Spatial learning, discrimination learning, paw preference and neocortical ectopias in two autoimmune strains of mice

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NZB and BXSB mice were given a battery of behavioral tests including paw preference, water escape, Lashley III maze, and discrimination learning. Their brains were then evaluated for cortical ectopias. The incidence of ectopias was 40.5% in NZBs and 48.5% in BXSBs. In the NZB strain left-pawed ectopic mice (both male and female) had the fastest swimming time in the water escape test, while right-pawed ectopies were the slowest. The same findings were obtained for left- and right-pawed ectopic BXSB males, but not for the females. However, on discrimination learning the BXSB males had the exact opposite pattern: right-pawed ectopies were the best learners while left-pawed ectopics were the worst. Male BXSBs and both male and female NZBs were manifesting autoimmune disease at the time of testing, while female BXSBs were not, suggesting that autoimmunity is a necessary background condition for the differential expression of ectopias and paw preference upon learning processes. The finding that the left-pawed ectopic BXSB mice, who were the poorest learners in the non-spatial discrimination learning test, learned best in the spatial water escape test is in agreement with the Geschwind hypothesis that pathological events during brain development may, in some instances, produce superiority of function.

INTRODUCTION

The NZB and BXSB mouse strains are being used as animal models for developmental learning disorders because they have some of the brain anomalies seen in individuals with developmental dyslexia. Dyslexia has been associated with a lack of structural asymmetry in the brain. For example, the planum temporale, a language related region on the superior surface of the temporal lobe, which is usually larger on the left side in control populations, is symmetrical in size in the dyslexic brain. In addition, left handedness may be increased in the dyslexic population. Autopsy studies of the brains of dyslexics also have revealed a variety of cortical malformations that include focal microgyria, molecular layer ectopic nests of neurons and glia, and neuron-free myelinated gliotic scars. NZB and BXSB mice also have molecular nests of cortical ectopic neurons and aberrant cortical lateralization.

In addition to the anatomical disturbances, dyslexics are also more likely to have autoimmune disorders. Both NZB and BXSB mice are autoimmune strains and have been used as models of systemic lupus erythematosus (SLE).

Because of the similarities between these mouse strains and developmental dyslexia at the anatomical and immunological levels, in the present study we looked for relationships at the functional level. We measured pawedness since behavioral laterality is a key factor in developmental dyslexia; and we tested the mice on three learning measures, one which required utilization of spatial information, a second which required associative learning independent of spatial cues, and a third which had both sets of cues. The results obtained are remarkably congruent with current knowledge and theory on developmental dyslexia.

MATERIALS AND METHODS

Subjects

The NZB and BXSB mice used in this study were born in the Developmental Psychobiology Laboratory at the University of Connecticut from foundation stock (NZB/BINJ and BXSB/MpJ) originally obtained from the Jackson Laboratory. All animals were given a series of behavioral tests, including paw preference, water escape, discrimination learning, and Lashley III maze, which are described below.
Paw preference and paw asymmetry

This is a test of degree and direction of laterality. The mouse was food deprived (water ad libitum) and then placed into a small cube containing a cylindrical feeding tube projecting at chest height when standing on its hind legs. The mouse reaches for grains of sweetened rolled wheat (Maypo) inside the tube and is observed until 50 paw reaches have been made. The paw preference score is the number of right paw entries (RPE). If the animal quits before 50 reaches, the score is adjusted appropriately (e.g. 22 RPEs in 35 attempts is adjusted to a score of 31).

The RPE score combines two dimensions of laterality: direction and degree of asymmetry. These were separated as follows. Paw preference (direction of asymmetry) was obtained by classifying as 'left' any animal with an RPE score of 0–25; and 'right' any mouse with a score of 26–50. To obtain degree of asymmetry, 25 was subtracted from the RPE score and the algebraic sign was dropped. The distribution was then split at the median; those at or below the median were classified as 'low asymmetry' while those above were called 'high asymmetry.' They were tested when 6 weeks old.

