Implications of Genetic Variation in Mouse Brain Structure for Electrode Placement by Stereotaxic Surgery

By: Douglas Wahlsten, William J. Hudspeth, and Kathryn Bernhardt

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Abstract:

The spatial locations of several forebrain and midbrain fibre tracts have been compared across seven commonly used inbred, hybrid and outbred mouse strains using a series of electrolytic lesions of known positions with respect to a stereotaxic instrument. Highly significant genetic variation was found in the positions of lambda, bregma and several major fibre tracts with respect to interaural zero; in the locations of fibre tracts with respect to bregma; and in the location of fibre tracts with respect to each other. It was demonstrated that stereotaxic coordinates of a structure for one strain could not be used for other strains. Errors resulting from improper alignment of the head in the instrument and histological artifact were also determined. These errors showed no significant group differences, but they revealed that within-group variation was substantially affected by such imperfections.

Article:

The major fibre tracts of the brain follow their appointed routes with great predictability. They are found in positions relative to one another which are often expressed in an atlas based upon a single representative of a species. Anomalous pathways do appear within common laboratory strains (e.g., Wahlsten, '74), and subtle intraregional variations in neural organization have been reported (Giolli and Creel, '74; Barber et al., '74). Nonetheless, the brains of most healthy species members are remarkably similar in structure.

Simply to speak of "great similarity," however, is not acceptable. It is imperative that the degree of regularity in brain structure be specified quantitatively. Only when this is done can the variability of brain structure be fully appreciated and the developmental and genetic origins of this variability be properly investigated.

In the present study the spatial locations of major fibre tracts in the mouse brain are measured precisely. Particular attention is devoted to genetic influences on spatial locations of brain structures.

The growing interest in genetic variation in neural function is making the mouse more attractive as an experimental subject. The mouse is without question the best known mammal genetically (Green, '66), and numerous inbred strains with widely ranging characteristics are readily available (Staats, '72). Several researchers have demonstrated the suitability of mice for stereotaxic surgery (Hudspeth and Wilsoncroft, '69; Zornetzer, '70; Slotnick, '72; Greenstein and Glick, '72).

The genetic variation in mouse brains which makes them so interesting, however, also raises the problem of placing electrodes and cannulae into the comparable structure in all genotypes. The seriousness of this problem is not clear at the present time. Two studies which employed stereotaxic techniques with several inbred mouse strains reported no problems in hitting major areas such as the amygdala (Leech, '69) or the septum (Oliverio et al., '73a) when the same coordinates with respect to bregma were used. On the other hand, it is well established that the brains of different mouse strains vary widely in weight (Storer, '67; Roderick et al., '73). It is therefore

likely that genotypic differences in the spatial locations of brain structures exist. The situation is complicated by the observation that a large brain is not simply an expanded version of a smaller brain. Wimer et al. ('69) reported that the Spearman correlation between relative brain volumes occupied by neocortex and hippocampus across nine inbred mouse strains was — 0.83, and hence all structures were not magnified by an equal factor in a larger brain. In addition to variation in the brain, the mouse skull which is used in practice as a reference to align electrodes is itself subject to genetic variation (Gruneberg, '63). It remains to be established just how large these genetic differences are, compared to the degree of accuracy demanded by various applications of the stereotaxic method.

METHOD

Methods were employed in the study which would resemble closely the techniques normally used in mouse brain surgery. Eight small lesions were placed in a standard array via electrodes passed through holes in the skull. All lesions were placed using the rigid stereotaxic instrument as zero reference point, thereby allowing great accuracy of placement. After histology was done on each brain, the spatial distances of major fibre tracts were measured with respect to the lesions. Because the lesions were placed in a regular, accurate array, corrections for many artifacts of alignment and shrinkage were possible.

Subjects

Five male and five female mice of each of seven strains underwent surgery at 77 or 78 days of age, having arrived in the laboratory at about 50 days of age. All mice were maintained five per standard cage on a 12-hour light-12-hour dark cycle with free access to water and dry lab chow. Strains selected for research encompassed a variety of genetic compositions, from highly inbred strains with negligible genetic variation but somewhat reduced viability because of inbreeding depression, to F_1 hybrids with no genetic variation but excellent viability owing to hybrid vigor, to outbred strains of unknown genetic composition but nonetheless in common use. The inbred strains A/J, C57BL/6J and DBA/2J, and the F_1 hybrids B6AF_1/J (C57BL/6J mother and A/J father) and B6D2F_1/J (C57BL/6J mother and DBA/2J father) were purchased from The Jackson Laboratory, Bar Harbor, Maine. The outbred CF #1 strain was obtained from Carworth Farms, New City, New York, while the outbred Swiss strain came from Simonsen Laboratories, Gilroy, California.

Apparatus

Surgery was done with the aid of a Kopf professional model stereotaxic instrument using a microelectrode carrier calibrated in 10 μ intervals, 30 degree ear bars, and a tooth bar notched for mice; interaural zero and the top surface of the tooth bar were level. Zeroing was done to an accuracy of ± 10 μ with the lesion electrode in place using electrical continuity readings. Vertical (dorsal-ventral, DV) zero was determined at a standard place on the top surface of the tooth bar, while horizontal (anterior-posterior, AP) zero was similarly obtained from the topmost edge of the ear bar. Midline or medial-lateral (ML) zero was taken to be the average of lateral measures of the lambda and bregma points determined visually by the experimenter.

