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Isolation Rearing Decreases Opiate Receptor Binding in Rat Brain[1]

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SCHENK, S., M. D. BRITT, J. ATALAY AND S. CHARLESON. *Isolation rearing decreases opiate receptor binding in rat brain.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 841–842, 1982.—Male hooded rats were reared from weaning either in isolation or in groups (3–4 per cage) for 45 days. Their brains were then analyzed for opiate receptor binding. Results indicated that the isolation reared rats had a lower density of opiate receptors than those rats reared in aggregation. These findings are discussed in terms of the relevance of opiate receptor binding to the behavioral efficacy of opiate drugs.

Opiates Receptor binding Rearing

EARLY environmental influences can alter the mature animal's response to the analgesic properties of opiates. Mice reared in isolation from weaning until approximately six months of age show differential analgesic responses to morphine injections when compared to group reared mice [3, 5, 6]. Although these differences are strain dependent with C57B1/6J mice showing a decreased analgesic effect [3] and Swiss albino mice showing an increased analgesic effect [5,6]; in both strains there is a positive correlation between the number of opiate receptor binding sites and the efficacy of opiate produced analgesia. Thus it appears that in mice the degree of antinociception induced by morphine is related to the amount of opiate binding which in turn can be influenced by the early developmental environment.

There is evidence that the relationship between the level of opiate receptor binding and opiate efficacy may hold for rats as well. Pharmacological manipulations can produce alterations in the number of opiate binding sites. Chronic treatment with the narcotic antagonist naloxone in rats results in an increase in opiate receptor binding [7]. This increase was also associated with an increased sensitivity to the analgesic effects of morphine.

In addition to the similarity between rats and mice with respect to opiate receptor binding and opiate produced analgesia, there is an indication that early environment may influence a rat's sensitivity to opiates. Isolation housed rats are less susceptible to the pain reducing effects of morphine than are those reared in aggregation [6]. We were therefore interested in determining whether isolation rearing could alter opiate receptor binding in the rat brain.

METHOD

Male hooded rats of the Royal Victoria strain were bred and reared in the animal colony at Concordia University. At weaning (21–23 days) the rats were either isolated in metal cages (20×25×18 cm) or group housed 3–4 per metal cage (41×25×18 cm). These conditions were maintained for 45 days. Accordingly the rats were 66–68 days of age when the present experiment was conducted. The animals were decapitated and the brains rapidly dissected out and analyzed for differences in (^{3}H) naloxone binding.

The brains, minus the cerebellum were weighed and prepared according to the method of Pert *et al.* [9]. Each brain was minced separately in 15 ml of ice-cold 0.05 M tris-Cl buffer, pH 7.7, and homogenized using a Polytron PT 10 at setting No. 4 for 30 sec. After centrifugation at 49,000 g for 15 min, the supernatant fluid was discarded and the pellet resuspended in 15 ml of buffer, and homogenized for 20 sec. The homogenate was then diluted with 145 ml cold buffer and the suspension incubated for 40 minutes at 37°C. After cooling on ice with gentle stirring for 15 minutes, the suspension was incubated in triplicate for 30 minutes at 25°C with (^{3}H) naloxone (3.0×10^{-9} M). After incubation, samples were placed in ice water for 15 minutes; the mixture was then filtered onto Whatman GF/B filters under vacuum. Control incubations contained 1×10^{-6} M levallorphan tartrate. The tubes and filters were washed with two 5.0 ml portions of cold buffer. The damp filters were counted in 3.0 ml Biofluor scintillation fluid in Beckman biovials after a 3 hour stabilization period. Specific binding of (^{3}H) naloxone to homoge-

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TABLE 1

RECEPTOR BINDING OF (^{3}H) NALOXONE (3×10^{-9} M) TO WHOLE BRAIN HOMOGENATES IN CHRONICALLY ISOLATED OR GROUP HOUSED RATS

	fmoles/mg protein	
Grouped (N=7)	114.0 ± 2.7*	210.3 ± 4.4*
Isolates (N=8)	104.0 ± 3.2	197.9 ± 3.7

Values are the means ± standard error of the means.
*$p<0.05$ 2-tailed Student's t-test.

nates was calculated as the difference between incubations in the presence and absence of 1×10^{-6} levallorphan tartrate with or without 200 mM NaCl.

RESULTS

Table 1 shows opiate receptor binding, in the presence and absence of NaCl, for the group and isolation reared rats. In both cases the isolation reared rats had less binding than the aggregates ($t(13)=2.3805, p<0.05$; +NaCl; $t(13)=2.1767$, $p<0.05$; −NaCl). As well, the brain weights of the isolates were significantly greater than those of the grouped rats (isolates—1.6075 g, grouped—1.51429 g, $t(13)=3.4241, p<0.01$).

DISCUSSION

Opiate receptor binding was significantly lower in the isolation reared rats than in those that were group housed. These data, coupled with Kostowski *et al.*'s [6] findings that rats housed in isolation are less sensitive to the analgesic properties of morphine, are consistent with the notion that opiate receptor binding is an important factor in a rat's responsiveness to opiates [7].

Isolation rearing can alter not only the analgesic efficacy of opiates but also other opiate-related behavioral measures. Isolation housed rats are less sensitive to certain aspects of the naloxone-precipitated withdrawal syndrome than group housed rats [1]. Further, isolation reared rats consume more morphine solution than those reared in aggregation [2]. This may be due to a decreased sensitivity of these rats to the rewarding impact of morphine. To compensate for this decreased effectiveness of opiates, these rats may be self-administering more drug to achieve the same reward value. Our findings suggest that these differences in the withdrawal syndrome, analgesic response to opiates, and oral consumption of morphine may be due to differential opiate receptor binding resulting from the environmental manipulation.

In the present study isolation was begun at approximately 22 days of age. It is interesting that in the studies of Kostowski *et al.* [6] and Adler *et al.* [1] the rats were approximately 150 days of age at the onset of isolation. Thus it appears that the behavioral effects of isolation can be induced relatively late in development. Although it has been shown that the opiate system does develop gradually until adulthood [4], further investigations are needed to determine the critical developmental time period of the housing effect. It is even possible that isolation housing could alter the opiate receptor binding in the mature central nervous system.

Developmental increases in binding have been shown to result from increases in the number of receptors rather than from increases in receptor affinity [4]. Thus it is likely that our results are due to changes in the number of receptors. However, this possibility should be confirmed by a Scatchard analysis [9] on the binding characteristics of the tritiated ligand. Further, more detailed studies delimiting which of the various opiate receptor subpopulations could be influenced by this purely behavioral, nonpharmacological manipulation would be of interest.

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