Testosterone Fails to Reverse Spatial Memory Decline in Aged Rats and Impairs Retention in Young and Middle-Aged Animals

E. GOUDSMIT, N. E. VAN DE POLL, AND D. F. SWAAB¹

Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

Recently, the vasopressin (AVP) innervation in the rat brain was shown to be restored in senescent rats following long-term testosterone administration. In order to investigate whether this restoration is accompanied by an improvement in learning and memory, both sham- and testosterone-treated young (4.5 months), middle-aged (20 months), and aged (31 months) male Brown-Norway rats were tested in a Morris water maze. All animals learned to localize a cued platform equally well, indicating that the ability to learn this task was not affected by sensory, motoric, or motivational changes with aging or testosterone treatment. There were no significant differences in retention following cue training. Subsequent training with a hidden platform in the opposite quadrant of the pool (place training) revealed impaired spatial learning in middle-aged and aged animals. Retention following place training was significantly impaired in the sham-treated aged rats as compared with sham-treated young rats. Testosterone treatment did not improve spatial learning nor retention of spatial information, but, on the contrary, impaired retention in young and middle-aged animals. The present results confirm earlier reports on an impairment of spatial learning and memory in senescent rats but fail to support a role of decreased plasma testosterone levels and central AVP innervation in this respect. © 1990 Academic Press, Inc.

Deficits in both short-term and long-term memory have been shown with aging in humans, monkeys, and rodents, and might be related to the numerous neuroanatomical, neurophysiological, and neurochemical

¹ This research was supported by the Foundation for Medical and Health Research (MEDIGON, Grant 900-552-056). Rats were made available by the Netherlands Organization for Scientific Research and the European Communities Concerted Action on Cellular Aging and Disease (EURAGE). The authors thank Ms. Tj. Van Der Woude, Ms. P. Boon, Ms. A. Louwerse, and Mr. T. Y. A. Van Groenestein (AMC) for technical assistance; Mr. H. Stoffels and Mr. G. Van Der Meulen for preparing the illustrations; and Drs. J. J. Hagan (Organon, Oss), Ch. W. Pool, H. Nyborg, and J. M. Ruijter for valuable suggestions. Correspondence and reprint requests should be sent to E. Goudsmit, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam (ZO), The Netherlands.

changes which take place in senescence (for a review see: Kubanis and Zornetzer, 1981). Such changes include a reduction in extrahypothalamic vasopressin (AVP) fibers in a number of areas in the senescent rat brain (Fliers, De Vries, & Swaab, 1985). In addition to the hormonal function of AVP in the periphery, this peptide probably acts as neurotransmitter or neuromodulator in central brain regions (Buijs, De Vries, Van Leeuwen, & Swaab, 1983). Extrahypothalamic AVP projections may be involved in the regulation of blood pressure (Pittman, Lawrence, & McClean, 1982), body temperature (Naylor, Ruwe, & Veale, 1986), and nociception (Kordower, Sikorszky, & Bodnar, 1982). In addition, central administration of AVP was shown to affect passive avoidance behavior (Bohus, Conti, Kovács, & Versteeg, 1982), active avoidance behavior (Koob, Dantzer, Bluthé, Lebrun, Bloom, & Le Moal, 1986), and social memory (Le Moal, Dantzer, Michaud, & Koob, 1987). Meck, Church, and Wenk (1986) showed that peripheral injection of AVP can restore temporal memory in aged animals.

The central AVP innervation is dependent on circulating levels of sex steroids (De Vries, Buijs, Van Leeuwen, Caffé, & Swaab, 1985). Testosterone (T) levels decline progressively with age in the rat (Kaler & Neaves, 1981; Ravid, Fliers, Swaab, & Zurcher, 1987) and, recently, long-term peripheral T supplementation was shown to restore AVP innervation in various areas in the senescent rat brain including the substantia nigra, the locus coeruleus, and the hippocampus (Goudsmit, Fliers, & Swaab, 1988). These results led to the question of whether restoration of central AVP innervation following T administration might be accompanied by an improvement in memory in aged rats. In this paper, this question was investigated using a Morris water maze (Morris, 1981). A testing procedure for spatial memory was selected, because spatial memory deficits are a constant finding in aged rodents (Barnes, Nadel, & Honig, 1980; Gage, Dunnett & Björklund, 1984; Ingram, London, & Goodrick, 1981; Rapp, Rosenberg & Callagher, 1987). In addition, spatial learning and memory are seriously disrupted following lesioning or deafferentation of the hippocampus (Morris, Garrud, Rawlins, & O'Keefe, 1982; Dunnett, Gage, Björklund, Stenevi, Low & Iversen, 1982), an area exhibiting extensive degenerative changes in the rat with aging (Landfield, Braun, Pitler, Lindsey, & Lynch, 1981) including a decrease in AVP innervation (Fliers et al., 1985), which can be restored by peripheral T administration to aged animals (Goudsmit et al., 1988). The Morris water maze not only selectively measures spatial information processing but also allows for the direct evaluation of confounding factors such as sensory, motoric, and motivational capabilities (Morris, 1984; Gage et al., 1984). Therefore, this procedure was used in order to see whether spatial memory could be improved following T administration to aged rats.