Lesion electrodes were of 0.22 mm tungsten coated twice with Epoxylite and having an exposed hemispherical tip. One electrode was used for all eight successive lesions in a single mouse. Lesion current (0.5 ma for 20 seconds) was supplied by a Nuclear-Chicago stimulator and monitored by an oscilloscope.

Surgery

After the mouse was in the stereotaxic instrument with the skull properly exposed, measurements were made of the lambda and bregma points, and the "mid-line" coordinate was calculated. The eight skull holes were made by lowering the electrode in the appropriate positions near the skull, marking carefully the place on the skull with a needle, and then drilling with a No. 69 (0.75 mm) drill. After this the eight lesions were placed in standard order, starting 1.50 mm left of midline at interaural zero and progressing anteriorly to 2.00 mm, 4.00 mm and 6.00 mm anterior to interaural zero, then moving to 1.50 mm right of midline and 6.00 mm anterior to zero and progressing in 2.00 mm steps back to interaural zero; depth of all lesions was 3.00 mm above interaural zero. Immediately following the last lesion, the mouse was removed from the instrument and perfused intracardially with 10% formalin in physiological saline.

Histology

Following extraction from the skull, the brain was allowed at least one month further fixation in 10% formalin. Parts which were sometimes damaged in extraction were carefully cut away to a standardized extent in all brains; these parts included anterior olfactory bulbs, optic nerves, trigeminal nerves, paraflocculi, and the spinal cord. The wet weight of the fixed brain was then measured to the nearest mg after blotting on filter paper. Next the brain was encased in 10% gelatin and placed in formalin until sectioning. Serial coronal sections were cut with a freezing microtome throughout the area of interest at 25 μ and every other section was mounted on a glass slide and stained with Sudan Black B for myelin; about 125 sections were stained from each brain, representing 50 μ intervals.

Measurement of lesions and landmarks

Stained, serial sections of each brain were first viewed microscopically at 40 × magnification in a rostral-caudal sequence in order to determine the section in which major landmarks were located. The cytoarchitectonic atlas of the mouse brain by Sidman et al. ('72) was used to identify all structures. Abbreviations for most structures, specified in table 1, are consistent with those given by Konig and Klippel ('63). For each of the eight lesions, the section in which the center of the spherical lesion occurred was determined; in most cases this was also where the lesion was of greatest diameter. The following six lateral landmarks were viewed for each half of the brain:

(a) First appearance of VL;

(b) CAP at the point where it was midway between midline and the CE;

(c) The FH where it formed a clear bridge between dorsal and ventral hippocampi;

(d) First appearance of a distinct CC;

(e) First appearance of LL;

(f) Last appearance of mcgm.

Fourteen structures at midline were also located, as indicated in figure 2. In most cases the section number in which the structure was *first* present at midline was recorded; this indicated the most rostra! location of the structure. For two structures, SCC and CFV, the section in which they *last* appeared was determined as well.

TABLE 1

Abbreviations of brain structures [1]

BO, Bulbus olfactorius
CAA, Commissura anterior, pars anterior
CAP, Commissura anterior, pars posterior
CC, Crus cerebri
CE, Capsula externa
CFV, Commissura fornicis ventralis
CH, Commissura habenularum
CP, Commissura posterior
DPCS, Decussatio pedunculorum cerebellarium superiorum
DSUP, Decussatio supramammillaris
DVT, Decussatio ventralis tegmenti
FH, Fimbria hippocampi
FO, Fornix
GCC, Genu corporis callosi
LL, Lemniscus lateralis
LT, Lemniscus trigeminalis
mcgm, Nucleus marginalis corporis geniculati medialis
PONS, Pons
SCC, Splenium corporis callosi
VL, Ventriculus lateralis

[1] Adapted from Konig and Klippel ('63).

Subsequently, the above lesions and landmarks were viewed with a projecting microscope at 47.1 × magnification. Distances of lesion centers from midline and from the dorsal surface of the cerebral cortex were

measured in mm. Likewise, distances of the lateral landmarks from midline and of the midline landmarks from the dorsal surface of cerebral cortex were recorded.

For the purposes of quantification, the location of each structure in the anterior-posterior (AP) dimension was determined by multiplying the section number by 50 μ. The medial-lateral (ML) and dorsal-ventral (DV) coordinates were found by dividing the raw distances in mm by 47.1. Corrections for shrinkage were later applied to these AP, ML and DV values.

RESULTS

Statistical analysis was done using parametric analysis of variance and correlation. Within-group variances were homogeneous for most measures. All groups had five male and five female subjects, except for B6AF$_1$/J where one male died prior to completion of surgery. Hence, unweighted means analysis of variance was used with a Groups (7) by Sex (2) design.

