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The Interstitial Nuclei of the Human Anterior Hypothalamus: An Investigation of Variation with Sex, Sexual Orientation, and HIV Status

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The interstitial nuclei of the human anterior hypothalamus (INAH1–4) have been considered candidates for homology with the sexually dimorphic nucleus of the preoptic area of the rat. Volumetric sexual dimorphism has been described for three of these nuclei (INAH1–3), and INAH3 has been reported to be smaller in homosexual than heterosexual men. The current study measured the INAH in Nissl-stained coronal sections in autopsy material from 34 presumed heterosexual men (24 HIV− and 10 HIV+), 34 presumed heterosexual women (25 HIV− and 9 HIV+), and 14 HIV+ homosexual men. HIV status significantly influenced the volume of INAH1 (8% larger in HIV+ heterosexual men and women relative to HIV− individuals), but no other INAH. INAH3 contained significantly more neurons and occupied a greater volume in presumed heterosexual males than females. No sex difference in volume was detected for any other INAH. No sexual variation in neuronal size or density was observed in any INAH. Although there was a trend for INAH3 to occupy a smaller volume in homosexual men than in heterosexual men, there was no difference in the number of neurons within the nucleus based on sexual orientation.

Key Words: **hypothalamus; preoptic area; sex difference; human; homosexuality; sexual orientation; AIDS; HIV.**

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Examination of the human hypothalamus for morphological sex differences comparable to those described in animals (Bleier *et al.*, 1982; Commins and Yahr, 1984; Gorski *et al.*, 1978; Hines *et al.*, 1985; Tobet *et al.*, 1986) has produced discrepant results. Swaab and Fliers (1985) examined a hypothalamic cell group previously designated as the intermediate nucleus (Brockhaus, 1942) and found it to be larger in males than in females. They suggested that the nucleus may correspond to the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the rat (Gorski *et al.*, 1978). Consequently, they redesignated it as the SDN-POA or, more simply, the sexually dimorphic nucleus (SDN) (Swaab and Hoffman, 1988, 1995). Allen *et al.* (1989) were unable to verify a sex difference in that nucleus; however, they identified two other nuclei which they called the second and third interstitial nuclei of the anterior hypothalamus (INAH2 and INAH3), both of which they found to be larger in males. They designated the SDN of Swaab and Fliers as the first interstitial nucleus of the anterior hypothalamus (INAH1). Thus, the terms intermediate nucleus, SDN-POA, SDN, and INAH1 correspond to a single cell group in the human. Allen *et al.* (1989) also described a nucleus which they called INAH4 for which they found no sexual dimorphism. Like Allen *et al.* (1989), LeVay (1991) found INAH3 to be larger in males than in females and found no sex difference in

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INAH1 or INAH4. LeVay (1991), however, found no sex difference in the volume of INAH2.

To address the discrepancies in the literature regarding sexual dimorphism of the INAH, we assessed the volume of each of the INAH in serial sections from postmortem specimens obtained at autopsy and also examined each nucleus for sex differences in neuronal density, total neuronal number, and mean neuronal size. In agreement with two prior studies (Allen *et al.*, 1989; LeVay, 1991), we found INAH3 to be sexually dimorphic, occupying a significantly greater volume in males than females. In addition, we determined that the sex difference in volume was attributable to a sex difference in neuronal number and not in neuronal size or density (Byne *et al.*, 2000). We found no evidence for sexual dimorphism of any other INAH.

LeVay (1991) examined the volumes of the INAH for variation with sexual orientation in men. He found the volume of INAH3 in homosexual men to be smaller than that of presumed heterosexual men and comparable in size to that of presumed heterosexual women. On the basis of that result, he hypothesized that INAH3 is dimorphic, not only with sex, but also with sexual orientation, at least in men (LeVay, 1991). LeVay's characterization was done only for volume and not for cell number.