METHODS

Animals

Male Brown-Norway (BN/BiRij) rats, 16 young (3.0 months), 16 middle-aged (18.3 months), and 19 aged (29.8 months), were obtained from IVEG TNO (Rijswijk, The Netherlands). Fifty percent survival of this strain is about 30 months (Mos & Hollander, 1987). All animals were housed in individual cages together with a 2.5-month-old ovariectomized female Wistar rat (HSD CPB, Zeist, The Netherlands) in order to reduce variability in T levels due to differences in social status in the male rats. Temperature was kept at 21°C and lights were on from 7.00 AM to 7.00 PM. Food and water were supplied ad lib.

Treatment

After a 2-week period to recover from transportation, half of the male animals in each age group were randomly given a subcutaneous implant of 2.5-cm Silastic tubing (SR3, Talas BV, Ommen, The Netherlands; 1.5 mm I.D.; 2.1 mm O.D.) under light ether anesthesia. Implants were filled with testosterone (Steraloids, Inc., Wilton, NH) and sealed at both ends with silicone. All other male rats received empty tubing. Decapitation blood of young, middle-aged, and aged animals was collected at the end of the experiment, 7 weeks after implants were given, between 9.00 AM and 12.30 PM and plasma T levels were determined by radioimmunoassay (Pratt, Wiegman, Lappöhn, & Woldering, 1975). All animals were inspected for gross pathology. Testes were removed and weighed.

Testing Apparatus

The water maze was adapted from Morris (1981). A circular pool made of light-blue PVC (1.45 m diameter and 0.68 m height of which the upper 28 cm was transparent) was filled with water to a depth of 28 cm. The water was made opaque by the addition of 1 kg milk powder (Completa, Domo, Beilen, The Netherlands). The temperature was maintained at $29 \pm 1^\circ\text{C}$.

The pool was placed at the far end of a rectangular room. The walls were equipped with a variety of apparatus used in behavioral testing. Two parallel rows of neon lights were dimmed to half intensity. In addition, the number of extra-maze cues was increased by a desk lamp illuminating part of one of the walls. A videocamera was mounted above the center of the pool. Events in the pool were monitored, timed, and recorded on videotape by the experimenter in a section of the room screened off by a curtain.

The pool was divided into four quadrants by markings on the rim which were not visible from the inside and which were used as entry points during testing (see below). A removable circular white escape

platform (10 cm diameter) which remained invisible 2 cm below the opaque water surface could be placed on four sockets which were placed in the center of each quadrant. A red plastic rod (0.7 cm diameter, 10 cm length) could be fixed vertically on the platform as a visual cue.

Testing Procedure

Behavioral testing started 1 month following implantation, when animals were age 4.5, 19.8, and 31.3 months, respectively. On Day 1 all animals were placed in the water maze for 60 s with no escape platform present in order to allow for habituation to the apparatus.

Cue training. From Day 2 to Day 6 all animals were trained in 4 daily trials for a total of 20 trials to locate the cued platform. For each rat the platform location was fixed in a particular quadrant throughout training and platform locations were counterbalanced across and within the age and treatment groups in a quasi-random way. This part of the experiment was designed in order to evaluate differences in sensory, motoric, and motivational capability (Morris, 1984) and to minimize neophobic responses in aged rats (Brennan, Blizard, & Quartermain, 1982; Collier & Sladek, 1984).

Testing was started each day at 10.00 AM and lasted until approximately 4.00 PM. Each animal was placed in the pool facing the wall at one of the four entry points and allowed to escape onto the platform and to stay there for 30 s. Immediately thereafter the animal was placed again in the water at a different entry point. The order of the four entry points was varied each day in a quasi-random fashion. If an animal failed to escape onto the platform within 60 s it was placed on the platform for 30 s. The animals were tested in a fixed order each day, four animals of one age group followed by four of another, each group of four containing two T-treated and two sham-treated animals in an order unknown to the experimenter. Escape latencies of each trial were recorded at once. For each animal the sum of the escape latencies of the four daily trials was used in the statistical analyses.

