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## Projection from the prefrontal cortex to histaminergic cell groups in the posterior hypothalamic region of the rat. Anterograde tracing with *Phaseolus vulgaris* leucoagglutinin combined with immunocytochemistry of histidine decarboxylase

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We investigated the projection from the infralimbic division of the prefrontal cortex (area 25) to histaminergic neurons in the posterior hypothalamic area. *Phaseolus vulgaris*-leucoagglutinin (PHA-L) was injected in the prefrontal cortex of rats. Frozen brain sections were subjected to combined PHA-L and histidine decarboxylase (HDC)-peroxidase immunocytochemistry, using nickel-enhanced diaminobenzidine (blue reaction product) to visualize the transported PHA-L, and diaminobenzidine (brown reaction product) to visualize simultaneously the HDC-containing neurons. PHA-L-labeled fibers could be seen coursing in the capsula interna, leaving the telencephalon via the anterior thalamic radiation and the medial forebrain bundle. In the lateral and posterior hypothalamic areas, PHA-L-labeled fibers leave the medial forebrain bundle and traverse the nuclei containing HDC-immunoreactive neurons. Varicosities on the PHA-L-labeled fibers, the majority of which occur en passant, could be observed in close association with the HDC-immunoreactive neurons. The results suggest that the hypothalamic histaminergic neurons receive afferent synaptic input from neurons of the infralimbic division of the prefrontal cortex.

The neurochemistry, morphology and pattern of anatomical connectivity of the neuronal system in the central nervous system (CNS) containing the neurotransmitter candidate histamine<sup>6,19</sup> have recently received broad attention. Histaminergic perikarya occur only in the posterior hypothalamic region<sup>22,23,32,35</sup>, including the nucleus tuberalis magnocellularis (TM, nomenclature of Bleier et al.<sup>4</sup>), nucleus caudalis magnocellularis (CM) and nucleus caudalis magnocellularis postmammillaris (PCM). Histaminergic fibers form extensive plexuses in all regions of the CNS, with the highest fiber densities in the hypothalamus and in forebrain areas<sup>23</sup>. The organization of the ascending and descending projections from the histaminergic nuclei has been determined<sup>14,24,28</sup>. Both histaminergic perikarya and fibers have been examined at the ultrastructural level<sup>10,27,34,35</sup>. Colo-

calization of histamine with various other neuroactive substances has been suggested<sup>14,15,17,20,29,31,34</sup>. In sharp contrast with this wealth of data, very little is known of the afferent connections of the histaminergic neurons. The main reason for this lack of data is that the majority of the histaminergic neurons appear spread in a thin sheet at the ventrolateral surface of the posterior hypothalamus. This architecture severely restraints experimental approaches with retrogradely transported markers.

Our present knowledge of the afferent connections of the hypothalamic areas containing the histaminergic neurons is only indirect, i.e., in various anterograde tracing studies termination zones have been described in areas adjacent to or partially overlapping TM, CM and PCM, of fibers originating in the olfactory tubercle<sup>11</sup>, the subiculum<sup>3,16,25</sup>, the sep-

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tum<sup>26</sup>, the dorsal tegmental region<sup>8</sup>, and the prefrontal cortex<sup>2,18</sup>. Unfortunately, the nuclei containing histaminergic neurons were neither cytoarchitectonically nor chemically identified in these studies.

With the anterograde tracing technique based on injection, uptake and transport of the plant lectin *Phaseolus vulgaris*-leucoagglutinin (PHA-L), it is possible to study in detail the cell population of origin as well as the course and the terminations of nerve fibers in the CNS<sup>7,30,33</sup>. With a recently developed double-label immunocytochemical technique for visualizing anterogradely transported PHA-L and, simultaneously, neurons containing histidine decarboxylase, which is the rate-limiting synthesizing enzyme of histamine<sup>36,37</sup>, it is possible to determine the afferent connections of histaminergic neurons. Since the projections of the infralimbic division of the prefrontal cortex to the posterior hypothalamic region are well documented<sup>2,18</sup>, we chose to study this possible input to the histaminergic neurons.

