Immune Response to *Trypanosoma lewisi*, Cerebral Asymmetry, Behavioral Activity and Laterality in the Female Alcohol-Preferring P and Alcohol Nonpreferring NP Rat Lines*

John R. Lakey, Karen J. Ott, Kit C. Helm, David A. Koehler Jr., Shannon D. Sandall, and Jennifer P. Thomas
University of Evansville

Abstract

The alcohol-preferring (P) and alcohol-nonpreferring (NP) lines of female Wistar rats were compared for the course of infection with the rodent blood parasite *Trypanosoma lewisi*, behavioral laterality biases and general activity, and cerebral hemisphere measurements of weight, length and height. Significant differences between the lines were obtained for both the infection response and activity measurements: the P line exhibited a less effect immune response and was more active than the NP line. Various tests failed to find significant differences for behavioral laterality biases in both lines, the left hemisphere was significantly longer than the right, yet no hemisphere asymmetry differences were found between lines. However, the brains of the P line were both significantly higher and heavier than those of the NP line. These results indicate a relationship between immunity, activity, brain size, and a genetic preference for alcohol.

**KEY WORDS:** P and NP Rats, Immune System, *Trypanosoma lewisi*, Laterality, Activity.

Introduction

The children of alcoholic fathers have been found to be at higher risk for alcoholism with indications of genetic predisposition.1,2 Among many factors which may bear some relationship to alcoholic predisposition, two have particular relevance to this study. (1) The children of alcoholic fathers are frequently characterized with having development disorders, usually conduct disorder and hyperactivity.3,4 (2) Familial left-handedness (sinistrality) has been reported disproportionately high among alcoholics, and individual left-handedness has been considered a negative indicator in treatment of alcoholism.5,6

Geschwind & Galaburda2,8 have proposed left-handedness to be related to both immune and developmental disorders, and their hypothesis has received some mixed support.9,10 Generally, high embryonic testosterone levels might account for diminished neuronal growth in the left cerebral hemisphere (the hemisphere which most frequently dominates and gives rise therefore to right handedness) with concurrent suppression of thymus development (a gland critical to normal immune function). London et al.11 have suggested that the laterality expressed by left-handedness, immune dysfunction, and increased risk for alcoholism may be related to a common metabolic lesion. The relationship among these parameters is worth examining as such studies may provide important insight into the physiological bases and comorbidity risks of alcoholism.

The objective of our study was to examine immune responsiveness, behavioral laterality biases, general activity, and cerebral hemisphere asymmetries in unique lines of alcohol-preferring (P) and alcohol-nonpreferring (NP) Wistar rats. These rats provide an intriguing model of alcoholism,12,13,14 and the P line has been reported to be more active than the NP line.15 Their assessment for possible immunologic differences, laterality biases and cerebral asymmetries had not yet received research attention.

Immune responsiveness of the P and NP rats were examined by following the course of an infection with *Trypanosoma lewisi*. This trypanosome was among the first of the rodent parasites discovered and it has been extensively studied for the last sixty years.16,17,18,19 *T. lewisi* causes a blood infection which
can be easily quantified by use of blood smears or by hemacytometric methods. Typically, the outcome of the infection is that rats acquire immunity to the parasites, and they survive. Over the course of a few weeks, reproduction of the parasite is eventually halted by IgG, then marked by IgM, and subsequently eliminated by IgG. Differences in the immune system that might involve one or more of these specific factors could be selectively identified by comparison of the growth, peak, and decline phases of parasite counts.

The lateral preference of the P and NP lines were examined with three standard behavioral tests, two of which provided indices of general activity. Cerebral hemispheres were weighed and measured for height and length to identify possible asymmetries. Behavioral laterality and neuroanatomical asymmetries have been reported for the rat, but both appear related to sex, age, handling, environmental richness, and other factors. 20,21 Various tests have been devised to measure behavioral laterality, but results are highly variable usually revealing themselves only with the statistical power of large samples. The open-field test and the more stressful swim-test rotation test appear to yield the most reliable laterality measurements, 22 and both can provide indices of general activity. The standard unbailed T-maze test provides less reliable laterality results, but with minimal stress to the animal.