We now report our examination of the INAH for possible variation with sexual orientation in men. Sexual orientation was determined from a review of medical records available at autopsy. No information regarding sexual orientation was available for subjects that died from causes unrelated to HIV infection. In the absence of such information, all individuals who were not known to be HIV positive (HIV+) at the time of death were classified as heterosexual because of the low rate of homosexuality in the population (Hamer *et al.*, 1993; Michael *et al.*, 1994). Specimens were obtained from HIV+ men only if the autopsy record listed a single HIV risk factor (i.e., intravenous drug use or homosexual behavior). Those for whom intravenous drug use was the only known risk factor were presumed to be heterosexual. Those for whom homosexual behavior was listed as the risk factor were presumed to be homosexual. Thus, as in the study of LeVay (1991), all specimens from homosexual men came from individuals who died from opportunistic infections associated with HIV infection. It has, therefore, been necessary to examine possible effects of HIV infection on the INAH. To date, we have examined all four INAH for volumetric variation with sexual orientation and HIV status. In addition, we have examined INAH3 for such variation in neuronal number, size, and packing density. In the absence of a source of brains from women of known sexual orientation, it has not been feasible to address the possibility that one or more of the INAH exhibit variation with sexual orientation in women.

The present sample includes the specimens from HIV-negative (HIV−) individuals who were included in our earlier study (Byne *et al.*, 2000) in which all of the morphometric methods were fully described. Throughout the text, data are presented as mean ± standard error of the mean (SEM). The mean age of subjects did not differ significantly across groups: HIV+ presumed heterosexual males 47.1 ± 10, $n = 10$; HIV− presumed heterosexual males 49.5 ± 2.9, $n = 24$; HIV+ presumed heterosexual females 40.6 ± 3.2, $n = 9$; HIV− presumed heterosexual females 49.9 ± 2.5, $n = 25$; and HIV+ homosexual males 41.8 ± 2.5, $n = 14$.

HIV Influence on INAH1 and Sexual Influence on INAH3

For the initial analysis (Table 1), HIV+ and HIV− heterosexual males and females were analyzed in a two-way ANOVA for the volumes of INAH1–4. Only INAH3 revealed a significant influence of sex. There was a highly significant effect of sex ($P < 0.0001$), with no influence of HIV status [$F(1, 61) = 1.61$, $P > 0.20$]. Only INAH1 revealed a significant influence of HIV status, as there was a statistically significant effect on volume ($P = .047$). Volume was increased in HIV+ males and HIV+ females by about 8% (0.369 ± 0.012 vs 0.401 ± 0.017). Since there was no significant influence of HIV status on INAH3, the influence of sexual orientation was analyzed by a one-way ANOVA collapsing across HIV status to yield three groups: male heterosexual, female heterosexual, and male homosexual subjects. This analysis yielded a significant effect of group: $F(2, 76) = 13.95$, $P < 0.0001$, with the majority of the effect due to the male–female difference. The size of INAH3 in homosexual males (0.096 ± 0.007, $n = 14$) was intermediate between the heterosexual males (0.121 ± 0.007, $n = 31$) and females (0.073 ± 0.005, $n = 34$), but the differences did not reach statistical significance relative to either group ($P > 0.05$, post hoc Tukey–Kramer HSD) (Fig. 1).

Influences on Brain Weight

Excluding the homosexual male group, there were significant sex ($P < 0.001$) and HIV ($P < 0.05$)

TABLE 1
Volumes (in Cubic Millimeters) of the INAH[a]

	Heterosexual males		Heterosexual females		Homosexual males, HIV+
	HIV−	HIV+	HIV−	HIV+	
INAH1	.364 ± .017 (n = 21)	.424 ± .033 (n = 9)	.372 ± .018 (n = 20)	.409 ± .003 (n = 8)	.379 ± .026 (n = 12)
INAH2	.059 ± .004 (n = 23)	.058 ± .010 (n = 10)	.055 ± .002 (n = 24)	.058 ± .013 (n = 8)	.059 ± .005 (n = 13)
INAH3	.123 ± .009 (n = 22)	.108 ± .009 (n = 9)	.077 ± .006 (n = 25)	.067 ± .012 (n = 9)	.096 ± .007 (n = 14)
INAH4	.101 ± .010 (n = 22)	.103 ± .011 (n = 8)	.091 ± .010 (n = 25)	.083 ± .013 (n = 9)	.085 ± .012 (n = 14)

[a] Some INAH were incomplete in some specimens precluding volume determination. The number of specimens for which volumes could be determined, therefore, varied among the nuclei as indicated. An ANOVA (excluding the homosexual males) revealed a significant main effect of HIV status only for INAH1 [$F(1, 55) = 4.02$, $P < 0.05$] and a significant main effect of sex only for INAH3 [$F(1, 61) = 20.7$, $P < 0.0001$]. There was no significant sex × HIV interaction for any nucleus.