Free swim trial 1. On Day 7 the platform was removed and subjects were placed in the pool at an entry point opposite the former platform location for a free swim trial of 60 s. The time spent in each of the four quadrants (quadrant time) and the number of times the animals crossed each of the four possible platform locations (annulus crossing) were determined from videotape. The quadrants were designated TR (training quadrant), RA (right adjacent quadrant, clockwise), LA (left adjacent quadrant, counter clockwise), and OP (opposite quadrant).

Place training. Place training was started following a 7-day interval and lasted from Day 15 to Day 19. The platform was moved to the quadrant opposite the one where it had been during cue training and the visual cue was removed. Thus, in contrast to previous cue training,

information available for the localization of the platform consisted exclusively of extra-maze cues. The animals were trained each day to escape onto the platform during four trials as described for cue training.

Free swim trial 2. On Day 20 each subject was placed in the pool at a point opposite the former platform location. Quadrant time and annulus crossings were determined as in free swim trial 1. The quadrants were designated TR, RA, LA, and OP (see above) relative to the position of the platform during place training.

Statistics

The effects of age and T treatment on plasma T levels and testicular weight were investigated with analysis of variance (ANOVA). The effects of age and T treatment on escape latencies during cue and place training were analyzed using ANOVA with the factor Training Day as a repeated measure (5 days).

Retention of spatial information acquired during cue training and during place training was investigated for each experimental group at free swim trials 1 and 2, respectively, by testing the null hypothesis that there were no differences in preference of the animals for the four quadrants. Since time spent in one quadrant was related to time spent in the other quadrants and since the data were not assumed to be normally distributed a nonparametric test for several related samples was used [Friedman's test (Conover, 1980)]. If the null hypothesis was rejected the rank sums of time spent in the individual quadrants were compared pairwise as described by Conover (1980, p. 300–303). The number of annulus crossings in each of the four quadrants was compared in the same way. In addition, the effects of age and T treatment on the time spent in TR and the number of annulus crossings in TR during free swim trials 1 and 2 were analyzed using ANOVA. If appropriate, multiple comparisons were made using the Newman–Keuls *a posteriori* test.

Homogeneity of variance, which is a major requirement for ANOVA, was tested using the Bartlett Box *F* test or using Box's *M* (in designs with repeated measures). If the hypothesis of homogeneity was rejected ANOVA was applied to rank-transformed data rather than the original data (cf. Conover, 1980). A 0.05 level of significance was used in all statistical tests.

RESULTS

Morbidity and Mortality

Three aged animals were excluded from the experiment because they were dead or moribund before cue training started. In addition, one aged rat was excluded because of a massive venous thrombosis of a hind leg, severely impairing its mobility. Gross pathological examination of all

remaining animals at the end of the experiment revealed tumor growth in the liver (2), in the lung (2), and in the pituitary (2), a grossly enlarged heart (1), renal cysts (2), and hydronephrosis (2). These findings were evenly distributed over T-treated and sham-treated animals and were consistent with data in the literature for the Brown-Norway rat at 30–33 months (Burek, 1978; Mos and Hollander, 1987).

Testosterone Levels and Testicular Weight

T levels fell progressively with age ($F(2, 41) = 5.46, p < .01$) and were elevated by T treatment ($F(1, 41) = 43.90, p < .001$) (Table 1). No Age $\times$ Treatment interaction was observed ($F(2, 41) = 0.69, p = .507$). T levels of T-treated middle-aged and aged animals were restored to levels within the range of those of sham-treated young animals. Testicular weight also declined with age ($F(2, 41) = 18.84, p < .001$). T treatment resulted in a reduction in testicular weight ($F(1, 41) = 10.61, p < .003$) (Table 1). There was no Age $\times$ Treatment interaction in this respect ($F(2, 41) = 0.83, p = .442$).

Cue Training

Due to a disturbance in the closed television circuit, latencies of three animals (one aged sham-treated and two aged T-treated) were not recorded on one of the training days. Therefore, these three animals were omitted from this part of the analysis. Statistical analyses of the latencies during the 5 days of cue training (Fig. 1) revealed a main effect of Age on Latency throughout cue training ($F(2, 38) = 17.10, p < .001$). Treatment as main effect did not affect Latency ($F(1, 38) = 0.02, p = .887$) nor was there an Age $\times$ Treatment interaction ($F(2, 38) = 0.05, p = .955$). Separate comparisons showed the aged animals had longer latencies than young ($F(1, 26) = 30.43, p < .001$) and middle-aged ($F(1, 26)$

