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Increased Brain Size and Cellular Content in Infant Rats Treated with an Opiate Antagonist

Abstract. *From birth to day 21, rat offspring received daily injections of naltrexone at a dosage that blocked morphine-induced analgesia 24 hours a day. At 21 days, body, brain, and cerebellar weights of naltrexone-injected animals were 18, 11, and 5 percent greater than corresponding control weights. In addition, morphometric analysis of the cerebrum revealed a somatosensory cortex that was 18 percent thicker than that of the controls. The cerebellum of naltrexone-treated rats was 41 percent larger in total area and contained at least 70 percent more glial cells and 30 percent more granule neurons. Neurons derived prenatally were unaffected by drug treatment. These results show that naltrexone can stimulate body and brain growth in rats and suggest a role for the endorphin and opiate receptor system in development.*

Opioid compounds, in addition to having analgesic and behavioral effects, are known to alter cell function and growth, particularly in developing neural systems (*1–3*). Clinical observations of infants and children exposed in early life to opiates such as heroin and methadone reveal a retardation in somatic and neurobiological development (*4*). Similar perturbations in growth have been reported in laboratory animals subjected to opioids perinatally (*1–3*, *5*) and in cells in culture treated with exogenous and endogenous opioids (*6*). This interference in growth is stereospecific and is blocked by coadministration of narcotic antagonists (*2*, *5*), with the locus of opioid action postulated to reside at the opiate receptor (*2*, *7*). We administered naltrexone, a potent narcotic antagonist, to infant rats at a dosage that continuously blocks the opiate receptor from interaction with endogenous opioid peptides. The prolonged naltrexone exposure stimulated brain development, indicating that the opiate receptor is related to mechanisms of neurobiological growth and that endogenous opioids serve in the regulation of nervous system development.

Newborn Sprague-Dawley rats, reared in litters of eight pups per mother, were given daily subcutaneous injections of naltrexone (50 mg/kg) or sterile water until 21 days of age (weaning). By that time, the naltrexone-treated offspring had body, brain, and cerebellar weights that were 18, 11, and 5 percent greater than the control weights (Table 1). Macroscopic dimensions of the brain and cerebellum in the naltrexone-treated animals were 2 to 11 percent larger than those of the control animals (Table 1). Morphometric analysis of histological sections from the somatosensory cortex and cerebellum showed an enlargement of both regions in naltrexone-treated offspring (Table 2). In particular, areas of cerebellum analyzed were 41 to 45 percent larger than in controls. Further analysis of the cerebellum revealed increases in cellular content in sections of the pyramidal lobe (Table 3). The number of internal granule neurons per section was increased 30 percent and the number of glial cells (oligodendrocytes and astrocytes) in the medullary layer was increased 70 percent. The total population of glial, basket, and stellate cells in the molecular layer was increased 169 percent (Fig. 1). Furthermore, the effect of naltrexone on cell number in the cerebellum appeared to be directed solely toward cell populations derived during the treatment interval, since Purkinje cells, which are generated prenatally, did not change in number with perinatal naltrexone exposure (Table 3).

These results demonstrate that naltrexone can markedly stimulate the course of somatic and neurobiological development in the rat. The dose of naltrexone mediating these effects (50 mg/kg) represented 2 to 3 percent of the median lethal dose for adult rats (*8*). This low dose antagonized morphine-induced analgesia completely and effectively. Measurement of nociceptive responses (Analgesia Meter, Technilabs) 30 minutes after challenge with morphine sulfate (0.2 mg/kg) showed that the naltrexone blocked opiate receptors 24 hours a day.

Opiate receptors have been identified in brain and body tissues during ontogeny (*9*), endorphin immunoreactivity has been recorded in fetal brain and spinal cord cells (*10*), and endorphins have been found in the plasma and brain tissues of developing organisms (*11*). Our experiments show that when opiate receptors are continuously blocked, presumably preventing the interaction of these receptors with endogenous opioid peptides, larger animals with correspondingly bigger brains develop. This effect occurred in both sexes. Enlarged brain size was accompanied by an increase in the number of neurons and glia, particularly those arising postnatally. The number of neurons derived prenatally did not appear to be affected by exposure to naltrexone. The larger animals also showed an acceleration in neurobehavioral ontogeny and in the appearance

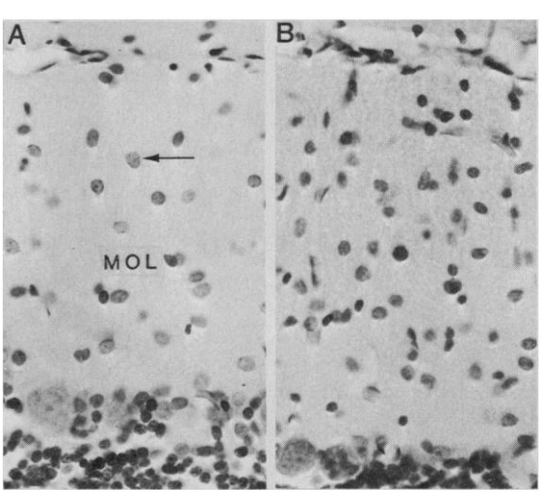

Fig. 1. Molecular layer (*MOL*) from the cerebellar pyramidal lobe of 21-day-old control (A) and naltrexone-treated (B) rats. The number of neural cell nuclei (arrow) is greater in the naltrexone-treated animals. Rats were fixed by cardiac perfusion with 10 percent neutral buffered Formalin and processed in polyester wax, and tissue was stained with hematoxylin and eosin (×830).

of physical characteristics (such as eye opening).

