Neocortical Ectopias in BXSB Mice:
Effects upon Reference and Working Memory Systems

BXSB mice have an ~40–60% incidence of neocortical ectopias in layer I of the prefrontal/motor cortex. Prior studies have found major behavioral differences between those with ectopias and their non-ectopic littermates. Some of these findings indicate that the two groups differ with respect to spatial reference and working memory. The purpose of this study was to measure reference and working memory in the same animals to test the hypothesis that the ectopias would have better reference memory but less effective working memory. The Lashley III maze has cul-de-sacs which must be eliminated, and T-choices where the animal has to decide whether to go left or right. Ectopic and non-ectopic mice were equally able to learn the maze and did not differ on cul-entry or T-choice errors. Then the maze was inverted and the animals were retested. Turning the maze upside down did not change the relative status of the blind alleys. Therefore, the reference memory knowledge from the prior week’s training could be used to avoid entering the culs. However, inverting the maze caused a left–right mirror image reversal of the T-choices. Therefore, prior reference memory information would interfere with learning the new path through the maze, whereas working memory would enable the mouse to eliminate T-choice errors. Ectopic mice made less cul-entry errors and more T-choice errors than their non-ectopic littermates, as predicted.

Dyslexic individuals have developmental learning difficulties, a higher incidence of autoimmune problems and a number of brain anomalies, including ectopic nests of neurons in the molecular layer of the neocortex (Galaburda and Kemper, 1979; Geschwind and Behan, 1982; Galaburda et al., 1985; Pennington et al., 1987; Humphreys et al., 1990; Gilger et al., 1992; Wood and Cooper, 1992). We have been using two inbred mouse strains, BXSB and NZB, as animal models of developmental learning disorders because the strains have lupus-like autoimmune conditions (Murphy and Roths, 1978, 1979; Dixon, 1982; Theofilopoulos and Dixon, 1985) and ~40–60% of them have ectopic collections of neurons in layer I of the cerebral neocortex, which are structurally similar to the ectopias found in dyslexics (Sherman et al., 1985, 1987, 1990a).

Neuronal ectopias in mice consist of a mushroom-shaped extrusion of neuronal cell bodies into the molecular layer of cortex, with distortion of the subjacent cortical laminae and a strikingly dense bundle of radially oriented axons underlying the ectopic cell cluster (Sherman et al., 1990b). In utero, these anomalies show aberrant radial glial fiber morphology and an apparent breach in the glial external limiting membrane (Sherman et al., 1992a). Recent work at the electron microscopic level (Boehm et al., 1995) has revealed ultrastructural abnormalities in the cells of ectopias which are suggestive of highly localized excitotoxic damage. Ectopias have been shown to form as early as E13–15 (Sherman et al., 1992b) and show a heritability pattern consistent with a single gene model (Sherman et al., 1994). Ectopias in BXSB mice generally occur in prefrontal/motor cortex, while NZB ectopias are present mostly in somatosensory cortex (Sherman et al., 1987, 1990a; Denenberg et al., 1991b; Schrott et al., 1993; Boehm et al., 1996).

Ectopic mice have been found to differ on a number of behavioral parameters from their non-ectopic littermates. On some behavioral tasks, ectopic mice learn less well than non-ectopics, but on other tasks the opposite finding has been obtained (Denenberg et al., 1991a,b; Schrott et al., 1992, 1993). These effects have been shown to occur whether affected mice have only a single ectopia or several ectopias, whether the ectopias are large or small and whether they are located in the right hemisphere, the left hemisphere or both. We have not yet found an effect of architectonic location although, because the large majority of these mice have ectopias in the prefrontal/motor region, we have been unable to obtain adequate numbers for proper statistical comparison.

Denenberg et al. (1996) investigated Morris maze learning in two groups of BXSB mice: one which had been conventionally reared and others which had been transferred into the uteri of non-autoimmune recipients at the eighth stage of cell division. In both groups, ectopic mice were superior to non-ectopics. They took less time to find the hidden platform, had greater speed, spent more time in annulus 2 and spent less time in annulus 3. The distance measure approached significance (P < 0.08), with ectopics swimming a lesser amount.

