intermix, contribute to such difficulty (Achim & Corballis, 1977; Beale, Williams, Webster, & Corballis, 1972; Bykov & Speranski, 1924).

Consistent with these lines of evidence is our recent demonstration (Noonan & Axelrod, 1991) that complete corpus callosum section reduced the number of trials required by rats to acquire a left–right response differentiation (LRRD) but did not affect performance in the same apparatus on tasks requiring brightness discrimination or consistent unilateral responses. The question now arises whether the difficulty in distinguishing left and right experienced by intact rats is mediated by specifiable portions of the callosum. If it is, then section limited to those portions should reproduce the effects of total callosotomy, and tracing the projections of those portions might then yield insight into the neural processes underlying the difficulty. Therefore, in a replication and extension of our prior research, we conducted an investigation to ascertain whether facilitatory effects on LRRD would result from selective sectioning of anatomical subdivisions of the callosum.

Procedure

Subjects

Adult male and female hooded (Long-Evans) rats were maintained on an alternating 12:12-hr white–red light cycle, with all testing occurring during the red phase. Room temperature was kept at 26 °C. The rats were randomly assigned to one of five surgical treatments: anterior corpus callosum section (*ANTSECT*), middle corpus callosum section (*MIDSECT*), posterior corpus callosum section (*POSTSECT*), total corpus callosum section (*TOTSECT*), or sham surgery (*SHAMSECT*).

Surgery

Our callosotomy procedure has been described in detail elsewhere (Noonan & Axelrod, 1991). Briefly, the scalp of the anesthetized rat was incised, and an area of bone on one side was thinned to allow a specially designed knife to be maneuvered under the dura through a lateralized puncture wound in the adherent osteodural tissue and, through a sequence of pivoting movements, into the longitudinal fissure below the intact superior sagittal sinus. Movement of the knife in the midsagittal plane then caused its sharpened leading edge to transect all or part of the corpus callosum (and dorsal hippocampal commissure).

For the ANTSECT group, the knife was pivoted into the longitudinal fissure 2 mm anterior to bregma, with the excursion of the cutting edge extending back to bregma. For MIDSECT, the knife was pivoted into the longitudinal fissure (and corpus callosum) just under bregma, making a 2-mm excursion posteriorly from that point. For POSTSECT, the knife was pivoted at a point 2 mm posterior to bregma, making a posterior excursion from that point back to lambda. For TOTSECT, the knife was pivoted 2 mm in front of bregma, making a posterior excursion to lambda. The SHAMSECT procedure mimicked the TOTSECT procedure but used a probe with a shortened leading edge that could traverse the longitudinal fissure without cutting callosal tissue.

A minimum of 6 weeks of postsurgical recovery was provided before beginning behavioral testing, and in the week preceding testing each rat was handled twice daily to habituate it to human contact.

Water Maze

We used two water mazes, which were T-shaped Plexiglas tanks, 46 cm deep. Water entered continuously (6.3 L/min at 24 °C) at the floor of the starting box, flowed out at the floor at the end of both arms, and was maintained at a depth of 25 cm. Each maze arm was 15 cm wide and extended for 30 cm laterally. The arms then turned 90° back, so that an escape ramp, extending down into the water at the end of the

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Correspondence concerning this article should be addressed to Michael Noonan, Department of Psychology, Canisius College, Buffalo, New York 14208.

appropriate arm, was out of sight to a rat at the choice point. The reinforcer was escape from the water, accomplished by climbing up the ramp.

The walls of the maze were white and could be back-illuminated. When not back-illuminated, the walls, as viewed from within the maze, appeared to the human observer as uniform and dark. When the walls were back-illuminated, alternating dark and light 3-cm-wide vertical stripes appeared.

LRRD Test

When the maze walls were illuminated, the escape ramp was placed in the right arm; when the walls were unilluminated, the ramp was placed in the left arm. A "dummy" ramp, which extended down only to 15 cm above the water surface and could therefore not be used for escape, was always placed in the opposite arm so that the escape ramp location could not be determined by a view from outside the tank. Pseudorandomly sequenced trials (maze illuminated–maze unilluminated) were presented at 4-min intertrial intervals. On each trial the rat was placed in the starting box and allowed to swim until it found the ramp; it was then returned to its home cage until the next trial. Each rat was tested for 25 trials per day until it reached the criterion of 10 successive correct first turns at the choice point (or for a maximum of 5 successive days). The number of trials taken to reach this criterion served as the index of difficulty in making the LRRD (cf. Noonan & Axelrod, 1989, 1990, 1991).