Our results imply that the opiate receptor is directly involved in growth-related cellular events, such as cell proliferation and differentiation. Moreover, the endorphins may serve to regulate growth through interactions with opiate receptors. This hypothesis is consonant with findings that high doses of naltrexone exacerbate the tumor response, particularly tumor growth, in mice inoculated with neuroblastoma cells (*12*). It is also consistent with the observation (*13*) that rats receiving twice-daily injections of naloxone on days 7 to 20 of gestation have offspring with accelerated somatic and behavioral development. In addition, if endogenous peptides regulate growth at the opiate receptor level, then exogenous opioids could have a similar action. Indeed, exogenous opioids such as heroin, methadone, and morphine inhibit growth of humans, laboratory animals, and cultured cells (*1–7*), and naloxone can reverse these effects (*2, 5*).

Table 1. Body, brain, and cerebellar measures for 21-day-old rats treated from birth with saline or naltrexone (50 mg/kg). Values are means ± standard errors for 21 to 30 animals per group with an equal number of males and females. Each value for naltrexone-treated animals differs from the corresponding control value at $P < 0.01$ (analysis of variance and Newman-Keuls test).

Measure	Control group	Naltrexone group
Body weight (g)	47.22 ± 0.72	55.17 ± 0.91
Brain		
Weight (g)	1.54 ± 0.02	1.71 ± 0.02
Height (mm)	9.68 ± 0.05	10.15 ± 0.05
Length (mm)	24.32 ± 0.16	26.23 ± 0.08
Width (mm)	15.53 ± 0.06	15.88 ± 0.06
Cerebellum		
Weight (mg)	196.13 ± 3.26	210.60 ± 3.45
Width (mm)	11.12 ± 0.11	11.64 ± 0.09

Table 2. Morphometric analysis of the somatosensory cortex and cerebellum of 21-day-old rats treated from birth with saline or naltrexone. Values are means ± standard errors for two sections per brain from 12 animals per group (equal numbers of males and females). Coronal sections (8-μm) of the somatosensory cortex [area 18 (*15*)] and midsagittal sections of the cerebellum were stained with hematoxylin and eosin, and areas were traced and computed with an Apple Graphics Tablet and Apple II computer. Cerebral width was measured as the distance along a line perpendicular to the midline and extending to the widest portion of the cortex. Cerebral area in each hemisphere encompassed the dorsal limit of layer II of the cortex and the area ventral to the anterior commissure. Cortical widths were measured as perpendicular depths from the dorsal surface of layer II to the corpus callosum. Measurements began at the most vertical point of the corpus callosum and proceeded laterally every 8 mm on the tracing until the cerebral width line was reached. Although measurements were made in both hemispheres of each brain, there were no differences and thus the data were combined.

Measure	Control group	Naltrexone group
Cerebrum		
Width (mm)	5.06 ± 0.10	5.54 ± 0.08*
Area (mm^2)	16.12 ± 0.70	18.82 ± 0.77†
Cortical thickness (mm)	1.29 ± 0.04	1.52 ± 0.03*
Cerebellum		
Total area (mm^2)	12.14 ± 0.60	17.15 ± 0.55*
Molecular layer area (mm^2)	5.97 ± 0.38	8.41 ± 0.34*
Internal granule layer area (mm^2)	5.08 ± 0.24	7.15 ± 0.27*
Medullary layer area (mm^2)	1.08 ± 0.06	1.57 ± 0.10*

*$P < 0.01$. †$P < 0.05$.

Table 3. Cellular content of cerebellar pyramidal lobe sections from 21-day-old rats treated from birth with saline or naltrexone. Differential cell counts were made with an ocular grid at ×630 magnification with a Nikon Biophot. All cells were counted from the pyramis, and the total number of cells per section was computed by multiplying the cell concentration by corresponding areal measurements. In the molecular and medullary layers all glial and neuronal cells were counted; endothelial cells were excluded. Only Purkinje cells with distinct nucleoli were counted. At least two sections per cerebellum from 12 animals per group (equal numbers of males and females) were counted. Values are mean numbers of cells ± standard errors.

Cells counted	Control group	Naltrexone group
Neural cells in molecular layer	1,527 ± 108	4,100 ± 368*
Internal granule neurons in internal granule layer	8,200 ± 615	10,623 ± 651*
Glial cells in medullary layer	175 ± 19	299 ± 42†
Purkinje cells	47 ± 3	52 ± 2

*$P < 0.01$. †$P < 0.05$.

Although these findings indicate that the endorphin and opiate receptor system mediates growth, it should be recognized that narcotic antagonists may have biological actions unrelated to opiate receptor blockade (*14*); these alterations may, by themselves or in conjunction with the endorphin and opiate receptor system, be responsible for naltrexone's actions.

In conclusion, this evidence that naltrexone can markedly alter the course of development supports the intriguing possibility that opiate receptors and endogenous opioid peptides play an integral role in cell proliferation, migration, and differentiation. These findings have potentially important implications for understanding the events involved in normal and abnormal growth.

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