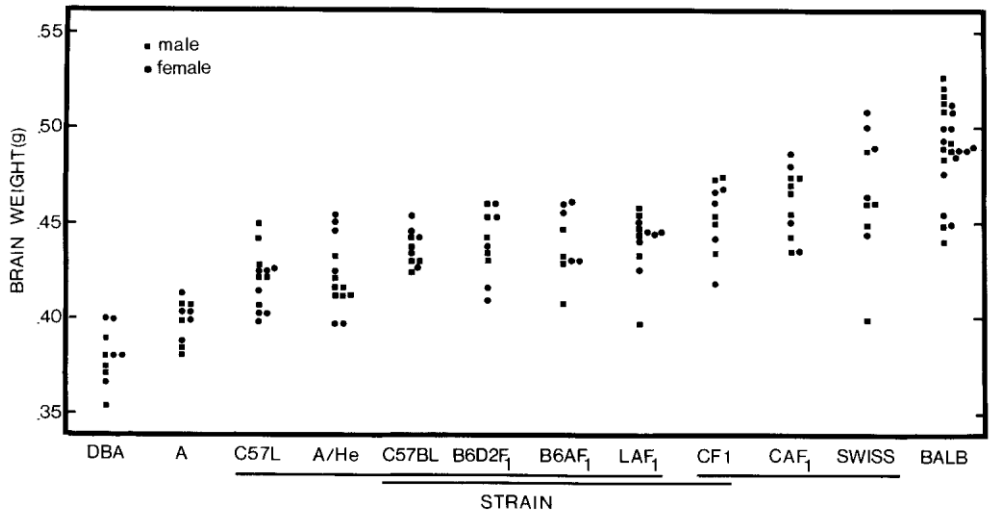

Fig. 1 Weight of the trimmed, fixed brain (g) for male (squares) and female (circles) mice of six inbred, four F_1 hybrid, and two outbred (CF #1, SWISS) strains. Strains are arranged in rank order of mean brain weight. Strains connected by an unbroken line are not significantly different using a Newman-Keuls test with $\alpha = 0.05$.

Brain weight

The weight of the trimmed brain is shown in figure 1 for each mouse in this study as well as for mice of five other strains measured with identical procedures in another project (Wahlsten, '74). Between-group differences were highly significant ($p < 0.0001$), but sex differences were only marginally significant ($p = 0.05$). Thus, it was clear that large differences in the overall size of the brain were present.

Skull landmarks

Significant group differences (all $p < 0.02$) in both the AP and DV dimensions were detected in the spatial locations of the lambda and bregma points with respect to interaural zero (figs. 2 through 8). Between-group variance in the AP measure was substantially greater for the lambda point (0.468) than for the bregma point (0.267). Sex differences were generally small and unreliable. The presence of such large genetic variation in the spatial structure of the skull suggests that interaural zero is probably not a satisfactory reference point for stereotaxic surgery.

The bregma point was elevated above lambda for all mice. This angle of elevation ranged from 3.9 degrees for CF #1 mice to 8.2 degrees for DBA mice, the mean elevation over all groups being 6.4 degrees; group differences were highly significant ($p < 0.0001$). The consequences of these differences can be calculated if it is assumed that brain structures of different strains are consistently aligned with the surface of the skull and not with the ear bar-tooth bar plane. For electrode placements through a hole in the vicinity of bregma, the actual DV value would be the nominal DV value times the cosine of the angle of skull elevation above the nominal. For a nominal placement 3.5 mm below bregma, this would amount to a difference between CF #1 and DBA strains (4.3 degree difference) of 0.01 mm in DV value, which is trivial. For the AP dimension, however, the

difference would be the nominal depth times the sine of the angle, which for CF #1 and DBA would amount to 0.26 mm difference in AP placement. Since this is a substantial difference, it may be worthwhile to adjust the position of each mouse in the instrument so that bregma is a standard angle with respect to lambda, perhaps, 6.5 degrees. This procedure would be relatively easy using a head holder which can be rotated, such as the one described by Slotnick ('72).