We used 22 young adult female Wistar rats (180 g b. wt.). Under deep anesthesia, *Phaseolus vulgaris* leucoagglutinin (PHA-L; Vector Labs, U.S.A.; 25  $\mu\text{g}/\mu\text{l}$ ) dissolved in 50 mM Tris-buffered saline, pH 7.4, was injected in the infralimbic cortex (area 25) through glass micropipettes (tip diameter 10–20  $\mu\text{m}$ ), using a positive-pulsed 6- $\mu\text{A}$  DC current (7 s on/7 s off) for 15–30 min. Five to 8 days after surgery, the rats were reanesthetized with sodium pentobarbital and perfused transcardially at a constant hydrostatic pressure<sup>13</sup> with 100 ml of physiological saline solution, of 38 °C, pH 6.9, followed immediately by 1500 ml of a mixture of 4% freshly depolymerized paraformaldehyde, 0.1% glutaraldehyde and 0.2% picric acid in 125 mM phosphate buffer, pH 7.3 (room temperature). Frozen sections of 40  $\mu\text{m}$  thickness were incubated according to a double peroxidase immunocytochemical procedure<sup>36,37</sup>. The sections were incubated in goat anti-PHA-L (Vector Labs, U.S.A. 1:8000) mixed with rabbit anti-histidine decarboxylase (anti-HDC, kindly provided by Drs. T. Watanabe and H. Wada, Tohoku University School of Medicine and Osaka University School of Medicine, Japan; final concentration 1:3000) in Tris-buffered saline (TBS) to which 0.5% Triton X-100 (Sigma, St. Louis, U.S.A.) was added. The incubation was continued with a mixture of donkey anti-goat whole serum and swine anti-rabbit whole serum

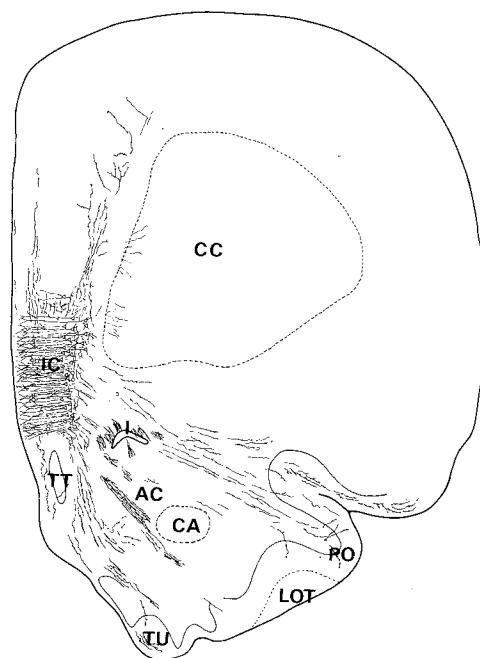


Fig. 1. Drawing of a transverse section through the site of PHA-L injection in the infralimbic cortex (IC), in a representative case (86183). Double-peroxidase immunocytochemistry for PHA-L and HDC. At this level, no HDC-immunoreactive structures are visible. The triangles and circles indicate PHA-L-labeled pyramidal and non-pyramidal neurons, respectively. PHA-L-labeled fibers can be seen intracortically, running towards the basal forebrain, and in the capsula interna. AC, nucleus accumbens; CA, commissura anterior; CC, corpus callosum; IC, infralimbic cortex; LOT, lateral olfactory tract; PO, primary olfactory cortex; TT, taenia tecta; TU, tuberculum olfactorium; I, lateral ventricle.

(Nordic Immunology, Tilburg, Netherlands; both 1:50, final concentration). Finally, the sections were incubated for 1 h with goat peroxidase-antiperoxidase (goat PAP, Nordic, 1:400) and reacted with Ni-enhanced diaminobenzidine<sup>9</sup>. After rinsing in TBS, the sections were incubated in rabbit-PAP (Nordic, 1:500 in TBS with 0.5% Triton X-100, pH 7.6) and reacted with diaminobenzidine. PHA-L-labeled fibers appear dark blue to black, while the HDC-immunoreactive cell bodies and dendrites show a light brown color. HDC-immunoreactive fibers, except in the hypothalamus, remain unstained. In parallel incubations, instead of the pooled primary antisera, we used control antisera: goat anti-PHA-L only, or rabbit anti-HDC only. Sections were also incubated in TBS without primary antisera. After the immunocytochemistry, the sections were mounted, dehydrated

