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CEREBELLAR CGMP LEVELS REDUCED BY NARCOTIC AND HYPNOTIC DRUGS: --ETC(U)  
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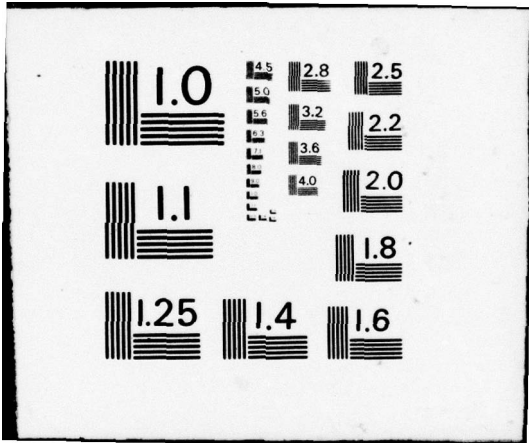
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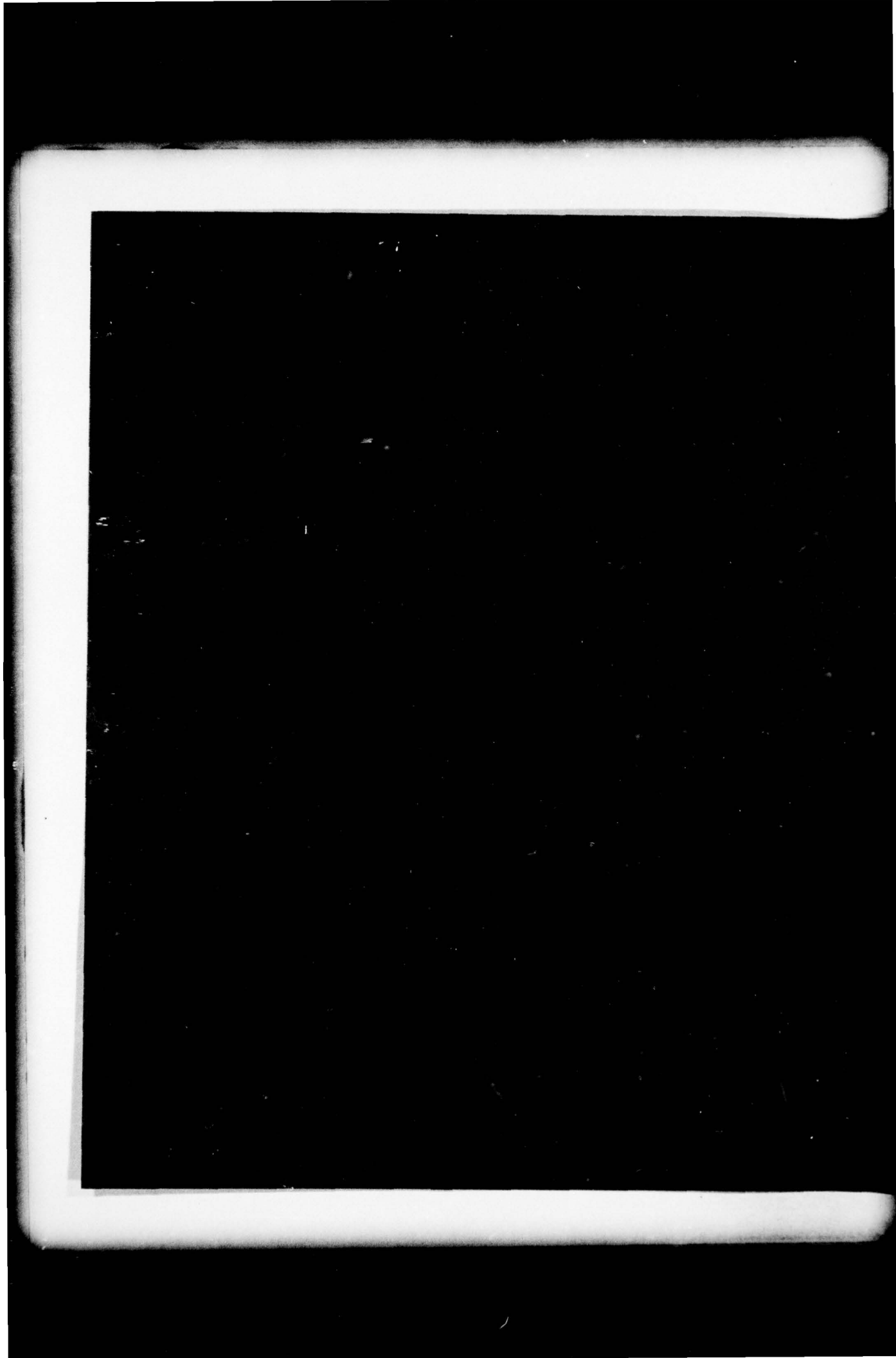


