BIOLOGY AND HOMOSEXUALITY

15. Brief Version of the Case Study

15.1 Problem Formulation

Is there a biological basis for sexual preference? Following up on research suggesting that certain cell clusters in the brain govern sexual behaviour, Simon LeVay measured the volumes of four cell groups in the interstitial nuclei of the anterior hypothalamus in post-mortem tissue from 41 subjects at autopsy from seven metropolitan hospitals in New York and California. The volumes of one cell cluster, INAH3 were measured for the 41 subjects. Subjects were classified into five groups according to three factors: gender, sexual orientation, and cause of death.

The goal of the study was to examine the relationship between sexual orientation and volume of INAH3. Specifically, the researcher hypothesized that INAH3 is large in individuals sexually oriented toward women (heterosexual men and homosexual women) and small in individuals sexually oriented toward men (heterosexual women and homosexual men).

The results of the research are described in the paper " A Difference in Hypothalamic Structure Between Heterosexual and Homosexual Men" published in *Science*, Vol. 253, pages 1034-1037, 1991.

The data are available in the SPSS file homo.sav located in the STAT 252 directory on the FTP server.

The following is a description of the variables in the data file:

Column	Name of Variable	Description of Variable
1	VOLUME	1,000 times the volume of INAH3 in mm^3
2	CODE	Group Number (an integer from 1 to 5) 1= Male, Heterosexual, AIDS Death 2= Male, Heterosexual, Non-AIDS Death 3= Male, Homosexual, AIDS Death 4= Female, Heterosexual, AIDS Death 5= Female, Heterosexual, Non-AIDS Death

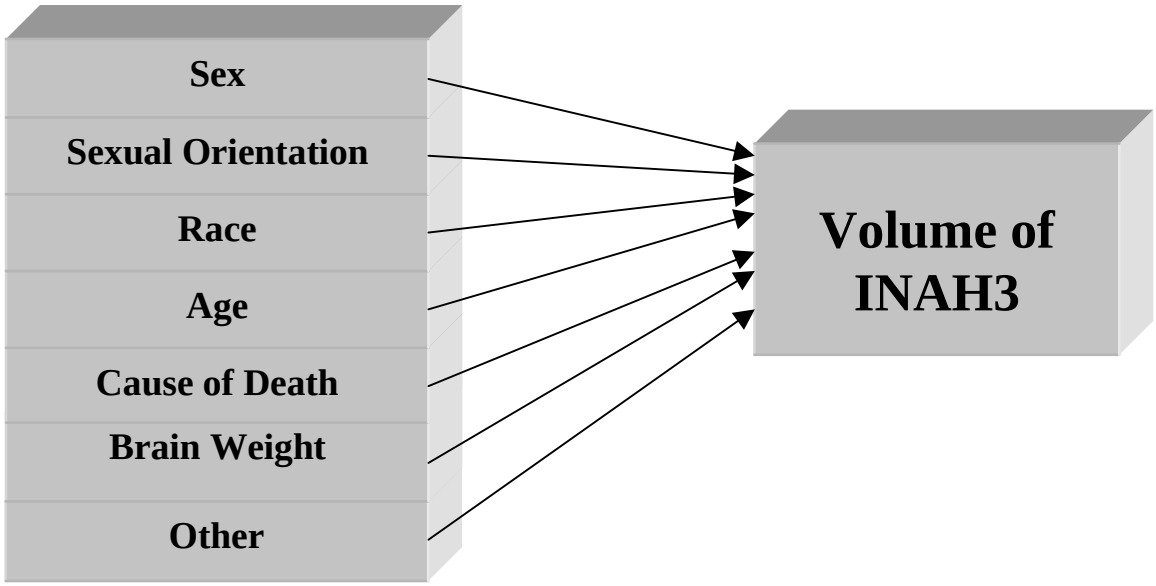
We will use SPSS to answer the following questions using the data:

1. Do heterosexual males tend to differ from homosexual males in the volume of INAH3?
2. Do heterosexual males tend to differ from heterosexual females in the volume of INAH3?
3. Do heterosexual females tend to differ from homosexual males in the volume of INAH3?