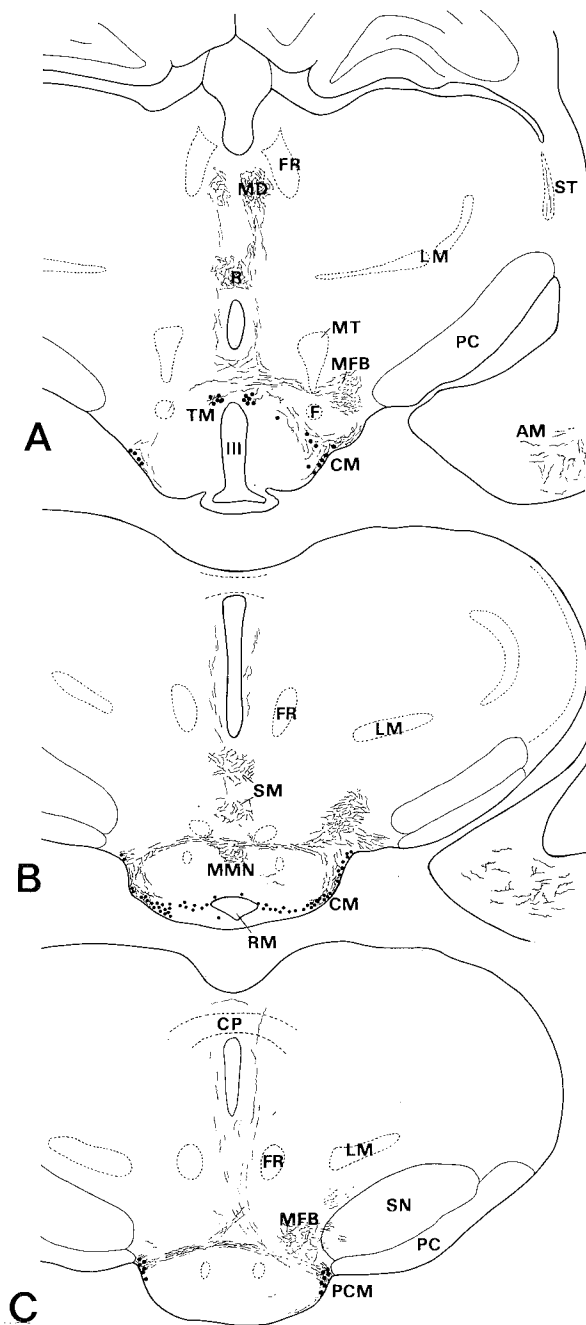


Fig. 2. Chartings of PHA-L-labeled fibers (fibers) and HDC-immunoreactive cell bodies (black dots) in transverse sections at 3 rostrocaudal (A–C) levels through the posterior hypothalamic region. Double-peroxidase immunocytochemistry for PHA-L and HDC, following PHA-L injection in the infralimbic cortex (rat 86183). AM, amygdaloid complex; CM, nucleus caudalis magnocellularis; CP, commissura posterior; F, fornix; FR, fasciculus retroflexus; LM, lemniscus medialis; MD, mediodorsal nucleus; MFB, medial forebrain bundle; MMN, medial mammillary nucleus; MT, mamillothalamic tract; PC, pedunculus cerebri; PCM, nucleus postmammillaris magnocel-

and coverslipped. Some sections were counterstained with Cresyl violet.

The majority of the injections (16 out of 22) appeared to be centered in the deep layers of the infralimbic cortex (area 25; Fig. 1). We restrict the present report to the PHA-L-labeled fibers leaving the telencephalon through the anterior thalamic radiation and the medial forebrain bundle (MFB). Most PHA-L-labeled fibers in the anterior thalamic radiation reach the mediodorsal nucleus and the anterior portion of the nucleus reuniens of the thalamus, and form dense terminal arborizations in both nuclei.

In the dorsal hypothalamic area, a cluster of histidine decarboxylase (HDC)-immunoreactive perikarya is present at the dorsal tip of the third ventricle (TM<sup>4</sup>, located ventral to the caudal portion of nucleus reuniens thalami, Fig. 2A). In the same transverse plane through the diencephalon, HDC-immunoreactive neurons appear near the hypothalamic pial surface. This is the rostralmost extension of the CM (Figs. 2A, B and 3A). At more caudal levels, the CM consists of a lamina of HDC-immunoreactive neurons located directly beneath the hypothalamic pial surface. The caudal part of the CM is continuous with the PCM (Fig. 3C), of which the main portion lies directly underneath the pial surface, lateral and slightly dorsal to the lateral mammillary nucleus. The PCM further extends to the caudalmost pole of the postmammillary nucleus. A few solitary HDC-immunoreactive neurons are present in the area between the TM and CM. The HDC-immunoreactive neurons are mostly bi- or tripolar with dendrites up to 150–200  $\mu\text{m}$  long (Fig. 3C, D; see also ref. 35). In the hypothalamus, a weakly stained plexus of HDC-immunoreactive fibers can be observed.