All animal handling and data collection were carried out by observers who were unaware of the animals' surgical-group assignment and recorded on videotapes that were then reviewed by a second observer who was also unaware of assignment. In this and related projects, we have also conducted a number of checks to confirm that our subjects' scores were not functions of confounding variables: When testing was continued beyond criterion, but by different handlers–observers, in different rooms, and in different mazes oriented differently with respect to compass heading, the subjects continued to demonstrate reliable LRRD.

Histology

After behavioral testing, the rats were perfused intracardially with saline, which was followed by formalin. Serial 8-μm coronal sections were made, and every 20th slice was stained with cresyl violet.

Thirty rats were prepared in each of the five surgical groups. LRRD data from 2 rats were spoiled by experimenter error during testing and were discarded. The LRRD data from 9 additional rats were subsequently excluded (without reference to LRRD scores) because the lesions revealed by postmortem examination did not conform to those appropriate to any of the intended subdivisions. The final group sample sizes were ANTSECT, 28; MIDSECT, 27; POSTSECT, 29; TOTSECT, 27; and SHAMSECT, 28. Fifteen rats (2 ANTSECT, 6 MIDSECT, 2 POSTSECT, 0 TOTSECT, and 5 SHAMSECT) failed to reach criterion after completing the 5 days of testing (125 trials); they were assigned trials-to-criterion LRRD scores equivalent to the lowest score possible given their ending performance. For example, if the response on the 125th trial was the last of five consecutive correct responses, the rat was given a score of 130. If the 125th trial was incorrect, the rat was given a score of 135.

Results

For each brain, we determined the number of stained sections in which callosal fibers would have crossed the midline had the brain remained intact, but in which the fibers were severed. For the TOTSECT subjects, this value averaged 15.3, representing 94% of the sections in which midline callosal fibers either appeared or would have appeared. For ANTSECT, MIDSECT, POSTSECT, and SHAMSECT subjects, these values were 5.3 (35%), 6.2 (40%), 8.0 (54%), and 0.0 (0%), respectively. Sections of representative brains in each group are presented in Figure 1.

Examination of the brain sections also showed that most animals suffered a degree of incidental surgical damage to the midline cortex and sometimes also to the septum, fimbria, hippocampus, thalamus, or tectum, comparable to that re-