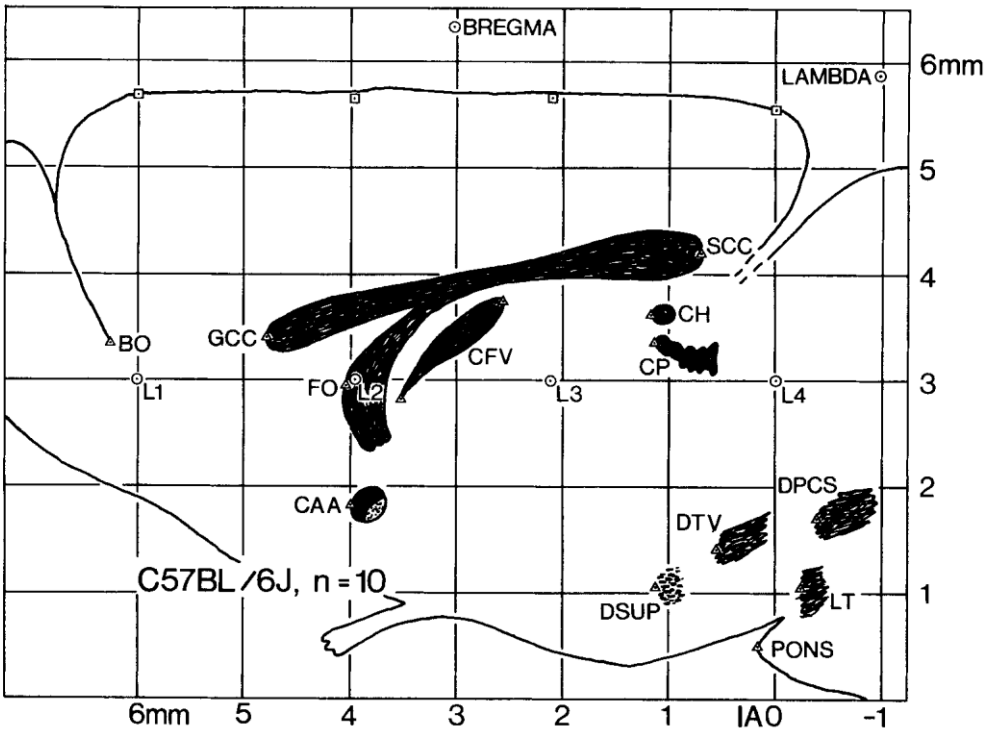

Fig. 2 Reconstruction of mid-sagittal section of the C57BL/6J mouse brain. Each measured point represents the mean location of the point across ten subjects. Points shown within circles were measured directly with reference to the stereotaxic device. Points within triangles were measured from serial, coronal brain sections stained for myelin; the raw measurements were corrected for several sources of artifact of surgery and histology to yield the final figures (see text). Points within squares measured the distance of the most dorsal surface of the cerebral cortex from the nearest lesion (L1, L2, L3, or L4); the measure was also corrected for several artifacts.

Brain landmarks

In order to convert the various measures of the brain sections into units (mm) of real brain size, a computer program was written to perform a variety of transformations. It compensated for section thickness, magnification of the overhead projector, and shrinkage artifacts. It also converted all measures to interaural zero as the origin, and it calculated angles of deviation of actual lesion axes from the three cardinal planes.

Alignment artifacts. Because lesions were placed in pairs 1.5 mm on either side of midline, the deviation of true brain midline from midline ascertained from lambda and bregma was found by averaging the difference between ML measures of left and right lesions across the four pairs. There were no significant group differences in midline displacement, and the mean displacement was quite small (0.02 mm to the left of true midline), but variability was relatively great; the standard deviation was 0.117 mm. Fortunately, this problem did not affect the measurements of loci of midline structures depicted in figures 2 through 8.

When the ML difference between each lesion pair was regressed upon the AP distance of each pair, the slope of the regression line equalled the tangent of the angle between lesion axis and true midline. No significant group differences were present, the mean deviation was small (rostral end 0.44 degree to the left of true midline), and the standard deviation was also small (1.02 degree).

When depths of right and left lesions below the dorsal surface of the cerebral cortex differed across the four lesion pairs, tilt of the head in the stereotaxic coronal plane was indicated which was probably a result of poor placement in the ear bars or asymmetry of the auditory meatus. No group differences existed; the overall mean

angle was small (0.36 degree); but the standard deviation was 2.60 degree, indicating that this angle was an important source of within-group variation.

Deviation of the electrode track from the coronal plane of cutting was found by comparing the section where each electrode entered dorsal cortex to the section of lesion center. Errors of this nature were small (mean = 0.86 degree; standard deviation = 1.48 degree).

The lateral brain landmarks mentioned in the section Measurement of lesions *and landmarks* enabled the angle of histological sectioning with respect to the perfectly symmetrical coronal plane to be estimated. An average angle was found for each brain based on the left and right positions of six lateral landmarks. Again, group differences were not significant. The mean angle was 1.14 degrees to the right (rostral end of brain to the right of the sagittal plane) with a standard deviation within groups of 1.76 degree.

Summarizing these results for deviations from stereotaxic and histological perfection, no systematic group differences were found. Average deviations were too small to affect coordinates based on group means, but within-group variability was influenced strongly by these errors. The development of instruments and techniques to compensate for these errors appears to be well worth the effort.

Shrinkage artifacts. Artifacts arising from shrinkage of the tissue during embedding and sectioning were determined separately for the AP and ML dimensions for each brain; shrinkage in the DV dimension was assumed to be the same as for ML, since cutting was in the coronal plane. Shrinkage factors were calculated by comparing mean separations of lesions in stained sections to actual separations in surgery (2.00 mm AP, 3.00 mm ML). The factor for each mouse was an average of six values for the AP dimension and four values for the ML dimension. Shrinkage in the AP dimension amounted to 16.3% with a standard deviation of 2.0%; no significant group differences were present. In the ML dimension shrinkage was 6.5% with a standard deviation of 2.8% and without significant between-group variation. Shrinkage factors used to correct all distance data for a brain were the separate AP and ML shrinkage factors calculated for that brain. It should be noted that these "shrinkage" factors also served to correct for errors of alignment in placing lesions or in sectioning the brain; since ML and DV measures would be greater in a brain that was not perfectly aligned, the shrinkage factor would tend to be somewhat less as a result. For this reason, no attempt was made specifically to correct for errors of alignment using trigonometric functions.

Two aspects of the above analyses are especially important for subsequent discussions. Of all the errors and artifacts described, only one showed any significant genetic variation. The angle of bregma above lambda differed sufficiently to have an effect on estimated location of structures several mm below bregma; however, only the AP dimension would be influenced substantially by this artifact. The other important point is that errors of asymmetry of the lesion pairs were eliminated for the purpose of reconstructing a midsagittal section by taking all AP and DV measures as the average of values of left and right lesions of each pair. Altogether these analyses suggest strongly that genetic differences in the spatial locations of midline fibre tracts do not result from genotype-dependent methodological artifacts.