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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 14 AFRRI-SR76-30	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) 6 CEREBELLAR cGMP LEVELS REDUCED BY NARCOTIC AND HYPNOTIC DRUGS: CORRELATION WITH DRUG-INDUCED ATAXIA	5. TYPE OF REPORT & PERIOD COVERED	
7. AUTHOR(s) 10 J. B./Katz and G. N./Catravas	6. PERFORMING ORG. REPORT NUMBER	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Armed Forces Radiobiology Research Institute (AFRI) Defense Nuclear Agency Bethesda, Maryland 20014	8. CONTRACT OR GRANT NUMBER(s)	
11. CONTROLLING OFFICE NAME AND ADDRESS Director Defense Nuclear Agency (DNA) Washington, D. C. 20305	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NWED QAXM C 912 04	12. REPORT DATE 11 Jun 76
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) 9 Scientific rept.	13. NUMBER OF PAGES 12p. 14	15. SECURITY CLASS. (of this report) UNCLASSIFIED
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) A		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Maintenance of normal coordination and smooth motor activity requires an intact Purkinje cell system in the cerebellum. Acute administration of morphine or pentobarbital results in a reduction in the cGMP pool of cerebellar Purkinje cells that is both dose and time dependent. The induction of ataxia by these drugs correlates with their ability to depress cerebellar cGMP levels. Animals tolerant to		

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20. ABSTRACT (continued)

→ morphine show neither the cGMP reduction nor ataxia. The study of changes in cerebellar cGMP levels induced by narcotic and hypnotic drugs provides information on basic mechanisms of function of the mammalian central nervous system. This information is of great value to studies of effects of other toxic agents including ionizing and nonionizing radiation.

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## INTRODUCTION

Regulation of Purkinje cell activity in the cerebellar cortex is a critical point in the cerebellar modulation of voluntary motor activity.<sup>1,6,7</sup> Elevation of climbing fiber input to the Purkinje cell from the inferior olivary nucleus by harmaline, a CNS stimulant and amine oxidase inhibitor, dramatically increases Purkinje cell firing rates, and elevates cerebellar levels of 3',5'-cyclic guanosine monophosphate (cGMP).<sup>10,11</sup> This increased Purkinje cell outflow exerts an inhibitory influence on deep cerebellar nuclei and lateral vestibular nuclei and is manifested ultimately through bulbar reticular nuclei as tremor.<sup>6,7</sup> By contrast, in infant rat cerebellum, in which synaptic contact has not been established from climbing fibers of the inferior olivary nucleus to the Purkinje cells, the level of cGMP is very low and harmaline has no effect upon either Purkinje cell activity or upon cGMP levels.<sup>15</sup> As synaptic contact develops, the level of cGMP increases to adult levels, and can be further elevated at that time by harmaline, or decreased by the skeletal muscle relaxant and tranquilizer diazepam.<sup>15</sup> As a harmaline antagonist, diazepam counters several harmaline effects, including spiking of climbing fibers and Purkinje cells, tremor, and elevation of cerebellar cGMP levels.<sup>10</sup> Furthermore, in mutant mice with specific degeneration of nearly all cerebellar Purkinje cells during the 2nd postnatal month, cGMP levels are greatly reduced, and the animals are ataxic.<sup>12</sup>

