The effects of bilateral cingulate cortex lesions on morphine withdrawal symptoms in rats

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In order to test the hypothesis that bilateral cingulate cortex lesions prevent opiate dependence and associated withdrawal symptoms, rats were given access to sucrose morphine solutions and subsequently challenged with nalorphine, an opiate antagonist. Animals with cingulate cortex lesions lost more weight following the nalorphine challenge than other animals, indicating that the lesions do not prevent withdrawal symptoms. On the contrary, the lesions are associated with more severe symptoms, and by inference, physiological dependence as well.

Cingulate cortex lesions are associated with decrements in morphine-oriented behavior by rats (Trafton & Marques, 1971). Such decrements in morphine intake are shown both by animals premedicated with morphine injections and by animals that are not premedicated (Trafton & Kahn, 1974). However, the mechanism whereby cingulate cortex lesions reduce morphine intake remains obscure. One explanation is that cingulate lesions render the pleasurable or positive reinforcing effects of morphine intake less intense, thus reducing the animal's consumption. An alternative argument is that, at least among premedicated animals, cingulate cortex lesions somehow prevent physiological dependence and, thus, withdrawal symptoms as well. Morphine intake, according to the latter argument, is at least in part the dependent animal's method of avoiding withdrawal symptoms. Thus, if lesions prevent withdrawal symptoms, morphine intake would be unnecessary.

The present study was designed to test the hypothesis that bilateral cingulate cortex lesions prevent physical dependence and withdrawal symptoms in rats. Following the procedure of Risner and Khavari (1973), oral ingestion premedication is followed by nalorphine challenge. Rats subjected to such conditions show severe weight loss due to diarrhea during the 48-h period after nalorphine injections and that weight loss is taken as an index of physiological dependence. Presumably, the diarrhea is one uncomfortable symptom among others associated with opiate withdrawal.

METHOD

Subjects

The subjects were 30 male albino rats of the Sprague-Dawley strain, weighing between 216 and 350 g at the beginning of the

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experiment. Each rat was housed individually and had ad-lib access to Purina Rat Chow throughout the experiment. Drinking solutions were provided in 100-ml graduated glass drinking tubes mounted on the front of the cages.

Surgical Procedure

The rats were randomly assigned to three groups of 10 each. Group CM (Cingulate-Morphine) received bilateral anterior cingulate cortex lesions. Sodium pentobarbital anesthesia (35 mg/kg, IP) was induced ½ h after atropine sulfate injections (1 cc) which were given to reduce respiratory tract secretions. The bilateral anterior cingulate cortex lesions were made electrolytically through closely spaced holes drilled in the skull .6 mm lateral from the midline. A current of 1.0 mA was applied for 15 sec at each electrode site. The lesion targets extended 5.5 mm anterior from the bregma and were designed to fuse to produce a continuous ablation of the anterior midline cortex. Group NCM (No Cingulate-Morphine) received sham operations. They were anesthetized, and skull holes were drilled as for Group CM, but no lesions were introduced. Group S (Sucrose) received no surgical treatment.

Procedure

Two weeks after the lesions were given, the water bottles were removed from the cages, and Groups CM and NCM were given bottles containing 1.0 mg of morphine HCl per milliliter of 10% sucrose solution. Group S received 10% sucrose only. No other fluids were available, and Groups CM and NCM were thus forced to consume morphine. Daily measurements of food, fluid intake, and body weight were recorded for 21 days. On the 21st day of the experiment, all rats were given a 16-mg/kg intraperitoneal injection of nalorphine. Food intake, liquid intake, and body weight were measured at 0 (baseline), 4, 8, 24, and 48 h after the nalorphine injection. Ad-lib access to the sucrose morphine and sucrose solutions for the experimental and control groups, respectively, was maintained throughout these tests. Following these tests, the rats in Group CM were killed; their brains were perfused with saline and Formalin, sectioned and stained with cresyl violet, and inspected to assess brain damage.

RESULTS

Seven rats died during the experiment. Two rats were from Group CM, while five rats were from Group NCM. The deaths were preceded and probably caused by a refusal to drink the only available fluid, sucrose morphine, and a subsequent decrease in body weight. No rats in Group S died.

Figure 1. Mean body weight (% of baseline) following 16 mg/kg nalorphine injections.

After perfusion of the Group CM brains, observations showed that three of the brains did not have visually detectable anterior cingulate cortex lesions. The three rats were thus considered to belong to the NCM group. So, finally, five rats remained in Group CM, eight rats comprised Group NCM, and Group S had 10 rats.

Group CM lost more body weight after the nalorphine injection than either Group NCM or Group S. Figure 1 shows the mean percentage of baseline body weight (weight at time of nalorphine injection) lost for the three groups at 4, 8, 24, and 48 h after the nalorphine injection. Group CM lost weight steadily until 24 h after the nalorphine injection; by 4 h postinjection, they had lost 6% of baseline weight. Eight hours after the injection, 9% of baseline weight had been lost. Twenty-four hours postinjection, 15% of mean body weight was lost. There was a return to baseline body weight at 48 h after the injection. Group NCM lost 4% of mean body weight 4 h after the nalorphine injection. At 8 and 24 h after injections, their weight loss was constant at 6% before returning to baseline. Group CM lost significantly more weight 24 h after nalorphine injections than either other group ($p < .05$) and lost significantly more weight than Group S at 4 and 8 h postinjection, as well ($p < .05$). No significant differences between any groups were found either at

baseline or at 48 h postinjection. Wilcoxon-Mann-Whitney tests were used on all comparisons.

Baseline weight and intake measures were defined by the averages obtained during Days 19, 20, and 21 of the preinjection period.

Analysis of the food and fluid intake data during the first 24-h period following injections reveals that there were no statistically significant differences between the groups for food or fluid intake. However, as Table 1 shows, Group NCM drank a significantly greater amount of fluid than Group S at 48 h postinjection.

The lesions induced were extensive in all five rats of Group CM. Most of the midline tissue from the anterior pole to the genu of the corpus collosum was destroyed, with the lesions extending about 1 mm lateral to the midline and from 2 to 4 mm below the dorsal surface of the cortex including the pre- and subgenual areas. One rat received damage to the corpus collosum and dorsal septum as well.

DISCUSSION

Cingulate cortex lesions do not lessen morphine dependence or withdrawal symptoms in rats. On the contrary, among rats with extensive morphine intake experience (Groups CM and NCM), the animals with lesions tend to lose more weight following nalorphine challenge than nonlesioned rats. Thus, the effectiveness of cingulate cortex lesions in reducing voluntary morphine intake (Trafton & Kahn, 1974; Trafton & Marques, 1971) cannot be explained by the absence of dependence and associated withdrawal symptoms.

The present results, coupled with those of Trafton and Kahn (1974) showing decreases in morphine ingestion by cingulate-lesioned but drug-naïve rats, seem most compatible with the argument that such lesions reduce the pleasurable or positively reinforcing effects of morphine.

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Table 1
Intake of Food and Fluids Following Nalorphine Injections (Percentage Baseline)

Group	Hours Following Injections			
	24		48	
	Food	Fluid	Food	Fluid
S	86	91	101	114
NCM	93	62	125	175*
CM	63	45	126	131

* $p < .05$ relative to Group S