Short communication

Increased light intensity prevents the age related loss of vasopressin-expressing neurons in the rat suprachiasmatic nucleus

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Abstract

We investigated whether increased light input can counteract the age-related decrease in vasopressin- (AVP) and vasoactive intestinal polypeptide (VIP)-expressing neurons of the suprachiasmatic nucleus (SCN) by determining the numbers of these neurons in rats of different ages, housed under low or high intensities of light. The significant age-related decrease for AVP was prevented in old animals after high light housing. For VIP, no effects were found.

Keywords: Suprachiasmatic nucleus; Aging; Vasopressin; Vasoactive intestinal polypeptide; Light intensity; Immunocytochemistry

The suprachiasmatic nucleus (SCN) is considered to be the principal component of the biological clock in the brain [11,15–17,24]. It contains two well characterized subdivisions: in the first place, vasoactive intestinal polypeptide (VIP) containing neurons are found in the ventral SCN that receive direct information about the environmental light–dark cycle via the retinohypothalamic projection [11,12,17]. These neurons are supposed to transduce information on environmental illumination [1,9,11,12,15–17,27]. In the second place, vasopressin (AVP) neurons are found in the dorsomedial SCN [11,12,16,17]. They are an important output system of the SCN [12,13,17]. During aging, the circadian system undergoes profound functional and morphological degenerative changes [3,7,10,19,22,25,30,31]. Earlier studies demonstrated significant decreases in the numbers of AVP (31%) and VIP (36%) immunoreactive neurons in the SCN of old rats [5,23]. Witting et al., [32] demonstrated that by increasing light intensity without changing the light period, age-related decreases in the amplitude of several functional sleep–wake parameters could be restored in old animals to the point of attaining the level of young animals. In order to study whether this increased light input would be able to counteract the decreases in AVP and VIP expression in the

senescent SCN, rats of different ages were housed under low or increasing high intensities of light during the light period for 16 days following which the AVP and VIP cell numbers were determined in the SCN.

Fourteen young (age: 3 months old, weight $\pm$ S.E.M.: 203.5 ± 3.4 g), 16 middle aged (24.5 months; weight: 425.3 ± 6.0 g) and 18 old (34–37 months, weight: 405.6 ± 7.0 g) male Brown–Norway (BN/BiRij) rats were obtained from TNO Rijswijk, The Netherlands, where they had been housed under a 12 h L/12 h D period of approximately 250 lux (lights on 07.00 h, lights off 19.00 h). After arrival at our Institute, they were housed together with 3 or 4 animals in standard laboratory cages (appr. 120–170 lux), divided over two rooms and left for 3 weeks of adaptation. The experiment lasted 16 days and was performed in January. Experimental lighting settings were installed consisting of small light bulbs (13498 Philips, 24 V/21 W) that were affixed outside the cages; light intensities were measured in the middle of the cage at a distance of 15 cm from the lamp using a luxmeter (Gossen Master-six). In one room, light intensity during the light phase was gradually increased, step by step every three days as described by Witting et al. [32] from 3.5 to 600 lux. The animals in the other room were housed under a fixed light intensity of approximately 18 lux during the light phase. In both rooms a dim red light at night provided approximately 0.5 lux.

After the experiment, animals were perfused ad random as described before [2] on two consecutive days between

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10.00 and 12.00 h. The brain was submerged in the same fixative and left overnight in the opened skull for postfixation. The next day the hypothalamus was dissected and, after 2 more weeks of fixation, dehydrated and embedded in paraffin. Serial 6 μm coronal sections were mounted on chrome-alum coated slides. Animals with obvious abnormalities of the eyes, optic nerves or chiasmatic region were excluded leaving 43 hypothalami that were used for further morphometric analysis. Immunocytochemical staining for AVP and VIP was performed as described earlier [5,23] although with a different antibody for VIP. Incubation took place with anti-AVP (Truus, 25-1-86) 1:800 or anti-VIP (Viper, 18-9-86) 1:1000, both raised at our Institute [4], as described previously [5,23]. Antibodies against glutaraldehyde-thyroglobulin present in the VIP antiserum causing

background staining in pilot experiments were removed by preadsorption according to Pool et al., [21].