influences on brain weight with males (1347.3 g) having greater brain weights than females (1187.3 g) and HIV− individuals (1299.1 g) having greater brain weights than HIV+ individuals (1235.5 g). Although the interaction of sex and HIV status was not significant ($P = 0.11$), the bulk of the HIV influence was due to larger brain weights in HIV− heterosexual males (1409.6 ± 22.9) vs HIV+ heterosexual males (1291.0 ± 41.1). This may not be due to the HIV infection itself, as the average brain weight for the HIV+ homosexual males (1409.3 ± 31.5) did not differ from that of the HIV− heterosexual males (Table 2); however, in the absence of an HIV− homosexual group, the main and interactive effects of sexual orientation and HIV status cannot be completely analyzed. None of the dependent variables examined exhibited significant covariance with brain weight. Similarly, no variables covaried with age, and mean ages did not differ across groups.

Because ANOVA revealed a significant group effect on brain weight, an additional ANOVA was run for INAH3 volume/brain weight. The results were the same as for the raw volumes of INAH3. There was a significant main effect of sex [$F(1, 61) = 12.07$, $P = 0.009$], but no effect of HIV [$F(1, 65) = .416$, $P = 0.5213$]. Post hoc Tukey–Kramer HSD failed to demonstrate a significant difference between homosexual men (0.069 ± 0.006) and either heterosexual men (0.087 ± 0.005) or women (0.061 ± 0.004) ($P > 0.05$, each test), although there was a trend for INAH3/brain weight to be reduced in the homosexual relative to heterosexual men ($P < 0.10$, two-tailed) (Table 3).

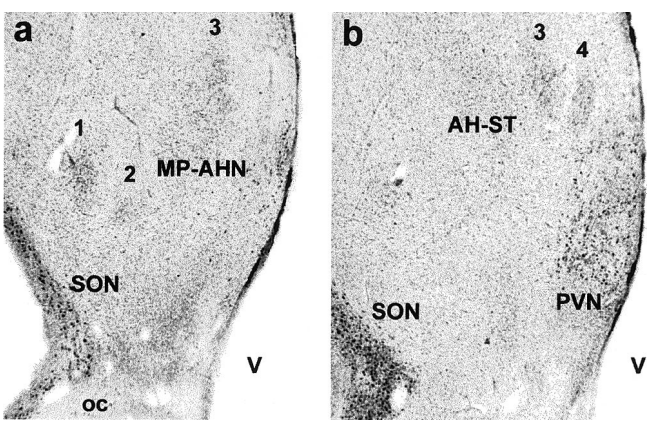

FIG. 1. Eighty-micron-thick coronal sections from specimen ms-136 (69-year-old presumed heterosexual male) illustrating the INAH, which are indicated by the corresponding numbers. INAH1 and INAH2 are seen clearly in level a, in which INAH3 begins to emerge as an area of increased density within MP-AHN. Level b is 880 μm posterior to level a. INAH3 and INAH4 are prominent. INAH3 is in close proximity to a cell group designated by Bleier as AH-ST (see Byne, 1998, for discussion). Abbreviations: AH-ST, confluence of the anterior hypothalamic nucleus (AH) with the bed nucleus of the stria terminalis (ST); MP-AHN, medial preoptic-anterior hypothalamic nucleus; PVN, paraventricular nucleus; oc, optic chiasm; SON, supraoptic nucleus; v, third ventricle.

INAH3 Neuronal Number Varies with Sex But Not Sexual Orientation

In our previous studies, the most robust cellular finding was an increased number of neurons in the INAH3 of males compared to females (Byne *et al.*, 2000). The present data set agrees with those findings in presumed heterosexual individuals with the male INAH3 containing on average approximately 60% more neurons than the female. The number of neurons in INAH3 from homosexual males did not differ from heterosexual males. For neuronal number, a one-way ANOVA revealed a significant group effect [$F(2, 53) = 7.21$, $P < 0.01$] that was attributable to the fact

TABLE 2
Brain Weights (Grams)[a]

HIV status	Sex/orientation		
	Male heterosexual	Female	Male homosexual
Positive	1291.0 ± 41.1 (n = 10)	1180.0 ± 30.1 (n = 9)	1409.3 ± 31.5 (n = 14)
Negative	1409.6 ± 22.9 (n = 24)	1194.6 ± 22.6 (n = 25)	