Along their trajectories in the MFB, the PHA-L-labeled fibers continuously give off collaterals and *en passant* and *terminal* varicosities. Bundles of fibers diverge from the MFB and reach the thalamus and the lateral, dorsal and posterior hypothalamic areas. The thalamic nuclei receiving MFB-fibers are the mediodorsal nucleus (Fig. 2A), which receives also PHA-L-labeled fibers via the anterior thalamic ra-

lularis; R, nucleus reuniens thalami; RM, recessus mammillaris of the third ventricle; SM, nucleus supramammillaris; SN, substantia nigra; ST, stria terminalis; TM, nucleus tuberalis magnocellularis; III, third ventricle.

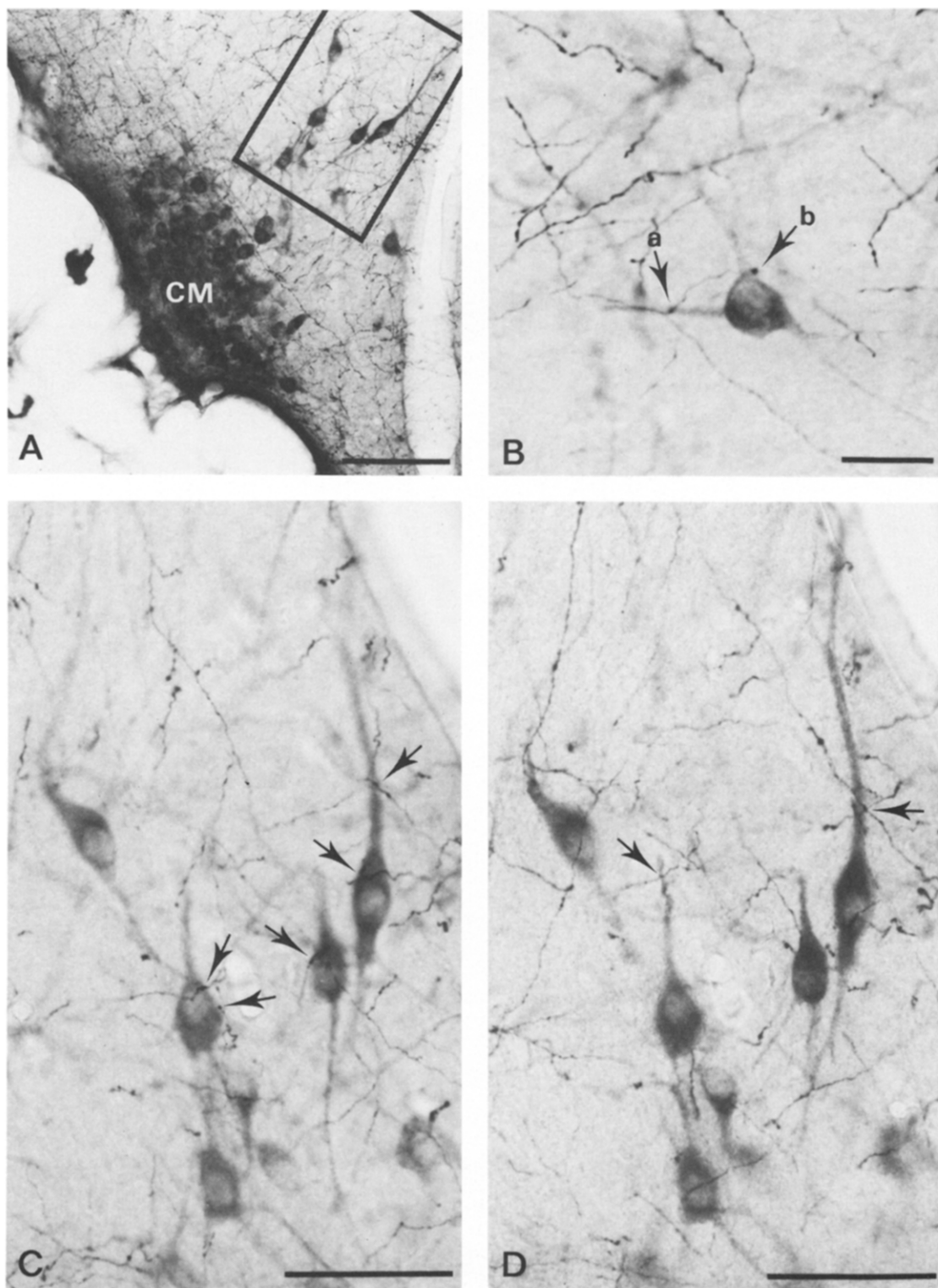


Fig. 3. A: transverse section through the rostral portion of the nucleus caudalis magnocellularis (CM). Double-peroxidase immunocytochemistry for PHA-L and HDC, following PHA-L injection in the infralimbic cortex. The cell bodies show HDC-immunoreactivity (DAB reaction product, brown), while all the fibers are PHA-L-labeled (nickel-enhanced DAB reaction product, blue). Notice that the main part of the CM receives relatively few PHA-L-labeled fibers. The boxed area is shown in C, D. Bar = 100  $\mu$ m. B: HDC-immunoreactive neuron in the TM. A PHA-L-labeled fiber forms an *en passant* varicosity (a) in close association with a dendrite, and forms also a *terminal* varicosity (b) in close apposition to the perikaryon of this neuron. Bar = 25  $\mu$ m. C and D: micrographs focused at two different levels of the same section (boxed area A), showing the delicate network of PHA-L-labeled fibers in the lateral hypothalamic area. At the sites indicated by an arrow, an *en passant* varicosity is present on the PHA-L-labeled fibers, in close association with a perikaryon or a dendrite of a HDC-immunoreactive neuron. Bar = 50  $\mu$ m.

diation, the caudal portion of the nucleus reuniens (Fig. 2A) and the nucleus gelatinosus. Beyond the hypothalamus, the MFB continues into the ventral tegmental area.