15.2 Study Design

According to pathologists, there are several factors that might affect the size of INAH3. The most important are sex, sexual orientation, race, cause of death (AIDS), age, brain weight, hospital of origin, length of time between death and autopsy. In general, it would be difficult or even impossible to consider all variables affecting the volume. Some variables may not be recognized or measured.

The variables that might affect volumes of INAH3 are visualized in the following diagram.



The five groups in the study are based on some combinations of sex, sexual orientation, and cause of death of the 41 subjects at autopsy. As none of the factors was decided by the investigator for each of the 41 subjects, the case study is an example of an observational study. In other words, allocation of the subjects to the five groups was not determined by any chance mechanism.

As the study is an observational study, we are not able to draw any causal conclusions from the statistical analysis alone. It is possible that some confounding variables are responsible for the disparity in the volumes of INAH3 between heterosexual and homosexual males. For example, as all homosexual males in the study died of AIDS, there is the possibility that the small size of INAH3 in the homosexual men is the result of AIDS or its complications and is not related to the men's sexual orientation.

Notice that the 41 subjects at autopsy in the five groups were not selected from any well-defined populations. Therefore, the observed pattern cannot be inferred to hold in some general populations, for example the population of all homosexual men unless we assume that the homosexual men in the study are representative of the population. However, all homosexual men in the study had AIDS. There is a possibility that AIDS patients constitute an unrepresentative subset of gay men.

15.3 Data Collection

Brain tissue was obtained from 41 subjects at routine autopsies of persons who died at seven metropolitan hospitals in New York and California. 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.

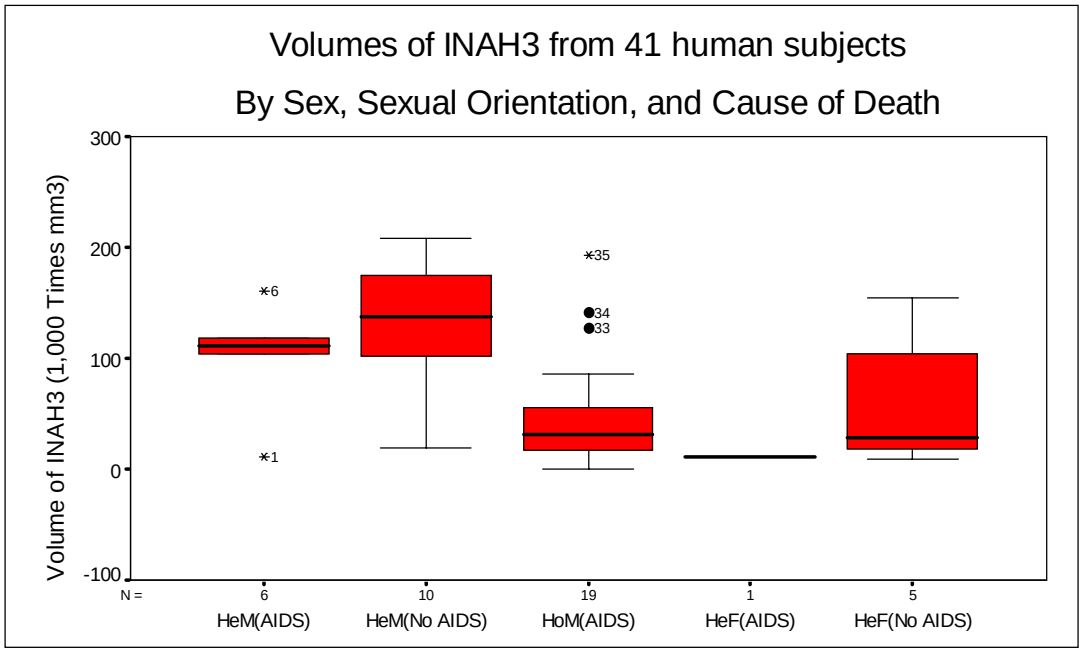
The areas of the traced outlines were determined with a digitizing tablet, and the volume of INAH3 was calculated as the summed area of the serial outlines multiplied by the section thickness. The procedure used to calculate the volumes is sufficiently accurate if the sections are very thin. In this 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).