Water escape

The purpose of this task was to obtain a simple measure of learning that required the animal to use spatial information. The mouse was put into an oval tub (51.2 x 102.4 cm) with water (17–19 °C) and had to swim to a submerged platform at the far end. It was allowed 120 s to find the platform. The platform was set away from the wall 10.2 cm so that the animal could not find it by swimming around the rim of the tub. If found, the animal remained on the platform for 20 s and was then placed into a mouse box with shavings under a heat lamp where it remained until all members of the squad had been tested. The water temperature was 17-19 °C.

The computer program for tracking and scoring an animal has been described. Each animal was randomly assigned black or white, and the other white. The maze was 7.7 cm wide. The stem was 35.8 cm long and each alley was 48.6 cm long. At the end of the alley designated as 'positive', hung a ladder made of wire mesh which the mouse could climb. The ladder was curved back toward the stem so that the mouse could not see the ends, thus insuring that it could not use the wire mesh as a cue to choose which alley to enter. The temperature of the water was 17–19 °C.

The purpose of this test was to measure nonspatial associative learning. The apparatus was a two-armed swimming T-maze, made of galvanized tin; the stem was painted grey, one alley was painted black, and the other white. The maze was 7.7 cm wide. The stem was 35.8 cm long and each alley was 48.6 cm long. At the end of the alley designated as 'positive', hung a ladder made of wire mesh which the mouse could climb. The ladder was curved back toward the stem so that the mouse could not see the ends, thus insuring that it could not use the wire mesh as a cue to choose which alley to enter. The temperature of the water was 17–19 °C.

The computer program for tracking and scoring an animal has been described. Each animal was randomly assigned black or white as the positive stimulus. The left-right location of the positive stimulus for each day's 10 trials was determined by selecting a semi-random sequence. A different sequence was used each day. The mouse was placed into the water at the stem end and allowed 60 s to find the ladder. If the mouse never left the stem of the T during the trial, it was stimulated after the default time had lapsed. This initiated swimming and the animal would then make a left-right choice at the T-junction. After arriving at the escape ladder, the mouse was removed and put into a dry mouse box under a heat lamp where it remained until all members of the squad had been tested. It was then given its second trial. Ten trials were given per day for 5 days. The measure of learning was the number of correct responses, defined as swimming down the stem of the T and making a correct choice without entering the incorrect alley. The mice were tested when 9 weeks of age.

Lashley III maze

This is a relatively complex maze which contains cul-de-sacs that the animal has to learn to avoid, and T-choices where the animal has to learn to make the correct left or right turn. The maze can be learned by use of extra-maze spatial cues or by memorizing the chain of correct left and right turns. We use a water version of the maze. The apparatus and scoring procedure has been described in detail. Briefly, the mice were given a single trial a day for 10 days. The mouse's path is typed into a computer where the program Observe Software analyses the data into correct entries and errors. The measure of learning is called the learning index and is defined as number of correct entries divided by total number of entries (which consists of correct plus error entries). The mice were tested on weeks 10 and 11.

Anatomical analysis of brains

When 13–14 weeks old, the mice were anesthetized and blood was drawn via heart puncture, plasma was removed, and frozen at -70 °C for immune assays. They were then transcardially perfused with 0.9% saline followed by 10% formalin. The heads were placed in 10% formalin, and sent to Beth Israel Hospital for histological processing and anatomical analysis.

The brains were removed from the skulls and placed into formalin for at least one week. They were then dehydrated in 80%, 95%, 100% ethanol and ethanol/ether. The brains were embedded in 3% colloidin for 3–4 days followed by 12% colloidin for 2–3 days or until hard. Afterwards they were cut into 30 μm coronal sections, and every fifth section was stained with Cresyl violet for Nissl bodies and mounted on glass slides. The slides were examined under the light microscope and the presence of cortical ectopias, dysplasias, and other types of brain abnormalities was noted. Ectopias were judged to be either large, moderately-sized, or small. Large ectopias were characterized by a mushroom-like extrusion of cells into the molecular layer, containing more than 50 neurons; moderately sized ectopias presented as collections of neurons in the molecular layer containing between 20 and 50 cells; small ectopias contained less than 20 neurons clustered in layer I. The architectonic and hemispheric location of the ectopias were also recorded. In addition, a number of brains had small areas of neuronal loss (referred to as neuron-free zones) located in the neocortex.