[a] An ANOVA, excluding the homosexual males, revealed a main effect of sex [$F(1, 65) = 27.3$, $P < 0.0001$] a main effect of HIV status [$F(1, 65) = 4.3$, $P = 0.04$] and no sex × HIV interaction ($P = 0.114$). The bulk of the HIV effect is accounted for by the reduction of brain weight in the HIV+ heterosexual males relative to the HIV− heterosexual males.

that both male groups had more neurons in INAH3 than did the female groups. The trend toward decreased INAH3 volume in the male homosexual group in the absence of a difference in total neuronal number suggested the potential importance of examining neuronal density. There was a strong trend for an influence of sexual orientation on neuronal density in INAH3 [$F(2, 53) = 3.03$, $P = 0.057$] that was attributable entirely to a higher neuronal density in INAH3 of male homosexuals versus heterosexual males and females (Table 3). There was no group effect on the cross-sectional area (μm^2) of neurons within INAH3 (heterosexual males 120.7 ± 3.4, n = 21; females 118.4 ± 3.1, n = 21; homosexual males 121.9 ± 3.8, n = 14).

Discussion

The present study provides further evidence that INAH3 occupies a larger volume (Allen *et al.*, 1989; LeVay, 1991) and contains more neurons (Byne *et al.*, 2000) in presumed heterosexual men than women. The primary sexually dimorphic cellular characteristic of INAH3, neuronal number, did not vary as a function of sexual orientation. The current study failed to provide evidence of sexual dimorphism in INAH1 (Swaab and Fliers, 1985; Swaab and Hoffman, 1995) or INAH2 (Allen *et al.*, 1989).

It has been proposed that hormones exert a global organizational influence on the developing brain, influencing the development of brain regions and cir-

TABLE 3
INAH3 Data[a]

	Heterosexual male	Female	Homosexual male
Volume (mm^3)			
HIV−	.123 ± .009 (22)	.077 ± .006 (25)	
HIV+	.108 ± .009 (9)	.067 ± .012 (9)	.096 ± .007 (14)
All	.121 ± .007 (31)	.073 ± .005 (34)**	
Volume/brain wt (mm^3/g)			
HIV−	.088 ± .006 (22)	.064 ± .005 (25)	
HIV+	.086 ± .007 (9)	.058 ± .011 (9)	.069 ± .006 (14)
All	.087 ± .005 (31)	.061 ± .004 (34)**	
Neurons per mm^3			
HIV−	14484 ± 1179 (13)	15912 ± 1113 (12)	
HIV+	17755 ± 1447 (8)	16167 ± 1224 (9)	18792 ± 881 (14)††
All	15730 ± 960 (21)	16021 ± 804 (21)	
Neuronal number			
HIV−	1737 ± 179 (13)	1123 ± 156 (12)	
HIV+	1887 ± 275 (8)	1122 ± 249 (9)	1831 ± 184 (14)
Total	1794 ± 149 (21)	1123 ± 135 (21)**†	

[a] Cellular parameters were assessed only if INAH3 was completely intact. These parameters were assessed in either all intact specimens per group or a randomly selected subset of intact specimens. ANOVAs are described in text.

** Different from heterosexual males, $P < .001$.

† Different from both heterosexual and homosexual males, $P < .001$.

†† Trend toward increased density in homosexual compared with heterosexual males, $P = .057$.

cuits inside and outside the hypothalamus such as the anterior commissure (e.g., Allen and Gorski, 1992). We also measured the anterior commissure in the same blocks of tissue used for the present hypothalamic study (data not shown) and were unable to replicate a report that its cross-sectional area is larger in women than in men (Allen and Gorski, 1992). Thus, while a variety of other structural sex differences have been described for the human brain, including differences in the corpus callosum, anterior commissure, and portions of the bed nucleus of the stria terminalis (reviewed in Byne, 1993; Fox *et al.*, 1999), the sexual dimorphism of INAH3 is thus far the only one that has been subjected to repeated and successful attempts at replication by independent laboratories in the absence of significant failures of replication.

