

Differential Lateral Septal Vasopressin in Wild-type Rats: Correlation with Aggression

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The vasopressin (VP)-containing projections from the cells of the bed nucleus of the stria terminalis to the lateral septum (LS) are sexually dimorphic and dependent on gonadal steroids. Recently, the difference in VP distribution found among both sexes was also demonstrated in male mice genetically selected for different levels of intermale aggression. In the present study we examined whether this differential VP distribution in males also exists in an outbred strain of wild-type rats. After the animals were tested for their level of aggression, the VP content and the fiber density of the LS were measured using radioimmunoassay and immunocytochemistry, respectively. In addition, basal levels of plasma testosterone (T) were measured. Both biochemical data and immunocytochemical data revealed a negative correlation between VP and intermale aggression. Aggressive rats exhibited low levels of VP whereas intermediate and nonaggressive animals showed higher levels. Differences in adult levels of T were not found. The results are in accordance with the observations previously found in male mice, reconfirming the correlation between lateral septal VP and aggression. © 1997 Academic Press

Neurons which express vasopressin (VP) in rats have been localized in both hypothalamic and extrahypothalamic nuclei (De Vries, Buijs, Van Leeuwen, Caffé, and Swaab, 1985). The extrahypothalamic areas include the bed nucleus of the stria terminalis (BNST) and the medial amygdala (MA), which have VP-expressing cells that are highly sensitive to circulating levels of gonadal hormones (De Vries, Buijs, and Swaab, 1981; De Vries, Buijs, Van Leeuwen, Caffé, and Swaab, 1985; Miller, De Vries, Al-Shamma, and Dorsa, 1992; De Vries, Buijs, and Sluiter, 1984; Miller, Vician, Clifton, and Dorsa, 1989; Van Leeuwen, Caffé, and De Vries, 1985). The VP-containing projections from the cells of the BNST terminate in the lateral septum (LS) (Van Leeuwen, Caffé, and De Vries, 1985; De Vries, Buijs, Van Leeu-

wen, Caffé, and Swaab, 1985). When adult male rats are gonadectomized, a significant decrease in the VP fiber density in the septum occurs within several weeks. This is preceded by a significant decrease in the expression of cytoplasmic and nuclear VP mRNA in the BNST (and MA) within 3 days (De Vries, Buijs, Van Leeuwen, Caffé, and Swaab, 1985; Szot and Dorsa, 1994a; Miller, De Vries, Al-Shamma, and Dorsa, 1992). All these effects can be restored by replacement of testosterone (T); both VP and its mRNA reach intact levels again (Van Leeuwen, Caffé, and De Vries, 1985; Szot and Dorsa, 1994a; De Vries, Buijs, Van Leeuwen, Caffé, and Swaab, 1985; Miller, Urban, and Dorsa, 1989; Miller, Vician, Clifton, and Dorsa, 1989). Furthermore, a sexual dimorphism exists in this T-dependent neural system. In male rats, more cells in the BNST express VP and, as a result, these cells provide a greater density of fibers projecting to the LS than is present in females (Wang, Bullock, and De Vries, 1993; Miller, Vician, Clifton, and Dorsa, 1989; De Vries, Wang, Bullock, and Numan, 1994; Van Leeuwen, Caffé, and De Vries, 1985; De Vries, Buijs, and Swaab, 1981). This sexual dimorphism is not restricted to rats, but can also be found in, for instance, opossums, jerboas, gerbils, and microtine rodents (Iqbal and Jacobson, 1995; Lakhdar-Ghazal, Dubois-Dauphin, Hermes, Buijs, Bengelloun, and Pévet, 1995; Crenshaw, De Vries, and Yahr, 1992; Bamshad, Novak, and De Vries, 1993; Wang, Smith, Major, and De Vries, 1994).

Although, at adult age, T is necessary for the integrity of BNST-LS projections, the sexual dimorphism is thought to be established by T acting neonatally (Wang, 1994). In both male and female rats, the first appearance of VP immunostaining in the LS occurs on Postnatal Day 10. On Postnatal Day 12 the sexual dimorphism becomes apparent (De Vries, Buijs, and Swaab, 1981). When focussing on VP mRNA expression, it is shown that the development of the VP system is delayed in females compared to the males. As early as Postnatal

Day 3 VP mRNA is detectable in the BNST in the male whereas in the female it is not detectable until Day 14 (Szot and Dorsa, 1993). Recently, it has been shown that T acts on the estrogen receptor thereby mediating the initial expression of VP mRNA in the BNST of both sexes (Szot and Dorsa, 1994b). The time difference in neonatal onset of VP expression is thought to be a factor explaining the sexual dimorphism in the VP system.

