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*Published in:*  
Brain Research

*DOI:*  
[10.1016/0006-8993\(92\)90266-C](https://doi.org/10.1016/0006-8993(92)90266-C)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1992

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

VANDERZEE, E. A., COMPAAN, J. C., LYNCH, C. B., Compaan, J. C., & Lynch, C. B. (1992). DIFFERENCES IN THE NUMBER OF ARGININE-VASOPRESSIN-IMMUNOREACTIVE NEURONS EXIST IN THE SUPRACHIASMATIC NUCLEI OF HOUSE MICE SELECTED FOR DIFFERENCES IN NEST-BUILDING BEHAVIOR. *Brain Research*, 578(1-2), 335-338. DOI: 10.1016/0006-8993(92)90266-C

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## Short Communications

# Differences in the number of arginine-vasopressin-immunoreactive neurons exist in the suprachiasmatic nuclei of house mice selected for differences in nest-building behavior

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(Accepted 21 January 1992)

**Key words:** Vasopressin; Suprachiasmatic nucleus; Paraventricular nucleus; Nest-building behavior; *Mus domesticus*; Selection; Immunocytochemistry;

Arginine-vasopressin (AVP) is a homeostatic modulator of body temperature during fever and may also be involved in normal body temperature control. In the present study the hypothalamus of mice bi-directionally selected for thermoregulatory nest-building behavior was immunocytochemically labeled for AVP. The low-selected mice had a 1.5-fold higher number of AVP-immunoreactive neurons in the suprachiasmatic nuclei (SCN) compared to the unselected control and the high-selected mice. No differences between the selected lines could be detected in the number of AVP-immunoreactive neurons in the paraventricular nuclei (PVN). The neuroanatomical data suggest a possible role of AVP in the SCN and control of thermoregulatory nest-building behavior. Our selected mice may prove to be a model system to study the role of AVP in the SCN.

The hypothalamus plays a key role in thermoregulatory control<sup>1</sup>. Additionally, arginine-vasopressin (AVP) is a homeostatic modulator of body temperature during fever<sup>6</sup> and Epstein et al. proposed that AVP may also be involved in normal body temperature control<sup>4</sup>. One useful approach for studying the relationship between AVP and thermoregulation is to examine AVP-immunoreactivity in the hypothalamus of widely divergent genetic strains for thermoregulation. We have such house mouse strains, selected for differences in thermoregulatory nest-building behavior<sup>7,8</sup>, available in our laboratory.

Forty-nine generations of bi-directional selection for thermoregulatory nest-building behavior have resulted in a 40-fold difference in the amount of cotton used for a nest between the low- and high-selected mouse lines. The unselected control lines show intermediate values (Fig. 1). Lower ambient temperatures induce higher nest-building behavior in all selected lines<sup>7</sup>. However, differences in nest-building behavior between the selected lines are maintained across changes in ambient temperature<sup>7</sup>. This suggests a different temperature threshold for the expression of nest-building behavior in these different lines. Schneider and Lynch have also proposed a difference in thermal set-point controlling prog-

esterone-mediated maternal nesting in these lines<sup>13</sup>. Importantly, the selected lines also differ in normal body temperature. The high-selected mice have a higher body temperature than control mice which in turn have a higher body temperature than low-selected mice<sup>13</sup>. Body temperature in mice is expressed in a circadian pattern with high temperatures during the night and low temperatures during the day<sup>13</sup>. In rats, this circadian rhythm is abolished after lesion<sup>3,12</sup> or isolation<sup>15</sup> of the suprachiasmatic nuclei (SCN), although in the squirrel monkey SCN lesions fail to abolish body temperature rhythms<sup>5</sup>.

In the present study, we employ bi-directionally selected mice for thermoregulatory nest-building behavior to determine if AVP differences exist in the hypothalamus.

Thirty male house mice, *Mus domesticus*, bi-directionally selected in our laboratory for differences in nest-building behavior, were used. The mice were kept under a 16:8 h light–dark cycle (lights on at 7.00 h), and each mouse was tested for nest-building behavior (after the methods of Lynch<sup>8</sup>). Nest-building behavior is expressed as the total nesting score, which is the total amount of cotton used for 4 consecutive nests during a 4-day testing period.