Although 3',5'-cyclic adenosine monophosphate (cAMP) is present in the cerebellum, its level is relatively unaffected by drug-induced, genetic, or environmental (cold stress) influences which affect the functional status of the cerebellum and the balance between tremor and ataxia-relaxation.<sup>12</sup> The central position of the olivocerebellar-bulbar reticular circuit and the Purkinje cell in modulating that balance is generally accepted. It has been further postulated that excitatory and inhibitory influences on the Purkinje cells (from climbing fibers, and basket and stellate cells respectively) may regulate cGMP pool size, which thus may be a neurochemical correlate indicative of the activity of the olivocerebellar circuit.<sup>10</sup> The presence of a specific, high-affinity

cGMP-binding protein in rat cerebellum, a distinct cGMP-dependent protein kinase in bovine cerebellum and the relatively high cGMP concentration in the cerebella of several species emphasizes the putative role of cGMP in mediating at least some of the cerebellar motor regulatory functions.<sup>13,14,16</sup>

#### METHODS

The mechanism of narcotic- and hypnotic-induced ataxia in young adult male Sprague-Dawley rats treated with either morphine or pentobarbital was investigated. Naive animals were injected (i.p.) with either drug, in varying dosages, and euthanatized 15 minutes postinjection by exposing the head to focused microwave irradiation (1.3 kW, 4.0-sec exposure) which inactivates brain enzymes and prevents postmortem changes in cyclic nucleotide levels (microwave source was a Litton Menumaster oven modified by Medical Engineering Consultants, Lexington, Massachusetts, model 70/50). cGMP was extracted from the cerebellum and the quantity measured with a competitive protein binding assay.<sup>2,9</sup>

#### RESULTS AND DISCUSSION

Figures 1 and 2 display the dose-response curves relating morphine and pentobarbital dose to cGMP level in the cerebellum. Ataxia was pronounced 15 minutes after injection in rats treated with 5 mg/kg morphine or 12.5 mg/kg pentobarbital, dosages which strongly depressed cerebellar cGMP content. Lesser dosages produced less obvious ataxia and reduced cGMP levels to a lesser extent. Figure 3 presents time course studies on the cGMP-depressant effects of morphine and of pentobarbital. Just as the rapid onset of ataxia in acutely treated animals correlates with an abrupt drop in cGMP levels, the resumption of normal, smooth motor activity is accompanied by a rise in cerebellar cGMP levels back to control levels in a few hours.

A second group of rats was made tolerant to morphine through twice daily injections of 60 mg/kg of morphine for 10 days; using a standardized noxious heat stimulus, tolerant rats were defined as responding as quickly to minimize

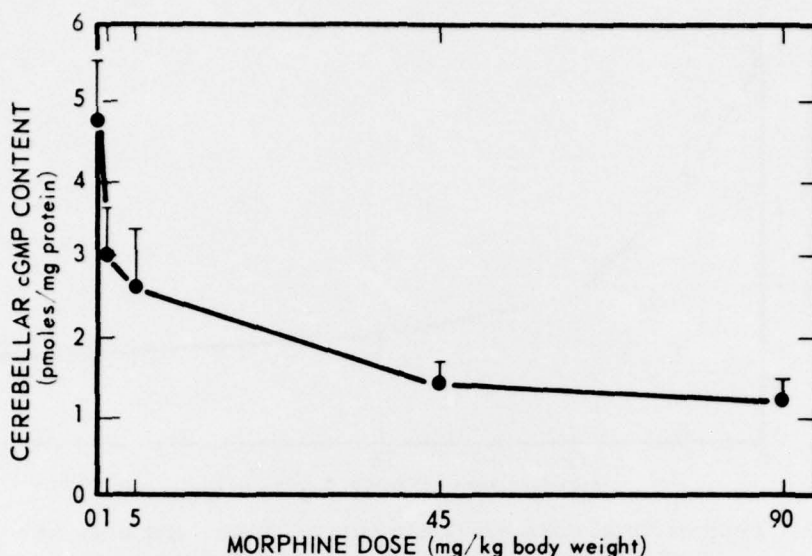


Figure 1. Morphine dose-response curve: dose-dependent reduction in cerebellar cGMP content. Groups of 150 g Sprague-Dawley rats ( $n = 7$ , means  $\pm$  S.E.) were injected with varying amounts of morphine sulfate (i.p.) and euthanatized 15 minutes later by focused microwave irradiation of the head (1.3 kW power density, 4.0-sec exposure). Cerebella were removed and their cGMP content assayed as previously described.<sup>2,9</sup> Cerebellar protein content was measured with the Lowry method and the level of cGMP in each cerebellum was expressed in picomoles per milligram (pmoles/mg) protein.<sup>8</sup> cGMP values not corrected for recovery during extraction and purification.