The plasma was sent to the Institute of Neurological Sciences at Glasgow, Scotland for immune evaluation. These findings will be the subject of a separate paper.

RESULTS

NZB mice

Eighty-one mice were tested (45 males and 36 females). Two died after the behavior tests but before perfusion. Table I summarizes the ectopia characteristics. Thirty-two NZBs had one or more ectopias, while 46 others did not show clear evidence of pathology and were classified as having no pathology. Thus, 40.5% of the 79 mice had ectopias. The ectopias were primarily unilateral and equally likely to occur on the right as on the left. The single ectopias were mostly large in size and were primarily in the somatosensory/sensorimotor area of cortex. There was no sex difference in ectopia incidence. The neuron-free animal was excluded from all behavior analyses since the purpose was to evaluate the effects of ectopias.

Not all animals performed in the paw preference test. Sixty-two (37 males and 25 females) had paw preference, water escape, and discrimination learning scores (45.2% were left pawed). Each animal was classified with respect to sex, ectopia (present or absent), and paw preference (left or right) and unweighted means analyses of vari-
Fig. 1. A: mean water escape time for NZB mice classified by paw and ectopia (n’s range from 11 to 19). B: mean water escape time for male BXSB mice classified by paw and ectopia (n’s range from 18 to 29). C: mean discrimination learning for male BXSB mice classified by paw and ectopia (same n’s as in B).

Fig. 2. Water escape learning of left- and right-pawed ectopic NZB mice (n’s of 11 and 15, respectively).

Table 1

<table>
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<tr>
<th>Characteristic</th>
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<th>BXSB</th>
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<td>Number of ectopias</td>
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<tr>
<td>None</td>
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<td>50</td>
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<tr>
<td>One</td>
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<tr>
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<td>Side of ectopia*</td>
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<td>10</td>
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</tr>
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<td>6</td>
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<tr>
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<tr>
<td>Without ectopia</td>
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<td>1</td>
</tr>
<tr>
<td>With ectopia</td>
<td>3</td>
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* Side information of one BXSB mouse lost.
mance was quite poor. Their learning curve is shown in Fig. 3.

**BXSB strain**

Nineteen mice were tested (50 males and 49 females). Anatomical analyses found that 48 had one or more ectopias, including 3 that had both an ectopia and a neuron free zone. Fifty mice did not show clear evidence of pathology. Thus 48.5% (48 of 99) of the brains had ectopias. As with the NZBs the ectopias were primarily unilateral and equally likely to occur on the right as on the left (Table I). The single ectopias were equally likely to be large or moderate in size, but their distribution did not differ from that of the NZBs (chi-square test). The single ectopias occurred primarily in the frontal/motor region of cortex, and the two mouse strains differed markedly on ectopia location ($\chi^2 = 19.45$, df = 1, $P < 0.001$). Sex was not a significant variable. The four mice with neuron-free zones were excluded from all analyses. Of the remaining 95 mice, 92 had paw preference scores. Of those, 51 (55.4%) were left-pawed.

Sex, ectopia, and paw preference were the classifications used to evaluate water escape and discrimination learning. After that, paw asymmetry replaced paw preference, and the ANOVAs were re-run. No significant effect was obtained from the paw asymmetry measure, and these analyses will not be further discussed.

**Water escape.** Significant effects were found for paw ($F_{1.84} = 6.72, P < 0.05$), sex × paw ($F_{1.84} = 4.46, P < 0.04$), and sex × ectopia × paw ($F_{1.84} = 4.83, P < 0.03$). Examination of the triple interaction revealed that the female groups did not differ among themselves in swimming time, while the male groups did. The profile of the male groups is shown in the center panel of Fig. 1 and is the same as that found for the NZB mice: left-pawed male ectopic mice were faster swimmers than right-pawed male ectopics, with the two non-ectopic groups in the middle. Fig. 4 shows the learning curves for these two groups.