Within a week after nest-building behavior was mea-

sured, at an age of  $54 \pm 3$  days, the mice were killed and each brain prepared for immunocytochemistry, as described below. Ten male mice from the low, high or control lines received (alternately between 10.30 h and 16.30 h) an overdose of sodium pentobarbital (100 mg/kg, i.p.) and were transcardially perfused with heparinized saline (6,000 U heparin/l) for 1 min followed by a 12 min perfusion with a phosphate buffered (pH 7.4) paraformaldehyde solution (3%) at a perfusion rate of 7 ml/min. The brains were removed and cryo-protected in 30% buffered sucrose at 4°C. The brain was cut at the level of the hypothalamus into 20  $\mu$ m frontal sections on

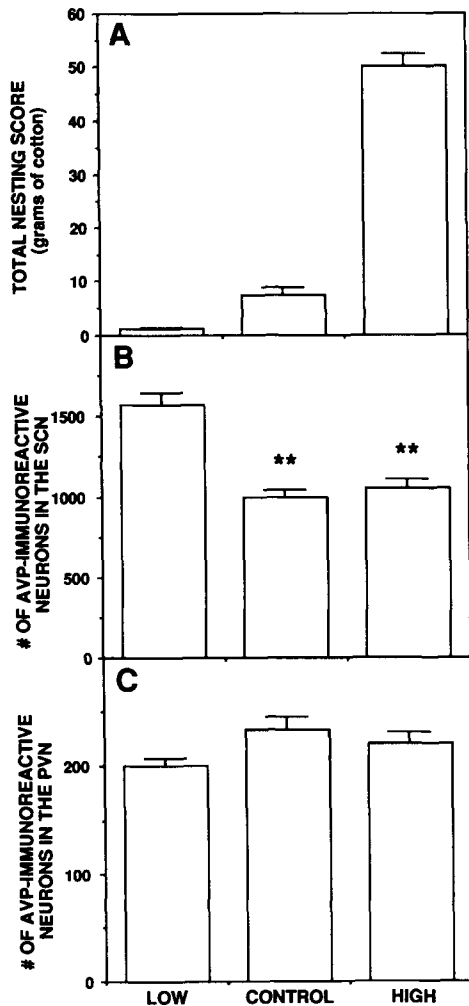


Fig. 1. The means  $\pm$  S.E.M. of 10 male mice of the low-selected, control, and high-selected lines for: A: the total nesting score. The selected lines diverged in nest-building behavior significantly after 15 generations of selection<sup>8</sup> and have been distinctly different since<sup>7</sup>. B: the number of arginine-vasopressin (AVP)-immunoreactive neurons in 6 frontal sections through the suprachiasmatic nuclei (SCN). One-way ANOVA:  $F_{2,27} = 29.600$ ,  $P < 0.001$ . The pair-wise comparisons, using the Tukey-Kramer method, yielded significant differences between the low- and high-selected lines and low-selected and control lines:  $**P < 0.01$ . C: the number of AVP-immunoreactive neurons in one frontal section medially through the paraventricular nuclei (PVN). One-way ANOVA:  $F_{2,27} = 2.798$ , NS.

