($p < 0.001$) in the muscle. We have termed this diameter variability exhibited by the type II fibers to be a 'centripetal neurogenic atrophy'. The type I fibers did not show any further atrophy, but did exhibit an increase in diameter distally after 28 days post-denervation. Fiber splitting was not observed in any of the muscle fiber populations sampled and therefore could not account for the atrophy observed.

Discussion. Both human and animal muscle can be broadly subdivided into 2 fiber types, type I fibers (red muscle fibers) and type II fibers (white muscle fibers). Morphologically, the type I fibers have more mitochondria and lipids than the type II fibers which are rich in glycogen and have more sarcoplasmic reticulum than the type I muscle fibers[9]. Physiologically, the type I muscle fibers have slow-twitch properties, whereas the type II muscle fibers have fast-twitch properties. There is a growing debate in the literature regarding which muscle fiber population atrophies following denervation. The present study is in agreement with those investigators which have described a preferential type II atrophy[3,10]. This implies that both fiber populations atrophy, however, the type II cells atrophy to a much greater extent than the type I fibers. The apparent susceptibility of the type II fibers to denervation atrophy suggest that they are in greater need of neural control than the type I fibers. The observed centripetal neurogenic atrophy of the type II fibers was completely unexpected and might possibly be due to several causes. It has been reported that 3-4 days following denervation, there is an increased synthesis of acetylcholine (ACh) receptors along the entire sarcolemma of the muscle fiber; this results in a concurrent sensitivity to ACh[11]. It has been shown that this chemosensitivity to ACh spreads from the endplate region towards the tendon ends[12]. This sensitivity appears to peak in about 2 weeks and after approximately 3 weeks declines in a centripetal direction, resulting in only the endplate region exhibiting any ACh sensitivity[13]. It is of interest to note in this regard, that it is at 3 weeks following denervation that we observed a centripetal neurogenic atrophy of the type II fibers. Another possible explanation for the observed systematic diameter variability of the type II fibers is that these cells might exhibit a differential stretch effect, whereby their proximal and distal thirds are under greater stretch than their middle third. It would appear that fiber size varies according to the area selected for biopsy and measurement. This variability of fiber size along the longitudinal axis of the muscle can cause considerable problems in interpretation of clinical severity and prognosis.

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Neuronal cell deficits following maternal exposure to methadone in rats[1]

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Summary. Rat offspring were maternally subjected to methadone hydrochloride during gestation or lactation. At 21 days of age, the area of the pyramis (cerebellar lobule VIII) in prenatally and postnatally exposed groups was reduced 45% and 36%, respectively, from control levels, and the total number of internal granule neurons/section was reduced 49% and 46%, respectively; the number of granule neurons/mm^2 in both groups was 75% of control values. Based on histological evaluation the timetable of cerebellar morphogenesis was unaltered in rats prenatally exposed to methadone, suggesting a permanent neuronal deficit, but cerebellar development was markedly delayed in animals subjected postnatally.

Methadone, a narcotic analgesic, is widely used in detoxification programs for narcotic-addicted pregnant women[2]. This synthetic opioid crosses the placenta and enters the fetal circulation of humans[3] and laboratory animals[4], and has been detected in the milk of lactating humans on methadone maintenance[3]. Clinical reports suggest that infants maternally subjected to narcotics are at risk for neurodevelopmental dysfunction[5,6], but the mechanisms underlying these problems have not been defined. The developing nervous system of laboratory animals also appears particularly sensitive to opiates, and a variety of physiological, biochemical, and behavioral abnormalities have been reported in drug-exposed offspring[7-12]. In an earlier study[7], conducted in rats at postnatal day 21 when cerebellar neurogenesis is normally completed, animals maternally subjected to methadone during either gestation or lactation had cerebella that weighed 25% less than that of controls and contained 11-18% fewer cells. In a morphological investigation, we now demonstrate a loss of cerebellar neurons in 21-day-old rats perinatally exposed to methadone. Moreover, we have found that the sequence of neuro-ontogenic events responsible for these neuronal deficits is dependent on the timing of drug exposure; the temporal course of cerebellar morphogenesis proceeds unaltered in rats prenatally subjected to methadone, but is markedly delayed in animals postnatally-treated with this synthetic opiate.

Female Sprague-Dawley rats (180-200 g) were housed

Areal analysis and granule cell population in the pyramis of cerebella from 21-day-old rats maternally exposed to methadone

	Control	Gestation	Lactation
Total area (mm^2)	1.80±0.09	1.04±0.06**	1.15±0.05**
MOL width (mm)	0.16±0.02	0.14±0.02*	0.14±0.02*
IGL area (mm^2)	0.75±0.05	0.46±0.02**	0.53±0.02**
Granule cells/ mm^2	3,795±267	2,864±90**	2,834±72**
Granule cells/ section	28,845±3407	14,720±928**	15,488±961**

Animals were maternally subjected to methadone during gestation or lactation. Values represent means ±SE. Data were evaluated using analysis of variance and the Newman-Keuls procedure[15]. Significantly different from control values. * $p<0.05$; ** $p<0.01$.