EXPERIMENT I

Methods

Animals:
Sixty alcohol-naive female rats, half P and half NP, were obtained from the Indiana University School of Medicine’s parent colony through the courtesy of Dr. Ting-Kai Li. Following shipment, rats were individually housed in 19x11x8-in cages shelved in alternating P-NP order. Animals were provided ad lib food (Purina Lab Chow) and tap water, and cedar-shaving litter was changed weekly. The animal room was maintained at 75 degrees F and 50% humidity with a 12-hr light-dark cycle. Five days were allowed the animals to habituate to our facilities before any testing. At the day of infection, 14 days later, when approximately 45 days of age, the animals were weighed: Mean P weight = 169.8g (SEM=4.6g), Mean NP weight = 141.0g (SEM=4.8g) [t=4.33, df=58, p<.0005]. At the termination of the study, 55 days later when approximately 100 days of age, the animals were again weighed: Mean P weight = 270.2g (SEM=4.5g); Mean NP weight = 269.4 g (SEM=3.2g) [t=0.14, df58, p=.887]. The early significant weight difference between the two lines may be attributed to litter size: Mean P litter size = 7.8 pups (SEM=1.4); Mean NP litter size = 12.9 pups (SEM=0.3) [t=7.54, df=58, p<.0005].

Procedures
At approximately 40 days of age, all animals were individually tested in an open field during a morning session and an unbailed T-maze during an evening session on each day of a consecutive five-day period.

The open field was an open 3-ft square wooden box with 18-in high sides, painted gloss gray, lined into 9 1-ft squares, and evenly lighted by a single 100w lamp 4-ft above the center square. The animal was placed into a corner 6-in square start box formed with a wooden angle shield. The shield was removed 10 sec later allowing a 3-min field exploration. The recorded data included the left or right line (the animal’s left or right) crossed in exiting the corner 1-ft square and the number of 1-ft squares fully entered (all four feet) in the exploration interval. Daily sessions began at 8:00am, used the same alternating P-NP order, and were scheduled to assure that individual rats were tested at the same time each day. The starting corner was counterbalanced across days, and the floor wet sponged between trials to control carryover odor cues. Only the data of the last four days were used in reported results. Laterality scores (LS) were computed with the formula R-L/SQR(R+L), and activity scores were simply the number of squares entered during the 3-min trial.

The T-maze consisted of interchangeable 18-in segments of 4-in diameter white PVC pipe joined with standard T couplings and arms closed with standard caps. The runway declined at a 30-deg angle into the T-joint. The animal was placed into the runway and
simply observed to turn into either the left or right arm. The animal was removed, allowed a 2-min intertrial period in its home cage, and the procedure repeated in a second maze for two daily trials. To control possible carryover odor cues, mazes were fully disassembled, washed, and dried between trials. In addition, three complete sets of interchangeable maze parts were available, and these parts were randomly selected to assemble the next trial's maze. Daily sessions began at 6:00pm, used the same alternating P-NP order, and were scheduled to assure that individual rats were tested at the same time each day. Only data of the last four days were used in reported results, and laterality scores (LS) were computed with the formula \( R - L / SQR(R + L) \).

At 45 days of age, 48 animals were inoculated with \( T. lewisi \), and 12 animals were injected with sterile medium as controls. Daily tail-tip blood samples were obtained over the next 18-day period, one for Monopet measurement and microscopic count of erythrocytes per cubic mm of blood, and a second for stained slides and microscopic counts of trypanosome/erythrocyte ratio. \( Trypanosoma \) per cubic mm of blood was computed from these measurements. \(^{23,24}\) Samples were also obtained on 23rd and 26th days for those animals with continuing infections.

At 75 days of age, all animals were retested in the open field in a morning session and tested for swimming rotational bias in an evening session of the same day. The swim rotation test employed an evenly lighted 18-in diameter circular tank filled with water at room temperature to a depth of 26in. Animals were individually placed in the center of the tank, and the number of quarter rotations left (counter-clockwise) and right (clockwise) counted for a 3-min swim. Daily sessions began at 6:00pm, used the same alternating P-NP order, and were scheduled to assure that individual rats were tested at the same time each evening. Starting head-orientation was counterbalanced across days, and only the data of the last four days we used for the reported results. Laterality scores (LS) were computed with the formula \( R - L / SQR(R + L) \), and activity scores were simply the total number of quarter rotations swam during the 3-min trial.

At 80 days of age, all animals were tested for actual preference of 1 ethanol. Ethanol alone was supplied for 3 days, then cages were provided with both ethanol and water bottles for the next five days. Bottles were weighed, refilled to a constant volume, and the positions alternated daily. Ethanol preference was computed by bottle weight differences (Ethanol - Water / Ethanol + Water).