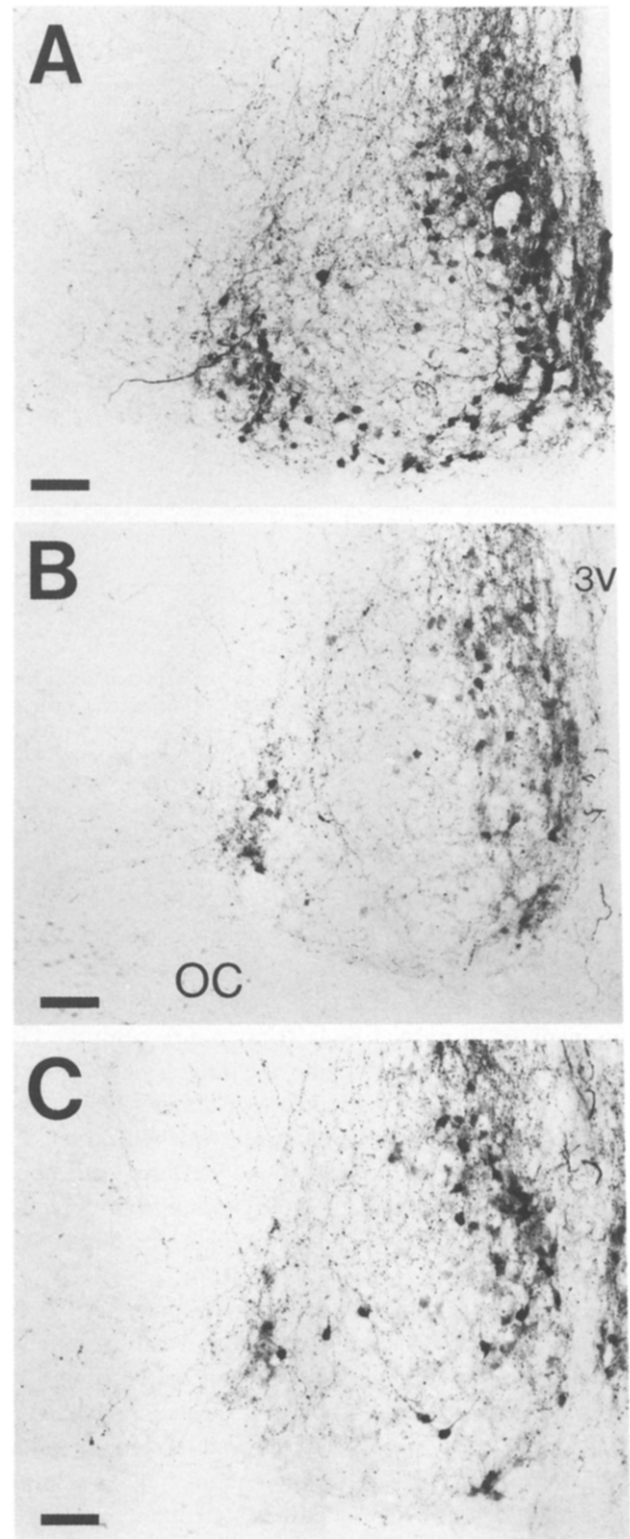


Fig. 2. Photomicrographs of AVP-immunoreactive neurons in a frontal section through the medial portion of the SCN of a low-selected (A), control (B) and high-selected (C) mouse. Compared to the control and high-selected lines, the low-selected lines show significantly higher numbers of AVP-immunoreactive neurons in the ventro-lateral and dorso-medial areas of the SCN as well as a denser axonal plexus of AVP-fibers throughout the SCN. AVP-immunoreactive neurons are absent in the central area at this level in the SCN. 3V, third ventricle; OC, optic chiasm. Bar = 50  $\mu$ m.

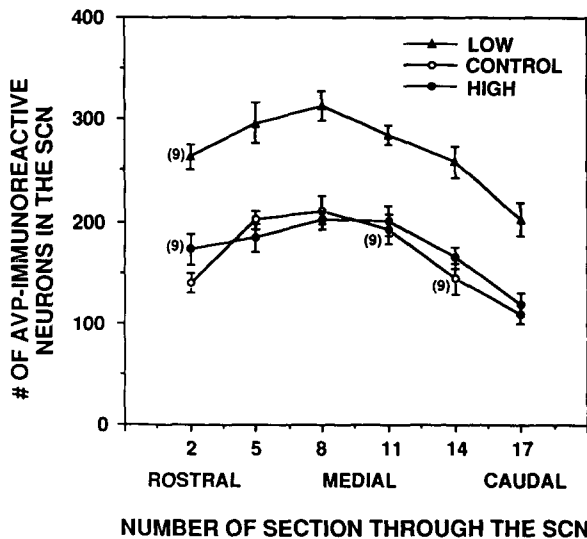


Fig. 3. The means  $\pm$  S.E.M. of 10 male mice (unless stated otherwise) of the low-selected, control and high-selected lines for the total number of AVP-immunoreactive neurons at different levels of the SCN from rostral to caudal.

a cryostat. For immunocytochemistry, the brain sections were rinsed in phosphate buffered saline (pH 7.4), pre-incubated with normal goat serum (5%) and incubated with the primary polyclonal IgG antibody rabbit anti-AVP (1:1,000) overnight at 4°C. The antibody was graciously supplied by Dr. R.M. Buijs of the Netherlands Institute for Brain Research. Subsequently, the sections were thoroughly rinsed, pre-incubated with normal goat serum (5%), and incubated with goat anti-rabbit IgG (Zymed; 1:100) for 2 h at room temperature. After rinsing, the sections were exposed to rabbit peroxidase anti-peroxidase (PAP) (Dakopatts; 1:500) for 2 h at room temperature. Finally, the tissue sections were processed with diaminobenzidine (DAB).

We quantified AVP in the hypothalamus by counting the number of AVP-immunoreactive neurons in the SCN in every third frontal section containing the SCN to a total of 6 sections per brain. The number of AVP-immunoreactive neurons in the paraventricular nuclei (PVN) were also counted in one medial section through the PVN.

Differences in the number of AVP-immunoreactive neurons between the selected lines were tested for significance using a one-way analysis of variance (ANOVA) with selected line as factor. When significance was found, pair-wise differences were tested for significance using the Tukey-Kramer method<sup>14</sup>. Differences in the number of AVP-immunoreactive neurons in the SCN of mice sacrificed between 10.30 h and 13.30 h compared to between 13.30 and 16.30 were tested for significance using a two-way ANOVA with perfusion time and selected line as factors.