INAH1 and INAH3 have each been considered as candidates for homology with the much-studied SDN-POA of the rat. We believe that, in addition to its sexual dimorphism, INAH3 more closely resembles the SDN-POA of the rat in a variety of ways, including its positional and cytoarchitectonic characteristics (Byne, 1998; Byne *et al.*, 2000). For example, like the SDN-POA, INAH3 is a component of the medial preoptic-anterior hypothalamic nucleus (MP-AHN) (Fig. 2). In contrast, INAH1 is situated completely outside and lateral to the MP-AHN. While it might be helpful to bring chemoarchitecture to bear on the issue of homology, little is known about specific markers in the human INAH. Moreover, homologous nuclei do not necessarily express the same peptides in all species. For example, the large cholinergic neurons of

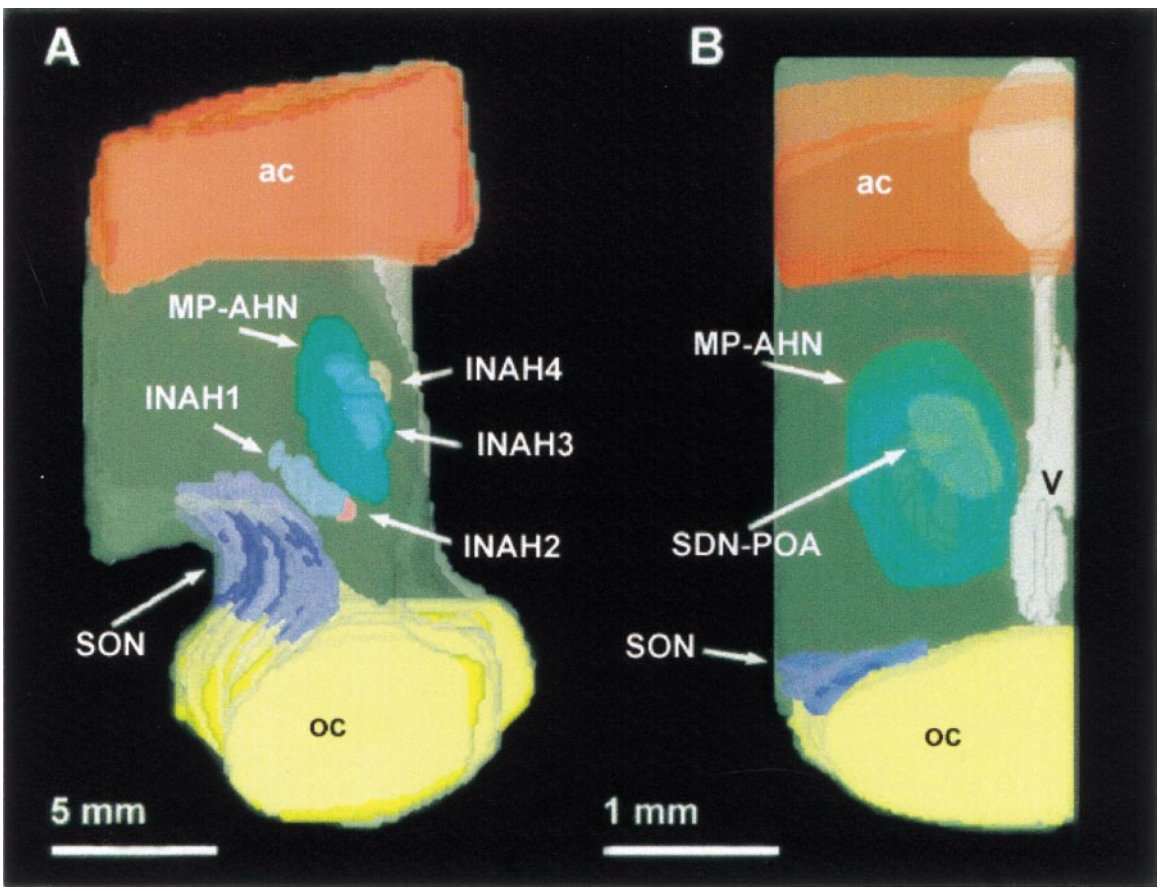

FIG. 2. Three-dimensional reconstructions of the medial preoptic-anterior hypothalamic continuum of the human (A) and rat (B). INAH3 in the human, like the SDN-POA of the rat, is a component of the MP-AHN. In contrast, the other INAH are situated outside the MP-AHN. In the rat, an expansion of the ventricle (V) is seen behind the anterior commissure (ac). In the human, the region of the reconstruction did not extend through ac posteriorly. Reconstructions were prepared from thionin-stained serial sections with the assistance of Application Visualization System software (Advanced Visual Systems, Inc., Waltham, MA). Abbreviations: ac, anterior commissure; INAH, interstitial nucleus of the anterior hypothalamus; MP-AHN, medial preoptic-anterior hypothalamic nucleus; oc, optic chiasm; SDN-POA, sexually dimorphic nucleus of the preoptic area; SON, supraoptic nucleus.