PHA-L-labeled fibers, branching from the MFB, are abundantly present in the TM, and form en passant varicosities in close proximity to the HDC-immunoreactive cell bodies and their dendrites (Fig. 3B). These close associations strongly suggest the presence of synaptic contacts. In the lateral and posterior hypothalamic regions, the majority of the PHA-L-labeled fibers form an extensive, loose plexus, with the highest density in a zone subjacent to the brain surface. This zone extends from the caudal border of the supraoptic nucleus to the caudalmost border of the mammillary body, with an interruption ventral to the mammillary recess of the third ventricle, and covers parts of the CM, and the entire PCM. The rostral and dorsal parts of the CM receive numerous PHA-L-labeled fibers of which many form en passant varicosities in the close proximity to HDC-immunoreactive perikarya and dendrites (Figs. 3A, C, D). The ventral half of the CM and a less dense bridge of HDC-immunoreactive cells between the main portion of the CM and the lateral sulcus of the mammillary recess of the third ventricle receive relatively few PHA-L-labeled fibers. The medial part of the medial mammillary nucleus and the supramammillary nucleus receive a dense innervation by PHA-L-labeled fibers (Fig. 2B). Labeled fibers cross also in the supramammillary decussation and innervate the contralateral PCM (Fig. 2C). In the PCM, numerous en passant varicosities on the labeled fibers closely appose HDC-immunoreactive perikarya or dendrites. The lateral and caudal aspects of the posterior mammillary nucleus are surrounded by a thin layer of PHA-L-labeled fibers.

The results of the present tracing study are in general agreement with previous autoradiographic tracing studies<sup>2,18</sup>. The small differences between these and our findings, e.g., the appearance of scattered PHA-L-labeled fibers in the stria terminalis, can be explained by the circumstance that by PHA-L-labeling individual fibers are directly labeled, with little or no background, while the autoradiographic technique is based on indirect detection, i.e., on exposing silver halide crystals to radiation emitted by fibers containing radioactive label. To raise the signal to a

significant detection level with this technique, a sufficient amount of label is necessary, often only obtained by the combined radioactivity emitted by several fibers<sup>30</sup>.