In the medial portion of the SCN AVP-immunoreactive neurons are mainly located in the ventro-lateral and dorso-medial areas of the SCN, whereas these neurons are absent in the central area at this level in the SCN (Fig. 2). Most rostrally and most caudally the AVP-immunoreactive neurons are evenly distributed throughout the SCN. The mean total number of AVP-immunoreactive neurons in the SCN in 6 frontal sections through the SCN of 10 low-selected, control, and high-selected male mice are  $1577 \pm 70$  (S.E.M.),  $999 \pm 47$  (S.E.M.) and  $1052 \pm 57$  (S.E.M.), respectively, revealing a 1.5-fold difference between the low-selected mice compared to the control and high-selected mice (Fig. 1). The number of AVP-immunoreactive neurons in the SCN of the low-selected mice is increased throughout the SCN (from rostral to caudal) compared to the control and high-selected mice (Fig. 3). Selection for nest-building behavior appears to have resulted in a correlated response increasing the number of AVP-immunoreactive neurons in the SCN in the direction of low nesting.

The time of perfusion did not have an effect on the number of detectable AVP-immunoreactive neurons in the SCN (perfused between 10.30 h and 13.30 h compared to between 13.30 h and 16.30 h; two-way ANOVA, indicating an effect of selected line [ $F_{1,24} = 29.36$ ,  $P < 0.001$ ] but no effect of perfusion time [ $F_{1,24} = 0.690$ , NS] and no interaction [ $F_{1,24} = 1.05$ , NS]).

In contrast to the SCN, the number of AVP-immunoreactive neurons in the PVN did not differ significantly between the selected lines (Fig. 1). Therefore, the differences observed in the number of AVP-immunoreactive neurons is not a general feature but a localized change within the SCN.

The role of AVP-immunoreactive neurons in the SCN of mammals is unknown. Selection for low nest-building behavior appears to have resulted in an increase in the number of AVP-immunoreactive neurons in the SCN (Fig. 1). Although the role of AVP in the control of the expression of nest-building behavior needs to be shown empirically, this correlated response suggests a possible role of AVP in the SCN in the control of thermoregulatory nest-building behavior, possibly through regulation of a thermoregulatory threshold controlling the expression of this behavior. Fig. 1 shows a large difference in the level of nest-building behavior between the control and high-selected mice but, surprisingly, no difference in the number of AVP-immunoreactive neurons in the SCN. However, other systems in the SCN, e.g. AVP-sensitive neurons, might account for the differences in nest-building behavior between the control and high-selected lines. The low-selected mice do not express any nest-building behavior. The complete elimination of the behavior may have required a change in both the

AVP and AVP-sensitive systems whereas a difference in the level of expression of the behavior, as observed between the control and high-selected mice, might only have required a change in the AVP-sensitive system in the SCN. We are currently studying the latter system.

The higher number of AVP-immunoreactive neurons in the SCN shown here and the lower body temperature of the low-selected mice compared to the control and high-selected mice<sup>13</sup> is consistent with the lowering effect of AVP on body temperature during fever<sup>6</sup> and supports Epstein et al.'s suggestion that AVP might be associated with normal body temperature control<sup>4</sup>.

The effect of tetrodotoxin on the release of AVP by the SCN in vitro<sup>2</sup> suggests that AVP neurons do not control nor are they a part of the pacemaker mechanism of the SCN, but should be viewed as an output system of the SCN. The differences in the number of AVP-immunoreactive neurons in the SCN in our selected lines may therefore also reflect differences in circadian rhythmicity between these lines. Preliminary data suggest differences between the selected lines in the entrainment

to the light-dark cycle and free-running period in constant darkness of running-wheel activity (A. Bult, unpublished data). We are currently investigating the differences in circadian rhythms between the selected lines in more detail.

The  $\tau$  mutant in the golden hamster<sup>10,11</sup> and selected lines for photo-responsiveness in the Djungarian hamster<sup>9</sup> have proven to be useful model systems in which gene differences are used to study circadian function. The role of AVP in the SCN remains unclear, but the behavioral and neuroanatomical differences between our selected lines potentially make them a model system to study the multi-functional role of the SCN in general (e.g. how the circadian mechanism integrates locomotor activity and behavioral thermoregulation) and the role of AVP in the SCN specifically.

We are very grateful to G. Robert Lynch, Frederick M. Cohan, Wolfgang Puchalski and Laurie Hiestand for their helpful comments on the manuscript. We thank R.M. Buijs for providing the AVP antibodies.

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