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# Effect of High Doses of Naloxone on Shuttle Avoidance Acquisition in Rats

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TURNBULL, B. A., D. L. HILL, L. H. MILLER, J. MCELROY AND R. S. FELDMAN. *Effect of high doses of naloxone on shuttle avoidance acquisition in rats.* PHARMACOL BIOCHEM BEHAV **19**(3) 423–426, 1983.—Administration of high doses of naloxone intraperitoneally (2.5–10.0 mg/kg) resulted in a dose-related impairment of avoidance response acquisition in a shuttle avoidance paradigm in rats. Naloxone in this dose range produced a significant decrease in the number of intertrial responses but did not result in a significant dose-response. Escape latencies were not affected by naloxone administration at any dose tested. The effect of naloxone on activity and nociception are implicated as possible causes of the observed behavior. The results are discussed as behavioral evidence supporting theories postulating multiple opiate receptors.

Naloxone  Avoidance acquisition  Opioid system  Intertrial response  Escape latency  Opioid receptors

ENDOGENOUS opioid peptides have been demonstrated to have a profound affect on memory and learning [3, 9, 11, 15, 19, 20]. It has been shown that rats receiving post-training injections of met-enkephalin into the lateral ventricles exhibit enhanced retention of inhibitory avoidance responding [3]. Systemic injection of either met- or leu-enkephalin prior to acquisition or prior to testing attenuates $CO_2$-induced amnesia [19], while intraperitoneal injection of met-enkephalin facilitates maze learning in rats [15]. However, other studies have found both met- and leu-enkephalin to impair acquisition of an active avoidance response [20], and beta-endorphin has been shown to impair shuttle avoidance acquisition in rats [9].

To further assess the role of the endogenous opioid system in memory and learning numerous studies have administered naloxone, a specific opiate antagonist [22], and observed its effects on various learning paradigms [8, 9, 10, 16]. Naloxone has been shown to impair shuttle avoidance acquisition and delay habituation to the rearing response when administered prior to training [9]. Naloxone administered post-training results in improved retention of both inhibitory and active avoidance responding [8,16]. Facilitation of memory consolidation of habituation to the rearing response has also been demonstrated when naloxone is administered post-training [10].

More recently, it has been demonstrated that high doses of naloxone (2–4 mg/kg) in humans result in alterations in behavior, systolic blood pressure and respiration rate not found with lower doses of the opiate antagonist [4,5]. In the past, few human studies have been conducted utilizing large doses of naloxone. This discrepancy may originate from the exceedingly small amount of naloxone (<5 μg/kg) which is required to precipitate withdrawal from exogenous opiates [17].

Studies which demonstrate differential behavioral or physiological effects when naloxone is administered in the mg/kg range verses the μg/kg range [4, 5, 18] would seem to support biochemical evidence of the existence of multiple opiate receptors differentially sensitive to naloxone. These studies clearly demonstrate that while complete opiate withdrawal can be initiated by infusion of very low doses of naloxone not all effects mediated by the opiate receptors can be blocked by administration of naloxone in the μg/kg range. The present study investigates the effect of naloxone at high doses (in the mg/kg range) on behaviors observed in the shuttle avoidance paradigm.

## METHOD

The animals used were 40 Holtzman albino rats, age 25 weeks, weighing 350–400 g. Animals were housed in stainless steel cages with water ad lib and received a daily ration of 20 g standard lab chow one hour after testing. They were maintained in a temperature controlled room on a 12 hr light-dark cycle. All testing took place in the dark component of the cycle.

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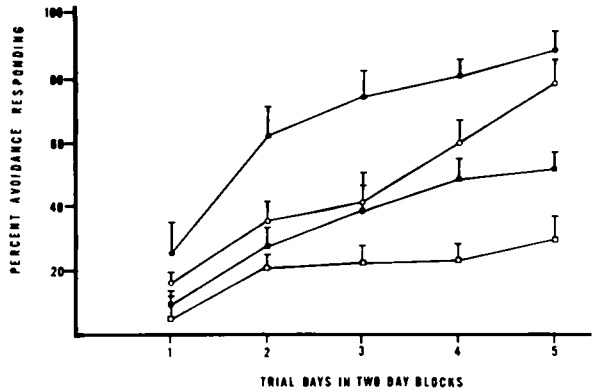


FIG. 1. Effects of high doses of naloxone on shuttle avoidance acquisition in rats. Data consists of mean percent avoids (±S.E.M.) in two day blocks. Groups are: ●—● control, ○—○ naloxone 2.5 mg/kg, ■—■ naloxone 5.0 mg/kg and □—□ naloxone 10.0 mg/kg.