At 100 days of age, animals were anesthetized with sodium pentobarbital and maximum blood samples were obtained intracardially. With aorta clamped, the animal was then perfused with physiologic saline followed by 10% Formalin. Livers and spleen were removed, trimmed of mesentery, a weighed. Brains were next removed, placed in 10% Formalin for 48 hours then 70% ethanol for storage. Following the approach of Kolb et al.,\(^{25}\) the pineal and cerebellar flocculi were removed, the olfactory bulbs were blocked off evenly, the brains sectioned into hemispheres, and the cerebellum and underlying brain stem removed (Stoelting 51425M tissue slicer). The brains were visually graded for damage, even sectioning of bulbs and cord, and clean sagittal section of the longitudinal fissure with symmetrical bisection of the infundibulum and cerebral aqueduct. The length and height of the cerebral hemispheres were measured with high-quality calipers to .0001-in (Smiec). These measurements were independently repeated, and differences reconciled by additional remeasurement. The tissues were rinsed with and stored in fresh 70% ethanol for 24-48 hours. Hemispheres were individual removed from the ethanol, blotted and drained for 30-sec on filter paper, transferred to distilled water in covered weight bottles, then weighed to 1 mg (Ohaus E120 scale).

**Data Analyses:**
Data were analyzed with SYSTAT 4.1 programs.\(^{26}\) In respect to the parasitic counts tracking the infection response, Bartlett’s test for homogeneity of group
variances revealed significant differences between lines for Days 1-3 and 12-18 (p<.05), thus the data were subjected to various power transformations. A cube-root transformation provided uniform homogeneity at all days (all p<.05), and these transformed data were used in reported analyses. These transformed data yielded more conservative results than those obtained with the untransformed data. Given significant different P-NP mean body weights at the day of infection, the daily parasitic counts were first analyzed with body weight as a covariate; however, neither weight (F=1.82, df=1/45, p=.184) nor weight x day (F<1) were found significant. Analysis of red blood cell counts found requisite homogeneity without transformation, and with body weight as a covariate, N-PN differences were again not significant. Both data sets were accordingly analyzed as a two-factor mixed design without consideration of any covariate. In respect to the behavioral and neuroanatomical indices, the equivalent results of the two experiments were not significantly different, therefore the data were combined in subsequently reported analyses.

**Results**

**Infection Response:**

Figure 1A presents the course of mean trypanosome count growth and decline in the two lines. For the P line relative to the NP line, initial parasitic growth is noticeably more rapid, peaks earlier, maintains consistently higher levels as the infection subsides, and sustains detectable levels even at the last day of study. A lines x days analysis of variance for Days 1-18 obtained a significant line effect (F=6.10, df=1/46, p=.017), a significant days effect (F=85.35, df=17/782, p<.0005), and a significant line x days interaction (F=3.50, df=17/782, p<.0005).

Supplemental t-tests obtained significant differences between P-NP lines for Days 2, 6 and 14-18 (p<.05). Thereafter, the P line exhibited detectable trypanosome levels through the 26th day, the last day of sampling; however the NP line was totally free of parasites at both the 23rd and 26th day.

Figure 1B presents the mean course of erythrocyte count decline and recovery. Within each infected
a significant days effect (F=25.91, df=17/952, p<.0005), and a significant control x days interaction (F=12.59, df=17/952, p<.0005). Other interaction terms were not significant (all p>.233). Supplemental t-tests obtained significant line differences for all days (all p<.026) and significant infected-control group differences for days 6-18 (all p<.010). The NP line would appear comparatively anemic (to other rat lines as well as the P line) magnifying their parasite-to-erythrocyte estimates, thus reducing the P-NP parasite count differences. The P-line's immune response is likely still less effective in dealing with the parasite challenge than indicated by our data.

Liver and Spleen Weights:
At 55 days post-infection, spleen and liver weights were relatively larger for the NP line compared to the P line, however, these respective organ-to-body-weight ratios (P=.00474 versus NP=.00489 and P.03980 versus NP=.04016) were not significantly different (all F<1). As expected, spleen and liver weights were larger for the infected animals than for the control animals (1.30g versus 0.71g and 10.78g versus 10.31g). Spleen-to-body-weight ratio of infected animals was significantly larger than that of control animals (.00481 versus .00262: t=9.89, df=58, p<.0005), however liver-to-body-weight ratio was not significantly different (.03980 versus .3821: t=1.76, df=58, p=.08).