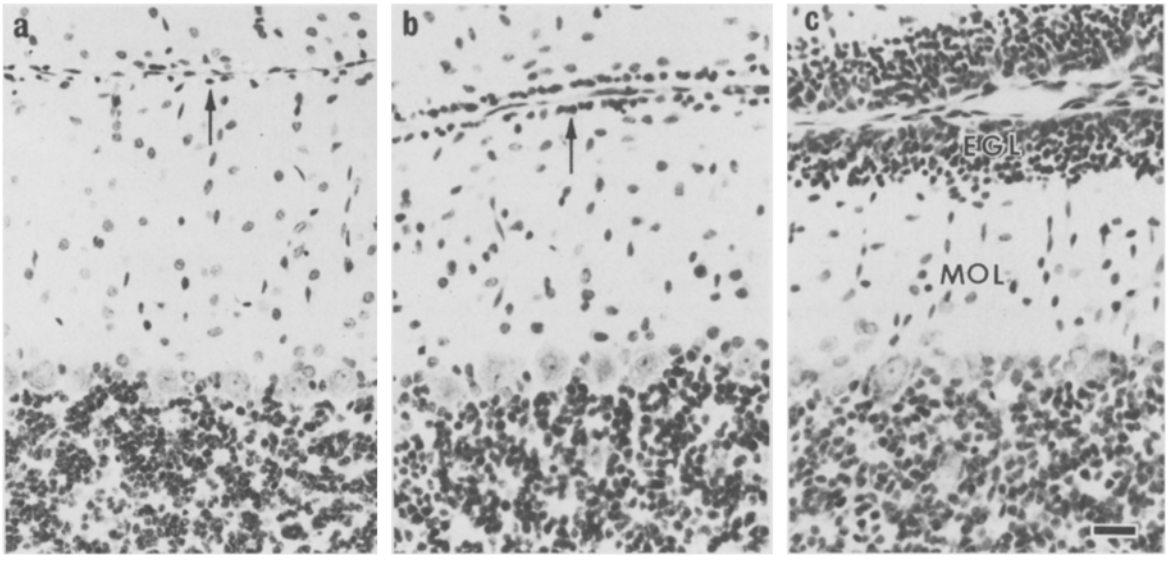
under standard conditions[7,12] and treated daily with an i.p. injection of either 5 mg/kg dl-methadone hydrochloride (Dolophine, Eli Lilly Company, Indianapolis, IN) or an equivalent volume of physiological saline. 5 days after the beginning of treatment, females were mated. All injections were continued throughout mating, gestation, and lactation, and terminated at weaning (day 21). Within 4 h after birth, litters were culled to 8 pups/litter and all pups were cross-fostered to form 3 treatment groups as described elsewhere[7,12]. One group of animals was exposed to methadone only during gestation, and another group received methadone only during lactation; a 3rd group of litters from saline-injected mothers served as controls. At 21 days, cerebella of 10 animals/treatment group were prepared histologically as previously described[13,14]. Areal measurements were made planimetrically on midsagittal sections of the pyramis (lobule VIII) that were projected at ×63 with a Bausch and Lomb microprojector; 2 sections/cerebella from each animal were utilized. The concentration of internal granule neurons was determined by counting cells within randomly selected grid areas (0.016 mm^2) at ×400; 3 grid areas/section were examined.

Areal analysis and cell counts revealed marked abnormalities in the pyramis of rats perinatally subjected to methadone (table). The total area of the pyramis of animals exposed to methadone during gestation or lactation was reduced 45% and 36%, respectively, from control values, whereas the area of the internal granule layer (IGL) was reduced 39% and 29%, respectively. The concentration of granule cells in these animals was also subnormal, with rats in both drug-treated groups having 75% of the cells normally concentrated in the IGL of this lobule. Because of these reductions in area and cell density, computation of the number of granule cells/section revealed significant cell deficits, with animals in the gestation and lactation groups having 51% and 54%, respectively, of the cells normally present. In addition, the molecular layer (MOL), composed of parallel axons from granule neurons, was considerably decreased in width in both gestation and lactation animals; these reductions were visible in histological sections (fig., b and c).

Although reductions in area and neuronal cell number were present in cerebella of 21-day-old rats exposed to methadone during either gestation or lactation, histological examination revealed totally different mechanisms responsible for these results. Profiles of the cerebellar cortex from rats exposed to methadone prenatally were similar to those of controls with respect to the presence of only a few external germinal cells scattered beneath the pial surface (fig., a and b).

Thus the schedule of neurogenesis in these animals was normal, and the deficits in granule neurons were presumably permanent in nature, since the external germinal cells are known to be the progenitors of the internal granule neurons. In the case of rats subjected to methadone during lactation, 6–12 layers of external germinal cells were present. In fact, the cerebellar cortex of lactation-treated animals resembled those of 10- to 15-day-old control rats. Therefore, the timetable of cerebellar neuro-ontogeny was retarded in animals exposed postnatally to methadone; a full assessment of the population of granule neurons in these animals awaits further study.

