The Supraoptic and Paraventricular Nuclei of the Human Hypothalamus in Relation to Sex, Age and Alzheimer's Disease

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GOUDSMIT, E., M. A. HOFMAN, E. FLIERS AND D. F. SWAAB. *The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease*. NEUROBIOL AGING 11(5) 529–536, 1990.— Volume and total cell number were determined in the supraoptic (SON) and paraventricular (PVN) nuclei of 14 male and 16 female subjects ranging in age from 10 to 93 years. In addition, 4 male and 6 female subjects suffering from Alzheimer's disease (AD) and ranging in age from 46 to 97 years were studied. Subjects were divided into two age groups, viz., "young" for subjects up to 60 years, and "old" for subjects older than 60. No sex differences in volume and in total cell number were observed in the SON and PVN in either age group. In addition, no significant correlation was found between total cell number in the SON and PVN and brain weight. No significant differences in volume and total cell number were found in either the SON or PVN between young and old control subjects or between AD cases and controls, indicating that these nuclei are spared from degenerative changes in senescence and AD. Determination of neuron numbers in the SON supported this view. In contrast, volume and total cell counts in the suprachiasmatic decreased in senescence and were dramatically reduced in AD. The present results indicate the occurrence of differential patterns of cell loss within the human hypothalamus with aging and in AD, which are proposed to be related to functional differences between the hypothalamic nuclei.

Aging	Alzheimer's disease	Hypothalamus	Supraoptic nucleus	Paraventricular nucleus
Suprachiasmatic nucleus				

HUMAN aging is accompanied by a number of morphological changes in the central nervous system including a decrease in brain weight (7,15) and brain volume (27). Apparently, not all parts of the brain are affected to the same extent, since changes in volume, cell number, neuronal size and lipofuscin content have been reported to vary widely among different structures (2,14). Age-related decreases in cell number were not only reported in cortical areas, but also in hippocampus, amygdala and cerebellum. In contrast, little change has been found in the majority of subcortical structures [for a review see (3)] with the distinct exception of the locus coeruleus which shows a marked cell loss after 63 years of age (46).

Differential patterns of cell loss have also been observed in Alzheimer's disease (AD). Cerebral atrophy is one of the obvious macroscopical abnormalities in this condition (22) and neuronal loss in excess of age-related cell loss has been reported in several cortical regions [for a review see (3)]. In addition, cell loss in several parts of the hippocampus and in a number of subcortical nuclei including the locus coeruleus and the cholinergic forebrain nuclei has been reported to exceed the changes which take place in

normal aging in different degrees (3).

Our group has studied alterations in cell numbers within the hypothalamus with aging and in AD in relation to (neuro)endocrine changes in these conditions. The suprachiasmatic nucleus (SCN), which is considered to regulate circadian rhythmicity shows a marked cell loss after 80 years of age in normal aging. In AD patients cell counts in this nucleus were about 60% lower than in age-matched controls (40). An even more dramatic cell loss which sets in during the fifth decade of life was observed in the sexually dimorphic nucleus of the preoptic area (SDN-POA) (17, 39, 41). The SDN-POA might be involved in aspects of masculine sexual behavior and reproductive endocrinological functions (4,43). Cell numbers in the SDN-POA of AD patients were not different from those of age-matched controls (41).

In the present study, volume and total cell number (i.e., including glial cells) were determined in the supraoptic (SON) and paraventricular (PVN) nuclei in the same series of human hypothalami used for the SCN and SDN-POA studies mentioned above. Additional neuron counts were performed in the SON. The SON and PVN are part of the hypothalamo-neurohypophyseal system

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TABLE 1

POSTMORTEM INTERVAL AND DURATION OF FIXATION IN YOUNG AND AGED CONTROL SUBJECTS AND ALZHEIMER CASES

Group	n	Postmortem Interval (hours)	Fixation Time (days)
Controls ≤ 60	17	19 ± 3	46 ± 9
Controls > 60	13	16 ± 2	39 ± 3
AD ≤ 60	2	14 ± 10	83*
AD > 60	8	21 ± 7	$73 \pm 31^\dagger$

Data are given as means $\pm$ S.E.M. No statistically significant differences were found in postmortem interval and duration of fixation between young and aged subjects (MW-U; $p > 0.30$) and between AD patients and controls (MW-U; $p > 0.14$).