Fibre tract locations. The corrected AP and DV coordinates of several major brain structures occurring at or near mid-line were averaged over the ten mice in each group to produce the mid-sagittal plots shown in figures 2 through 8. In the C57BL/6J panel (fig. 2) all points measured in this study are indicated. In viewing the composite maps of the six other strains, it is important to recognize that only the specific points indicated in figure 2 were measured precisely. The shape and diameter of various structures constitute approximations necessitated by illustrative convenience.

Spatial locations of most brain structures with respect to interaural zero showed substantial genetic variation. In the AP dimension all structures anterior to and including the posterior commissure (CP) possessed highly significant genetic variation (all $p < 0.0001$), while more posterior structures showed less reliable differences. Genetic differences in the DV dimension were much less reliable, even among forebrain fibre tracts. Only five

of 14 structures showed group differences significant beyond p = 0.01. Sex differences were marginally significant in almost half of the measures in both dimensions, but no sex difference was ever less probable than p = 0.01. There was no discernable pattern indicating which structure in which dimension would show a significant sex difference. Hence, there was no reason to present separately the mid-sagittal maps for males and females.

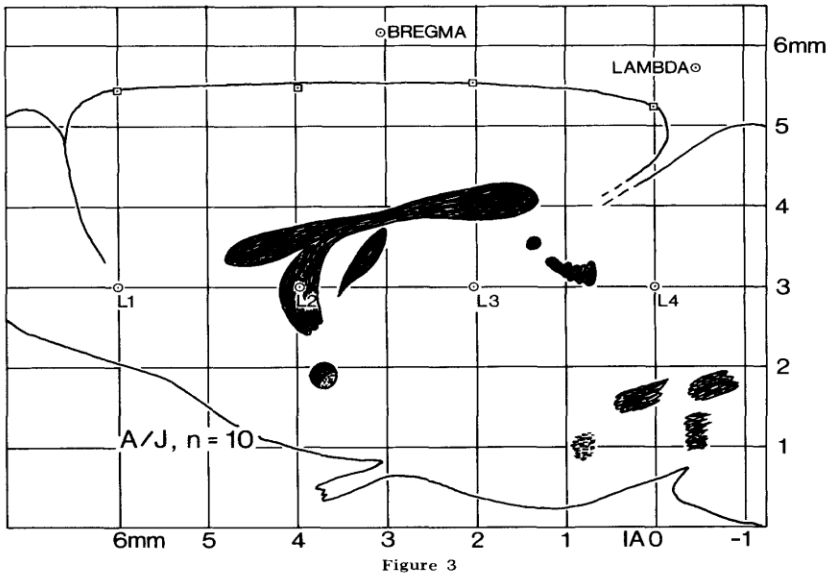

Figure 3

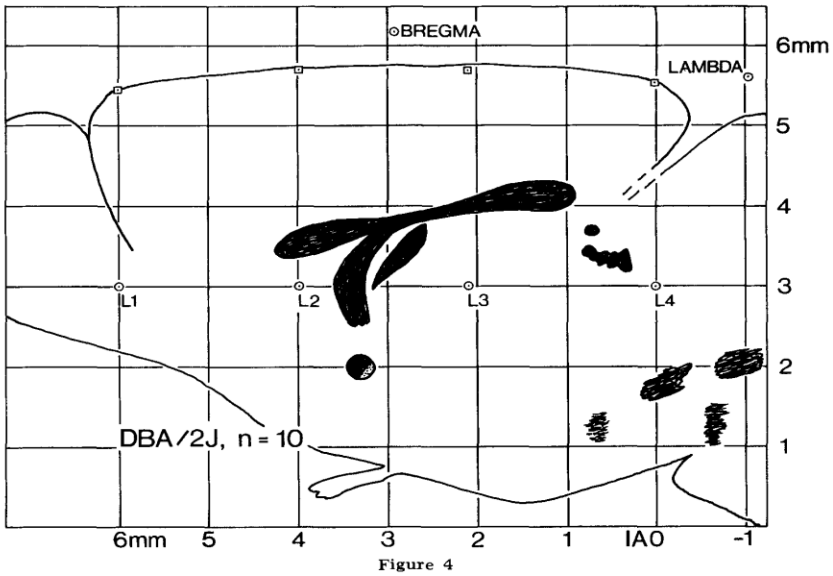

Figure 4

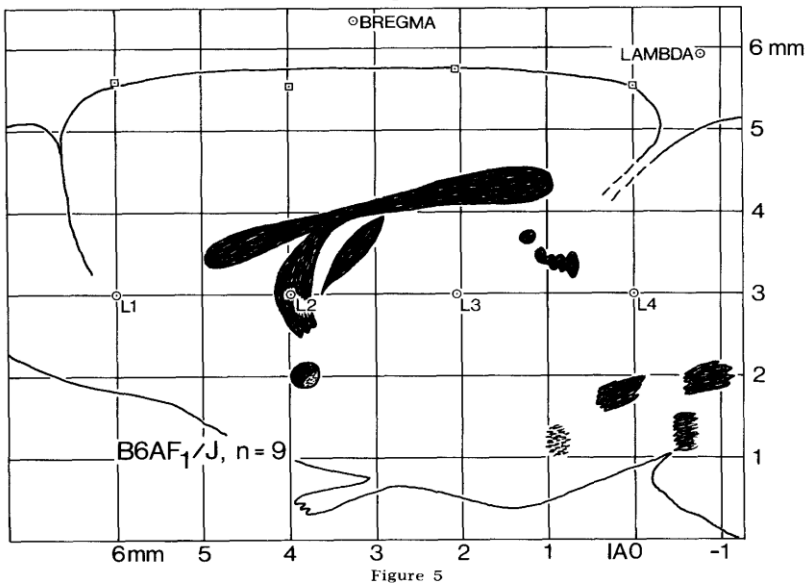

Figure 5

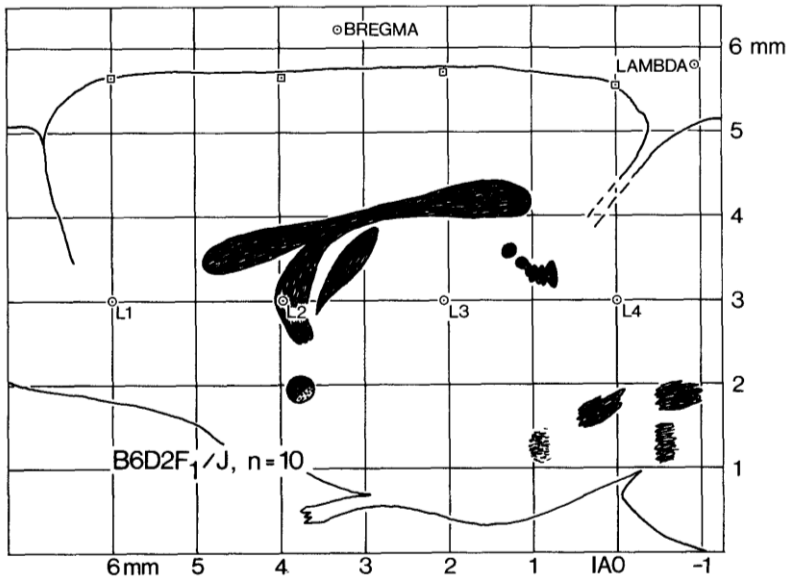

Figure 6

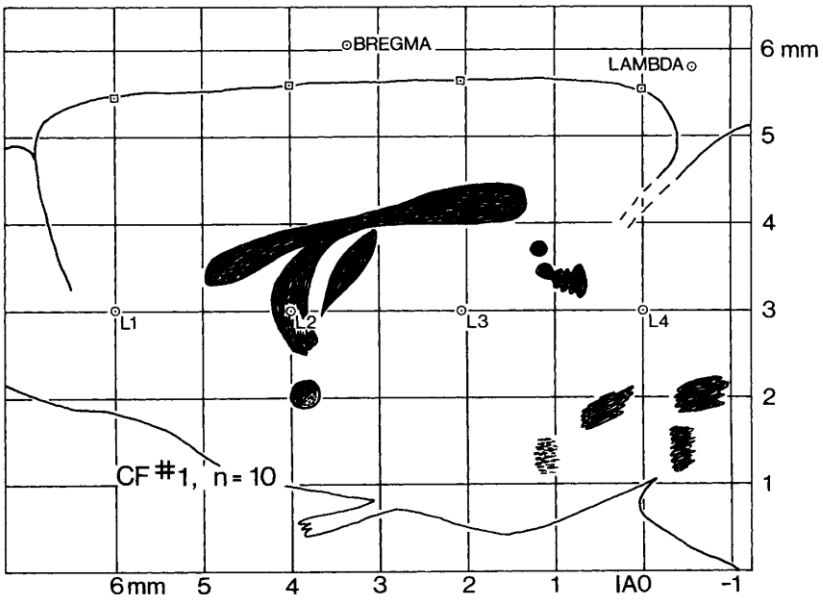

Figure 7

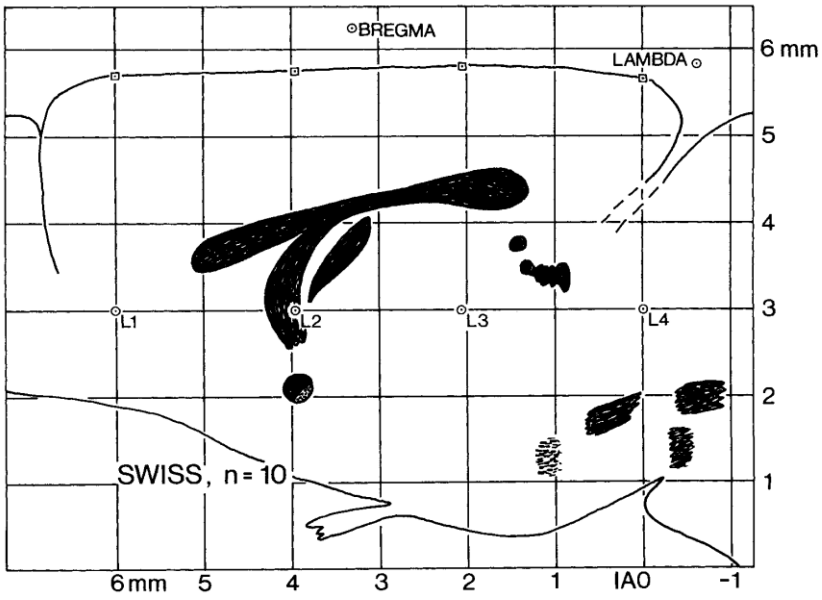

Figure 8

The proper interpretation of these substantial genetic differences in spatial location of structures with respect to interaural zero is made more difficult, of course, by the presence of genetic variation in locations of bregma and lambda. It is possible that brain structures are more closely related to the bregma point, for example, than to interaural zero. Although most mouse stereotaxic surgery is done using bregma as a reference point, no really good evidence is available in the literature to demonstrate that bregma is a better origin than lambda or interaural zero. The present data, on