Trials were significant as a main effect ($F_{4,336} = 32.2$, $P < 0.001$), and also in interaction with sex ($F_{4,336} = 4.84, P < 0.001$). Females were slower on the first trial and faster thereafter, thus showing a faster rate of learning.

**Discrimination learning.** Analysis of the number of correct choices found a significant sex × ectopia × paw interaction ($F_{1.84} = 4.61, P < 0.04$). Further analyses found that there were no differences among the female groups, while the males differed significantly. The third panel in Fig. 1 shows the profile for the males. Right-pawed ectopic males had the best learning scores of any of the eight groups while left-pawed ectopics had the worst scores ($P < 0.05$). Note that this is the exact opposite of what was found on the water escape test. Fig. 5 shows the discrimination learning curves for the left- and right-pawed ectopic males.

In addition, the triple interaction of sex × ectopia × days was significant ($F_{4,336} = 2.57, P < 0.04$). Further evaluation of the data found that ectopic females were significantly poorer in learning the discrimination than the non-ectopic females ($F_{4,184} = 3.62, P < 0.008$). Their
curves are shown in Fig. 6. The two male groups did not differ; their curves were similar to that of the non-ectopic females.

Lashley III maze. None of the independent variables was significant. Unlike the NZB mice, the BSXBs performed well on this task. Their learning curve is shown in Fig. 3.

Side of ectopia, sex, paw preference, and paw asymmetry

Within each strain the location of the ectopia (left, right, bilateral) was evaluated with respect to sex, paw preference, and paw asymmetry. χ² tests did not find any significant associations.

Are ectopia characteristics related to behavior?

To answer this question analyses were run within the ectopic groups, using the dimensions listed in Table I. Thus, mice with single ectopias were compared to those with multiple ectopias; left, right, and bilateral ectopic mice were compared; within single ectopic animals, the ectopia size was compared; and the location (somatosensory versus frontal/motor) was compared. This was done for each strain separately for the variables of paw preference, paw asymmetry, mean water escape time, total number of correct choices in discrimination learning, and mean learning index on the Lashley III maze. In no instance was a significant effect found.

Is degree of paw laterality related to behavior?

To answer this question the RPE scores were correlated against mean water escape time, total number of correct choices in discrimination learning, and mean learning index on the Lashley III maze within the NZB and BXSB strains. No significant correlation was obtained.

Left-right paw preference criterion

Neveu et al.¹⁸ have raised the question of the criterion to use to classify an animal as left- or right-pawed. In their study, they only used mice that had paw preference scores less than 20 or greater than 30, while we used all mice, regardless of score. When we re-analysed our data, using the same exclusion criterion as Neveu et al., we found only minor differences No significance level was changed.

DISCUSSION

NZB males and females, and BXSB males, had the same ectopia-paw associations in the water escape test: left pawed mice were superior to right-pawed ones (Fig. 1A,B). Given the distinctly different histories of these two mouse strains²,⁸,¹₅,²₅, the finding of the same brain-paw-behavior pattern establishes the replicability and generality of the findings, and indicates that this is a robust association.

A puzzle is why the BXSB females did not fit this pattern. One interesting possibility is that the BXSB females were the only group without SLE at the time of testing. Female BXSBs have a late onset variety of SLE. We have found this as well (anti-dsDNA levels of BXSB females do not differ from DBA controls, while the levels of the other three groups are significantly higher; unpublished data; this is consistent with other reports that SLE strains have significant levels of anti-dsDNA at 4–5 months of age²₅). This suggests the hypothesis that autoimmunity is necessary as a context variable to obtain differential behavioral expression of ectopias and paw preference.