EXPERIMENT II
Methods
Animals:
Forty additional alcohol-naïve female, half P and half NP, were obtained from the same source and housed in the same manner.

Procedures:
Testing procedures were identical to those of Experiment I: At approximately 40 days of age, all animals were individually tested in an open field during a morning session and in an unbaited T-maze during an evening session. At 75 days of age, all animals were individually retested during a morning session and tested for swimming rotational bias in an evening session. At 80 days of age, the animals were tested for ethanol preference, but not ethanol consumption. In contrast to the previous study, brains were removed for hemispheric measurements and weights at 260 days of age. The data from these 40 animals were combined with those of the previous experiment's 60 animals.

Combined Results

Behavioral Laterality Measurements:
Figure 2 presents the mean laterality scores obtained when approximately 40 days old (open-field start and T-maze turn) and when 75 days old

![Figure 2](image.png)

Fig 2. Behavioral Laterality Biases Open-field start bias and P-maze arm bias for the P and NP lines at 40 days of age Open-field start bias and swim-tank rotational bias for the P and NP lines at 75 days of age. None of these laterality scores for the P and NP lines were significantly different

(open-field start and swim rotation). The P line shows a right bias when younger and a left bias when older, while the NP line shows the reverse pattern, a left bias when younger and a right bias when older. While suggestive, significant differences were not obtained for any of the four laterality measurements (Wilk's Lambda = 0.93, F=1.68, df=4/95, p=.161: Field#1 F=1.24, p=.268; T-Maze F=.107, p=.107; Field#2 F<1, p=.354; and Swim Tank F<1, p=.431). A lines x age analysis of repeated open-field start preferences also failed to obtain any significant difference between the P-NP lines (F<1), as well as the two age groups (F<1) or the lines x age interaction (F=2.73, df=1/98, p=.102).
Behavioral Activity Measurements:

Figure 3 presents the mean open-field activity scores when approximately 40 days old and again when 75 days old, and an additional swim activity score also when 75 days old. P-line open-field activity increased from 34.9 to 38.7 squares/trial, and the NP-line activity increases from 26.4 to 35.4 squares/trial in the three-minute trial period. A lines x age analysis of open-field activity obtained significant differences between the P-NP lines (F= 12.85, df=1/98, p=.001) and age groups (F30.39, df=1/98, p<.0005) with a significant interaction (F=5.19, df=1/98, p=.025). The P line swam 14.0 quarter rotations and the NP line swam 10.6 quarter rotations in the three-minute trial period (t=3.28, df=98, p=.001). The P-line displays both consistent and significant greater overall activity than the NP-line.

Ethanol Preference:

Significant differences between the P and NP lines were obtained for initial 10% ethanol preferences for all 100 animals (P=+0.11; NP=-0.83: t=14.94, df=98, p<.0005) and initial ethanol consumption for the 60 animals of the first experiment (P=4.88 gmEtOH/kgBodyWt, NP=1.21 gmEtOH/kgBodyWt: t=5.60, df=58, p<.0005). A correlational analysis found significant correlations only between ethanol preference and two activity measurements: first-test open field activity (r= +.32; p=.001) and swimming activity (r= +.23, p=.020). Ethanol preference and second-test open-field activity were not significantly correlated (p=.573). The only other significant ethanol preference correlation was obtained with total brain weight (r +.26, p=.018).

Cerebral Hemispheres Measurements:

Table 1a presents the mean hemispheric lengths and heights obtained from 83 brains removed without damage at approximately 100 or 260 days of age, and the mean hemispheric weights obtained from 57 of those brains successfully processed with even sectioning of the olfactory bulbs and spinal cord and sagittal sectioning in the longitudinal fissure that symmetrically bisected the infundibulum and cerebral aqueduct. Each measurement was separately evaluated with a line x age x hemisphere analysis of variance, and results obtained are summarized in Table 1b.

The analysis of hemispheric lengths found a significant age effect (F=5.55, df=1/79, p=.021, EtaSq=.054) and a significantly longer left hemisphere (F=40.92, df1/79, p<.0005, EtaSq=.053). The analysis of hemispheric heights found only a significant line
effect (F=26.50, df=1/79, p<.0005, EtaSq=.209). The analysis of hemispheric weights found a significant line effect (F=7.82, df=1/53, p=.007, EtaSq=.100) and a significant age effect (F=11.17, df=1/53, p=.002, EtaSq=.143). No other main effect or interaction obtained significance in any of the analyses (all p>.084). Although not reported, analysis of hemispheric weights for all 87 undamaged brains, including those with asymmetric sagittal section, produced equivalent statistical results.