Morphometry was performed as described earlier [28] without knowing age or housing condition of the animal. Total AVP and VIP cell numbers were determined by multiplying the numerical AVP and VIP cell densities with the volumes of these particular cell groups. A two-way ANOVA was used to test effects of age and light and whether any interaction was present, followed by a Tukey test in order to determine age effects separately ($P = 0.05$ level of significance).

Immunocytochemical staining revealed characteristic distributions of VIP and AVP in the ventral and dorsomedial part of the SCN, respectively (Fig. 1A and B). No obvious changes with age in stainability for either of the

Fig. 1. Vasopressin (AVP) (A) and vasoactive intestinal polypeptide (VIP) (B) expressing neurons in the suprachiasmatic nucleus of a 24.5-month-old rat. Note the characteristic dorsomedial and ventral distribution for AVP and VIP, respectively. Scale bar = 75 μm ; III, third ventricle; OC, optic chiasm.

Fig. 2. Graph depicting numbers of vasopressin (AVP) (A) or vasoactive intestinal polypeptide (VIP) (B) expressing neurons in the suprachiasmatic nucleus of rats of different ages after housing under a low (hatched bars) or high (white bars) light intensity. Bars represent mean $\pm$ S.E.M. * $P < 0.05$ compared with both young and middle-aged animals under low light intensity. Note that the numbers of AVP-expressing neurons in the old group after high light treatment do no longer differ significantly from the young and middle-aged animals.

neurotransmitters could be observed. Age of the animals was found to have a significant effect on the number of AVP neurons in the SCN (ANOVA; $F_{2,37} = 5.01$; $P = 0.012$) (Fig. 2A). In addition, although nearly significant, no interactions were found between age and light ($F_{2,37} = 2.42$; $P = 0.1$). For the VIP cell number no such age effect ($F_{2,37} = 0.879$; $P > 0.4$) or interaction between age and light ($F_{2,37} = 0.513$; $P > 0.6$) was present. A subsequent one way Tukey B test revealed that the differences in AVP cell numbers between old and middle aged as well as between old and young animals in the low light group was significant ($P < 0.05$ in both cases), whereas after high light treatment, both these age groups no longer differed in a statistically significant way (Fig. 2A). For VIP, no statistical significant differences between young and old, middle aged and old age in the low light condition or after

high light treatment could be established (Fig. 2B). Data are depicted in Table 1 and Fig. 2.

The present study shows that under low light intensity, AVP cell numbers were significantly reduced in old animals as compared to young and middle aged ones, but were no longer significantly different after housing the animals under high light conditions, indicating that high light intensity indeed prevents the age related loss of AVP-expressing neurons in the SCN. The possibility that the decrease in lux rather than age itself caused the AVP loss in the SCN is not very likely, since this would have become apparent also in the young and middle aged groups, which was clearly not the case since cell numbers under the 18 lux and 600 lux conditions in these groups were similar (Fig. 2A and Table 1).

An interesting aspect of our study is, first of all, that considerable plasticity is present in the SCN of old rats which apparently is still able to respond morphologically to stimulation with increased light input. High light intensity was already shown to be effective also functionally, since Witting et al. [32] could restore the age related decreases in rhythm amplitudes in old animals after housing them under similar high light conditions as we used. Secondly, our results deviate from other studies on young animals that reported changes in peptide levels or immunoreactivity of VIP rather than AVP after light changes, thereby putting forward VIP as a candidate peptide for transduction of illuminational input to the SCN [1,9,11,12,20,27]. It should be noted, however, that these results are based on changes in period length or timing of a lightstimulus rather than on differences in light intensity during a light period that remains furthermore constant. From the present data it appears as if AVP neurons are more sensitive to light intensity changes at this circadian time point than VIP neurons, at least in old animals. Another possibility is that VIP neurons, that were not affected by the aging process in our present study, do not respond to the increased light input.

Earlier studies used, in contrast to the present study, conventional stainings [5,23] in stead of the AVP or VIP volume, or different methodology [6,18] for calculating the SCN volume and subsequently VIP and AVP cell numbers as well as total cell numbers. In spite of this, we found similar AVP cellnumbers in young animals as Roozendaal et al. [23]. In contrast, our VIP cellnumbers were lower than found earlier [5]. The fact that we could reproduce the data for AVP using the same antibody shows that also in a considerably higher constant day light setting (Roozendaal; 250 lux) than the present one (i.e. 18 lux) the age related loss of AVP expressing neurons persists.

