3. Data Collection

The inferences we may draw from the data depend also on the way the data were collected. We will describe the data collection process by answering the following questions:

3.1 How was the study carried out?

Brain tissue was obtained from 41 subjects at routine autopsies of persons who died at seven metropolitan hospitals in New York and California. Nineteen subjects were homosexual men who died of complications of AIDS. Sixteen subjects were presumed heterosexual men: six of these subjects died of AIDS and ten of other causes. Six subjects were presumed heterosexual women. One of these women died of AIDS and five of other causes. The mean age of homosexual men was similar to that of the heterosexual men.

The brains were fixed by immersion for 1 to 2 weeks in buffered formalin and then sliced by hand at a thickness of about 1 cm. Tissue blocks containing the anterior hypothalamus were dissected from these slices and stored for 1 to 8 weeks in 10% buffered formalin. These blocks were then given code numbers; all subsequent processing and analysis was done without knowledge of the subject group to which each block belonged. The blocks were frozen-sectioned at a thickness of 52μm in planes parallel to original slices. With the aid of a compound microscope, the outlines of INAH3 were traced in every section. The outline of INAH3 was drawn as the shortest line that included every cell of the type. INAH3 is spherical or ellipsoidal and its borders of INAH3 are not well demarcated; hence a blind procedure was used to reduce bias effects.

The areas of the traced outlines were determined with a digitizing tablet, and the volume of INAH3 was calculated as the summed area of the serial outlines multiplied by the section thickness. The procedure used to calculate the volumes is sufficiently accurate if the sections are very thin. In this case the sections were extremely thin (52 μm), and it is reasonable to assume that the volumes were obtained with high accuracy.

The 41 subjects were divided into five groups based on their sex (male or female), sexual orientation (heterosexual and homosexual), and cause of death (AIDS and non-AIDS). The five groups are: heterosexual males who died of AIDS, heterosexual males who died of other causes, homosexual males who died of AIDS, heterosexual females who died of AIDS, and heterosexual females who died of other causes. Including cause of death in the classification is a consequence of the fact that the volumes of INAH3 were obtained only for the homosexuals who died of AIDS. There is no data for homosexuals who died of other causes.

Moreover, observe that as there is no data about homosexual females, we are not able to make some other meaningful comparisons like the comparison of homosexual females with heterosexual females or homosexual females with heterosexual males (both groups have sexual orientation toward females).

3.2 Glance at the Data

At first glance, there are significant differences in the group sizes. Specifically, the group sizes vary from 1 to 19. Those large differences in the group sizes are a consequence of very strict criteria for inclusion of subjects in the study. According to the authors of the study, the criteria were as follows: (i) age 18 to 60, (ii) availability of medical records, (iii) in AIDS patients, statement in the records of at least one AIDS risk group to which the patient belonged (homosexual, intravenous drug abuser, or recipient of blood transfusions), (iv) no evidence of pathological changes in the hypothalamus, and (v) no damage to the INAH3 nuclei during removal of the brain. Fourteen specimens (over and above the 41 used in the study) were rejected for one of these reasons; in all cases the decision to reject was made before decoding.

There appears to be little difference among the five groups. However, there is a great deal of variability in each group, and it looks as though any differences among the groups can be attributed to this variability. The range of values in each group is very similar. Some presumed heterosexual men have small INAH3 nuclei, and some homosexual men have large ones. However, when we compare the distribution of observations in each group, a different view emerges. The group of homosexual men who died of AIDS shows a relatively high fraction of low values.

The existence both very large and very small observations in the group of heterosexual and homosexual men hints at the possibility that sexual orientation, although an important variable, may not be the sole determinant of INAH3 size. It is also possible, however, that these unusual observations are due to technical shortcomings or to misassignment of subjects to their subject groups.

3.3 What are the consequences of misclassification of the subjects and inaccurate data?

It is possible that some subjects were misclassified, that is they were classified as heterosexuals when in fact they were homosexuals. The researcher used the term *presumed* homosexual to indicate the possibility. The records of some patients contained no information about their sexual orientation. Those patients were assumed to have been heterosexual. This misclassification would support the claim of no difference in the volumes between the two groups.

The outlines of INAH3 are not well demarcated. Therefore, it is obvious that some volumes were underestimated and some were overestimated. As all processing and analysis was done without knowledge of the subject group to which each tissue belonged (blind procedure), we can expect that all five groups had similar fractions of underestimated and overestimated measurements. However, notice that this assumption may be not realistic taking into account very small sample sizes in some groups. Fortunately, the measurement errors are believed to be small due to significant advances in the technology in recent years.

3.4 Should the data be transformed to obtain meaningful comparisons?

Biological research shows that brain weight is an important factor affecting the size of INAH3. However, the brain weight changes as we move across the sexual orientation groups. The mean brain weight for women is smaller than that for either heterosexual or homosexual men. In the case study, the researchers obtain the following means, respectively: 1256 g, 1364 g, and 1392 g. Therefore, in order to make meaningful comparisons, the data should be normalized for brain weight.

The researchers found that normalizing the data for brain weight had no effect on the results.