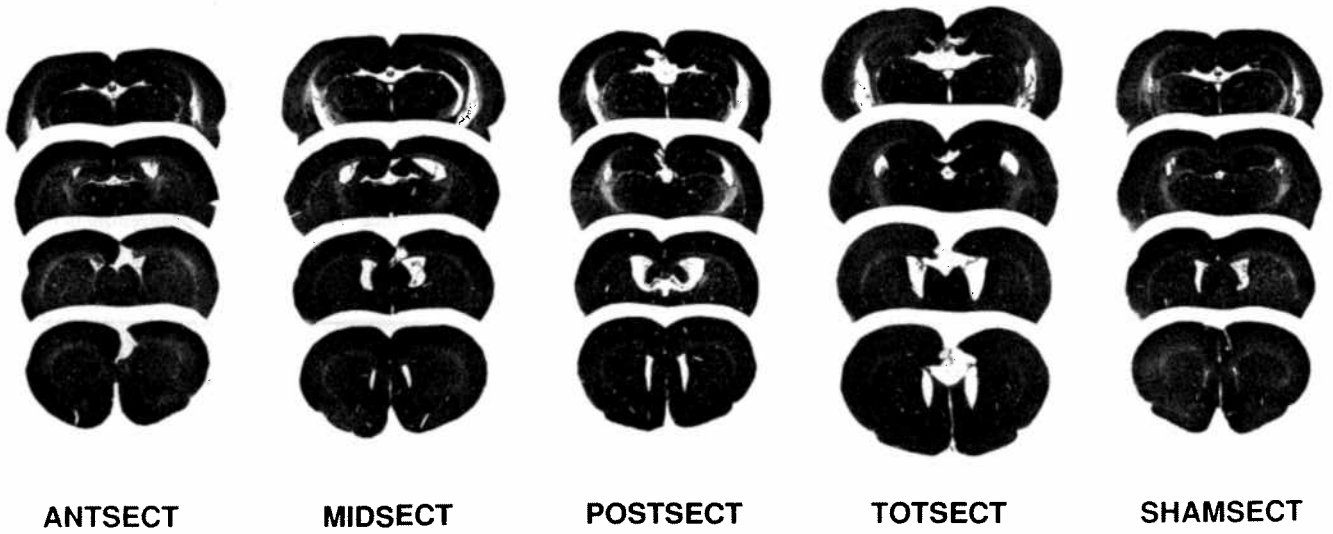

Figure 1. Brain sections from representative subjects. (ANTSECT = anterior corpus callosum section group; MIDSECT = middle corpus callosum section group; POSTSECT = posterior corpus callosum section group; TOTSECT = total corpus callosum section group; SHAMSECT = sham surgery group.)

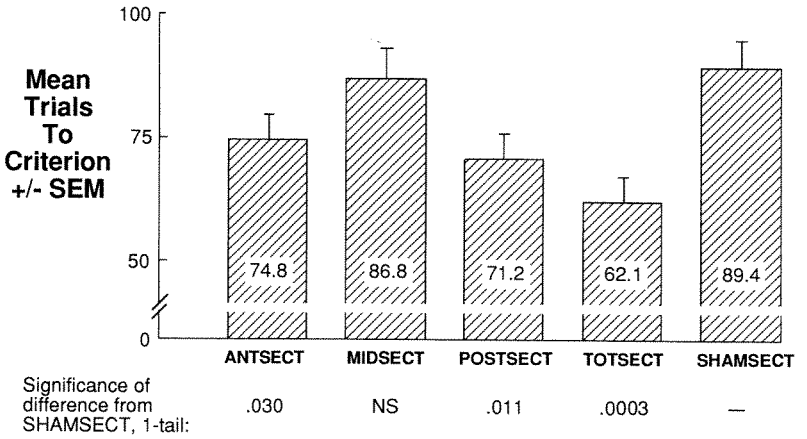

Figure 2. Performance on left–right response differentiation, by surgical group. (ANTSECT = anterior corpus callosum section group; MIDSECT = middle corpus callosum section group; POSTSECT = posterior corpus callosum section group; TOTSECT = total corpus callosum section group; SHAMSECT = sham surgery group.)

ported by Noonan and Axelrod (1991). Representative examples are provided in Figure 1. Analyses parallel to those presented in the earlier research (analyses of variance [ANOVAs] comparing the LRRD scores of those with and those without such damage, or Pearson correlations testing the relation between the extent of such damage and LRRD scores, within each group) showed, as did that study, that LRRD scores were not related to the presence or extent of damage to any of these structures.

The mean number of trials to reach criterion on the LRRD task for each group is shown in Figure 2. A one-way ANOVA showed that the five means differed reliably, $F(4, 134) = 4.21$, $p = .003$. Application of the Cochran C, the Bartlett-Box F, and the maximum–minimum variance tests indicated that the within-groups variances were not significantly different from homogeneous. Subsequent one-tailed t tests were applied, using the within-groups mean square from the ANOVA as the estimate of error variance, testing for each lesion-group mean the hypothesis that it was lower than the SHAMSECT mean. As in our earlier study, the TOTSECT group required significantly fewer trials than the shams, $t(134) = 3.51$, $p = .0003$. Both the ANTSECT group, $t(134) = 1.90$, $p = .030$, and the POSTSECT group, $t(134) = 2.38$, $p = .011$, likewise had significantly lower scores than the shams; the difference between MIDSECT and SHAMSECT, although in the hypothesized direction, was negligible, $t(134) = 0.33$.

The LRRD scores in this experiment were, on average, higher than those found in our earlier study (Noonan & Axelrod, 1991). For example, the mean scores of the complete-callosotomy and sham groups were 62.1 and 89.4, respectively, in the present study, compared with 49.3 and 65.4, respectively, in the earlier study. There were at least two differences between the samples in the two studies that might account for the differences in score. First, the rats used in the previous research were commercially bred and purchased at about 35 days of age, whereas the rats in the present experiment were bred in our laboratory. Second, the rats in the earlier study were tested at 150–170 days of age, in contrast to 102–133 days in the present study. Whatever the source of the difference between the studies in the general level of scores, for both, the improvement of the complete-callosotomy animals over their respective sham controls was significant and of approximately the same order (30.5% improvement in the present experiment and 24.6% in the previous study).