*Fixation times of the two young AD patients were 48 and 119 days, respectively.

† With the exception of one high value (292 days) fixation times of old AD patients were in the range of those of old controls. The median fixation time of old AD patients was 39 days.

(HNS), which releases vasopressin and oxytocin into the bloodstream and plays a role in the regulation of diuresis (13), vascular resistance (5), labor and lactation (38). The aim of the present study was to determine the amount of age-related cell loss in this anatomically and physiologically well-defined system in relation to the previously reported cell loss in another vasopressin containing nucleus, viz. the SCN, which lies in its immediate vicinity. In addition, volume and total cell number were determined in these nuclei in AD since degenerative changes in the central nervous system are generally more pronounced in this condition.

METHOD

Brains from 30 subjects (14 males; 16 females) ranging in age from 10 to 93 years were obtained at autopsy. Brains were weighed and fixed for 51 ± 7 days (mean $\pm$ S.E.M.) in 10% formaldehyde. Subjects were hospital patients without a neurological or psychiatric diagnosis who died from various causes [for details the reader is referred to (9)]. In addition, brains of 10 subjects (4 males and 6 females, ranging in age from 46 to 97 years) with clinical diagnosis of Alzheimer's disease were studied. This diagnosis was verified neuropathologically. AD patients showed extensive neocortical and hippocampal senile plaques and tangles. The areas examined were cortical areas: 4, 10, 17–18, 20, 38 and 42, corpus striatum, gyrus brevis, hippocampus, mesencephalon including substantia nigra, medulla oblongata, thalamus, locus coeruleus and cerebellum. The control brains displayed no neuropathological abnormalities. Postmortem delay and duration of fixation did not differ between controls younger than 60 years and old controls, or between controls and AD patients (Table 1). The hypothalamic area was dissected, dehydrated, embedded in paraffin and cut serially in 6 μ m frontal sections on a Leitz microtome. Every 50th section was mounted on a chrome-alum-coated object slide, deparaffinized, hydrated and stained with thionine (0.1% thionine in acetate buffer, pH 4).

Morphometry

Cross-sectional area measurements of the dorsolateral part of the SON (Fig. 1a) were performed unilaterally by means of a Calcomp digitizer using a Zeiss microscope with a $2.5 \times$ (PLAN) objective and $12.5 \times$ (PLAN) oculars. All sections containing 3 or more magnocellular neurons grouped together were included in the

measurements. The first and last sections that contained such a cluster were regarded as the rostral and caudal limits of the nucleus, respectively. Large blood vessels penetrating the SON from the side were excluded from the area measurements. This was not done for vessels lying completely within the nucleus.

Because of the larger dimensions of the PVN (Fig. 1b), cross-sectional areas of this nucleus were delineated using a $1 \times$ (PLAN) objective. The most rostral and caudal sections in which the nucleus could be recognized at this magnification were regarded as its limits. Large blood vessels were dealt with as in the SON.

Both the SON and the PVN were measured on the right-hand side of the brain except when the nucleus was not completely present within the dissected tissue on that side.

The volume of the SON and the PVN was determined by integrating all area measurements from the most rostral to the most caudal sections (44). The number of sections per subject in which the cross-sectional area was measured amounted to 15 ± 1 (mean $\pm$ S.E.M.) for the SON and 13 ± 1 for the PVN. Ten measurements of the same cross-sectional area on consecutive days revealed a measuring error of 1.6% and 3.5% (S.E.M. expressed as percentage of the mean) for the SON and PVN, respectively.

Numerical cell density in the SON and PVN was estimated by counting the total number of nuclear profiles per unit area followed by a discrete "unfolding" procedure (47) with the modification proposed by Cruz-Orive (6) and a correction for section thickness (6 μ m). For this purpose nuclear profile areas of all cells (including glial cells) were measured by manually delineating their outline using the digitizer and microscope described above with a $40 \times$ (PLAN) objective. In addition, nuclear profiles of all neurons containing Nissl substance were measured and counted in the SON. No neuron counts were performed in the PVN because of the difficulty in discriminating the many small neurons in this nucleus from astrocytes in Nissl-stained sections. Since cell density was found not to vary between the rostral and caudal poles in both the SON and the PVN, nuclear profiles were measured only in the section containing the maximal cross-sectional area of the SON and PVN, respectively. All nuclear profiles within a rectangular grid in one of the oculars which corresponded to 38,000 μ m² in the section were measured according to Gundersen (12). For the SON the grid was positioned twice at random over the maximal cross-sectional area of the nucleus; for the PVN three times. Measurements of the same section on 5 consecutive days revealed measuring errors of 3.3% and 2.6% (S.E.M. expressed as percentage of the mean) for the SON and PVN, respectively. The computer programmes for these procedures were developed by Dr. R. W. H. Verwer at our institute.