Recent evidence suggests that male mice, genetically selected for their level of intermale aggression, may exhibit a variability in VP expression which is as large as that found when males and females are compared (Compaan, Buijs, Pool, De Ruiter, and Koolhaas, 1993; Van Oortmerssen and Bakker, 1981). Nonaggressive males were characterized by a more dense VP immunoreactive innervation of the LS and a higher VP neuron number in the BNST compared to aggressive males. In addition, a radioimmunoassay for VP confirmed the findings that nonaggressive males have higher lateral septal VP content compared to aggressive male mice. Together these results indicated the existence of a negative correlation between lateral septal VP and intermale aggression. This was somewhat surprising since a number of studies have indicated an activating role for VP in aggression in hamsters (Ferris, Delville, Irvin, and Potegal, 1994; Ferris, Delville, Grzonka, Luber-Narod, and Insel, 1993), microtine rodents (Winslow, Hastings, Carter, Harbaugh, and Insel, 1993; Wang, Ferris, and De Vries, 1994; Wang, 1995), and rats (Koolhaas, Moor, Hiemstra, and Bohus, 1991).

Therefore, in the present study, we examined the generality of variation in lateral septal VP in relation to aggression. We did so by studying whether the differentiation in fiber distribution as observed in mice also exists in a strain of wild-type rats. Although not genetically selected for the trait, males of this strain show a large variability in intermale aggression. Plasma levels of T were measured as well to study the possibility of a correlation between adult VP and circulating T.

MATERIALS AND METHODS

Animals

Male wild-type rats (*Rattus norvegicus*) were obtained from our own breeding facilities and maintained at a 12:12 hr light/dark cycle (lights off at 12:30 P.M.) in a temperature-controlled room (19–21°C). They were housed in perspex cages in groups of five to eight animals with free access to water and lab chow (Hope

Farms, Woerden, The Netherlands). When the animals reached the age of 14 to 15 weeks, intermale aggression (of all animals used in this study) was measured in a standard resident–intruder test based on the method described by van Oortmerssen and Bakker (1981). Males were housed individually with an ovariectomized female for 10 days in a large cage (50 × 75 × 50 cm). The cage contained sawdust bedding and a PVC tube as hiding place (20 cm). The test started after 5 days of cohabitation with the female. It was performed by replacing the female by a naive, male Wistar rat (300 g), after which an agonistic encounter could take place. This encounter was terminated immediately after an attack by the resident, or, in the absence of an attack, after 10 min. An attack is defined as a biting attack to the back and the rump of the intruder, which in almost every case results in a full clinch in wild-type rats. The highly aggressive individuals develop attacks without any preceding behavioral signs such as lateral threat or keep down. Immediately after the test, females were returned to the males. The mean of the tests, the attack latency score (ALS, in seconds), was calculated from tests performed on 4 consecutive days; each test was performed with a different intruder. Low values of ALS indicate aggressive individuals; high values (up to 600 sec) indicate nonaggressive animals. All tests were carried out between 13:00 and 17:00 hr under dim light conditions. One day after the fourth test, males were regrouped with original littermates until they were operated upon or sacrificed. Three different experiments were carried out. A radioimmunoassay (RIA) was performed on lateral septal VP and plasma levels of T (experiment I, $n = 61$ and $n = 29$, respectively). In a second experiment, animals were used for repeated blood sampling to determine basal T levels, also measured by RIA (experiment II, $n = 15$). In the third experiment, the density of VP-containing fibers in the lateral septum and the number of VP-expressing cell bodies in the BNST were studied using quantitative immunocytochemistry (ICC) (experiment III, $n = 30$).