15.4 Displaying and Describing the Data

SPSS produces the following side-by-side boxplots of the volumes of INAH3 for the five combinations of sex (M-male or F-female), sexual orientation (He-heterosexual, Ho-homosexual), and cause of death (AIDS, No AIDS) on the original scale of measurement.



The positions of medians indicate that the median volume of INAH3 was a much smaller for the homosexual males and heterosexual woman than for the heterosexual men. Moreover, the boxplots indicate that volumes are associated with sexual orientation but not with cause of death.

As you can see there is some skewness in some of the groups. In particular, the distribution of volumes for heterosexual females who died from other causes than AIDS is highly skewed to the right. Moreover, the distribution of volumes for heterosexual males who died from other causes than AIDS is skewed to the left.

Nevertheless, the skewness is exhibited in the groups consisting of a relatively small number of observations (5 and 10), it is difficult to detect nonnormality in such cases.

SPSS can be also used to obtain the descriptive statistics for the volumes of INAH3 for the five groups in the study.

MEASURES OF	STATISTICS	MALES		
		Heterosexual		Homosexual
		AIDS	Non-AIDS	AIDS
CENTER	MEAN	103.3333	128.0000	50.3158
	MEDIAN	111.5000	137.5000	32.0000
	5% TRIM MEAN	105.2037	130.3889	45.1287
	95% CI FOR MEAN	(51.636, 155.030)	(85.34,172.26)	(25.41, 75.23)
SPREAD	STANDARD DEV.	49.2612	60.7523	51.6710
	STD ERROR	20.1108	19.2116	11.8541
	VARIANCE	2426.667	3690.844	2669.895
	IQR	47.7500	89.5000	48.0000
	MINIMUM	12.0000	20.0000	1.0000
	MAXIMUM	161.0000	209.0000	193.0000
	RANGE	149.0000	189.0000	192.0000
SHAPE	SKEWNESS	-1.4132	-0.8069	1.6956
	ST. ERROR SKEW	0.8452	0.6870	0.5238
	KURTOSIS	3.3489	-0.0942	2.3049
	ST. ERROR KURT	1.7408	1.3342	1.0143
COUNT		6	10	19

MEASURES OF	STATISTICS	FEMALES	
		Heterosexual	
		AIDS	Non-AIDS
CENTER	MEAN	12	63.0000
	MEDIAN	12	29.0000
	5% TRIM MEAN	12	61.5000
	95% CI FOR MEAN		(-15.241, 142.442)
SPREAD	STANDARD DEV.	0	63.4965
	STD ERROR	0	28.3965
	VARIANCE	0	4031.8000
	IQR	0	115.5000
	MINIMUM	12	10.0000
	MAXIMUM	12	155.0000
	RANGE	0	145.0000
SHAPE	SKEWNESS		0.8958
	ST. ERROR SKEW		0.9129
	KURTOSIS		-1.3584
	ST. ERROR KURT		2.0000
COUNT		1	5

The numerical summaries are consistent with the conclusions we have reached while examining the side-by-side boxplots. The mean volume of INAH3 was more than twice as large in the heterosexual men as in the homosexual men. Similarly, the mean volume of INAH3 was twice as large in the heterosexual men as in the women. The cause of death does not seem to have any effect on the volumes.