Discussion

The decision to subdivide the corpus callosum into three anteroposterior portions was a reasonable, if arbitrary, first step in our effort to narrow the facilitatory effect of callosotomy on LRRD to a specifiable callosal subdivision. These callosal subdivisions were not, however, equal in terms of the proportion of callosal tissue included; the anterior and posterior portions of the callosum (the genu and splenium) are thicker than the trunk. In light of this, the negligible and nonsignificant improvement of the MIDSECT group in relation to the SHAMSECT group might have been due to the relatively small proportion of the total callosum having been sectioned in this group. It might be hypothesized that the facilitatory effect on LRRD performance of callosotomy is simply a function of the proportion of callosal fibers sectioned, irrespective of the location of the cut fibers.

A direct test of such a "mass-action" hypothesis would require deliberate variation of lesion size at a number of callosal loci. However, the following post hoc analysis of the present data would appear to make such an account unlikely. We calculated the callosal midline area present in those sections of each sham brain corresponding to those that had been severed in each callosotomy subject, and we then assigned the mean of these calculated areas to each callosotomy subject. For example, if an ANTSECT brain showed the corpus callosum to have been severed in the first 5 of the 15 sections in which it either crossed or would have crossed the midline, we calculated for each of the 28 sham brains the sum of the midsagittal thicknesses of the corpus callosum in each of the first one third of the sections in which the corpus callosum

crossed the midline (in cases in which this procedure did not yield an integral number of sections, the sum was adjusted by linear interpolation). This sum, indexing the midline callosal area in the sham brain corresponding to the callosal portion that had been severed in the sectioned brain, was then divided by the sum of the midline callosal thicknesses from all sections, indexing the sham brain's total callosal area. The 28 proportions thus produced were averaged to yield an estimate of the proportion of the callosum severed in the sectioned brain. (The mean values of these estimates were 44.8% for the ANTSECT group, 29.7% for MIDSECT, 44.1% for POSTSECT, and 92.4% for TOTSECT.)

LRRD performance turned out not to be a function of these estimates: The Pearson product-moment correlations between LRRD score and proportion of callosum cut across all the partial callosotomy subjects and, within each partial callosotomy group, were nonsignificant and variable in sign. It appears, then, that the facilitatory effect of callosal section does not depend on the location-independent magnitude of the section. Rather, facilitation was produced by sectioning of the anterior and posterior callosal fibers specifically.

That the ANTSECT and POSTSECT groups both had LRRD scores intermediate between those of the TOTSECT and SHAMSECT groups suggests that the facilitative effect on LRRD of full callosotomy derives from two separate contributions, one associated with severing the anterior callosal fibers and the other with severing the posterior fibers. It is possible that the callosal intermixing of left- and right-specific information contributing to the difficulty of distinguishing left and right occurs at both the motor (anterior) and sensory (posterior) levels of processing. Whatever their nature, the contributions appear to be additive: The magnitude of the facilitatory effect of complete callosotomy (expressed as the difference between the TOTSECT and SHAMSECT means; 27.3 trials) is close to the sum (32.8) of the facilitatory effects of anterior sectioning (14.6) and posterior sectioning (18.2). It now remains to be determined whether these partial contributions can be further subdivided and whether our ability to localize them anatomically can be further refined so that the origins and terminations of the implicated callosal fibers can be identified.

References

Achim, A., & Corballis, M. (1977). Mirror-image equivalence and the anterior commissure. *Neuropsychologia, 15,* 475–478.

Beale, I., Williams, R., Webster, D., & Corballis, M. (1972). Confusion of mirror images by pigeons and interhemispheric commissures. *Nature, 238,* 348–349.

Bykov, K., & Speranski, A. (1924). Sobaka s pererezannym corpus callosum [Dog with transected corpus callosum]. *Trud. Fiziol. Lab. Pavlova, 1,* 47–59.

Corballis, M., & Beale, I. (1976). *The psychology of left and right.* Hillsdale, NJ: Erlbaum.

Noonan, M., & Axelrod, S. (1989). Behavioral bias and left-right response differentiation in the rat. *Behavioral and Neural Biology, 52,* 406–410.

Noonan, M., & Axelrod, S. (1990). Failure of induced asymmetries to improve left-right response differentiation in the rat. *Journal of General Psychology, 117,* 197–202.

Noonan, M., & Axelrod, S. (1991). Improved acquisition of left-right response differentiation in the rat following section of corpus callosum. *Behavioural Brain Research, 46,* 135–142.

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