The two hemispheres of the P line were found to be both significantly higher and heavier (8.84mm and 432.0mg) than those of the NP line (8.38mm and 404 6mg) irrespective of age, with line accounting for 20.9% of the height variance and 10.0% of the weight variance. In both lines, the two hemispheres of older animals were both significantly longer and heavier (13.03mm and 433.4mg) than those of younger animals (12.79mm and 402.5mg), with age accounting for 5.4% of the length variance and 14.3% of the weight variance. In both lines, the left hemisphere (13.03mm) was found to be significantly longer than the right (12.78mm), with this asymmetry accounting for 5.3% of the length variance.

Discussion

Both open-field and swim-rotation results confirm higher P than NP activity as previously reported by Waller et al. In respect to our laterality results, consistent P-NP differences were observed to vary with age, yet none were statistically significant despite the power provided by 100 animals.

Obviously, these data are highly variable, and the tests of questionable reliability. Our unique T-maze assembly produced the greatest P-NP differences, and the results are intuitively less ambiguous than the other tests. A repeated T-maze testing might have been informative Nonetheless, we have no support for behavioral laterality differences between the lines.

In respect to our cerebral hemisphere measurements, the P-line has relatively larger brains than the NP-line, and this difference is related to hemispheric height--not hemispheric length that appears to account more for brain growth with age. P brains would appear different, not just more mature, than NP brains. Smaller P litter sizes, perhaps better nutrition, might be related to these cerebral differences

Regardless, we have no support for neuroanatomical asymmetry differences between the lines

In respect to our immune-response findings, our results clearly indicate less effective P than NP immune response to the blood parasite. This impaired immunity is observed in both the initial parasitic population growth (Ig Factor?) as well as elimination of the infection (Ig Factor?). In respect to the erythrocyte counts, significant differences between the P-NP line are found for all but the first sample day, and this may be attributed to prolonged bleeding of the NP animals during daily sampling. Apparently, the NP line has developed slower blood clotting factors with their non-preference for ethanol. Nonetheless, this smaller-brain, possibly less well-nourished, and certainly anemic NP-line demonstrates superior immune response to the P-line. Comparison with the NP line underscores the poor immune response found in the P-line. Congruent with our results, Petitto et al. have recently reported natural killer cell activity to be markedly lower in the Short-Sleep than the Long-Sleep mouse lines (originally bred for their response to injected ethanol). Selection for either greater alcohol preference or lesser alcohol effect both appear positively correlated with less efficient immunity. If confirmed, there are meaningful implications For example, the association of alcohol abuse, contracting HIV, and more rapid development of AIDS, may be related to an impaired immune response coexisting with genetic risk for alcoholism and linked conduct disorders--in addition to alcohol's direct suppression of immune resistance. Those at high risk for alcoholism may be at high risk for AIDS.

Conclusion
These results may be interpreted as supportive of the suspected relationship between genetic alcohol preference, a less efficient immunity and higher levels of behavioral activity. An unexpected relationship was obtained between alcohol preference and relative brain size, specifically higher brain height and heavier brain weight. Our results do not support a relationship between genetic alcohol preference and either behavioral laterality biases or cerebral hemispheric asymmetry.

References

24. Ott KJ Course of infection of Trypanosoma lewisi-infected rats Workshop at the Association for Biology Laboratory Education, Southwest Missouri State University, June 4-8, 1990.

* From the Departments of Biology (KJO, JTT), Chemistry (DAK) and Psychology (JRL, KCH, SDS), University of Evansville. Evansville, Indiana. Presented at the 3rd Annual Meeting of the American Psychological Society, Washington, DC, June 1991. This study was supported with an Undergraduate Research Grant funded by the Lilly Foundation and Research Fellowships funded by the University of Evansville Alumni Association. The animals were provided through the generosity of Dr. T-K Li and the Indiana University School of Medicine.
Reprint requests: John R. Lakey, Ph.D., Department of Psychology, or Karen J. Ott, Ph.D., Department of Biology; University of Evansville, Evansville, IN 47722