In the present study we observed that PHA-L-labeled fibers originating in the infralimbic cortex form varicosities in the lateral and posterior hypothalamus in close apposition with HDC-immunoreactive neurons. Recently it has been observed electron microscopically that varicosities of PHA-L-labeled fibers may contain synaptic vesicles and presynaptic membrane specializations<sup>33</sup>. Thus, it is likely that the varicosities of the PHA-L-labeled fibers seen in the light microscope actually represent synaptic axon terminals. It must be noted that the far majority of the varicosities on the PHA-L-labeled fibers were seen en passant in close vicinity to HDC-immunoreactive cell bodies and dendrites. Complex or specialized terminations in close proximity to HDC-immunoreactive neurons were not observed. The number of PHA-L-labeled varicosities apposing HDC-immunoreactive profiles is only a fraction of the total number of observed varicosities on PHA-L-labeled fibers in this area. Further, among the HDC-immunoreactive neurons of the lateral and posterior hypothalamic regions, those located in the TM and the PCM seem to receive the most extensive fiber input from the infralimbic cortex. A major portion of the CM remains virtually free of PHA-L-labeled fibers. Thus, only part of the histaminergic hypothalamic neurons seems to receive synaptic input of prefrontal cortical origin. Taken together, this suggests that HDC-immunoreactive neurons are not specific targets of the prefrontohypothalamic fibers. We must therefore assume that, in addition to histaminergic neurons, also non-histaminergic neurons in the posterior hypothalamic area receive synaptic contacts of prefrontohypothalamic fibers. While the chemical identity of the majority of the neurons present in the posterior hypothalamus is still undocumented, immunoreactivity against glutamic acid decarboxylase<sup>31,34</sup>, adenosine deaminase<sup>17,20</sup>, galanin<sup>15</sup>, substance P and Met-enkephalyl-Arg<sup>6</sup>-Phe<sup>7</sup> heptapeptide<sup>14</sup> has been observed in neurons in the termination area of the PHA-L-labeled fibers. In a number of these neurons, however, these substances appear to be co-localized with HDC<sup>14,15,17,20,29</sup>.

It is apparent from previous studies<sup>23,24,28,32</sup> that

the histaminergic fibers form extensive networks in the telencephalon. It is further assumed that histamine has widespread neuromodulatory effects<sup>19</sup>. Following intracerebral injections of histamine, a sedative effect has been measured on the electroencephalogram<sup>5</sup>. Also normal locomotor activity<sup>1</sup> or methamphetamine-induced locomotor hyperactivity<sup>12</sup> can be suppressed by injection of histamine. Further, in hippocampal slice preparations, histamine perfusion reduces the inhibitory postsynaptic potential of pyramidal cells<sup>21</sup>. Since it is likely that the varicosities formed between the PHA-L-labeled prefrontohypo-

thalamic fibers and the HDC-immunoreactive neurons represent synaptic terminals<sup>33</sup>, the infralimbic cortex, by its anatomical connections, may stimulate or regulate the activity of histaminergic neurons.