15.5 Comparing the Mean Volumes of INAH3

We would like to know whether there are significant differences in the volumes of INAH3 for the five groups. An appropriate statistical technique to examine the differences is one-way ANOVA.

SPSS produces the following output:

Variable VOLUME
By Variable CODE

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	4	49611.3712	12402.8428	4.0763	.0079
Within Groups	36	109536.2386	3042.6733		
Total	40	159147.6098			

The analysis of variance F-statistic is F=4.0763, with 4 and 36 degrees of freedom, giving a p-value of 0.0079. That small p-value indicates strong evidence against the null hypothesis of no difference among the mean volumes for the five groups. In other words, there is strong evidence of differences among the group means. The within-group mean square is 3042.6733, so the pooled estimate of a common standard deviation is the square root of the value, which is equal to 55.16043 (0.05516043 mm3).

However, the conclusions based on the ANOVA model are valid only if the underlying assumptions are satisfied. Specifically we assume that the volumes have normal distributions for each of the five groups, and the standard deviations of volumes are all the same. The analysis carried out in Section 6 indicates that the assumptions of normality and equal standard deviations might be violated for some of the groups.

Under these circumstances, the nonparametric Kruskal-Wallis test procedure provides a very good alternative. The Kruskal-Wallis test output in SPSS for our experiment is displayed below.

Kruskal-Wallis 1-Way Anova

VOLUME
by CODE

Mean Rank	**Cases**	**Group Code**
25.08	6	CODE = 1
30.35	10	CODE = 2
16.50	19	CODE = 3
5.00	1	CODE = 4
17.70	5	CODE = 5
Total	41	

Chi-Square	D.F.	Significance
11.6451	4	.0202

The p-value of the test is reported as 0.0202 indicating strong evidence against the assumption of no differences in the group means. This is consistent with the results obtained with the F-test.

15.6 Contrasts

The goal of the study is to examine the relationship between sexual orientation and volume of INAH3. Specifically, we wish to answer the following three questions: Do heterosexual males tend to differ from homosexual males in the volume of INAH3? Do heterosexual males tend to differ from heterosexual females in the volume of INAH3?
Do heterosexual females tend to differ from homosexual males in the volume of INAH3?

We will answer the above questions by looking at contrasts.

Let us define the mean volumes for the five groups by μ_i, where i=1,2, 3, 4, 5. We define the contrasts in the following way:

NO.	CONTRAST	GROUP				
		1	2	3	4	5
		MALE HET AIDS	MALE HET NON	MALE HOM AIDS	FEM HET AIDS	FEM HET NON
		CONTRAST COEFFICIENTS				
1	$\mu_1-\mu_3$	1	0	-1	0	0
2	$\mu_5-\mu_4$	0	0	0	-1	1
3	$\mu_2-\mu_1$	-1	1	0	0	0
4	$(\mu_2-\mu_5)-(\mu_1-\mu_4)$	-1	1	0	1	-1
5	$(\mu_2-\mu_1)/2 + (\mu_5-\mu_4)/2$	-1/2	1/2	0	-1/2	1/2
6	$\mu_1-\mu_4$	1	0	0	-1	0
7	$\mu_2-\mu_5$	0	1	0	0	-1
8	$(\mu_1-\mu_4)/2 + (\mu_2-\mu_5)/2$	1/2	1/2	0	-1/2	-1/2
9	$(\mu_1+\mu_2)/2-\mu_3$	1/2	1/2	-1	0	0
10	$\mu_3-\mu_4$	0	0	1	-1	0
11	$\mu_3 - (\mu_4+\mu_5)/2$	0	0	1	-1/2	-1/2

Contrast 1 compares the mean volume of heterosexual and homosexual males who died of AIDS. Contrasts 2 and 3 measure the NON-AIDS versus AIDS differences for heterosexual females and males, respectively. Contrast 4 asks whether the death cause differences are equal for heterosexual men and women. If there is no evidence that they differ, it is reasonable to proceed to contrast 5, the average of the two cause-of-death differences. Contrasts 6 and 7 measure the Male-Female differences for AIDS victims and victims of other causes, respectively.