Experiment I

Twelve to fourteen days after the last resident–intruder test, animals were sacrificed for biochemical determination of septal VP content. Rats were deeply anesthetized with halothane and killed by decapitation. The brains were removed from the skull and the septum was rapidly dissected, frozen in liquid nitrogen, and stored at –70°C until assayed. When present, fimbria tissue was removed from the sample to ensure that only septal tissue was assayed. Weighed samples were

sonified in 1 ml 0.1 M HCl, and centrifuged (2000g, 10 min, 4°C) before extraction. The VP content of the extraction samples was measured by RIA using rabbit anti-VP ("Peter," 3-10-80, from the Netherlands Institute for Brain Research, Amsterdam) in a final dilution of 1:50,000. Standard curves ranged from 0.25 to 64 pg VP/50 μ l and the assay had a detection limit of 0.25 pg/ml. Cross reactivity with VP¹⁻⁸ was 2.3%, with vasotocin it was 0.04%, and with oxytocin it was <0.01%. The intra- and interassay coefficients of variation were 7.7 and 13.4%, respectively. Since the septal area contains a dense network of VP immunoreactive fibers, whereas the surrounding areas contain no or only scattered fibers, the data reflect the total amount of VP per septum.

Blood samples were obtained from 29 of the 61 animals in order to measure plasma T levels. This was done by cardiac puncture just before the animals were decapitated. The blood samples were centrifuged (5000 rpm, 4°C) and plasma samples were stored at -20°C until assayed. Plasma concentrations of T were determined by a standard RIA for human T (Medgenix Diagnostics). Cross-reactivity with DHT and androstenedione was 1.0 and 1.2%, respectively. The sensitivity of the test reached a minimum of 0.044 ng/ml and the intra- and interassay variation coefficients were 4.7 and 8.1%.

Experiment II

To obtain a more accurate relation between levels of plasma T and aggression, and excluding accidental peak levels of T (Schuurman, 1980), 15 rats were selected on the basis of their ALS (five animals with ALS <200 sec (aggressive), five animals with 200 > ALS < 600 (intermediate), and five animals with ALS > 600 sec (nonaggressive)). Heart cannula surgery was performed 2 weeks after the last resident-intruder test. A cannula was inserted in a branch of the right jugular vein under halothane anesthesia. This allowed stress-free and repeated sampling of blood in freely moving rats (Steffens, 1969). Immediately after this surgery the animals were housed individually in perspex cages (25 \times 25 \times 30 cm) under identical temperature and food conditions as before. Lights went off at 13:30. Each animal received a penicillin injection (sc; 0.25 ml, 50,000 KIE) twice a week to prevent bacterial infections. Blood sampling was performed 3 weeks after surgery between 10:00 and 13:30 hr. Seven blood samples were collected at intervals of 30 min. Plasma concentrations of T were determined by RIA as described in experiment I. These

animals were not used for measuring lateral septal VP content or VP-ICC.

Experiment III

Thirty animals were tested for their ALS as described before. Two weeks after the last resident-intruder test, all animals were stereotaxically injected with colchicine to visualize the VP immunoreactive (ir) cells in the BNST. The colchicine (25 μ g/300 g body weight) was injected into the left lateral ventricle under halothane anesthesia. Two days later, all animals were perfused transcardially under deep anesthesia with 0.9% saline followed by 5% acrolein in 0.1 M phosphate buffer (pH 7.4). The brains were removed and stored in 20% sucrose for 2 nights, and 30- μ m sections were cut on a freezing microtome. Free-floating sections were used for VP immunocytochemistry according to the method described by Bamshad *et al.* (1993).

Briefly, sections were pretreated with 0.1% sodium borohydride for 15 min and rinsed three times for 15 min each in 0.05 M Tris-HCl (pH 7.6) with 0.9% NaCl (Tris-NaCl). This procedure was repeated for 0.1% H₂O₂. This was followed by incubation in the following solutions: (I) Tris-NaCl with 0.3% Triton X-100 (Tris-Triton) and 5% normal goat serum, 15 min of incubation; (II) anti-VP serum (ICN, Biomedicals, Costa Mesa, CA) 1:5000 in Tris-Triton containing 2% normal goat serum (Tritrigo), 1.5 hr at 37°C; (III) Tritrigo, 3 \times 15-min rinses at 37°C; (IV) biotinylated goat anti-rabbit in Tritrigo, 45 min of incubation at room temperature; (V) Tritrigo, two 15-min rinses, followed by one 15-min rinse in Tris-NaCl; (VI) ABC complex in Tris-NaCl, 45 min of incubation; (VII) 3 \times 15-min rinses, Tris-NaCl; (VIII) 0.05% 3-3'-diaminobenzidine in Tris-NaCl with 0.0015% H₂O₂, 25 min of incubation. Finally, sections were rinsed three times in Tris-NaCl, mounted on slides, air-dried, and coverslipped. No specific staining was observed in control sections incubated without the primary antibody. All sections were coded to ensure blind measuring of fiber density (LS) and cell number (BNST).