TABLE 1
Plasma Testosterone Levels and Testicular Weight in Relation to Age and Treatment

Age	Plasma T level (nmol/liter)		Testicular weight (g)	
	Sham-treated	T-treated	Sham-treated	T-treated
Young	9.55 $\pm$ 4.55	13.30 $\pm$ 1.52	3.44 $\pm$ 0.06	1.73 $\pm$ 0.11
Middle-aged	4.25 $\pm$ 1.13	8.60 $\pm$ 0.68	2.25 $\pm$ 0.38	1.49 $\pm$ 0.09
Aged	2.60 $\pm$ 0.25	10.64 $\pm$ 1.21	1.46 $\pm$ 0.11	1.23 $\pm$ 0.12

Note. Values represent means $\pm$ standard error of the mean. Plasma T levels and testicular weight declined progressively with age (ANOVA; $p < .01$). T levels were elevated following T treatment (ANOVA; $p < .001$), whereas testicular weight was reduced following T administration (ANOVA; $p < .003$).

FIG. 1. Mean latencies of sham- and T-treated young, middle-aged, and aged rats during 5 days of cue training. Latencies are the sum of four daily trials. Young and middle-aged animals reached the platform earlier than aged animals throughout cue training. However, the ability to learn the position of the visually cued platform was similar in all age groups and was not affected by T treatment.

= 26.13, $p < .001$) animals, which did not differ from one another ($F(1, 30) = 1.58$, $p = .218$) in this respect.

A highly significant effect of Training Day on Latency was observed ($F(4, 152) = 99.84$, $p < .001$), which showed no interactions with either Age ($F(8, 152) = 1.32$, $p = .236$) or Treatment ($F(4, 152) = 0.20$, $p = .937$), indicating that the training effect was the same for all age groups. In addition, there was no Training Day $\times$ Age $\times$ Treatment interaction ($F(8, 152) = 1.02$, $p = .426$).

Free Swim Trial 1

No significant preference for any of the quadrants and no significant variation in the number of annulus crossings between the four quadrants was observed during free swim trial 1 for any of the experimental groups with the exception of a moderate preference for the training platform by the T-treated middle-aged rats ($T3 = 12.65$, $p < .05$).

No main effects of either Age or Treatment were observed on Quadrant Time in TR ($F(2, 41) = 3.02$, $p = .060$; and $F(1, 41) = 0.34$, $p = .561$, respectively) or on the number of annulus crossings in TR ($F(2, 41) = 0.67$, $p = 0.517$; $F(1, 41) = 0.43$, $p = .518$, respectively). In addition there were no Age $\times$ Treatment interactions ($F(2, 41) = 1.09$, $p = 0.345$;

$F(2, 41) = 1.04$, $p = .361$ for quadrant time and annulus crossings in TR, respectively.)

Place Training

One sham-treated middle-aged animal managed to cling to one of the supporting points of the transparent part of the wall of the pool during some of the escape trials and was excluded from further analyses. Statistical analysis of latencies during 5 days of place training showed a main effect of Age ($F(2, 40) = 21.28$, $p < .001$) but not of Treatment ($F(1, 40) = 1.71$, $p = .199$) on Latency throughout place training (Fig. 2). There was no Age $\times$ Treatment interaction in this respect ($F(2, 40) = 1.54$, $p = .227$). Separate comparisons showed that aged animals had longer latencies than middle-aged ($F(1, 28) = 16.87$, $p < .001$) and young animals ($F(1, 29) = 38.52$, $p < .001$). The latencies of middle-aged animals were also longer than those of young animals ($F(1, 29) = 4.51$, $p < .05$).

A highly significant effect of Training Day was observed on Latency ($F(4, 160) = 24.09$, $p < .001$) (Fig. 2). In addition, there was a Training $\times$ Age interaction ($F(8, 160) = 3.90$, $p < .001$), but no Training $\times$ Treatment ($F(4, 160) = 0.28$, $p = .894$) or Training $\times$ Treatment $\times$

FIG. 2. Mean latencies of sham- and T-treated young, middle-aged, and aged rats during 5 days of place training. Latencies are the sum of four daily trials. Young animals had the shortest latencies throughout place of training, followed by the middle-aged and aged animals. The ability to learn the position of the hidden platform was reduced in middle-aged and aged rats. T treatment had no effect on Latency in any of the age groups.