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- Alvarez, E.O. and Guerra, F.A., Effects of histamine microinjections into the hippocampus on open-field behavior in rats, *Physiol. Behav.*, 28 (1982) 1035–1040.
- Beckstead, R.M., An autoradiographic examination of corticocortical and subcortical projections of the mediodorsal projection (prefrontal cortex) in the rat, *J. Comp. Neurol.*, 184 (1979) 43–62.
- Beech, J.N. and Raisman, G., Extended projections induced by partial deafferentation of the medial mammillary nucleus, *Brain Research*, 188 (1980) 347–355.
- Bleier, R., Cohn, P. and Siggelkow, I.R., A cytoarchitectonic atlas of the hypothalamus and hypothalamic third ventricle of the rat. In P.J. Morgane and J. Panksepp (Eds.), *Anatomy of the Hypothalamus, Vol. 1*, Dekker, New York, 1979, pp. 137–220.
- Delbarre, B. and Delbarre, G., Similar effects of clonidine and histamine on the central nervous system, *Soc. Neurosci. Abstr.*, 11 (1985) 51.
- Garbarg, M., Barbin, G., Bisschoff, S., Pollard, H. and Schwartz, J.C., Dual localization of histamine in an ascending pathway and in non-neuronal cells evidenced by lesions in the lateral hypothalamic area, *Brain Research*, 106 (1976) 333–348.
- Gerfen, C.R. and Sawchenko, P.E., An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, *Phaseolus vulgaris*-leucoagglutinin, *Brain Research*, 290 (1984) 219–238.
- Groenewegen, H.J. and Van Dijk, C., Efferent connections of the dorsal tegmental region in the rat, studied by means of anterograde transport of the lectin *Phaseolus vulgaris*-leucoagglutinin (PHA-L), *Brain Research*, 304 (1984) 367–371.
- Hancock, M.B., Visualization of peptide-immunoreactive processes on serotonin-immunoreactive cells using two-color immunoperoxidase staining, *J. Histochem. Cytochem.*, 32 (1984) 311–314.
- Hayashi, H., Takagi, H., Takeda, N., Kubota, Y., Tohyama, M., Watanabe, T. and Wada, H., Fine structure of histaminergic neurons in the caudal magnocellular nucleus of the rat as demonstrated by immunocytochemistry using histidine decarboxylase as a marker, *J. Comp. Neurol.*, 229 (1984) 233–241.
- Heimer, L., The olfactory connections of the diencephalon in the rat, *Brain Behav. Evol.*, 6 (1972) 484–523.
- Itoh, Y., Nishibori, M., Oishi, R. and Saeki, K., Neuronal histamine inhibits methamphetamine-induced locomotor hyperactivity in mice, *Neurosci. Lett.*, 48 (1984) 305–309.
- Jonkers, B.W., Sterk, J.C. and Wouterlood, F.G., Transcardial perfusion fixation of the CNS by the use of a compressed-air driven device, *J. Neurosci. Methods*, 12 (1984) 141–149.
- Köhler, C., Swanson, L.W., Haglund, L. and Wu, J.-Y., The cytoarchitecture, histochemistry and projections of the tuberomammillary nucleus in the rat, *Neuroscience*, 16 (1985) 85–110.
- Köhler, C., Erickson, H., Watanabe, T., Polak, J., Palay, S.L., Palay, V. and Chan-Palay, V., Galanin immunoreactivity in hypothalamic histamine neurons: further evidence for multiple chemical messengers in the tuberomammillary nucleus, *J. Comp. Neurol.*, 250 (1986) 58–64.
- Nauta, W.J.H., An experimental study of the fornix system in the rat, *J. Comp. Neurol.*, 104 (1956) 247–271.
- Patel, B.T., Tudball, N., Wada, H. and Watanabe, T., Adenosine deaminase and histidine decarboxylase coexist in certain neurons of the rat brain, *Neurosci. Lett.*, 63 (1986) 185–189.
- Room, P., Russchen, F.T., Groenewegen, H.J. and Lohman, A.H.M., Efferent connections of the prelimbic (area 32) and infralimbic (area 25) cortices: an anterograde tracing study in the cat, *J. Comp. Neurol.*, 242 (1985) 40–55.
- Schwartz, J.-C., Pollard, H. and Quach, T.T., Histamine as a neurotransmitter in mammalian brain: neurochemical evidence, *J. Neurochem.*, 35 (1980) 26–33.
- Senba, E., Daddona, P.E., Watanabe, T., Wu, J.-Y. and Nagy, J.I., Coexistence of adenosine deaminase, histidine decarboxylase, and glutamate decarboxylase in hypothalamic neurons of the rat, *J. Neurosci.*, 5 (1985) 3393–3402.
- Springfield, S.A. and Geller, H.M., Effect of histamine on IPSPs in rat hippocampal CA1 pyramidal neurons, *Soc. Neurosci. Abstr.*, 11 (1985) 575.
- Steinbusch, H.W.M. and Mulder, A.H., Immunohistochemical localization of histamine in neurons and mast cells in the rat brain. In A. Björklund, T. Hökfelt and M.J. Kuhar (Eds.), *Handbook of Chemical Neuroanatomy, Vol. 3, Classical transmitters and transmitter receptors*, Elsevier, Amsterdam, 1984, pp. 126–140.