If the contrasts 2 and 3 indicate that volumes are not associated with cause of death, then it is reasonable to ignore the cause of death in any other comparisons.

The contrast 8 is the average of the contrasts 6 and 7 and measures the overall differences between the volumes of heterosexual men and women when cause of death is ignored. The contrast 9 measures the differences between heterosexual males and homosexual males when cause of death is ignored.

The contrasts 10 and 11 address the question whether there is evidence that heterosexual females differed from homosexual males. At first glance, it may appear that this comparison makes no sense - the groups differ both in sex and in sexual orientation. However, both groups have sexual orientation toward males, so the question asks whether - among persons oriented toward males - there was a difference in the volumes between males and females.

The homosexual males in the study all died of AIDS. If one insists on strict control for cause of death, the only comparison available is between the groups 3 and 4. This is done by the contrast 10. However, there is only one subject in the group 4. If the contrasts considered above support an assumption of no cause of death influence, we can measure the difference by combining the groups 4 and 5. This leads to the contrast 11.

Now le us look at the results. SPSS produces the following output:

Variable VOLUME
By Variable CODE

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	4	49611.3712	12402.8428	4.0763	.0079
Unweighted Linear Term	1	15008.0277	15008.0277	4.9325	.0327
Weighted Linear Term	1	20109.5723	20109.5723	6.6092	.0144
Deviations from Linear	3	29501.7989	9833.9330	3.2320	.0335
Within Groups	36	109536.2386	3042.6733		
Total	40	159147.6098			

The analysis of variance F-statistic is F=4.0763, with 4 and 36 degrees of freedom, giving a p-value of 0.0079. That small p-value indicates strong evidence against the null hypothesis of no difference among the mean volumes for the five groups.

Observe that the above ANOVA table provides more information about the variation between groups than the ANOVA table provided in the previous section. More precisely, a linear pattern in the group means is investigated. The table indicates moderate evidence of a linear pattern in the group means.

Now let us look at the analysis of the contrasts. All the contrasts obtained below are based on the assumption that the pooled estimate of standard deviation from all five groups was used.

Pooled Variance Estimate

	Value	S. Error	T Value	D.F.	T Prob.
Contrast 1	53.0175	25.8312	2.052	36.0	.047
Contrast 2	51.6000	60.4252	.854	36.0	.399
Contrast 3	25.4667	28.4847	.894	36.0	.377
Contrast 4	-26.1333	66.8026	-.391	36.0	.698
Contrast 5	38.5333	33.4013	1.154	36.0	.256
Contrast 6	91.3333	59.5801	1.533	36.0	.134
Contrast 7	65.2000	30.2126	2.158	36.0	.038

Contrast 8	78.2667	33.4013	2.343	36.0	.025
Contrast 9	65.7509	19.0522	3.451	36.0	.001
Contrast 10	38.3158	56.5934	.677	36.0	.503
Contrast 11	12.5158	32.7558	.382	36.0	.705

The p-value of 0.047 for the contrast 1 provides moderate evidence of the difference between the volumes of homosexual and heterosexual males who died of AIDS or its complications. The difference between the two groups is estimated based on the samples of size 6 and 19.

The high p-values of the contrasts 2 and 3 indicate that there is no evidence of any effect of cause of death on volumes for heterosexual females and males. The p-value of 0.698 for the contrast 4 shows that the cause of death had no impact on the differences in volumes between heterosexual males and females. Contrast 5 is the average of the two cause-of-death differences. The p-value of 0.256 indicates that there is no evidence that volumes are associated with cause of death.

The p-value of 0.134 for the contrast 6 shows no evidence of the differences between heterosexual males and females that died of AIDS or its complications. However, notice that the contrast is based on two groups of size 6 and 1.