The total cell number in the SON and PVN was computed by multiplying the numerical cell density with the corresponding volume of the nucleus.

Data on volume and total cell number of the SCN in the same series of human hypothalami are an extension of the data published by Swaab *et al.* (40,42). Analysis of these data using linear regression analysis was performed in order to compare changes in total cell number in this nucleus with those in the SON and PVN (see below).

Statistics

Since a number of aspects of brain morphology begin to change substantially in the sixth decade of life (7, 15, 27), the "control" subjects were divided into two age-groups, one from 10 to 60 years (designated "young"; $n = 17$) and the other from 61 to 100 years (designated "old"; $n = 13$). Differences in postmortem delay and duration of fixation between groups were tested using the Mann-Whitney U test (MW-U). Sex differences in brain

FIG. 1. The supraoptic (a) and paraventricular (b) nuclei in frontal sections (6 μm) stained with thionine. OC, optic chiasm; PVN, paraventricular nucleus; SA, subarachnoid space; SON, supraoptic nucleus; V, third ventricle. Bar represents 200 μm .

weight and in volume and cell numbers in the SON and PVN were tested separately for each age-group using the MW-U test. If no differences were observed data of both sexes were pooled for further analyses. Differences between young and old control subjects and between AD cases and controls were tested using the MW-U test. The relationship of total cell number with age was determined within each age-group using linear regression analysis (36). The relation between fixation time and brain weight was investigated for males and females separately.

Bivariate linear models were used to study the relationships between total cell numbers in the SON, PVN and SCN and between total cell numbers in these nuclei and brain weight. The corresponding regression coefficients were estimated by the standard major axis method (16). A 0.05 level of significance was used in all statistical tests.

RESULTS

The average brain weight in male control subjects (1408 ± 24 g) was significantly higher than in female controls (1218 ± 36 g; MW-U; $p < 0.001$). However, in controls up to 60 years of age this sex difference failed to reach statistical significance (MW-U; $p = 0.075$; Table 2). In contrast, a statistically significant sex difference was observed in the old controls (MW-U; $p < 0.01$). Therefore, the effects of age and AD on brain weight were studied

separately for each sex. Duration of fixation showed no significant correlation with brain weight in either males ($p = 0.104$) or females ($p = 0.391$). No significant difference in brain weight was found between young and old males (MW-U; $p > 0.69$). In contrast, old females had lower brain weights than young ones (MW-U; $p < 0.01$). Male AD patients had lower brain weights than male controls (MW-U; $p < 0.02$ for old subjects). Although brain weights of female AD patients were also somewhat lower than in controls, this difference did not reach statistical significance (MW-U; $p > 0.51$ for old subjects). In summary, brain weights declined much more with normal aging in females than in males. In contrast, the additional weight loss in AD patients was more pronounced in males.

No data on total cell number in the PVN could be obtained in two cases (one young male control and one male AD patient), because the most caudal part of this nucleus extended beyond the dissected tissue block. Likewise, the rostral part of the SCN was missing in one old male control subject.

No sex differences were observed in volume and cell numbers in the SON and PVN of young (MW-U; $p > 0.10$), and old (MW-U; $p > 0.77$) controls. In addition, no significant linear relationships were found between volume and cell numbers in the SON and PVN and brain weight ($p > 0.34$). Therefore, data of both sexes were pooled for further analyses.

FIG. 2. Linear regression between total cell number in the SON and age. Data of male (Δ) and female ($\circ$) control subjects did not differ and were pooled. The correlations are not statistically significant in either young or old control subjects. Values of male ($\blacktriangle$) and female ($\bullet$) AD patients which are delineated by a minimum convex polygon were within the range of values of control subjects (for details see Table 3).