Although we did confirm the loss in AVP- [23], we could not duplicate the decrease in VIP-expressing neurons in old animals [5]. Several possibilities may underlie this difference. First, besides a different fixative, also a different antibody for VIP was used, possibly differing in its sensitivity to VIP epitopes. Secondly, the conditions of the

Table 1
Morphometrical parameters of the SCN of rats of different ages after low or high light intensities

	Young (3 months)			Middle aged (24.5 months)			Old (34–37 months)		
	Cell nr	Volume (mm ³)	Density ($\times 10^3$ /mm ³)	Cell nr	Volume (mm ³)	Density ($\times 10^3$ /mm ³)	Cell nr	Volume (mm ³)	Density ($\times 10^3$ /mm ³)
AVP									
Low	813 $\pm$ 106	0.0065 $\pm$ 0.0009	132.7 $\pm$ 11.0	807 $\pm$ 53	0.0061 $\pm$ 0.0006	135.8 $\pm$ 7.2	477 $\pm$ 62 **	0.0042 $\pm$ 0.0004	115.6 $\pm$ 13.7
High	847 $\pm$ 130	0.0065 $\pm$ 0.001	134.5 $\pm$ 11.9	719 $\pm$ 35	0.0057 $\pm$ 0.0005	130.3 $\pm$ 8.7	712 $\pm$ 26 ns	0.0055 $\pm$ 0.0004	131.4 $\pm$ 7.7
VIP									
Low	495 $\pm$ 72	0.0037 $\pm$ 0.0005	130.9 $\pm$ 10.9	543 $\pm$ 85	0.0041 $\pm$ 0.0006	131.0 $\pm$ 9.0	397 $\pm$ 85	0.0036 $\pm$ 0.0006	99.4 $\pm$ 15.5
High	441 $\pm$ 98	0.0034 $\pm$ 0.0008	152.5 $\pm$ 22.7	443 $\pm$ 81	0.0038 $\pm$ 0.0008	124.1 $\pm$ 13.9	427 $\pm$ 70	0.0038 $\pm$ 0.0006	115.6 $\pm$ 6.9

Data represent mean $\pm$ S.E.M. per group ($n = 7$, except for the old group under low light conditions ($n = 8$)). Abbreviations used: SCN: suprachiasmatic nucleus; AVP: vasopressin; VIP: vasoactive intestinal polypeptide; Low: low light intensity; High: high light intensity during the light phase (see text for details); ns: not significant; ** $P < 0.05$ compared to both the young and the middle aged group (One-way Tukey B -test).

experimental settings differed with respect to the time of perfusion, which took place between 10.00 and 12.00 h in the present study and was from 9.00 till 17.00 h in the earlier study [5] (T. van der Woude, pers. comm.). At the present time of perfusion, the immunocytochemical circadian profile of VIP was reported not to be steeply rising or falling [11,12,20]. For AVP, peak values in AVP content are to be expected around circadian time 4 [11,12]. This may yield different results than in a situation where time-points are randomly chosen throughout the circadian day [5]. Whether VIP cell numbers respond to increased light intensity when determined at a different circadian time of perfusion awaits further research.

Another important methodological difference was the physical condition of the animals used. In the two former studies [5,23], animals had been housed before in enriched environment cages and were operated on in order to implant sleep–wakefulness recording electrodes [29]. As a consequence, the condition of the old animals was much worse in view of their age (32–33 months) as compared to the ones used in the present study (34–37 months), which were in fact difficult to distinguish from the middle aged animals at the moment of perfusion. As the SCN is expected to degenerate only in the oldest age group [25,28,30,31], physical condition might also interfere with age-related changes in this nucleus, rendering the rats from the previous studies not necessarily comparable to the old animals used in the present study, that were in excellent shape.

In conclusion, housing of Brown–Norway rats under increasing high light intensity influences AVP- but not VIP-expressing neurons in the senescent SCN at this circadian timepoint. Our data indicate that high light intensity prevents the decrease in AVP-expressing neurons in the senescent SCN. This may be a relevant factor in the positive effects of light treatment reported in Alzheimer patients [8,14,26].

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