the stimulus as did naive controls.<sup>3,4</sup> Acutely morphine-treated rats responded much more slowly to the stimulus, and as tolerance to morphine was acquired over the 10-day schedule, response time decreased until it equaled that of the controls. As shown in Figure 4, the cGMP content of cerebella from tolerant rats challenged with 45 mg/kg of morphine was not significantly different from that of naive controls. Furthermore, tolerant rats undergoing precipitated withdrawal induced by the narcotic antagonist naloxone (2.5 mg/kg, i.p.) had cerebellar cGMP levels equal to those of morphine-challenged rats and naive controls. This observation is surprising in light of the behavioral

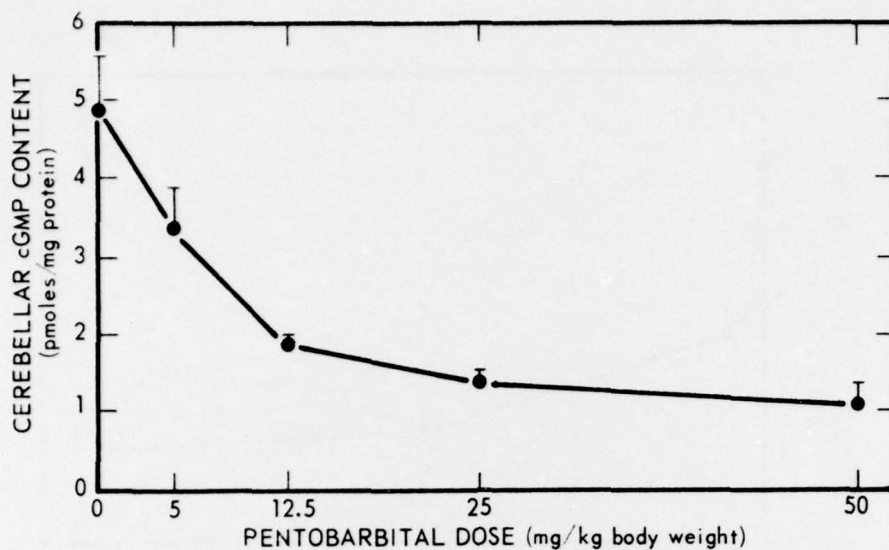


Figure 2. Pentobarbital dose-response curve: dose-dependent reduction in cerebellar cGMP content. Groups of 150 g Sprague-Dawley rats ( $n = 6$ , means  $\pm$  S.E.) were injected with varying amounts of sodium pentobarbital (i.p.) and euthanatized 15 minutes later. Techniques identical to those in Figure 1.