the other hand, provide sound bases from which to evaluate these alternatives. These data also allow an estimate to be made of inherent variability in spatial structure of the brain itself as measured by positions of fibre tracts with respect to each other independent of the locations of bregma and interaural zero. By comparing variances of certain brain landmarks about interaural zero, bregma, lambda and fibre tracts, potential sources of error in electrode placement arising from both between-group (genetic) and within-group variation can be identified.

Pearson correlations between the AP distances from interaural zero for GCC, CFV, CP, lambda and bregma are presented for each group in table 2. Clearly, bregma was much more highly correlated with the three structures than was lambda for SWISS mice, while the opposite was true for C57BL/6 and $B6AF_1$ mice. For A, DBA/2 and $B6D2F_1$ mice both skull points were highly correlated with the fibre tract positions, whereas for CF #1 neither point showed large correlations. Correlations between lambda and bregma AP values themselves also revealed a high degree of strain specificity. It was therefore obvious that neither point was universally superior as a stereotaxic reference point. Nonetheless, correlations were sufficiently large that considerable improvement over inter-aural zero as origin could be achieved by using skull landmarks.

TABLE 2

Within-group and between-group correlations between AP dimensions of skull and brain landmarks

	Bregma with			Lambda with			
	GCC	CFV	CP	GCC	CFV	CP	Bregma
A/J	0.76 [1]	0.80 [1]	0.72 [1]	0.71 [1]	0.76 [1]	0.57 [1]	0.90 [1]
C57BL/6J	0.27	0.40	0.54	0.75 [1]	0.63 [1]	0.51	0.57 [1]
DBA/2J	0.80 [1]	0.84 [1]	0.76 [1]	0.88 [1]	0.95 [1]	0.98 [1]	0.74 [1]
$B6AF_1/J$	0.27	0.18	0.25	0.83 [1]	0.90 [1]	0.71 [1]	0.06
$B6D2F_1/J$	0.87 [1]	0.89 [1]	0.90 [1]	0.70 [1]	0.75 [1]	0.82 [1]	0.82 [1]
CF #1	0.66 [1]	0.48	0.45	0.45	0.60 [1]	0.51	0.41
Swiss	0.71 [1]	0.66 [1]	0.52	0.22	0.35	0.11	0.34
Between groups	0.83 [1]	0.82 [1]	0.65	0.59	0.63	0.69 [1]	0.38

[1] $p < 0.05$, one tailed.

