d.f. = 3, 60; $P > .10$) was significant. We recognize that across-age comparisons are tenuous because of difficulties in equating motivational or activity consequences of a fixed period of deprivation. Nonetheless, even the youngest animals learned the tasks. Lack of age-related improvements does not equate the associative capacities of 7- and 21-day-old animals. Lack of improvement may be task-specific and differences may emerge with more complex tasks.

With regard to retention, all age groups (7, 12, 17, and 21 days) exhibited significantly fewer trials to criterion upon retesting after a 2-day interval. A repeated-measures analysis of variance showed that the task factor was highly significant ($F = 44.17$; d.f. = 2, 28; $P < .001$), but that neither age ($F = 1.20$; d.f. = 3, 88; $P > .10$) nor the age-task interaction ($F = 1.53$; d.f. = 6, 88; $P > .10$) was significant. Post hoc analyses indicated no significant difference in trials to criterion for acquisition versus reversal (Sheffé, all P's $> .10$) but that significantly fewer trials to criterion were required for retention as compared with either acquisition or reversal at each age (Sheffé, all P's $< .01$). Moreover, significantly fewer trials to criterion were required by rats 7, 12, 17, and 21 days old on retention as compared with acquisition or reversal tasks for all age groups (Sheffé, all P's $< .01$). Savings are therefore attributable to retention rather than maturational improvement in learning ability. As before, statements regarding age-related improvements are inappropriate. In this case the only retention interval used was 2 days. Others (*1*, *10*) have found that retention does improve during ontogeny.

These data are noteworthy in at least two respects. (i) The nonlactating nipple provides sufficient incentive for initiating and maintaining learned performance; milk letdown is not a necessary reinforcer. Accordingly, we may now determine when and how the appetitive stimuli that support learning will change during the course of ontogeny. (ii) From the point of view of learning and retention, these data make clear that neonatal rats can form and retain a simple left-right discrimination. Whether they are capable of forming other associations remains open, as does the development of complex discriminations during ontogeny.

These considerations are relevant for identifying the functional relationships between developing neurological and neurochemical systems and emerging associative behaviors. Despite recent advances in developmental neurology (*3–5*) and neurochemistry (*2*), the relationships between these systems and the learning capacities of very young animals have not been delineated—in part, because of difficulties in identifying neonatal learning abilities. The present report has provided a potentially powerful behavioral assay.

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Prolactin-Like Immunoreactivity: Localization in Nerve Terminals of Rat Hypothalamus

Abstract. *Antibodies to rat prolactin were used in immunohistochemical studies of the hypothalamus and preoptic area of the rat. Evidence was obtained that a protein immunochemically related to prolactin was stored in networks of nerve terminals of many hypothalamic areas such as the arcuate nucleus, the dorsomedial hypothalamic nucleus, and periventricular regions of the hypothalamus and preoptic area. The neuronal storage of a prolactin-like protein in the hypothalamus was unaffected by hypophysectomy.*

Immunohistochemical studies have demonstrated the existence of luliberin-, thyrotropin-, and somatostatin-containing nerve terminals in various parts of the brain, particularly in the median eminence of the hypothalamus (*1*). The morphological studies support the view that these or closely related peptides may not only control the hormone secretion from the anterior pituitary but may act as transmitters or modulators in the central nervous system (*2*). It was therefore of interest to evaluate whether large proteins such as adenohypophyseal hormones could also be stored in nerve terminals in the central nervous system. Prolactin was chosen, since it has been identified in the cerebrospinal fluid of rabbits and rats (*3*). In the present investigation the hypothalamus was analyzed by the indirect immunofluorescence technique.

Male albino Sprague-Dawley rats (150 to 200 g) were used. In one experiment five rats were hypophysectomized 1 month earlier to remove all peripheral stores of prolactin. The rats were given Nembutal (60 mg per kilogram of body weight) intraperitoneally and were immediately perfused with 300 to 500 ml of ice-cold formalin through the aortic artery. The brains were removed and, after thorough rinsing, sections (10 μm thick) of the hypothalamus and preoptic area were cut on a cryostat. The immunohistochemical procedure was that described by Coons (*4*), including incubation with antibodies against rat prolactin [rat prolactin 5-A, diluted 1:16, supplied by the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD)], rinsing, incubation with fluorescein isothiocyanate–conjugated sheep antibodies against rabbit antiserum (diluted 1:4), rinsing, mounting in a mixture of buffer and glycerin, and examination in a Zeiss Junior fluorescence microscope. Prolactin antiserum that had been treated with rat prolactin served as control serum. Ovine prolactin does not cross-react with rat prolactin, and in some experiments prolactin antiserum was first treated with ovine prolactin or synthetic corticotropin (1-24; 1-10; 4-10) as a specificity test. According to the NIAMDD, the antibodies against rat prolactin show no cross-reactivity with follitropin, lutropin, thyrotropin, or somatotropin. As a further test for specificity, sections were also incubated with antibodies against follitropin, lutropin, thyrotropin, and