The contrast 7 with its p-value of 0.038 indicates a difference in the volumes for the males and females who died of other causes. The p-value for the contrast 8 that measures the overall sex difference is 0.025. It shows that the effect of sex on volumes is significant.

In summary, it appears that volumes are associated with sex but not with cause of death. Therefore, it is reasonable to pool over causes of death. Pooling over causes of death the groups 1 and 2 and comparing their average to the average for the combined groups 4 and 5 produces the contrast 8. The p-value 0.025 shows convincing evidence of the differences in volumes between heterosexual males and heterosexual females.

Pooling over causes of death the groups 1 and 2 and comparing their average to the group 3 produces the contrast 9. Thus the contrast measures the sexual orientation difference. The small p-value of 0.001 indicates convincing evidence of the differences in volumes between heterosexual and homosexual males.

Observe that the pooling process provides stronger evidence (p-value=0.001) of the differences in volumes between heterosexual and homosexual males than does the contrast 1 (p-value=0.047).

The final question was whether there is evidence that heterosexual females differed from homosexual males. The difference is measured by the contrasts 10 and 11. The two-sided p-values of 0.503 and 0.705 respectively indicate that there is no evidence for this difference.

15.7 Final Comments

The goal of the study is to examine the relationship between sexual orientation and volume of INAH3. Specifically, the answers to the following three questions were sought: Do heterosexual males tend to differ from homosexual males in the volume of INAH3? Do heterosexual males tend to differ from heterosexual females in the volume of INAH3? Do heterosexual females tend to differ from homosexual males in the volume of INAH3?

The 41 subjects in the study 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 side-by-side boxplots of the data show that there is some skewness in some of the groups, but the scale of volume, untransformed, appears best (although not ideal). An appropriate statistical technique to determine whether the observed differences among the five groups are statistically significant is one-way ANOVA. The p-value of the F-test equal to 0.0079 indicates strong evidence of differences among the group means.

In order to answer the above questions, we looked at the contrasts. The analysis of contrasts showed there is no evidence of any effect of cause of death on volumes for heterosexual females and males. Therefore, it is reasonable to pool over causes of death.

The analysis of contrasts (contrast 9) showed that there is convincing evidence of the differences in volumes between heterosexual and homosexual males. There is also convincing evidence of the differences in volumes between heterosexual males and heterosexual females (contrast 8). The analysis showed also that there is no evidence that heterosexual females differed from homosexual males (contrast 11).

As none of the factors was decided by the investigator for each of the 41 subjects, the case study is an example of an observational study. In other words, allocation of the subjects to the five groups was not determined by any chance mechanism.

As the study is an observational study, we are not able to draw any causal conclusions from the statistical analysis alone. It is possible that some confounding variables are responsible for the disparity in the volumes of INAH3 between heterosexual and homosexual males. For example, as all homosexual males in the study died of AIDS, there is the possibility that the small size of INAH3 in the homosexual men is the result of AIDS or its complications and is not related to the men's sexual orientation.

Until tissue from homosexual men dying of other causes becomes available, the possibility cannot be rigorously excluded. The results do not allow us to decide if the size of INAH3 in an individual is the cause or consequence of that individual's sexual orientation.

Notice that the 41 subjects at autopsy in the five groups were not selected from any well-defined populations. Therefore, the observed pattern cannot be inferred to hold in some general populations, for example the population of all homosexual men unless we assume that the homosexual men in the study are representative of the population. However, all homosexual men in the study had AIDS. There is a possibility that AIDS patients constitute an unrepresentative subset of gay men.

The existence of exceptions in the data (that is, presumed heterosexual men with small INAH3 nuclei, and homosexual men with large ones) 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 exceptions are due to technical shortcomings or to misassignment of subjects to their subject groups.

Summarizing, the analysis indicates that INAH3 is dimorphic with sexual orientation, at least in men, and suggests that sexual orientation has a biological substrate.