When correlations between mean AP values across the seven genotypic groups were calculated for all 14 midline structures, an interesting pattern emerged. Structures anterior to bregma were all significantly correlated ($p < 0.05$) with bregma, but only one of four was significantly correlated with lambda. Among the eight most posterior structures five were correlated ($p < 0.05$) with lambda, and only two were significantly correlated with bregma. Thus, there was a general trend for a structure to be more highly correlated with the skull landmark nearest it. However, the trend was not so strong that the bregma point would be totally useless in improving accuracy for more posterior structures; all correlations with bregma were positive, and only three were less than 0.5. If it were necessary to choose just one point for use in all stereotaxic surgery, bregma would be superior.

TABLE 3

Statistical summary of variances of AP measures of GCC and FO about interaural zero (0), Bregma (BR) and CFV

	GCC-0	GCC-BR	GCC-CFV	FO-0	FO-BR	FO-CFV
MS_B	0.823	0.301	0.076	0.522	0.249	0.050
MS_W	0.039	0.041	0.010	0.042	0.042	0.003
Est. ω^2	0.637	0.354	0.362	0.498	0.298	0.613

In order to understand fully the implications of using bregma or even a brain landmark as origin, further statistical analysis was deemed necessary. The distance of a structure from bregma was found simply by taking the difference between AP distances of the structure and bregma from interaural zero. A brain structure chosen as origin for similar purposes was CFV (first appearance), which was fairly close to the average location of bregma. A one-way analysis of variance across the seven groups was computed for the AP distance of GCC and FO from interaural zero, bregma and CFV (table 3). The variance between groups (MSB), which reflected the

magnitude of genetic differences in AP distance, was substantially reduced by taking bregma as origin, and it became quite small indeed when CFV was used as origin. The variance within groups (MSw), on the other hand, was not affected at all by using bregma as origin, but it was greatly reduced when CFV was the origin. The net result of these changes in variance was that the proportion of the total variance in AP distance which was attributable to group differences, as estimated by ω^2 (Hays, '63), decreased by nearly half when bregma was used as origin instead of interaural zero. Using CFV as origin led to a similar reduction in ω^2 for GCC, but for FO it actually led to a much larger ω^2 value. In spite of these various changes, genetic differences in AP distance were highly significant ($p < 0.0001$) regardless of which point was used as origin.