The density of the VP-ir fibers was measured bilaterally at three levels in the lateral septum (+0.48 mm, 0.00 mm, and -0.26 mm to bregma (Paxinos and Watson, 1986)) based on the results found in the aggressive and nonaggressive mice (Compaan, Buijs, Pool, De Ruiter, and Koolhaas, 1993). Differences in density and distribution of VP-ir fibers in these mouse brains were found in the most caudal part of the septum. Density measurements were performed using the Leica Quantimet 600 automated image processing and analysis sys-

tem. Images of fibers were obtained with the 20X objective and a CCD camera (Sony Model DC10.5). The measurements were standardized by keeping camera and light settings constant across all brain sections. The number of pixels representing images of the VP-ir fibers was determined bilaterally in two adjacent sampling areas immediately bordering the ventricular lining (1 area: 512×736 pixels), covering a total area of $330 \times 960 \mu\text{m}$.

Cell numbers of VP-ir cells in the BNST were counted in three consecutive sections within the posterior division of the BNST, both left and right, using bright-field microscopy.

Statistics

Spearman rank correlations were calculated to determine the relationship between ALS and septal VP content, between ALS and plasma T concentrations, and between VP content and T concentrations. One-way analysis of variance (ANOVA) on group differences in septal VP was performed after the rats were divided into three aggression groups (aggressive, intermediate, and nonaggressive, see experiment II). The T data of experiment II were analyzed using ANOVA with repeated measures, followed by calculations of Spearman rank correlations for mean, maximum, and minimum of T per animal in relation to individual ALS. Results obtained in experiment III were analyzed by calculating Spearman rank correlations between ALS and septal VP-ir in all three brain levels. This was followed by ANOVA on group differences in septal VP-ir at each brain level (between subject factor), combined with left-right difference and brain level as within-subject factors. The same procedures were followed for the number of VP-ir cells in the BNST in the three studied levels. All results from ANOVA were further examined by Bonferroni's post hoc tests.

RESULTS

Experiment I

There were no correlational or group differences in the size of the dissected septal tissue used in the VP RIA. Data obtained from the RIA for lateral septal VP revealed a positive correlation between ALS and VP content (indicating a negative correlation between intermale aggression and lateral septal VP). VP concentrations ranged from 6 to 36 pg per milligram of assayed

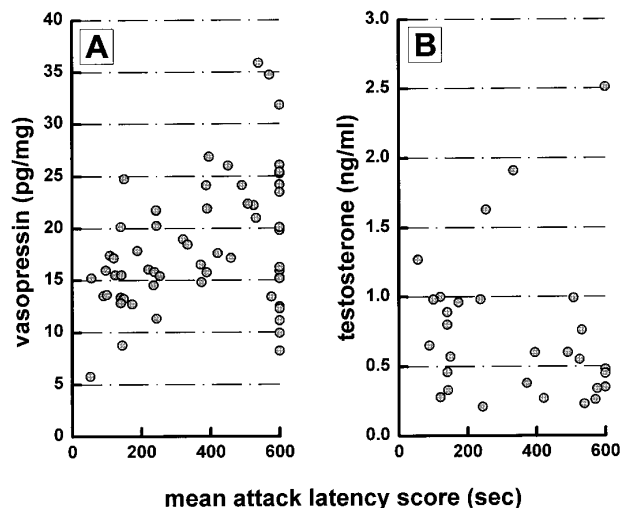


FIG. 1. Individual mean attack latency score (ALS) as determined in the resident-intruder test in relation to lateral septal vasopressin (VP; $n = 61$; A) and basal plasma testosterone levels (T; $n = 29$; B). A significant correlation was found between ALS and VP ($r_s = 0.279$, $P = 0.029$, two-tailed), but not between ALS and basal T. Animals with an ALS of 600 showed a large variability in lateral septal VP.

septal tissue (Fig. 1A). Statistical testing confirmed this relation ($r_s = 0.279$, $P = 0.029$, $n = 61$, two-tailed). However, the results illustrated in Fig. 1A also indicate that the group with an average ALS of 600 sec showed a large variability in VP content. The low correlation coefficient was partly explained by this variability of the nonaggressive group. For illustration, when omitted from statistical testing, significance levels improved to $r_s = 0.577$ and $P < 0.001$ ($n = 43$, two-tailed). ANOVA testing for group differences in VP content did show a significant effect ($F(2, 58) = 4.65$, $P = 0.013$). Post hoc analysis (Bonferroni) revealed a significant difference between the aggressive and the intermediate groups ($P < 0.05$). The comparison between aggressive and nonaggressive just missed the significance level ($P = 0.064$) due to the variability in the latter group.