- 23 Steinbusch, H.W.M. and Mulder, A.H., Localization and projections of histamine-immunoreactive neurons in the central nervous system of the rat. In J. Gannelin and J.-C. Schwartz (Eds.), *Frontiers in Histamine Research, Advances in Biosciences, Vol. 51*, Pergamon, Oxford, 1985, pp. 119–131.
- 24 Steinbusch, H.W.M., Sauren, Y., Groenewegen, H., Watanabe, T. and Mulder, A.H., Histaminergic projections from the premammillary and posterior hypothalamus region to the caudate putamen complex in the rat, *Brain Research*, 368 (1986) 389–393.
- 25 Swanson, L.W. and Cowan, M.W., An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat, *J. Comp. Neurol.*, 172 (1977) 49–84.
- 26 Swanson, L.W. and Cowan, M.W., The connections of the septal region in the rat, *J. Comp. Neurol.*, 186 (1979) 621–656.
- 27 Takagi, H., Morishawa, Y., Matsuyama, T., Hayashi, H., Watanabe, T. and Wada, H., Histaminergic axons in the neostriatum and cerebral cortex of the rat. A correlated light and electron microscopic immunocytochemical study using histidine decarboxylase as a marker, *Brain Research*, 364 (1986) 114–123.
- 28 Takeda, N., Inagaki, S., Taguchi, Y., Tohyama, M., Watanabe, T. and Wada, H., Origins of histamine-containing fibers in the cerebral cortex of rats studied by immunohistochemistry with histidine decarboxylase as a marker and transection, *Brain Research*, 323 (1984a) 55–63.
- 29 Takeda, N., Inagaki, S., Shiosaka, S., Taguchi, Y., Oertel, W.H., Tohyama, M., Watanabe, T. and Wada, H., Immunohistochemical evidence for the coexistence of histidine decarboxylase-like and glutamate decarboxylase-like immunoreactivities in nerve cells of the magnocellular nucleus of the posterior hypothalamus of rats, *Proc. Natl. Acad. Sci. U.S.A.*, 81 (1984b) 7647–7650.
- 30 Ter Horst, G.J., Groenewegen, H.J., Karst, H. and Luiten P.G.M., *Phaseolus vulgaris*-leucoagglutinin immunohistochemistry. A comparison between autoradiographic and lectin tracing of neuronal efferents, *Brain Research*, 307 (1984) 379–383.
- 31 Vincent, S.R., Hökfelt, T. and Wu, J.-Y., GABA neuron systems in hypothalamus and the pituitary gland. Immunohistochemical demonstration using antibodies against glutamate decarboxylase, *Neuroendocrinology*, 34 (1982) 117–125.
- 32 Watanabe, T., Tachugi, Y., Hayashi, H., Tanaka, J., Shiosaka, S., Tohyama, M., Kubota, H., Terano, Y. and Wada, H., Evidence for the presence of histaminergic neuron system in the rat brain: an immunohistochemical analysis, *Neurosci. Lett.*, 39 (1983) 249–254.
- 33 Wouterlood, F.G. and Groenewegen, H.J., Neuroanatomical tracing by use of *Phaseolus vulgaris*-leucoagglutinin (PHA-L): electron microscopy of PHA-L-filled neuronal somata, dendrites, axons and axon terminals, *Brain Research*, 326 (1985) 188–191.
- 34 Wouterlood, F.G., The ultrastructure of histaminergic neurons in rat brain: immunoelectron microscopy and Golgi-electron microscopy. In H.W.M. Steinbusch (Ed.), *Monoaminergic neurons at the light and electron microscopic level, IBRO Handbook series: Methods in the Neurosciences, Vol. 9*, Wiley, Chichester, 1986 pp. 315–339.
- 35 Wouterlood, F.G., Sauren, Y.M.H.F. and Steinbusch, H.W.M., Histaminergic neurons in rat brain: correlative immunocytochemistry, Golgi impregnation and electron microscopy, *J. Comp. Neurol.*, 252 (1986) 227–244.
- 36 Wouterlood, F.G., Steinbusch, H.W.M. and Bol, J.G.J.M., Anterograde neuroanatomical tracing with *Phaseolus vulgaris*-leucoagglutinin (PHA-L), combined with labeling of the target neurons by peroxidase- or alkaline phosphatase immunocytochemistry, *Abstr. Soc. Neurosci.*, 12 (1986) 444.
- 37 Wouterlood, F.G., Steinbusch, H.W.M. and Bol, J.G.J.M., Double-label immunocytochemistry: combination of anterograde neuroanatomical tracing with *Phaseolus vulgaris*-leucoagglutinin (PHA-L) and enzyme immunocytochemistry of the target neurons, *J. Histochem. Cytochem.*, submitted.