TABLE 4

Newman-Keuls tests [1] of differences in ordered strains means ($\alpha = 0.05$) for measures in AP dimension

Measure	Rank order						
	Largest						Smallest
GCC-0	SW [2]	CF	BA	BD	AJ	CJ	DJ
GCC-Bregma	SW	AJ	CJ	BA	CF	BD	DJ
GCC-CFV	BA	SW	BD	CF	AJ	CJ	DJ
FO-0	SW	CF	AJ	BA	BD	CJ	DJ
FO-Bregma	AJ	SW	CJ	BA	CF	BD	DJ
FO-CFV	AJ	CF	BA	CJ	SW	BD	DJ

[1] Strains connected by an unbroken line are not significantly different using *a posteriori* criteria.
[2] Abbreviations: A/J-AJ; C57BL/6J-CJ; DBA/2J-DJ; B6AF$_1$/J-BA; B6D2F$_1$/J-BD; CF #1-CF; SWISS-SW.

In summary, genetic differences in AP distances can be reduced by using bregma as origin, but they cannot be eliminated. The reason for this is that the spatial locations of various structures of the brain with respect to one another possess highly significant genetic variation which is completely independent of skull characteristics.

The nature of these genetic differences is perhaps more easily visualized by comparing strain rank orders for AP distances of GCC and FO about the three origins, as shown in table 4. It is obvious that many reversals of rank ordering occurred when a different origin was used, some of which were significant using the conservative, *a posteriori* Newman-Keuls test. Consequently, the reduced MSB did not represent simply a reduction of absolute difference in AP distance by using bregma or CFV, but rather the direction of the relative positions of strains frequently changed. A very important conclusion follows from these observations: *No monotonic transformation of stereotaxic coordinates about any origin can make an atlas based on one strain serve adequately for a wide range of genetic strains.*

DISCUSSION

The findings of this study have important implications for the application of stereotaxic surgery to mouse brain research. It should be evident that the use of the same stereotaxic coordinates to place electrodes in the brains of different strains of mice is not an acceptable practice because the electrodes will be in different structures for the various strains. The extent of this problem can be easily seen for the case of septal lesions. Three previous studies have all used the same coordinates with respect to bregma to place septal lesions in the mouse strains A/J (Oliverio et al., '73b), C57BL/6J and DBA/2J (Oliverio et al., '73a), and B6D2F$_1$/J (Carlson, '70). The lesions were all 0.8 mm anterior and 3.5 mm ventral to bregma. The expected center of this kind of lesion can be determined for each strain using the midsagittal diagrams (figs. 2, 3, 4, 6) in this report. The lesion center would range from 0.1 mm anterior to FO for the DBA strain to 0.4 mm posterior to FO for A/J mice. Hence, different regions of the septum would be destroyed in different strains. Although a large lesion would probably destroy the fornix and the columns of the fornix in all four strains, it might damage the hippocampal commisure only in A/J and C57BL/6J• There might be strain-specific effects on the accumbens nucleus as well. Whether the strain differences in response to septal lesions observed by Oliverio et al. ('73a,b) or by Gonsiorek et al. ('74) can in fact be attributed to differences in electrode placement is not known, of course.

It is unlikely that anyone will undertake the ponderous task of constructing a complete, precise stereotaxic atlas of many mouse strains in the future. Instead, a technique of successive approximations for each strain appears to be more applicable using available material (figs. 2 through 8; also Sidman, et al., '72).

In addition to precautions to insure homology of placements across strains, care should also be taken to reduce variability of placements within strains. Results of this study suggest that variability can be reduced by improved uniformity of alignment of the skull in the instrument; this will require the construction and use of spatial reference devices. Since the left and right bregma sutures frequently do not meet the saggital suture at the same point, a determination of the most reliable bregma reference point should also improve accuracy.

When these painstaking procedures have been incorporated into the stereotaxic routine, substantial variability within a strain will unfortunately persist. The major source of variance will be incongruence between the bregma point and the brain. From table 3 it is evident that the standard deviation of forebrain structures (AP dimension) with respect to bregma is about 0.2 mm. This problem may be visualized by passing an insect pin directly through bregma; a surprising degree of inconsistency between mice of the same inbred strain exists. This source of variance is reduced in human neurosurgery using direct visualization of ventricles via X-rays (Dawson et al., '69), but this procedure does not lend itself to work with the mouse brain. The other source of variability is that which is inherent in the brain itself. Relative spatial locations of forebrain fibre tracts have a standard deviation of about 0.1 mm (table 3). Since this value is very close to the limit of accuracy imposed by the methods of this study, the exact degree of variability cannot be stated with confidence. The spatial size of fibre tracts such as corpus callosum has a standard deviation of about 0.15 mm (Wahlsten, '74, fig. 3). This kind of variability is truly irreducable.

Note:
Figs. 3, 4, 5, 6, 7, 8 Mid-sagittal diagram for several other mouse strains. Only the points indicated in figure 2 were used to reconstruct the diagrams.

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