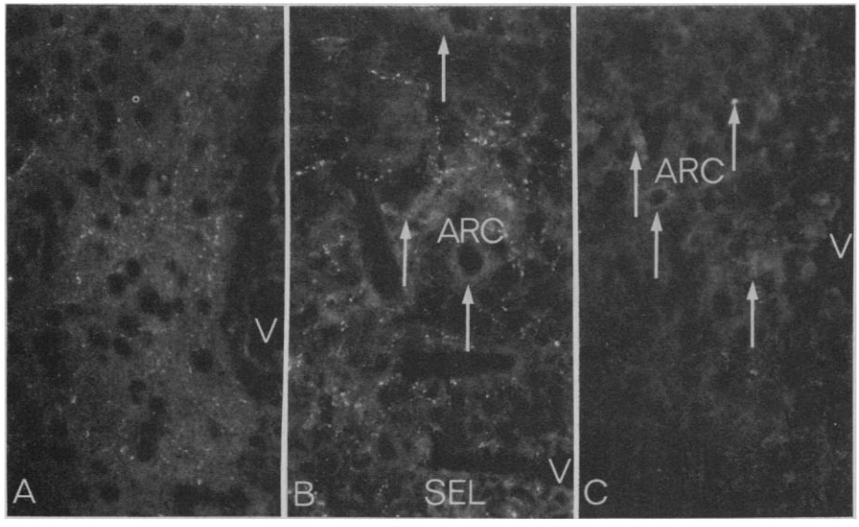

Fig. 1. Immunofluorescence micrographs of the rat dorsomedial hypothalamic nucleus (A) and of the rat arcuate nucleus (*ARC*) with the adjacent parts of the subependymal layer (*SEL*) of the median eminence (B). A plexus of prolactin-positive nerve terminals of medium density is seen in both pictures. Prolactin-positive terminals are present in the subependymal layer. In (C) the section from the arcuate nucleus has been incubated with prolactin antiserum that had first been treated with rat prolactin; no prolactin-positive terminals are present. Arrows point to nonspecific fluorescence; *V*, third ventricle (× 230).

somatotropin (supplied by NIAMDD). None of these antibodies cross-react with the other adenohypophyseal hormones mentioned above.

In the anterior and particularly the posterior periventricular region of the hypothalamus and the preoptic area, networks of varicose, fiber-like structures exhibiting prolactin-like immunoreactivity were observed. The diameter of the varicose enlargements mainly ranged from 0.5 to 1.5 μm and exhibited a strong immunofluorescence. Distinct networks of beaded fibers of medium density were also observed in the dorsomedial hypothalamic nucleus (Fig. 1A), the arcuate nucleus (Fig. 1B), the ventral hypothalamus, the subependymal and inner layers of the median eminence (Fig. 1B), the area ventral to the fornix, the ventral and especially dorsal premammillary nuclei, the preoptic suprachiasmatic nucleus, the area dorsal to the supraoptic nucleus, and the area immediately dorsal to the roof of the third ventricle. The density was low to moderate. Networks of medium density were also observed in the paraventricular rotundocellular thalamic nucleus, the supramammillary commissure, especially medial to the fasciculus mammillotegmentalis, and within Forel's field H_2.

None of the terminals described above were observed after treatment of prolactin antiserum with rat prolactin (Fig. 1C). However, the specific immunofluorescence did not disappear when the prolactin antiserum had first been treated with ovine prolactin or the synthetic corticotropins. Furthermore, the prolactin-like immunoreactive material was still present 1 month after hypophysectomy. No specific immunofluorescence was observed after incubation with antibodies against lutropin, follitropin, thyrotropin, or somatotropin.

The present results give evidence for the existence of hypothalamic nerve terminals containing a prolactin-like protein. The close approximity of this terminal system to the ventricles makes it likely that part of the prolactin in the cerebrospinal fluid (*3*) is derived from this neuronal pool of hypothalamic prolactin-like material. In agreement with the present morphological findings, Clemens *et al.* (*5*) have shown that prolactin-responsive neurons exist in the periventricular rabbit hypothalamus and preoptic area. Therefore, a prolactin-like protein may have a role in synaptic function. An interesting question that now arises is whether different neurons are involved (i) in the inhibitory feedback action of prolactin on its own secretion (*6*) and (ii) in the possible neurotransmitter role of prolactin-like proteins.

These results are evidence for the storage of prolactin-like material in nerve terminals of the mammalian brain and have implications for the role of such proteins in brain function. These initial data must be regarded with caution particularly since it is not known how other peptides, not yet tested, react with the present antiserum.

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Heteromorphic Sex Chromosomes in Male Rainbow Trout

Abstract. *A pair of subtelocentric chromosomes differs in the size of the short arm in male, but not female, rainbow trout (*Salmo gairdneri*). The morphological similarity of the X and Y chromosomes, and the observation of Y chromosomes intermediate between the X and normal Y, suggest that the sex chromosomes are at an early stage of differentiation in this species.*

Heteromorphic sex chromosomes are believed to evolve from morphologically identical homologs (*1*). Although most fish have undifferentiated sex chromosomes, a number of examples of heteromorphic sex chromosomes have been found (*2–4*). In the rainbow trout (*Salmo gairdneri*), I have found what appears to be an early stage in the differentiation of the X and Y chromosomes.

Sea-run rainbow trout (steelhead) returning to the Washington Department of Game hatcheries on the Cowlitz and Washougal rivers were studied. Chromosome preparations were made from white blood cell cultures as previously described (*5*). At least five cells with the modal configuration were counted in all individuals. Previous work (*5, 6*) has shown that a Robertsonian