The abnormalities in cerebellar neurogenesis observed in this investigation need to be evaluated in other areas of the brain. The permanent loss of neurons (i.e., gestation group)



Comparison of sagittal sections of the pyramis from the cerebellar cortex of animals maternally exposed to saline (a) or methadone during gestation (b) or lactation (c). Photomicrographs were taken in a region midway between the gyrus of lobule VIII and sulcus separating lobules VII and VIII; a portion of lobule VII has been included in each micrograph. A prominent external germinal layer (EGL) was recorded in the lactation group, but only a few EGL cells (arrows) were observed in the control and gestation group. Note a reduction in MOL width for gestation and lactation groups. Scale bar, 20 µm.

and/or delays in neuro-ontogeny (i.e., lactation group), however, may disturb the temporal and spatial sequence of brain development, thereby affecting such factors as dendritic orientation and synaptic connectivity. Ultimately, these changes may be of significance in neurobiological functioning. Some evidence of neurally related disorders has in fact been reported in rats perinatally exposed to methadone[8-11]. Finally, the clinical ramifications of the present results need to be considered, since methadone maintenance is commonly employed for the treatment of narcotic-addicted pregnant women[2,3,5]. Within the context of evidence gathered in our study, and given differences in routes of drug administration and/or pharmacokinetics between exposure in humans and laboratory animals, it appears that offspring subjected to methadone in early life may be compromized in terms of their physiological integrity and intellectual achievement. In fact, some functional problems in children of narcotic-addicted mothers have already been documented[5,6].

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Rodlet-cells: Gland cell or protozoon?

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Summary. In the dense core of the inclusions of the rodlet cells in fish, DNA is indicated by the method of Bernhard and by DNase digestion. The inclusions, the rodlets, seem to be transport forms of genetical material. This finding supports the theory of the protozoan nature of these cells.

In 1892, 90 years ago, Thélohan[1] discovered a peculiar cell in various organs of teleosts and described it as sporozoan parasite (*Rhabdospora thélohani*, Laguesse 1906[2]). This cell is characterized by a fibrillar capsule and conspicious inclusions, the sporozoites. Usually it is located within an epithelium (e.g. intestinum, kidney tubules, gallbladder, endothelium, gill epithelium). Its apex is always oriented towards the surface of the epithelium. Occasionally it is seen free in the bloodstream but also in the connective tissue. Developmental stages have been reported[3-6]. At last, the inclusions are released and the rest of the cell degenerates.

Only few years after Thélohan, the same cell was interpreted as baffling, but normal fish cell ('Stäbchendrüsenzelle' or 'rodlet cell'), presumably with a secretory function[7]. The inclusions, the rodlets, are seen to be secretory material.

The rodlet cells have been considered as regulatory elements associated with special functions of the epithelia such as ion transport, osmoregulation etc.[3,8], and even as blood cells (granulocytes)[9-12]. (For reviews, see[6,8,13,14]).

To date, this problem is still undecided. Morphological features (e.g. capsule, desmosomes), as well as the varying distribution of these cells in some organs of fish, may be taken as support for either theory. An endogenous nature is suggested by the absence of a pathological tissue reaction and the well developed desmosomes between epithelial cells and rodlet cells, but junctional complexes also occur between parasitic protozoa and e.g. erythrocyte membranes[15]. The theory of parasitic origin is supported by the capsule and by histochemical investigations. Light microscope tests have shown that the cortex of the rodlets consists of proteins and non-acid polysaccharides[16,17]. The dense core is Feulgen negative[16,17], but gives a positive reaction for RNA[6,17].

In the present paper an attempt is made to clarify whether the dense core consists of genetic (DNA, RNA) or secretory material by the use of electron microscopical routine, histochemical and enzymehistochemical methods.

Material and methods. Routine preparations were made from kidney tissue of *Cyprinus carpio* L., fixed in phosphate-buffered glutaraldehyde (2%, 0.1 M, pH 7.4) with or without postfixation in Palade-buffered OsO_4, dehydrated with ethanol and embedded in Epon 812.

Reactions on DNA. For the EDTA-method after Bernhard[18], blocks were fixed only in glutaraldehyde. Sections (grey to silver) were stained with 5% aqueous uranyl acetate for 5 min, then floated on 0.2 M ethylene diamine tetraacetic acid (EDTA) at pH 7.0, for 30-60 min and poststained with Reynold's lead citrate for 5 min.

DNase digestion was done on ultrathin sections, floating on 0.01% DNase I, type I from bovine pancreas, approximately 2000 Kunitz units per mg protein, substantially free of RNase, Mg-ion concentration: 4.2 mM (Sigma), in 0.05 M Tris-HCl buffer pH 7.6 for 30 min-6 h at 37 °C. After washing in Aqua destillata and mounting on grids, the sections were stained with uranyl acetate and lead citrate.

For the Feulgen-silver methenamine method after Peters and Giese[19], ultrathin sections of only glutaraldehyde-fixed