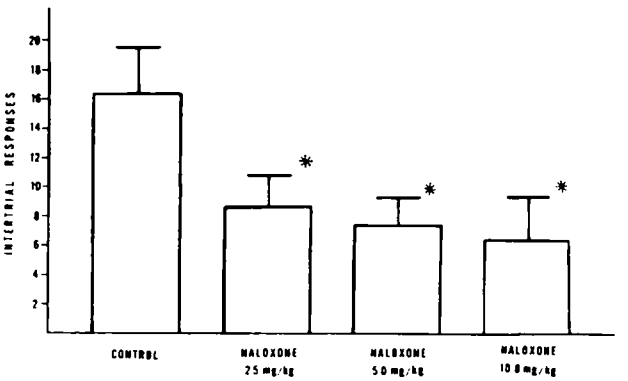


FIG. 2. Effect of high doses of naloxone on intertrial responding in the shuttle avoidance paradigm. Data is presented as mean number of intertrial crossings (+S.E.M.) collapsed across the 10 days of trials (*significantly different from control, $p<0.05$).

The shuttle avoidance apparatus was a Lehigh Valley Electronics model 146-04, a Plexiglas chamber measuring 50×20×20 cm divided by two center bars electrified at 2 mA by a Lehigh Valley Electronics Model 113-02 constant current shocker during testing. The conditioned stimulus consisted of a 10 second tone provided by a Sonlert model SC 628 mounted in the midpoint of the ceiling of the chamber. Floor current of 1.5 mA was delivered by a Lehigh Valley Electronics model 133-33 constant current shocker-scrambler. Testing was done in a dimly lit soundproof room.

Naloxone hydrochloride (Endo Laboratories, Garden City, NJ) was dissolved in physiological saline (0.9%), which alone served as vehicle control. All solutions were administered intraperitoneally (IP) in a volume of 1 ml/kg, five minutes before testing.

After 10 days of habituation to their home cages, the animals were randomly allocated to one of four treatment conditions: vehicle control, 2.5, 5.0 or 10 mg/kg naloxone HCl. The day before testing began each animal was allowed to explore the experimental chamber for fifteen minutes without tone or shock. Testing was conducted on the next ten consecutive days. Each animal received 20 trials per day on a 45 second variable interval schedule (min 5 sec; max 75 sec). The animals could avoid shock by shuttling from one side of the center bars to the other during a 10 second tone period. If the animal did not cross the center barrier during this period, the tone remained on and the floor shock was delivered to the half of the chamber the animal occupied until 10 sec of shock was delivered or the animal escaped to the other side. Automatic counters recorded the number of avoidance responses, escapes and intertrial crossings, while a running time meter recorded the total daily shock duration for each animal.

Data was statistically analyzed by means of a two-way analysis of variance (ANOVA). Post-hoc analysis for between-group differences was carried out by employing the Newman-Keuls method for multiple comparisons.

## RESULTS

Analysis of shuttle avoidance acquisition data yielded a high significance between treatment groups, $F(3,360)=54.32$, $p<0.0001$. A significant interaction between treatment and trial day was not found, $F(27,360)=0.84$, $p>0.50$. Comparisons between individual treatment groups yielded significance between any 2 given treatments ($p<0.05$), demonstrating a dose response impairment of shuttle avoidance learning produced by naloxone (see Fig. 1).

The difference between groups in the number of intertrial responses made was highly significant, $F(3,360)=20.62$, $p<0.0001$. The treatment-trial day interaction failed to demonstrate significance, $F(27,360)=0.64$, $p>0.50$. Post-hoc comparisons yielded significance between control and any of the naloxone treatment doses ($p<0.05$), but failed to demonstrate significance between any naloxone treatment groups (see Fig. 2). Thus, the effect of naloxone on intertrial responding did not demonstrate a dose response at the doses tested.

Average duration of shock per escape, which is obtained by dividing the daily shock time by the number of escapes on that trial day, did not differ significantly between treatments, $F(3,338)=0.93$, $p>0.40$. As previously stated, an upper limit of 10 sec of shock per trial was set. The average shock duration per escape (±S.E.M.) for the four treatments were: control—1.5 sec (±0.4), naloxone 2.5 mg/kg—1.6 sec (±0.3), naloxone 5.0 mg/kg—1.6 sec (±0.3) and naloxone 10.0 mg/kg—1.8 sec (±0.4). The ability to escape shock, as defined by average shock duration, was not affected by naloxone in this paradigm, demonstrating that the effect of naloxone on avoidance acquisition was not due to interference with the escape response.