Age ($F(8, 160) = 0.80, p = .604$) interaction. Separate comparisons revealed that the Training $\times$ Age interaction was statistically significant for the young and middle-aged ($F(4, 116) = 6.45, p < .001$) and the young and aged ($F(4, 116) = 5.15, p < .002$) animals, but not for the middle-aged and aged animals ($F(4, 112) = 0.68, p = .605$), indicating that the effect of training was less pronounced in both middle-aged and aged rats than in young animals.

Free Swim Trial 2

Training to localize a hidden platform resulted in a preference for the quadrant where the platform had been for some of the experimental groups, whereas others showed no retention (Fig. 3). The time spent in the four quadrants differed significantly for sham-treated young ($T_3 = 10.05, p < .05$) and middle-aged ($T_3 = 8.47, p < .05$) animals, but not for sham-treated aged animals ($T_3 = 5.74, .10 < p < .25$). Pairwise comparison of rank sums of time spent in the individual quadrants revealed that sham-treated young and middle-aged rats spent more time in TR than in any of the other quadrants ($p < .05$). In contrast, T-treated young and middle-aged rats showed *no* preference for TR ($T_3 = 5.74, .10 < p < .25$; $T_3 = 2.55, 0.25 < p < .50$, respectively). T-treated aged animals, however, did show a preference for TR (Fig. 3) ($T_3 = 9.45, p < .05$), but the rank sum of time spent in TR differed significantly only from that in LA ($p < .05$), and not from that in RA or OP.

Analysis of the number of annulus crossings in the four quadrants showed a comparable picture (Fig. 3). Both the sham-treated young and middle-aged animals showed significantly more annulus crossings in TR ($T_3 = 16.48, p < .001$; $T_3 = 10.71, p < .05$, respectively) as opposed to the sham-treated aged animals ($T_3 = 1.27, .50 < p < .90$). Pairwise comparisons of rank sums of the number of annulus crossings in the individual quadrants revealed that sham-treated young and middle-aged rats showed more annulus crossings in TR than in any of the other three quadrants ($p < .001$ and $p < .05$, respectively). The preference for annulus crossings in TR was less pronounced in the T-treated young and middle-aged animals than in their sham-treated counterparts but still statistically significant ($T_3 = 8.39, p < .05$; $T_3 = 8.46, p < .05$, respectively). However, pairwise comparisons of rank sums of the number of annulus crossings in the individual quadrants showed that the number of annulus crossings in TR of the T-treated young animals differed only from the number in LA ($p < .05$) and that the number of annulus crossings in TR of T-treated middle-aged animals differed only from the number in LA and RA ($p < .05$). The number of annulus crossings of T-treated aged animals was the same in all quadrants ($T_3 = 1.27; .50 < p < .90$).

No main effects of Age and Treatment on Quadrant Time in TR were observed ($F(2, 41) = 1.42, p = .253$; $F(1, 41) = 0.58; p = .451$, re-

FIG. 3. Free swim trial 2. (A) Mean time ($\pm$ SEM) spent in each of the quadrants of the water maze and (B) mean number of annulus crossings ($\pm$ SEM) in each of the quadrants during a 60-s free swim trial following place training for sham- and T-treated young, middle-aged and aged rats. TR, training quadrant; LA, left adjacent quadrant; RA, right adjacent quadrant; OP, opposite quadrant. *significantly different from all other quadrants ($p < .05$).

spectively). However, the Age $\times$ Treatment interaction approached statistical significance ($F(2, 41) = 2.97$, $p = .063$). Separate analysis of the sham- and T-treated animals revealed a statistically significant Age effect in the sham-treated animals ($F(2, 20) = 3.87$, $p < .05$), with young animals differing from aged ones ($p < .05$). No such effect was observed in the T-treated animals ($F(2, 21) = 0.16$, $p = .856$).

Statistically significant main effects of both Age and Treatment on the number of annulus crossings in TR were observed ($F(2, 41) = 12.64$, $p < .001$; $F(1, 41) = 5.61$; $p < .03$, respectively). There was no Age $\times$ Treatment interaction for this parameter ($F(2, 41) = 0.91$, $p = .412$).

The mean number of annulus crossings in TR in the T-treated animals (2.33 ± 0.27) was lower than in sham-treated animals (3.26 ± 0.35). Separate comparisons showed that the Age effect was significant in the sham-treated animals ($F(2, 20) = 9.08, p < .002$), the number of annulus crossings of aged animals being lower than those of young and middle-aged animals ($p < .05$). An Age effect was also observed in the T-treated animals ($F(2, 21) = 3.85, p < .04$), the aged animals differing significantly from the young ones ($p < .05$).