Boehm et al. (1996) confirmed these findings in an independent study. Boehm’s ectopic mice took less time to find the platform, swam a shorter distance and spent less time in the quadrant diagonally opposite the platform. Eight weeks after original learning, Boehm et al. retested their mice. On the first 3 days of retesting, ectopic mice took less time and swam a shorter distance than non-ectopics; the non-ectopics caught up on the last 2 days.

All these findings indicate that ectopic BXSB mice are better than non-ectopics at learning a spatial task and also have better long-term retention. We may conclude that ectopic BXSB mice have a better spatial reference memory system, both immediate and long-term, than their non-ectopic littermates. It was of interest, therefore, to investigate this strain’s spatial working memory system to determine if ectopics and non-ectopics differed. Waters et al. (1996) studied working memory in the Morris maze by using a spatial delayed-matching-to-sample procedure. To convert the Morris maze from a reference memory task to one involving working memory, the location of the hidden platform was systematically shifted to create new ‘problems’ for the mouse to solve. A problem was defined as a trial with the platform in a constant location but in a different quadrant than on the previous four trials. Thus, the first trial of a new problem was an information trial giving the quadrant location of the platform. The mouse could use this information (working memory) on trials 2–4 to locate the platform. Waters et al.
al. found, for the first five problems, that non-ectopic BXSB mice took less time and swam a shorter distance on trial 2 than did ectopics.

These data led to the hypothesis that ectopic BXSB mice are better in spatial reference memory and worse in working memory than non-ectopic littermates. However, these measures were obtained with different animals. The hypothesis would be strengthened if we could show, in the same animals, that ectopic mice had better reference memory and poorer working memory. We have developed a procedure for obtaining independent measures of reference and working memory using the Lashley III maze and have found evidence supporting our hypothesis.

Materials and Methods

Lashley Maze Errors and Their Relationships to Reference and Working Memory

Figure 1 is a schematic of the Lashley maze. We have previously described in detail our computer-aided scoring procedure and the definitions of errors (Denenberg et al., 1991c). For this paper, we are concerned with errors in the forward-going direction (i.e. toward the goal). There are two classes of errors: cul-entries and T-choices. A forward-going cul-entry error occurs when the animal swims past a T-choice and into a blind. The four possible cul-entry errors are shown in Figure 1. A T-choice error occurs when an animal makes the wrong turn (left or right) at a T-junction. There are also four of these errors, as shown in Figure 1. We have previously found that cul-entry errors have a lower frequency of occurrence and are eliminated more quickly than T-choice errors (Denenberg et al., 1991c). This is probably because the T-choice opening occurs before the blind alley and the simple rule, 'turn at the first opening', is sufficient to prevent entry into a cul.

The combination of cul-entry and T-choice errors offers an opportunity to independently assess reference and working memory. This occurs by first training animals in the conventional manner. The next step is the critical one. The maze is turned upside down and the mice are retested (see Figure 2). When the maze is turned over, (i) the location of the goal box is shifted in space; (ii) the T-choices are now mirror images; but (iii) the relative status of the culs have not changed (compare Figs 1 and 2). That is, the reference memory rule to avoid entering culs ('turn at the first opening') is equally valid when applied to the upside-down maze. However, the reference memory information for making T-choices cannot be used because they are now left-right reversed. Therefore, the animal has to use working memory to learn the new T-choices. Upon starting the retest phase, the initial trial is an information trial and does not reflect working memory processes. Based upon our prior findings, we hypothesized that, with the maze inverted, ectopic mice would be more competent at elimination of cul errors because they have a better reference memory system, but would make more T-choice errors since their working memory system is not as effective as in those without ectopias.

Subjects

Subjects were 25 female BXSB-Yaa mice bred at the Developmental Psychobiology Laboratory from BXSB/MpJ-Yaa foundation stock obtained from The Jackson Laboratory. They were housed in groups of five or six until ~1 year of age, when they were housed individually in clear Plexiglas laboratory cages (18 x 28 x 12 cm). Behavioral testing began 1 week thereafter.