The plasma levels of T obtained by cardiac puncture revealed no correlation with mean ALS (Fig. 1B). Although three points (above 1.5 ng/ml) may reflect peak values due to the pulsatile nature of T secretion, most data can be considered as basal levels of T ranging from 0.21 to 1.29 ng/ml, with a total group average of 0.75 ng/ml. Furthermore, there was no correlation between VP content and plasma T levels in the present data.

Experiment II

Results from the experiment with jugular vein cannulated rats confirmed the data obtained from cardiac

puncture (experiment I). Cannulas of two animals (aggressive and nonaggressive) were obstructed and no blood samples could be taken from them. Testosterone levels for each group over 3 hr of sampling time are illustrated in Fig. 2. One nonaggressive animal exhibited relatively high levels of T up to 6 ng/ml during these 3 hr. This explained the large standard errors for this group depicted in Fig. 2. No significant effect was found for blood sampling time, group, and group versus time interaction using ANOVA with repeated measures. This indicated that basal plasma T levels were measured and that T remained relatively constant over time. Comparing group means of both separate and averaged samples (per animal) did not result in significant differences.

Experiment III

The results obtained from the image analysis of VP-ir fibers in the septum revealed a significant difference between left and right parts of the septum at the two most rostrally located levels (ANOVA, $P < 0.01$). This difference is most likely due to the unilateral colchicine infusions. Since testing separately for both septal sides resulted in identical statistical results, averaged data for total septum will be presented here. The level of VP-ir was positively correlated with ALS in all three

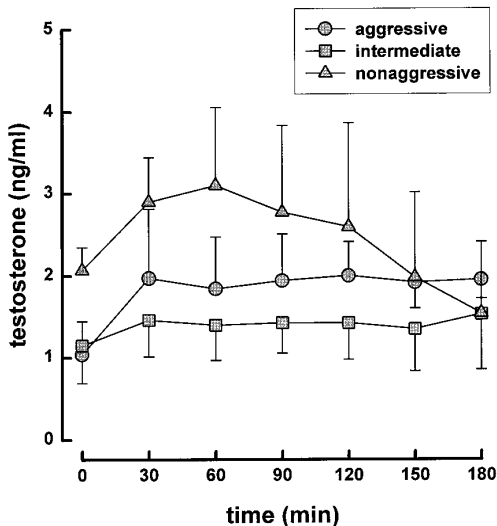


FIG. 2. Plasma testosterone (T) levels obtained by repeated blood sampling every 30 min for 3 hr. Although the T levels of nonaggressive animals ($n = 4$) seem somewhat elevated compared to aggressive ($n = 4$) and intermediate scoring rats ($n = 5$), no significant differences were found between groups or over time. Thus, basal levels of T did not differ between these animals.

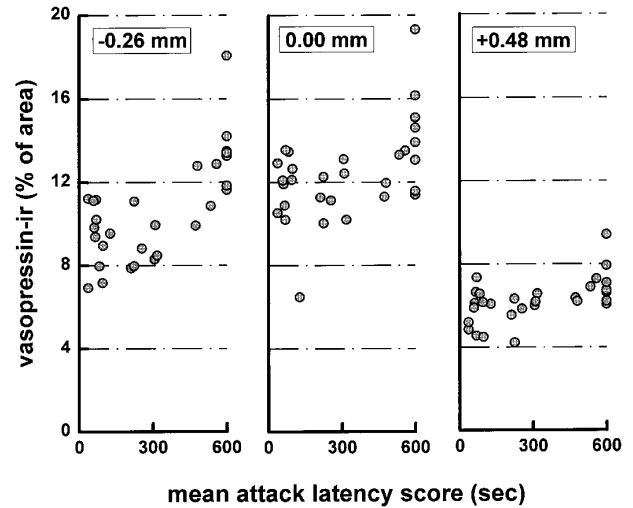


FIG. 3. Individual data ($n = 30$) of ALS in relation to the percentage of area covered by VP-ir fibers measured at three levels in the lateral septum. The highest level of immunoreactivity overall was found at the level right beneath bregma (0.00 mm). Lowest levels were measured at the level 0.48 mm rostral to bregma. At all three levels a positive correlation was found between ALS and the density of VP-ir fibers (see Table 1 for statistics).