Volume and total cell numbers in the SON and PVN were unchanged in senescence (Figs. 2, 3; Table 3). In addition, no significant correlations between cell numbers and age were found in either age group for the SON and PVN (Figs. 2, 3). Neuron numbers in the SON also remained unaltered in senescence (Fig. 4; Table 3) and showed a significant correlation with total cell numbers in this nucleus (Fig. 5). Although volume and total cell numbers in the SCN of old controls were not significantly decreased as compared with young subjects (Fig. 6; Table 3), significant negative correlations were observed in old subjects between volume and age ($r = -.790$; $p < 0.01$) and between total cell number and age ($r = -.681$; $p < 0.02$; Fig. 6). This negative correlation was due to the low values in subjects between 80 and 100 years of age. If this group was considered separately volume and total cell number were reduced as compared to young subjects (MW-U: $p = 0.06$ and MW-U: $p < 0.04$ for volume and total cell number, respectively) and as compared to subjects between 60 and 80 years of age (MW-U: $p < 0.02$).

Volume, total cell number and neuron number in the SON of

FIG. 3. Linear regression between total cell number in the PVN and age. No statistically significant correlations were observed in either young or old control subjects. AD patients were within the range of control subjects (for details see Table 3). Symbols as in Fig. 2.

the two young AD patients were extremely high as compared with those of both controls and those of old AD patients (Table 3). In the old AD patients these variables were within the range of the old controls. No differences were observed in the PVN between AD patients and controls in either age group. In the SCN volume and total cell number were markedly reduced in AD patients (Table 3).

Total cell numbers in the SON and PVN of controls showed a significant correlation ($r = .583$; $n = 29$; $p < 0.01$), which was even more pronounced if the old subjects were considered separately ($r = .720$; $n = 12$; $p < 0.01$; Table 4). In contrast, no significant correlations were found between total cell numbers in these two nuclei with total cell number in the SCN in either young or old subjects (Table 4). Total cell number in none of the three nuclei showed a significant correlation with brain weight in either age group (Table 4). The same applied for neuron number in the SON ($p > 0.63$).

DISCUSSION

The results of the present study show that total cell numbers in

TABLE 2
MEAN AGES AND BRAIN WEIGHTS FOR MALE AND FEMALE SUBJECTS

Group	Males			Females		
	Mean Age (years)	(n)	Brain Weight (gram)	Mean Age (years)	(n)	Brain Weight (gram)
Controls ≤ 60	37 $\pm$ 4	(8)	1411 $\pm$ 37	39 $\pm$ 6	(9)	1298 $\pm$ 42
Controls > 60	72 $\pm$ 4	(6)	1405 $\pm$ 31	84 $\pm$ 4	(7)	1116 $\pm$ 38*†
AD ≤ 60	46	(1)	1130	56	(1)	1180
AD > 60	81 $\pm$ 10	(3)	1245 $\pm$ 23‡	81 $\pm$ 5	(5)	1034 $\pm$ 73

Data are given as means $\pm$ S.E.M.

*Lower than old male subjects ($p < 0.01$).

†Decreased compared with young female controls ($p < 0.01$).

‡Decreased compared with old male controls ($p < 0.02$).

TABLE 3
VOLUME AND CELL NUMBERS IN THE SON, PVN AND SCN IN YOUNG AND OLD CONTROLS AND AD PATIENTS

Group	n*	Supraoptic Nucleus			Paraventricular Nucleus		Suprachiasmatic Nucleus	
		Volume (mm ³)	Total Cell Number ($\times 10^3$)	Neuron Number ($\times 10^3$)	Volume (mm ³)	Total Cell Number ($\times 10^3$)	Volume (mm ³)	Total Cell Number ($\times 10^3$)
Controls ≤ 60	17	2.83 $\pm$ 0.24	683 $\pm$ 68	43 $\pm$ 4	5.54 $\pm$ 0.37	1057 $\pm$ 70	0.25 $\pm$ 0.02	47 $\pm$ 4
Controls > 60	13	3.07 $\pm$ 0.26	715 $\pm$ 53	38 $\pm$ 3	6.72 $\pm$ 0.65	1299 $\pm$ 133	0.22 $\pm$ 0.02	42 $\pm$ 5
AD ≤ 60	2	7.05 $\pm$ 1.49	1357 $\pm$ 204	79 $\pm$ 22	8.78 $\pm$ 1.19	1143 $\pm$ 90	0.10 $\pm$ 0.05	14 $\pm$ 6
AD > 60	8	4.01 $\pm$ 0.44	636 $\pm$ 94	38 $\pm$ 12	6.98 $\pm$ 0.46	1294 $\pm$ 105	0.11 $\pm$ 0.02	16 $\pm$ 2†