In summary, the results of free swim trial 2 indicate that both young and middle-aged animals showed significant retention of the platform position following 5 days of place training. In contrast, aged animals showed no retention. The aged animals spent less time in the quadrant where the platform had been and showed less annulus crossings in that quadrant than young animals. T treatment resulted in impaired retention for the training quadrant and T-treated animals showed less annulus crossings over the former platform location than their untreated counterparts.

DISCUSSION

The present experiment was designed in order to determine whether peripheral T administration to senescent rats which was previously shown to restore AVP innervation in a number of brain regions (Goudsmit et al., 1988) might result in an amelioration of age-related cognitive impairment. Although an age-related impairment in spatial memory was confirmed (cf. Barnes et al., 1980; Gage et al. 1984; Ingram et al., 1981; Rapp et al., 1987) no restoration of spatial memory was observed following T treatment.

Cue Training and Free Swim Trial 1

The results of training with a visually cued platform are in agreement with those reported earlier by Rapp et al. (1987) and show that although aged rats are slower than middle-aged or young animals, they are as capable of learning to escape onto the platform as younger animals. Apparently, the ability to learn this task is not confounded by sensory, motoric, or motivational differences between the age groups. The fact that none of the experimental groups showed significant retention of the location of the platform indicates the animals did not use extra-maze cues when a visual cue on the platform itself was offered. However, Rapp et al. (1987) using a highly comparable task and procedure reported a significant retention of the platform position in young but not in aged rats following cue training. This discrepancy might be due to the fact that the free swim trial in their experiment immediately followed the last training session, whereas in the present experiment a 24-h interval was observed.

Place Training

During place training, when no visual cue was offered for the (reversed) platform position, a significant Age effect became apparent. Not only were the younger animals faster than the older ones (as they were during cue training), but the effect of five subsequent days of training was also significantly larger than in both middle-aged and aged animals, suggesting the young animals made more efficient use of extra-maze cues during this task. The less efficient performance of the middle-aged and especially of the aged animals is most probably not related to persistent neophobia, since no significant differences in the ability to learn the location of the platform were observed during the preceding cue training. Therefore, the most plausible explanation for the observed deficit seems to be an actual impairment in the processing of spatial information in middle-aged and aged rats (cf. Rapp et al., 1987).

Free Swim Trial 2

In view of the deficit in spatial learning demonstrated by the aged rats during place training, it was not surprising that the young animals showed a significantly stronger retention of the platform position during free swim trial 2 than the aged animals. However, the middle-aged animals which showed impaired spatial learning during place training were able to locate the former platform position during the free swim trial as well as the young animals. This discrepancy might be explained by the relatively short latencies of the middle-aged animals on the first day of place training (see Fig. 2), which attenuated the effect of the 5-day training period. One might speculate that the middle aged rats remembered the former (cued) platform position less well than the younger animals and consequently were not hampered by any bias toward that quadrant. However, this explanation seems improbable, since none of the age groups showed any retention for this quadrant during free swim trial 1. Alternatively, the middle-aged rats might have benefited more from their prior experience in the maze on the first day of place training than the other groups.

T Treatment

As opposed to our working hypothesis T treatment did not improve spatial learning or retention of a spatial localization task in any of the age-groups. On the contrary, the present results show for the first time a rather surprising decrease in retention in T-treated rats, which was most pronounced in the young and middle-aged animals. Since no adverse effects of T treatment were observed during place training, these results seem to imply that T treatment specifically impedes retention of spatial information.

General Considerations

The present results do not support our working hypothesis that restoration of AVP innervation in the senescent rat brain following peripheral T treatment (Goudsmit et al., 1988) is accompanied by an improvement in spatial learning and memory. Of course, an enhancement of other aspects of cognitive function cannot be excluded. Central administration of AVP was previously shown to enhance both passive and active avoidance behavior (cf. Bohus et al., 1982; Koob et al., 1986). However, the interpretation of these experiments in terms of memory was challenged by the demonstration of behavioral inhibition on various tasks following peripheral AVP administration (Van Haaren, Heinsbroek, Louwerse, & Van De Poll, 1986; Van Haaren, Van Zanten, & Van De Poll, 1986). Since the T-treated animals used in the present study showed elevated AVP concentrations in the medial amygdala in combination with a trend toward elevated AVP levels in the lateral septum and hippocampus (Goudsmit, Luine, & Swaab, 1989) our results raise additional doubt on a role of central AVP in spatial learning and memory.