brain levels (Fig. 3). Animals characterized as aggressive exhibited lower densities of VP-ir fibers compared to nonaggressive individuals. Statistics showed that the correlation and group differences were most pronounced at the level with the highest fiber density (see Table 1). Although present, the variability in VP-ir in the nonaggressive group (ALS = 600) was much smaller compared to the VP-RIA data. The fiber density throughout the three levels of the LS showed a significant group effect ($F(2, 27) = 13.27$, $P < 0.001$), while the area covered by VP-ir fibers increased significantly from rostral to caudal levels ($F(2, 54) = 191.19$, $P < 0.001$). A group \times brain level interaction was also found ($F(4, 54) = 3.09$, $P = 0.023$). Figure 4 shows digital photomicrographs of lateral septal VP-ir of two extreme cases (chosen for illustrative purposes).

Unfortunately, it was impossible to reliably count the cell numbers of VP-ir cell bodies in the BNST. Unlike the results from a small pilot experiment, the colchicine treatment did not result in the effect we anticipated. VP-ir cells were visible only in the brain side ipsilateral to the colchicine injection side. Although scattered fibers were present both contra- and ipsilaterally, BNST cells labeled for VP were barely visible on the contralateral side. Therefore, the quality of the colchicine treatment let us to discard the cell-counting results from further analysis.

TABLE 1

Results of Statistics on the VP-ir Data at Three Brain Levels in the LS

Brain level to bregma	Spearman rank correlation	ANOVA one-way	Bonferroni $P < 0.05$
-0.26 mm	$r_s = 0.65, P < 0.01$	$F(2,27) = 13.45, P < 0.001$	aggr < nonaggr interm < nonaggr
0.00 mm	$r_s = 0.45, P = 0.014$	$F(2,27) = 5.72, P < 0.01$	aggr < nonaggr interm < nonaggr
+0.48 mm	$r_s = 0.46, P = 0.01$	$F(2,27) = 3.88, P = 0.03$	aggr < nonaggr

Note. Spearman rank correlations were followed by one-way analysis of variance (ANOVA). Significant interactions were further examined by Bonferroni post hoc analysis.

DISCUSSION

The biochemical data obtained in this experiment revealed a negative correlation between lateral septal VP and intermale aggression as previously found in mice (Compaan, Buijs, Pool, De Ruiter, and Koolhaas, 1993). The same correlation was present in the data obtained for VP-ir fiber densities. Overall, aggressive rats (low ALS) exhibited low levels of VP whereas intermediate and nonaggressive animals (high ALS) showed higher levels. Again it is demonstrated that variation in lateral septal VP is present among males. In contrast to earlier experiments with mice we did not use animals genetically selected for aggression, but instead used random

bred rats. This strengthens the general significance of the present results. Since results on VP-ir cells in the BNST could not be obtained due to the unsatisfactory colchicine treatment, we cannot draw any conclusions on this subject. Based on the results of Compaan, Buijs, Pool, De Ruiter, and Koolhaas (1993) we might have expected lower VP cell number in the aggressive rats compared to intermediate and nonaggressive animals.

Although VP plays a role in intermale aggression, a direct causal link with lateral septal VP in rats seems unlikely. Several studies showed that VP, when applied to the LS, is able to increase aggression-related flank marking in golden hamsters (Ferris, Delville, Irvin, and Potegal, 1994; Ferris, Delville, Grzonka, Lubner-Narod,