Data are given as mean $\pm$ S.E.M. No significant changes were observed in volume and cell numbers in the SON and PVN between young and old controls (MW-U; $p > 0.16$). The two young AD patients had extremely large volumes and cell numbers in the SON, but not in the PVN. The individual values for SON volume in these patients were 5.56 and 8.54 mm³. Corresponding cell numbers were 1152 and 1561 $\times 10^3$ for total cell number and 56 and 100 $\times 10^3$ for neuron number in the SON. The old AD patients did not differ from controls of the same age for any of the morphological variables measured in the SON (with the exception of SON volume which tended to be larger in this group; MW-U; $p = 0.082$) or PVN (MW-U; $p > 0.45$). No changes were observed in volume and total cell number in the SCN between young and old controls (MW-U; $p > 0.40$). However, these variables were dramatically reduced in both young and old AD patients. Individual values for the two young AD patients were 0.05 and 0.15 mm³ for SCN volume and 8 and 16 $\times 10^3$ for total cell number in the SCN.

*n = 16 for controls ≤ 60 in the PVN and n = 12 for controls > 60 in the SCN.

†Reduced as compared to controls (MW-U; $p < 0.01$).

the SON and PVN remain constant during the aging process. The stability of these nuclei is in sharp contrast to the decrease in total cell number in the SCN, another vasopressin-containing nucleus in the anterior part of the hypothalamus. The present method of analysis was based on the distinction of two age-groups, one with ages lower than 60 years and the other with ages above 60 in combination with regression analysis. In the SCN no significant relation between total cell number and age could be demonstrated by regression analysis applied to pooled data from all ages (18). The present separate analyses of young and old subjects revealed a sharp decline in SCN cell numbers and volume in the old subjects only, which was due to low values in subjects older than 80 years, indicating that degenerative changes in this nucleus do not develop gradually during the life span, but set in rather abruptly in senescence. This view is in line with the original

presentation of SCN data by Swaab *et al.* (40,42). Another nucleus in the immediate vicinity of the SON and PVN, the SDN-POA, was also shown to undergo degenerative changes from about age 50 onwards (17, 39, 41). Apparently, these four nuclei in the human hypothalamus are affected in differential ways by the aging process, as they are in the rat (20, 21, 31, 34). These different aging patterns might be related to functional changes in the SON, PVN, SCN and SDN-POA. Degenerative changes in the SCN might be a causal factor in the changes in circadian rhythmicity in senescence and AD (45,49) and changes in the SDN-POA might be related to changes in reproductive functions (17,41). The stability of the SON and PVN with aging goes together with an activation of the vasopressin cells in these nuclei in senescence as shown by morphological studies in humans and Wistar rats (8, 9, 19). In addition, an increase in vasopressin

FIG. 4. Linear regression between neuron number in the SON and age. No statistically significant correlations were observed in either young or old control subjects. AD patients were within the range of control subjects (for details see Table 3). Symbols as in Fig. 2.

FIG. 5. Linear regression between total cell number and neuron number in the SON. Significant correlations were observed for both control subjects and AD patients. The residual bivariate deviation (16) from the regression line of the control group was not different for AD patients as compared to control subjects (MW-U; $p > 0.82$). Symbols as in Fig. 2.

FIG. 6. Linear regression between total cell number in the SCN and age. The correlation was not statistically significant for young control subjects. In old control subjects a significant negative correlation was observed ($p < 0.02$). Values of AD patients were significantly lower than control values (for details see Table 3). Symbols as in Fig. 2.

synthesis and release in senescence has been reported (10, 11, 25, 26, 29, 32, 33). This activation is probably related to the decreased sensitivity of the senescent kidney to vasopressin (1, 11, 28, 33). A number of observations indicate that neuronal loss with aging and in neurodegenerative disease might be prevented by neuronal activation (37). Therefore, the present results might suggest that age-related cell loss in the SON and PVN is prevented by an activation of the vasopressin cells in these nuclei. Since decreases in neuron numbers might be masked by reactive gliosis when total cell numbers are considered an additional determination of neuron numbers was performed in the SON. In the PVN no reliable distinction could be made between the many small neurons in this nucleus and other cell types (e.g., astrocytes) in the thionine-stained sections. The results indicate that neuron numbers in the SON remain indeed stable in senescence and in AD and show that total cell numbers reliably reflect neuron numbers in this nucleus. In addition, determination of the number of immunocy-

tochemically identified vasopressin neurons in the SCN, SON and PVN in the same material shows a decrease in the SCN in senescence (40,42) but not in the SON and PVN (Purba *et al.*, in preparation).