nucleus basalis of Meynert express galanin in baboons but not in humans (Walker *et al.,* 1991). Inferences regarding homology should, therefore, be based on a consideration of a wide variety of markers. The connections and functions of the nuclei in question are also relevant to the issue of homology, but again, little is known with regard to the INAH.

By analogy to hypothalamic sex differences in animals (e.g., Byne *et al.,* 1987; Gorski *et al.,* 1978; Tobet *et al.,* 1986) the sex difference in the human INAH3 may depend at least in part on sex differences in developmental exposure to gonadal hormones. The prevailing hypothesis concerning the development of the SDN-POA in rats is that gonadal steroids cause more neurons to survive in males than females (Davis *et al.,* 1996). As the sex difference in INAH3 is in the number of neurons, differences in neuronal survival between males and females may also contribute to the sexual dimorphism of INAH3. In addition, sex related differences may also emerge later in development as the neurons that survive become part of functional circuits (Hutton *et al.,* 1998). The current results may provide a suggestion of such a later emerging influence. Thus, while the difference in volume between the sexes was accounted for by an increased number of neurons in men, the trend toward a difference between homosexual and heterosexual men was accounted for by a difference in volume without a difference in neuron number (i.e., increased neuronal density). Such a difference in volume could be accounted for in at least two ways. First, it might simply reflect shrinkage during tissue fixation. While that possibility cannot be ruled out, it seems unlikely since no other INAH was similarly affected and there is no evidence of neuronal shrinkage in the homosexual specimens (i.e., neuronal size did not vary between homosexual and heterosexual men). Alternatively, if validated by independent replication, the present findings could reflect a reduction of neuropil within INAH3 in the homosexual group. Work in animals suggests that the elaboration of synaptic connections continues after cytoarchitectonic patterns (i.e., the positioning of neurons) are determined (Byne *et al.,* 1987; Hutton *et al.,* 1998). It is known that postnatal experience in animals can influence the elaboration of neuropil in some brain regions (e.g., Bhide and Bedi, 1984; Turner and Greenough, 1985). In humans, the major expansion of the brain occurs postnatally while the individual is in constant interaction with the environment (for references see Byne and Parsons, 1993). Thus, the elaboration of neuropil may be influenced by postnatal experience in humans. At present, however, nothing is known about the potential importance of either hormonal exposure or postnatal experience on neuropil development in INAH3.

Considerable speculation has addressed the possible functions of INAH3, particularly regarding a potential role in regulating male-typical sexual behaviors (Allen *et al.,* 1989; LeVay, 1991). At present, however, we can neither ascribe any function to INAH3, nor can we interpret the functional significance of its sexual dimorphism. If INAH3 is a site related to the functional circuitry of sexual orientation, then the current data suggest that measures other than simple nuclear volume are needed to discern the relationship. Based on the results of the present study as well as those of LeVay (1991), sexual orientation cannot be reliably predicted on the basis of INAH3 volume alone.

The finding of an increase in INAH1 volume associated with HIV infection in the heterosexual groups was not anticipated and should be viewed cautiously until replicated. Whether the increase in volume is related to a change in neuronal size or number in the present specimens remains to be determined. Since all of the HIV+ heterosexual males as well as some of the HIV+ females had histories of intravenous drug abuse, the increase in INAH1 volume may be related more closely to drug use than to HIV infection. Since INAH1 is rich in message for preproenkephalin, it may conceivably play a role in the brain reward systems that are dysregulated in drug addiction. In contrast to INAH1, we found no evidence for an influence of HIV on INAH3, lending credence to LeVay's (1991) contention that HIV infection did not account for the disparity in INAH3 volume he observed between homosexual and heterosexual men.

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