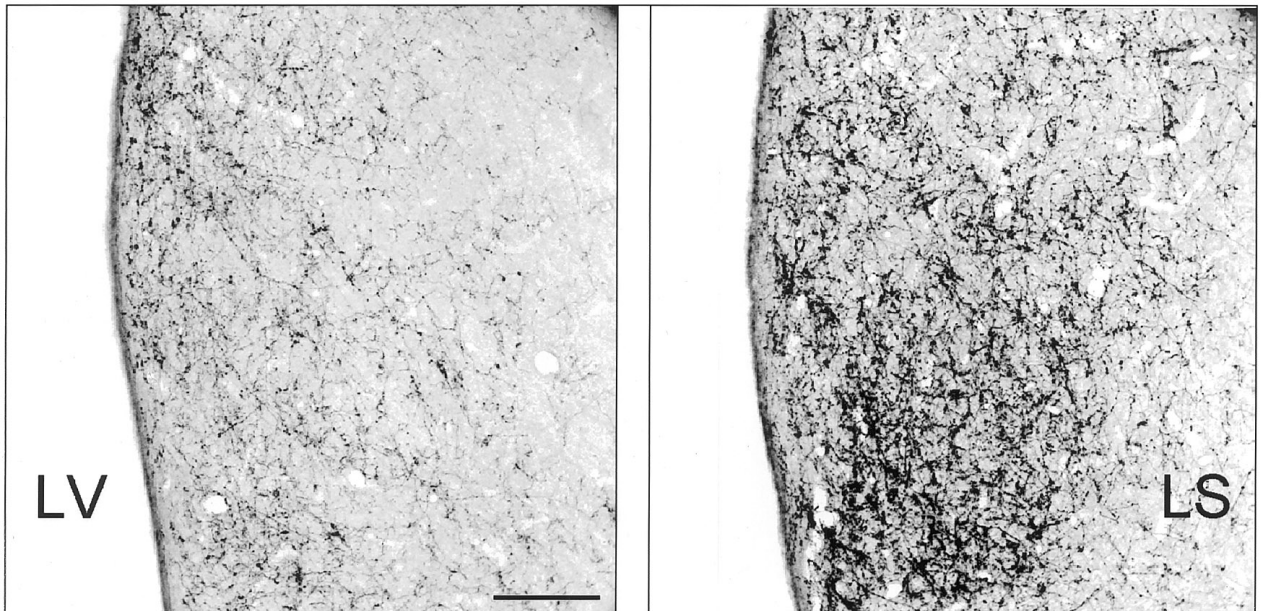


FIG. 4. Digital photomicrographs displaying two extreme cases of relatively low (left, aggressive animal, ALS = 95 sec) and high (right, nonaggressive, ALS = 600 sec) density of VP-ir in the lateral septum at -0.26 mm to bregma. LV and LS, lateral ventricle and lateral septum, respectively. Bar, 100 μ m.

and Insel, 1993). However, this is difficult to interpret in view of the fact that in golden hamsters the LS is almost devoid of VP immunoreactive fibers (Ferris and Delville, 1994). Another example in which lateral septal VP is implicated in aggression is found in microtine rodents. In the monogamous prairie vole lateral septal VP is strongly related to intermale aggression (Winslow, Hastings, Carter, Harbaugh, and Insel, 1993; Wang, Ferris, and De Vries, 1994; Wang, 1995). In this species VP antagonism seems to block the transition to aggression after mating. Furthermore, intracerebroventricular VP administration by osmotic minipumps (VP in LS is increased threefold) is able to induce intermale aggression within 12 hr. This effect of VP is strongly related to the process of pair bonding since in meadow voles, a species devoid of parental care, no changes in lateral septal VP were found after cohabitation with females or after becoming fathers (Bamshad, Novak, and De Vries, 1993; Wang, Smith, Major, and De Vries, 1994). Also, prairie voles exhibit high levels of both lateral septal VP and aggression compared to meadow voles (Wang, 1995). Administration of VP into the septum of rats has also been shown to induce aggression (Koolhaas, Moor, Hiemstra, and Bohus, 1991). Nevertheless, all these data seem in contrast with the present results. All reports relate relatively high levels of aggression with a large amount of VP present in the LS, whereas we found that animals with high levels of VP have low intermale aggression levels. This leads us to the conclusion that the differential distribution of basal VP in wild-type rats has no direct causal link to intermale aggression.

The lack of a causal link might explain why the correlation coefficients, though statistically significant, are low and thus do not completely account for the variances observed in the data. Obviously, some unknown factors are obscuring a strict correlation between aggression and lateral septal VP. This seems to be the case in the group of animals assigned with an ALS of 600 sec in particular. They showed a large variability in VP content (Fig. 1A), although the variability is less pronounced in VP-ir densities (Fig. 3). It is uncertain why the animals in this group refrain from attacking intruders within 600 sec. Some animals may eventually attack while others never will, indicating that this group is rather heterogeneous with respect to (the absence of) intermale aggression. Nevertheless, we chose to present the results of all behavioral phenotypes (regarding aggression) found in a group of random-bred wild-type rats.