Procedure

We use a water version of the Lashley maze. A mouse is released in the start box and given 2 min to swim to the exit box. If unable to successfully navigate the maze, subjects were guided to the exit box. Animals were tested in squads of 4-6 animals and returned to their heated home cage following each trial. The mice were given two trials a day, one in the morning and one in the afternoon, for 5 days. The two daily trials were pooled, yielding five trial blocks. Following the first week of testing, the maze was turned upside-down and all subjects were retested, using the same procedures described above.
Anatomical Analysis of Brains
Following anesthesia, cardiac puncture and perfusion were performed at -59 weeks of age. Sera were frozen for immunological analysis. Following exsanguination, animals were perfused with physiological saline followed by 10% formalin. The brains were removed from the skulls and placed into formalin for at least 1 week. They were then dehydrated in 80, 95 or 100% ethanol and ethanol/ether. The brains were embedded in 3% celloidin for 3-4 days, followed by 12% celloidin for 2-3 days or until hard. Afterward, they were cut into 30 μm coronal sections and every fifth section was mounted, in series, on a glass slide and stained with cresyl violet for Nissl bodies. The slides were examined under a light microscope for the presence of cortical ectopias, dysplasias, callosal agenesis and other types of brain anomalies (such as hydrocephalus, hippocampal abnormalities and gliotic neuron-free cortical patches). Ectopias were judged to be either large, moderately sized or small. Large ectopias were characterized by a mushroom-shaped extrusion of cells into the molecular layer, containing >50 neurons; moderately sized ectopias were visualized as collections of neurons in the molecular layer containing between 20 and 50 cells; small ectopias contained <20 neurons clustered in layer I. The architectonic and hemispheric locations of the ectopias and other abnormalities were also recorded.

Table 1
Ectopia characteristics of the BXSB mice

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of animals (total n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopias</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>15 (60% of total)</td>
</tr>
<tr>
<td>One</td>
<td>7 (28% of ectopias)</td>
</tr>
<tr>
<td>Multiple</td>
<td>3 (12% of ectopias)</td>
</tr>
<tr>
<td>Side of ectopia(s)</td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>5 (50% of ectopias)</td>
</tr>
<tr>
<td>Right</td>
<td>3 (30% of ectopias)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>2 (20% of ectopias)</td>
</tr>
<tr>
<td>Size of single ectopias</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>0 (0% of singles)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (14% of singles)</td>
</tr>
<tr>
<td>Small</td>
<td>6 (86% of singles)</td>
</tr>
<tr>
<td>Location of single ectopias</td>
<td></td>
</tr>
<tr>
<td>Prefrontal/Motor</td>
<td>6 (86% of singles)</td>
</tr>
<tr>
<td>Somatosensory</td>
<td>1 (14% of singles)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0% of singles)</td>
</tr>
</tbody>
</table>

*Of the three animals which had multiple ectopias, two had three ectopias and the other had two. Of these animals, one (33%) had at least one large ectopia and three (100%) had at least one ectopia in prefrontal/motor cortex.

Results

Neuropathology
Ten of the 25 mice had ectopias. This incidence is not significantly different from what has been reported elsewhere (Denenberg et al., 1991b; Schrott et al., 1993; Boehm et al., 1996). In this sample it turned out that 9/10 mice had ectopias in the prefrontal/motor region. Relevant neuropathology data are summarized in Table 1. None of the mice had other pathology which might warrant exclusion from the behavioral analyses. A low-power micrograph of an ectopia in motor cortex is shown in Figure 3.

Learning Behavior
Ectopic and non-ectopic mice did not differ on original learning of the Lashley maze. When the mice were retested with the maze inverted, there were less cul-entries than T-choice errors \( F(1,23) = 129.8, \ P < 0.001 \) and the interaction of Ectopia × Error Type × Days was significant \( F(4,92) = 3.08, \ P < 0.02 \). After the first test day, ectopic mice made less cul-entries and more T-choice errors.