For BXSB males the ectopia-paw combination was also extended to discrimination learning. However, the pattern was inverted: right-pawed ectopics, the slowest in the water escape task, had the best discrimination scores; whereas left-pawed ectopics, the best water escape learners, were the poorest discrimination learners of any of the eight groups (Fig. 1B,C). The requirements for successful performance in these two tasks are distinctly different. Water escape is learned by using spatial information. But spatial cues are not available in the discrimination test since the correct stimulus is equally often on the left and the right. Instead, the animal must learn to associate brightness (black or white) with escape, regardless of location.

It should not be overlooked that the ectopic mice were more extreme, both better and worse, than those without ectopias. It might appear paradoxical for ectopias, a cortical anomaly, to confer an advantage over those without the anomaly. This, however, is exactly what Geschwind¹₃ predicted. He suggested that pathological events during brain development may, in some instances, produce superiority in function. He has ar-
argued that it is possible that similar developmental pathology is responsible for superiority of talent in one activity and deficiency in another. It is conceivable, therefore, that developmental pathological insults occurring in the context of a certain underlying brain organization which manifests itself as variable lateralization, lead to reorganization of the cortical circuits such that some functions subserved are favored while others are handicapped.

In this model a developmental cortical abnormality produces opposite behavioral effects on animals according to laterality. We found that the side of the hemisphere containing the ectopia is not related to paw preference. This suggests that left- and right-pawed animals are not mirror images of each other since in that case, with randomly lateralized lesions, the functional effects on the two laterality groups should not differ. In human populations, from the anatomic point of view, left- and right-handers are not mirror images. Right-handers tend to show leftward asymmetry in the Sylvian fissure, while left-handers tend to be more symmetric.13

Other evidence that left- and right-pawed mice are not mirror images is found in the studies of Neveu and his colleagues, who showed that left-pawed mice had higher mitogen-induced lymphocyte proliferation than did right-pawed animals, and that left-pawed NZB females had earlier onset of antibodies against erythrocytes and double-stranded DNA than right-pawed females.18

The failure to find, within ectopic mice, any association between side, size, or site of the anomaly and behavioral events is not surprising. The ectopias are present during the prenatal period and may represent widespread developmental cortical disorganization. For example, neuropeptide organization in areas adjacent to the ectopias is altered and dense fiber bundles stained with neuropeptide antibodies are seen in conjunction with the ectopias and may join the corpus callosum.23

Another finding, independent of the above discussion, is that within the BXSB females those with ectopias were inferior in discrimination learning to those without ectopias (Fig. 6). Since these animals were not manifesting SLE at the time of testing, and no correlation was found with the paw preference measure of lateralization, the deficit appears to be attributable to the presence of the ectopia and its attendant cortical disturbances. It would be interesting to examine the learning behavior of these females after onset of SLE to find out if the ectopias, here associated with poor learning, would turn out to have beneficial effects for those who are left-pawed, as predicted from our own data and Geschwind’s hypothesis.

The behavior test most often used with the NZB and BXSB mice is avoidance learning, and both strains are notably bad learners.9,16,20,24 However, in this study both strains learned the water escape test and the black-white discrimination effectively. We have also found that both strains do well on the Morris maze (ref. 20 and unpublished data). Studies have shown that NZBs and other autoimmune mice are effective at learning a taste aversion response with lithium chloride as the reinforcer.1,24 However, the two strains perform very differently on the Lashley III maze, with the NZBs having very poor learning. Therefore, for both strains the poor learning in the avoidance apparatus, and the poor performance of the NZBs in the Lashley maze, appear to be evidence for a specific learning deficit, rather than a generalized learning difficulty. Ader et al.,1 from their taste aversion findings, have concluded that lupus-prone mice do not have a learning deficit, per se.

This is the first report showing differential learning behavior in autoimmune mice as a joint consequence of autoimmunity, handeness, cortical ectopia, and learning task requirements. The similarities between the NZB and BXSB mouse strains and developmental dyslexics at the anatomical, immunological, and functional levels are remarkable and strongly support our thesis that there are major biological determinants of this disorder.

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