Although a relation between plasma T and aggression has been reported in adult wild house mice (Van

Oortmerssen, Dijk, and Schuurman, 1987) and laboratory rats (Schuurman, 1980), we were not able to replicate the relation between plasma T and aggression in wild-type rats. The lack of a significant correlation between T and ALS, as well as between T and VP, indicates that adult T does not explain the differential VP distribution in the LS in this instance. To determine the origin of the observed variation in VP distribution, we suggest focusing on organizational processes occurring pre- and postnatally. It is known that T exerts an organizational role in VP distribution at neonatal age, rather than at adult age (Wang, Bullock, and De Vries, 1993; Wang, 1994). This proved to be an important factor in the selection lines of the wild house mice. A single injection of T at Postnatal Day 1 reduced the VP-ir in adult nonaggressive males until levels similar to those in control aggressive mice were reached (Compaan, Buijs, Pool, De Ruiter, Koolhaas, and Bohus, 1997, submitted for publication). Other studies suggested an endogenous difference in neonatal T sensitivity and peak levels (Compaan, De Ruiter, Koolhaas, Van Oortmerssen, and Bohus, 1992; Compaan, Hutchison, Wozniak, De Ruiter, and Koolhaas, 1994; De Ruiter, Koolhaas, Keijser, Van Oortmerssen, and Bohus, 1992), indicating a possible role for T neonatally. This closely resembles mechanisms which are thought to underlie sexual differentiation in the same VP network (De Vries and Al-Shamma, 1990; De Vries, Wang, Bullock, and Numan, 1994; Szot and Dorsa, 1993; Szot and Dorsa, 1994b). It remains to be established whether these processes are the cause of the observed variation in VP or whether later life experience is influencing VP expression in the wild-type rats. The mice data showed that at least the variation in the length of the period between the resident-intruder test and the day of sacrifice is not a factor that interferes with the results of the VP measurements.

There are a few other reports which have found differences in VP content in brain areas of untreated male animals. Ermisch *et al.* (1986) found VP levels to be significantly higher in the septum/striatum of rats with high performance in a brightness discrimination task compared to the group of low performers. However, in a later study this could not be replicated. Instead, a difference in hippocampal VP content was reported (Hess, Lesser, and Landgraf, 1992). Roman high- and low-avoidance rats, selected and bred for rapid acquisition versus nonacquisition in two-way, active avoidance behavior, have also been found to express differential basal levels of VP (Aubry, Bartanusz, Driscoll, Schulz, Steimer, and Kiss, 1995). These differences were found in the parvocellular neurons of the paraventricular nucleus, i.e., the low-avoidance group showed high

levels of VP. In view of the fact that these low-avoidance animals also show low levels of aggression, it seems that the observed differentiation in VP neurons might be more widespread throughout the brain.

It remains to be seen whether high peptide content as measured via RIA also indicates a higher rate of peptide release. It is more likely that the higher fiber density per septum is responsible for the higher levels of VP measured by RIA. The data would suggest that per VP-ir fiber there is at least an equal amount of peptide present. Optical density measurements obtained from mice (Compaan, Buijs, Pool, De Ruiter, and Koolhaas, 1993) showed that more VP is present in fibers of nonaggressive animals compared to those of aggressive males. However, the use of colchicine in the present study does not allow measuring optical density. It is known that VP binding sites (V1_a subtype (Shewey and Dorsa, 1988; Szot, Bale, and Dorsa, 1994; Ostrowski, Lolait, and Young, 1994)) are not sexually dimorphic and that gonadectomy or T treatment at adult age does not affect the density or affinity of the VP binding sites (Tribollet, Audigier, Dubois-Dauphin, and Dreifuss, 1990; Poulin and Pittman, 1991). Therefore we expect no differences in VP receptor sensitivity and distribution to occur within male wild-type rats. Whether equal amounts of receptors and a difference in the number of VP supplying fibers also mean differential (behavioral) effects remains a difficult and unanswered question. Again we should stress that we merely found a correlation between VP and aggression. Additional research is needed to gain more insight into a possible mechanism. So far, only very few studies have been able to correlate VP release with behavior by using microdialysis (Engelmann, Ludwig, and Landgraf, 1994; Landgraf, 1995; Kalsbeek, Buijs, Engelmann, Wotjak, and Landgraf, 1995). At this moment, measuring individual differences in peptide release upon an identical stimulus has yet to be accomplished.

In summary, we have described a negative correlation between intermale aggression and lateral septal VP content and immunoreactivity in wild-type rats. This is in accordance with earlier data on aggressive and nonaggressive mice, indicating that differences normally found between both sexes can also be found among males. No differences in basal plasma T levels were found. Future experiments will focus on the possible role of neonatal influences on adult expression of differential lateral septal VP and on the functionality of the observed difference in rats.

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