## DISCUSSION

Results of the present study demonstrate that administration of the opiate antagonist naloxone produced in a dose-related impairment of shuttle avoidance acquisition at the doses tested. This effect may be mediated by blockade of the endogenous opioid system. Hyperalgesia created by naloxone in animals previously exposed to footshock [12, 14, 21] may partially account for this observed impairment. It has been previously shown that animals trained with higher amperage acquire the shuttle avoidance response at a slower rate than those trained at a lower level of shock [2,24]. Administration of naloxone may thus be equivalent to increasing the level of shock administered to the animal. The effect of

naloxone on activity, as defined as intertrial crossings, was significant at all doses tested, however, no dose-relationship at the doses tested was found. This observed effect is most likely a summation of both the effect of naloxone on shock-induced freezing [6] and the indirect effect of naloxone on general exploratory/locomotor behavior [1, 7, 25]. In the present study, naloxone did not impair the escape response at any dose tested, demonstrating the lack of a direct effect on locomotor activity. Studies demonstrating that increased shock level in the shuttle avoidance paradigm also results in a decrease in intertrial responding while having no effect on escape latency [2, 6, 24] support the naloxone-induced hyperalgesia explanation of the observed results. Some investigators have found that the reversal of opioid mediated shock-induced analgesia by naloxone appears to be somewhat dose-related at the concentrations comparable to ones used in this study [12,21]. This would lend additional support to the concept of involvement of naloxone-induced hyperalgesia in the effect of naloxone on shuttle avoidance acquisition.

Naloxone enhancement of shock-induced freezing may assist in explaining the observed effect of naloxone on avoidance acquisition. At concentrations comparable to those used in the present study, naloxone produced a dose-related increase in the amount of time animals spent motionless after being shocked [6]. The effect of naloxone on shock-induced freezing was noted only in those animals previously exposed to shock, thus implicating a blockade of the shock-induced release of endogenous opioids as a cause of the observed effect. While this may be taken as alternative explanation for the results obtained in the present study, the effect of naloxone on shock-induced freezing may be a simple result of the hyperalgesia created by naloxone and not a separate effect of the opiate antagonist. Regardless of the cause, the effect of naloxone on shuttle avoidance acquisition represents the first dose-related impairment of learning due to the administration of an opioid antagonist reported in the literature to the best of our knowledge.

Cohen *et al.* have stressed the need for the inclusion of high doses of naloxone when assessing the effects of opioid blockade in humans [3,4]. We suggest that the need also exists when an animal model is being utilized. These observed effects support the biochemical evidence for the existence of multiple opiate receptors in the CNS with a differential affinity for naloxone and opiate receptor ligands [13, 23, 26]. We have shown that administration of doses in the mg/kg range results in an impairment of acquisition of a learned task which is dose related at the doses tested and that this dose-response occurs in a dose range more than 2000 times the dose that is that needed to fully reverse opiate overdose. Whether the reason for the effects demonstrated are multiple opiate receptors, differentially accessible opioid systems or complex feedback mechanisms in the opioid systems, a complete study on the effect of opioid blockade on physiological or behavioral measures should include high doses of naloxone (i.e., in the mg/kg range) to fully assess the role of the opioid system in the modulation of the studied response.