Total cell numbers in the SON, PVN and SCN did not show any correlation with brain weight, indicating that age-related changes in the hypothalamus do not depend on changes in brain weight in elderly subjects. In addition, differences in brain weight between males and females were not reflected by differences in total cell numbers in these three hypothalamic nuclei, indicating that the sexual dimorphism which was described for both cortical and subcortical structures (30, 39, 48), is not reflected in the SON, PVN and SCN. The age-related decline in brain weight reached statistical significance in females only, which is in line with Zilles' observation that brain weight decreases more in females than in males with aging (50). In addition, Jolicoeur *et al.* recently presented extensive data suggesting a more pronounced decline in brain weight in females than in males in senescence (24). However, the mean age of the old male subjects was more than 10 years lower than that of the old females, which might have contributed to the absence of a significant decline in brain weight in old males. The relatively high age of the old female controls, corresponding to a marked decrease in brain weight, might also have reduced the difference in brain weight between old female controls and AD patients.

The significant correlation between total cell numbers in the SON and PVN stresses the close relationship between these two nuclei. The deviant aging pattern of the SCN was also supported by the absence of significant correlations between total cell numbers in this nucleus and total cell numbers in the SON and PVN, especially in old subjects.

The present results indicate that, in contrast to the SCN, the SON and PVN do not degenerate in Alzheimer's disease. The dramatic decrease in total cell number in the SCN of AD patients which was reported earlier by Swaab *et al.* (40,42), was confirmed by the present analyses of the data. The observation that the SON and PVN are spared from degenerative changes in AD is in line with reports on the absence of Alzheimer type neuropathological changes in these nuclei (23,35) and might indicate that the activation of these nuclei in senescence mentioned above renders them resistant to the devastating effects of this ailment. To our surprise, the two presenile AD patients had very high total cell numbers in the SON, but not in the PVN. These high numbers were not due to gliosis since neuron numbers, which showed a strong correlation with total cell numbers in AD patients were also high in these two patients (Fig. 5). Whether a large SON with high cell numbers is a characteristic of presenile AD cases remains to be investigated. Preliminary immunocytochemical data indicate a moderate reduction in the number of vasopressin and oxytocin immunoreactive cells in the SON of AD patients, suggesting an alteration in peptide metabolism (Purba *et al.*, in preparation). In the PVN no differences were observed in numbers of vasopressin and oxytocin immunoreactive cells between AD patients and controls. In contrast, the number of vasopressin cells in the SCN was found to be dramatically reduced in AD (40,42) supporting the idea that this nucleus is much more affected in AD than the SON and PVN.

In conclusion, the present cell counts in the SON and PVN in combination with earlier work on the SCN and SDN-POA clearly show different patterns of cell loss in the human hypothalamus with aging and in AD, which might be related to functional changes in these nuclei. These different patterns might be explained by differences in neuronal activation in senescence and AD (37). Our hypothesis that the marked stability of the SON and PVN in senescence is related to the activation of vasopressin cells in these two nuclei is presently under investigation.

TABLE 4

RELATIONSHIPS BETWEEN TOTAL CELL NUMBERS IN THE SON, PVN AND SCN AND BRAIN WEIGHT IN YOUNG AND OLD CONTROL SUBJECTS AS REFLECTED BY PEARSON'S CORRELATION COEFFICIENT (r)

Group	Total Cell Number				Brain Weight
	SON	PVN	SCN	n	
Controls ≤ 60	SON	—	—	16	0.073
	PVN	0.258	—	17	0.118
	SCN	-0.012	0.466	16	-0.255
Controls > 60	SON	—	—	13	-0.244
	PVN	0.720*	—	12	-0.210
	SCN	-0.347	-0.072	12	0.021

Correlations were calculated using untransformed data.

* $p < 0.01$.

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