The question of how T treatment disrupts retention but not learning of spatial information in young and middle-aged rats cannot be answered at present. One might speculate on the consequences of an overall suppression of the hypothalamo-pituitary-gonadal axis by exogenous T as evidenced by the substantial decrease in testicular weight following T treatment. In addition, suppression of LH release following T administration was demonstrated in rats from young to old [Sartin & Lamperti, 1984]. Thus, T treatment probably depresses plasma levels of both gonadotropins and androgen precursors such as dehydroepiandrosterone and its sulfate (DHEAS), which was recently shown to improve active avoidance behavior in aging mice (Flood & Roberts, 1988). Such negative feedback effects would be expected to be most pronounced in the younger age groups as was indeed observed in our behavioral experiments. An alternative hypothesis might be formulated on the basis of the differences in performance between male and female rats in various complex mazes (for a review see Beatty, 1979). Although estrogen administration to adult male rats was reported to impair maze performance (Dawson, 1972), others failed to find effects following gonadectomy of adult male and female rats (Joseph, Hess, & Birecree, 1978). Effects of T administration to intact adult male rats on maze performance have not been reported so far. Since T is aromatized to estrogen in the brain (Hutchison & Steimer, 1984) it is conceivable that elevated blood levels of T might result in the less efficient "female" pattern of behavior in complex maze tasks.

In conclusion, the results of the present experiment confirm a decrease in spatial learning and memory in senescent rats, but fail to support a

stimulatory effect of peripheral T administration on spatial memory in aged rats and a role of central AVP in this respect. The surprising observation of a disruption of spatial memory by peripheral T administration in young and middle-aged rats needs further investigation.

REFERENCES

- Barnes, C. A., Nadel, L., & Honig, W. K. (1980). Spatial memory deficit in senescent rats. *Canadian Journal of Psychology*, **34**, 29–39.
- Beatty, W. W. (1979). Gonadal hormones and sex differences in nonreproductive behaviors in rodents: Organizational and activational influences. *Hormones and Behavior*, **12**, 112–163.
- Bohus, B., Conti, L., Kovács, G. L., & Versteeg, D. H. G. (1982). Modulation of memory processes by neuropeptides: Interaction with neurotransmitter systems. In C. A. Marsan & H. Matthies (Eds.), *Neuronal plasticity and memory formation* (pp. 75–87). New York: Raven Press.
- Brennan, M. J., Blizard, D. A., & Quartermain, D. (1982). Amelioration of an age-related deficit in exploratory behavior of preexposure to the test environment. *Behavioral and Neural Biology*, **34**, 55–62.
- Buijs, R. M., De Vries, G. J., Van Leeuwen, F. W., & Swaab, D. F. (1983). Vasopressin and oxytocin: Distribution and putative functions in the brain. In B. A. Cross & G. Leng (Eds.), *The neurohypophysis: Structure, function and control. Progress in brain research* (Vol. 60, pp. 115–122). Amsterdam: Elsevier.
- Burek, J. D. (1978). *Pathology of aging rats. A morphological and experimental study of the age-associated lesions in aging BN/Bi, WAG/Rij and (WAG×BN)F1 rats*. Boca Raton, FL: CRC Press.
- Collier, T. J., & Sladek, J. R. (1984). Increased neophobia: Behavioral identification of a subpopulation of memory-impaired norepinephrine-deficient aged F344 rats. *Society of Neuroscience Abstracts*, **10**, 772.
- Conover, W. J. (1980). *Practical nonparametric statistics*. New York: Wiley.
- Dawson, J. L. M. (1972). Effects of sex hormones on cognitive style in rats and men. *Behavior Genetics*, **2**, 21–42.
- De Vries, G. J., Buijs, R. M., Van Leeuwen, F. W., Caffé, A.R., & Swaab, D. F. (1985). The vasopressinergic innervation of the brain in normal and castrated rats. *Journal of Comparative Neurology*, **233**, 1–19.
- Dunnett, S. B., Gage, F. H., Björklund, A., Stenevi, U., Low, W. C., & Iversen, S. D. (1982). Hippocampal deafferentiation: Transplant-derived reinnervation and functional recovery. *Scandinavian Journal of Psychology, Suppl.* **1**, 104–111.
- Fliers, E., De Vries, G. J., & Swaab, D. F. (1985). Changes with aging in the vasopressin and oxytocin innervation of the rat brain. *Brain Research*, **348**, 1–8.
- Flood, J. F., & Roberts, E. (1988). Dehydroepiandrosterone sulfate improves memory in aging mice. *Brain Research*, **448**, 178–181.
- Gage, F. H., Dunnett, S. B., & Björklund, A. (1984). Spatial learning and motor deficits in aged rats. *Neurobiology of Aging*, **5**, 43–48.
- Goudsmit, E., Fliers, E., & Swaab, D. F. (1988). Testosterone supplementation restores vasopressin innervation in the senescent rat brain. *Brain Research*, **473**, 306–313.
- Goudsmit, E., Luine, V. N., & Swaab, D.F. (1989). Testosterone increases vasopressin content but fails to restore choline acetyltransferase activity in the senescent male rat brain. Submitted for publication.
- Hutchison, J. B., & Steimer, Th. (1984). Androgen metabolism in the brain: Behavioural correlates. In G. J. De Vries et al. (Eds.), *Sex differences in the brain: The relation between structure and function. Progress in brain research* (Vol. 61, pp. 23–51). Amsterdam: Elsevier.