## REFERENCES


1. Arnsten, A. and D. Segal. Naloxone alters locomotion and interaction with environmental stimuli. *Life Sci* **25**: 1035–1042, 1979.
2. Bauer, R. H. The effects of CS and US intensity on shuttlebox avoidance. *Psychon Sci* **27**: 266–268, 1972.
3. Belluzzi, J. D. and L. Stein. Enkephalin- and morphine-induced facilitation of long-term memory. *Soc Neurosci Abstr* **3**: 230, 1977.
4. Cohen, M. R., R. M. Cohen, D. Pickar, D. L. Murphy and W. E. Bunney. Physiological effects of high dose naloxone administration to normal adults. *Life Sci* **30**: 2025–2031, 1982.
5. Cohen, M. R.. R. M. Cohen, D. Pickar, H. Weingartner, D. L. Murphy and W. E. Bunney. Behavioral effects after high dose naloxone administration to normal volunteers. *Lancet* **2 (8255)**: 1110, 1981.
6. Fanselow, M. S. and R. C Bolles. Naloxone and shock-elicited freezing in the rat. *J Comp Physiol Psychol* **93**: 736–744, 1979.
7. File, S. Naloxone reduces social and exploratory activity in the rat. *Psychopharmacology (Berlin)* **71**: 41–44, 1980.
8. Izquierdo, I. Effect of naloxone and morphine on various forms of memory in the rat: Possible role of endogenous opiate mechanisms in memory consolidation. *Psychopharmacology (Berlin)* **66**: 199–203, 1979.
9. Izquierdo, I. Effect of B-endorphin and naloxone on acquisition, memory, and retrieval of shuttle avoidance and habituation learning in rats. *Psychopharmacology (Berlin)* **69**: 111–115, 1980.
10. Izquierdo, I. and M. Graudenz. Memory facilitation by naloxone is due to release of dopaminergic and beta-adrenergic systems from tonic inhibition. *Psychopharmacology (Berlin)* **67**: 265–268, 1980.
11. Izquierdo, I., R. D. Dias, D. O. Souza, M. A. Carrasco, E. Elisabetsky and M. L. Perry. The role of peptides in memory and learning. *Behav Brain Res* **1**: 451–468, 1980.
12. Jacob, J. and K. Ramabadran. Enhancement of a nociceptive reaction by an opioid antagonist in mice. *Br J Pharmacol* **64**: 91–98, 1978.
13. Jacquet, Y. F., W. A. Klee, K. C. Rice, I. Iijima and J. Minamikawa. Stereospecific and nonstereospecific effects of (+)- and (−)-morphine: evidence for a new class of receptors? *Science* **198**: 842–845, 1977.
14. Kaplin, R. and S. Glick. Prior exposure to footshock induced hyperanalgesia. *Life Sci* **24**: 2309–2312, 1979.
15. Kastin, A. J., E. L. Scollan, M. G. King, A. V. Schally and D. H. Coy. Enkephalin and a potent analog facilitate maze performance after intraperitoneal administration in rats. *Pharmacol Biochem Behav* **5**: 691–695, 1976.
16. Messing, R. B., R. A. Jensen, J. L. Martinez, V. R. Spiehler, B. J. Vasquez, B. Soumireu-Mourat, K. C. Liang and J. L. McGaugh. Naloxone enhancement of memory. *Behav Neural Biol* **27**: 266–275, 1979.
17. Pert, C. and B. L. Garland. The mechanism of opiate agonist and antagonist action. In: *Receptors and Hormone Action:* vol 3, edited by L. Birnbaumer and B. V. W. O'Malley. New York: Academic Press, 1978, pp. 535–551.
18. Pickworth, W. B. and L. G. Sharpe. EEG-behavioral disassociation after morphine- and cyclazocine-like drugs in the dog: further evidence for two opiate receptors. *Neuropharmacology* **18**: 617–622, 1979.
19. Rigter, H. Attenuation of amnesia in rats by systemically administered enkephalins. *Science* **200**: 83–85, 1978.
20. Rigter, H., T. J. Hannan, R. B. Messing, J. L. Martinez, B. J. Vasquez, R. A. Jensen, J. Veliquette and J. L. McGaugh. Enkephalins interfere with acquisition of an active avoidance response. *Life Sci* **26**: 337–345, 1980.
21. Satoh, M., S. Kawajiri, M. Yamamoto, H. Makino and H. Takagi. Reversal by naloxone of adaptation of rats to noxious stimuli. *Life Sci* **24**: 685–690, 1979.

22. Sawnok, J., C. Pinski and F. LaBella. Minireview on the specificity of naloxone as an opiate antagonist. *Life Sci* **25:** 1621–1632, 1979.
23. Squires, R. F. and C. Braestrup. Characteristics and regional distributions of two distinct (3H)-naloxone binding sites in the rat brain. *J Neurochem* **30:** 231–236, 1978.
24. Theios, A., A. D. Lynch and W. F. Lowe. Differential effects of shock intensity on one-way and shuttle avoidance conditioning. *J Exp Psychol* **72:** 294–299, 1966.
25. Walker, J. M., G. G. Berntson, T. S. Paulucci and T. C. Champney. Blockade of endogenous opiates reduces activity in the rat. *Pharmacol Biochem Behav* **14:** 113–116, 1981.
26. Zurkin, R. S. and S. R. Zurkin. Minireview—Multiple opiate receptor: emerging concepts. *Life Sci* **29:** 2681–2690, 1981.