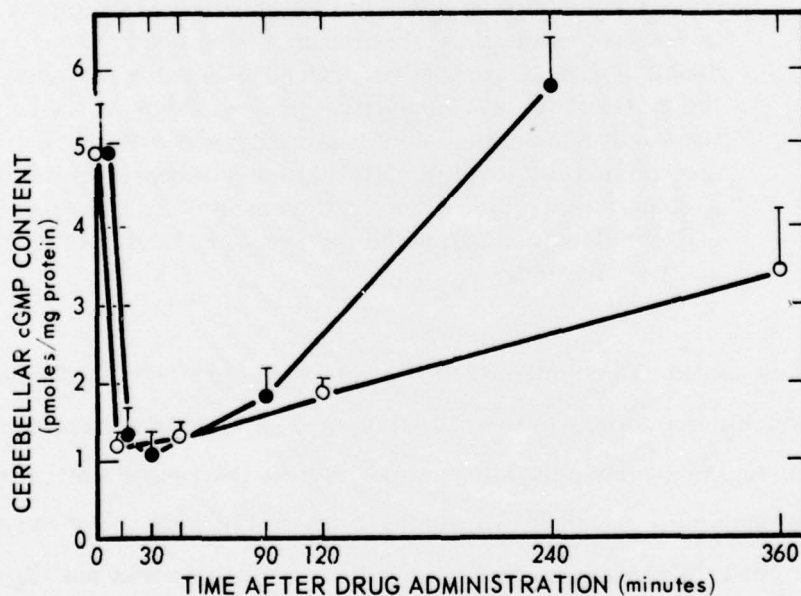


Figure 3. Time course studies: time-dependent effects of acute doses of morphine (45 mg/kg) or pentobarbital (25 mg/kg) on cerebellar cGMP content. All injections i.p. For morphine study,  $n = 7$ , means  $\pm$  S.E. For pentobarbital study,  $n = 5$ , means  $\pm$  S.E. Measurement techniques as in Figure 1. o: morphine time course; ●: pentobarbital time course.

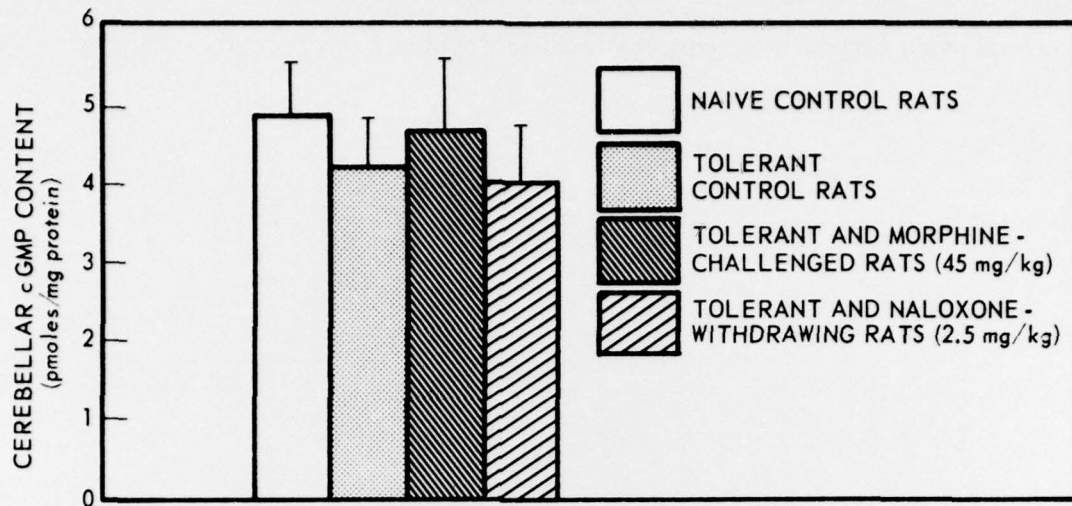


Figure 4. Effect of morphine tolerance, and morphine or naloxone challenge in morphine-tolerant rats on cerebellar cGMP levels. Rats were made tolerant to morphine as defined and determined by their response to minimize a standardized noxious heat stimulus.<sup>3,4</sup> Tolerant animals were defined as responding as quickly as animals never treated with morphine. Animals were made tolerant by twice daily injections of morphine sulfate (60 mg/kg, i.p.) for 10 days. A tolerant control group was euthanatized by focused microwave irradiation just prior to final scheduled morphine dose. Tolerant, morphine-challenged rats were injected with 45 mg/kg morphine, i.p., at this time, and tolerant, naloxone-withdrawing rats were injected with 2.5 mg/kg naloxone, i.p., at the same time. Both groups were euthanatized 15 minutes later, at which time naloxone-withdrawing rats exhibited the typical abstinence syndrome of diarrhea, shaking, and hypersensitivity to handling. Techniques identical to those in Figure 1; n = 7, means  $\pm$  S.E. for each group.

manifestations of induced withdrawal, which include shaking and shivering motions; a compensatory overshoot in cGMP content might have been expected during withdrawal, particularly after observing motor behavior associated with the abstinence syndrome. Because the rat cerebellum is singularly devoid of morphine receptors, which are widespread through the rest of the brain, effects of morphine and morphine withdrawal on cerebellar cGMP levels are probably

indirect.<sup>5</sup> Understanding the role and regulation of the cerebellar cGMP level may provide insight into neurochemical mechanisms of cerebellar function.

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