- Ingram, D. K., London, E. D., & Goodrick, C. L. (1981). Age and neurochemical correlates of radial maze performance in rats. *Neurobiology of Aging*, **2**, 41-47.
- Joseph, R., Hess, S., & Birecree, E. (1978). Effects of hormone manipulations and exploration on sex differences in maze learning. *Behavioral Biology*, **24**, 364-377.
- Kaler, L. W., & Neaves, W. B. (1981). The androgen status of the aging male rat. *Endocrinology*, **108**, 712-719.
- Koob, G. F., Dantzer, R., Bluthé, R.-M., Lebrun, C., Bloom, F. E., & Le Moal, M. (1986). Central injections of arginine vasopressin prolong extinction of active avoidance. *Peptides*, **7**, 213-218.
- Kordower, J. H., Sikorszky, V., & Bodnar, R. J. (1982). Central antinociceptive effects of lysine-vasopressin and an analogue. *Peptides*, **3**, 613-617.
- Kubanis, P., & Zornetzer, S. F. (1981). Age-related behavioral and neurobiological changes: A review with an emphasis on memory. *Behavioral and Neural Biology*, **31**, 115-172.
- Landfield, P. W., Braun, L. D., Pitler, T. A., Lindsey, J. D., & Lynch, G. (1981). Hippocampal aging in rats: A morphometric study of multiple variables in semithin sections. *Neurobiology of Aging*, **2**, 265-275.
- Le Moal, M., Dantzer, R., Michaud, B., & Koob, G. F. (1987). Centrally injected arginine vasopressin (AVP) facilitates social memory in rats. *Neuroscience Letters*, **77**, 353-359.
- Meck, W. H., Church, R. M., & Wenk, G. L. (1986). Arginine vasopressin inoculates against age-related increases in sodium-dependent high affinity choline uptake and discrepancies in the content of temporal memory. *European Journal of Pharmacology*, **130**, 327-331.
- Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, **11**, 47-60.
- Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, **12**, 239-260.
- Morris, R. G. M., Garrud, P., Rawlins, J. N. P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature (London)*, **297**, 681-683.
- Mos, J., & Hollander, C. F. (1987). Analysis of survival data on aging rat cohorts: pitfalls and some practical considerations. *Mechanisms of Ageing and Development*, **38**, 89-105.
- Naylor, A. M., Ruwe, W. D., & Veale, W. L. (1986). Thermoregulatory actions of centrally administered vasopressin in the rat. *Neuropharmacology*, **25**, 787-794.
- Pittman, Q. L., Lawrence, D., & McLean, L. (1982). Central effects of arginine vasopressin on blood pressure in rats. *Endocrinology*, **110**, 1058-1060.
- Pratt, J. J., Wiegman, T., Lappöhn, R. E., & Woldring, M. G. (1975). Estimation of plasma testosterone without extraction and chromatography. *Clinica Chimica Acta*, **59**, 337-346.
- Rapp, P. R., Rosenberg, R. A., & Callagher, M. (1987). An evaluation of spatial information processing in aged rats. *Behavioral Neuroscience*, **101**, 3-12.
- Ravid, R., Fliers, E., Swaab, D. F., & Zurcher, C. (1987). Changes in vasopressin and testosterone in the senescent Brown-Norway (BN/BiRij) rat. *Gerontology*, **33**, 87-98.
- Sartin, J. L., & Lamperti, A. A. (1984). FSH and LH response to testosterone and gonadotropin-releasing hormone in aging male rats. *Experimental Aging Research*, **10**, 183-187.
- Van Haaren, F., Van Zanten, S., & Van De Poll, N. E. (1986). Vasopressin disrupts radial-maze performance in rats. *Behavioral and Neural Biology*, **45**, 350-357.
- Van Haaren, F., Heinsbroek, R. P. W., Louwerse, A., & Van De Poll, N. E. (1986). Vasopressin has general rate-decreasing effects on schedules maintaining either high or low response rates. *Psychopharmacology*, **89**, 69-72.