Figure 3. Digitized composite (in Adobe Photoshop) showing a Nissl-stained coronal section containing a typical large ectopia (arrow) in layer I of the motor cortex of the BXSB mouse (Bar = 175 μm).
than non-ectopics. Even though the overall analysis found significance, the means and variance for cul-entries were less than those for T-choices. Therefore, separate analyses of variance were done on the two error types. When the cul-entries were evaluated, a significant Ectopia × Quadratic Days effect \( F(1,92) = 4.36, P < 0.05 \) was found. A similar analysis of the T-choice data found a significant Ectopia × Quadratic Days interaction \( F(1,92) = 5.37, P < 0.05 \). The curves are shown in Figures 4 and 5. These trend analyses reveal that the ectopic mice had the steeper learning curve for elimination of cul-errors, whereas the non-ectopics had the steeper curve for eliminating T-choice errors.

**Discussion**

In another study, long-term retention was investigated by training BXSB mice on the Lashley maze and retesting them 8 weeks later (Boehm et al., 1996). Both ectopics and non-ectopics had good retention, but they did not differ on any measure, including cul-entries and T-choices. In contrast, in the present study, marked differences were found when ectopics and non-ectopics were retested on the inverted maze, even though they were equally competent in original learning.

Ectopics took longer than non-ectopics to reduce their T-choice errors, but were faster in reducing cul-entries. The elimination of T-choice errors requires working memory, since turning the maze upside down causes a left-right reversal, thereby negating the effects of reference memory carried over from the original learning. Thus, the poorer scores of the ectopic mice indicate that they have a less effective working memory system than their non-ectopic littersmates. In contrast, reference memory could still be used to eliminate cul-entry errors, and here the ectopic mice were favored.

Previous experiments have established that ectopic BXSB mice are better than non-ectopics at learning the Morris maze (Boehm et al., 1996; Denenberg et al., 1996), leading us to conclude that these animals have a more effective reference memory system. The cul-entry data of the present experiment is consistent with and extends that conclusion.

In one prior experiment, ectopics were less effective than non-ectopics at a working memory task involving the Morris maze (Waters et al., 1996). The current study showed that ectopics were less effective than non-ectopics at a working memory task involving the Lashley maze.

The present findings are consistent with the hypothesis that ectopic BXSB mice are better in spatial reference memory and worse in working memory than non-ectopic littersmates. These two findings are unlikely to be coincidental and are both probably due to the development of the ectopias. The basis for this thesis comes from the findings of Goldman-Rakic (1987), who showed, via surgical intervention upon monkey fetuses, that the brain can create new connections in an attempt to compensate for a prenatal insult. We suggest that a similar process is occurring in the fetal mouse brain. Ectopias occur during cell migration and have been found as early as E13–15 (Sherman et al., 1992b). Additionally, the presence of abnormal neural connections associated with ectopias was confirmed by the immunocytochemical work of Sherman et al. (1990b), who showed the existence of dense, radially oriented fiber bundles spanning the thickness of the cortex immediately underlying ectopias. Further, electron microscopic analyses of neocortical ectopias show cytoskeletal damage to ectopic neurons, with ballooned dendrites, elevated numbers of lysosomes and other ultrastructural abnormalities indicative of excitotoxic damage distinctively localized to the area inside the ectopia (Boehm et al., 1995). Thus, the timing of occurrence and the neuroanatomical characteristics of ectopias are both consistent with the argument that their development may well be sufficient to bring about neural reorganization.

In BXSB mice, ~70–80% of ectopias occur in the frontal cortex (prefrontal and motor areas; Sherman et al., 1987; Sherman et al., 1990a; Denenberg et al., 1991b; Schrott et al., 1993; Boehm et al., 1996). In this study, 90% of the animals with ectopias (9/10) had them in this area. Because the frontal areas are connectionally related in the rodent (Zilles and Wree, 1995), we have not distinguished here between motor and prefrontal ectopias. Recent work has found that the prefrontal cortex is intimately involved with working memory processes in primates (Goldman-Rakic, 1987; Friedman and Goldman-Rakic, 1994; Funahashi and Kubota, 1994). We suggest, therefore, that the disruptions in cortical architecture that result in ectopia formation in frontal cortex of mice may be the basis for the less effective working memory system seen in ectopic mice, and can also be the basis for the better reference memory system, either...
directly, as a consequence of the disruption or indirectly, as part
of a reorganizational process due to the presence of ectopias.

Notes
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