

BRIEF COMMUNICATION

# Combined Opiate/Adrenergic Receptor Blockade Enhances Squirrel Monkey Vocalization

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HARRIS, J. C. AND J. D. NEWMAN. *Combined opiate/adrenergic receptor blockade enhances squirrel monkey vocalization.* PHARMACOL BIOCHEM BEHAV 31(1) 223-226, 1988.—This study provides evidence for the interaction between opiate and noradrenergic neuronal systems on primate vocal behavior. The rate of twitters produced by adult squirrel monkeys was increased in an additive or synergistic manner by the combined administration of the  $\alpha_2$  adrenergic antagonist yohimbine and the opiate antagonist naloxone. Similar effects were not demonstrated on the isolation call. In addition, the drug combination led to an increase of autonomic symptoms. The anatomical localization of these findings and mechanism for the production of increased twitters requires further investigation.

Opiate     $\alpha_2$  Adrenergic    Isolation call    Separation anxiety    Anxiety    Squirrel monkey    Vocalization

THERE is evidence for the interaction of opiate and noradrenergic neuronal systems at several levels in the central nervous system of laboratory animals (1,4). In a clinical study with human subjects, Charney and Heninger (2) found a synergistic increase in anxiety and associated autonomic symptoms when combining an opiate antagonist, naloxone, and an  $\alpha_2$  noradrenergic antagonist, yohimbine in comparison to each drug administered separately. As a test for possible synergism of these two systems at the behavioral level in a primate model, we have chosen a vocalization paradigm that takes advantage of the increased production of characteristic vocalizations from squirrel monkeys (*Saimiri sciureus*) when separated from familiar conspecifics (9). In previous studies (6,8), we have reported increases in the sonographically distinct separation vocalization or isolation call following administration of a noradrenergic or opiate antagonist and argued that this paradigm is a useful model to study the vocal component of separation anxiety. These studies have led to the current investigation of the effects of combined treatment with both agents on vocalization in the

context of social separation. The present study is, to our knowledge, the first effort to test for synergism of these systems in a nonhuman primate. Preliminary findings have been reported elsewhere (7).

## METHOD

These experiments followed a conventional repeated measures design commonly used in drug studies involving primates [e.g., (3,5)] where each subject is used as its own control. Four adult male squirrel monkeys who reliably emitted isolation calls for periods of up to one hour were selected as subjects for tests involving brief acoustic and physical separation from conspecifics. Each subject was captured and received an intramuscular injection of drug or sterile water in the lateral thigh before testing. After injection the animals were left undisturbed for 5 minutes in a transport cage to allow for drug absorption, then brought to an observation cage in a quiet room containing no other monkeys. Vocalization and activity were counted over a 15-minute period and

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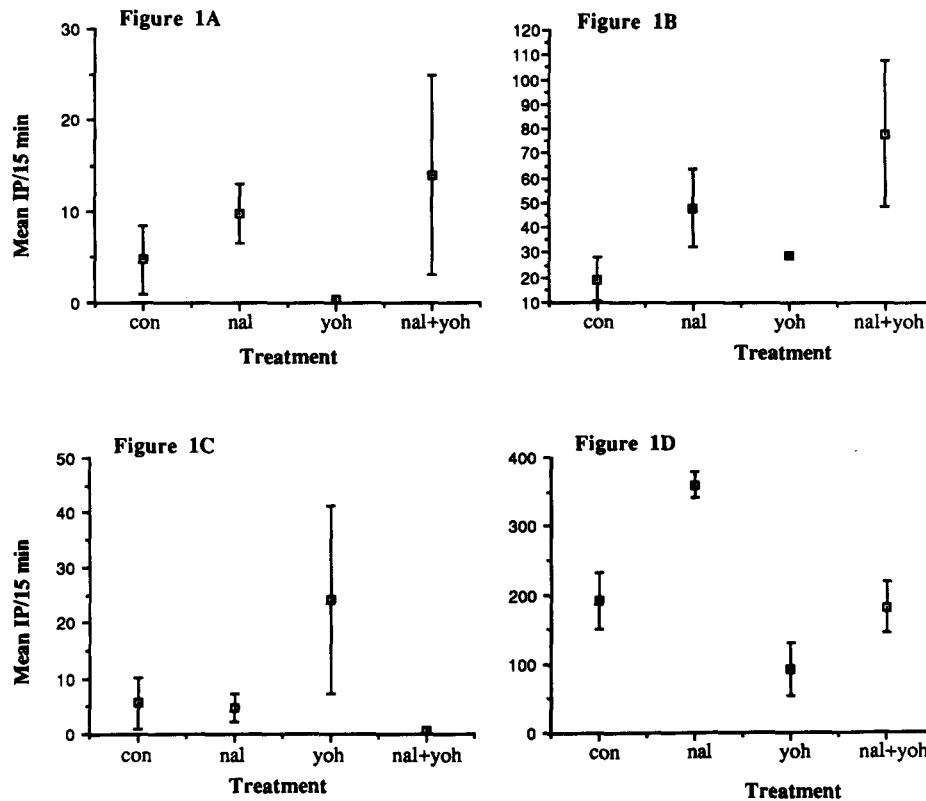


FIG. 1. (A-D) Drug effects on isolation call (IP) rate of production over three separate experiments. Results for subjects 1-4 respectively.

the entire session was videotaped for more detailed observation of autonomic and other behavioral symptoms. Activity scores consisted of the total number of counts recorded as the monkeys moved between eight different sectors. After testing the animal was returned to the colony room. Experiments were conducted at intervals of one or more weeks. The same order of treatment (vehicle, naloxone, yohimbine, combined naloxone/yohimbine) was used for each of three separate drug trials with at least one month between trials. Sonographic analysis was routinely carried out to compare drug trials with control sessions. Vocalizations were characterized according to the diagnostic scheme of Newman (9).

The choice of doses for the drug trials was based on our prior findings; Yohimbine hydrochloride at a dose of 0.2 mg/kg was shown in an earlier study (6) not to increase separation calls over control levels and therefore was chosen to make any synergistic effects more apparent and to reduce the possibility of a ceiling effect on calling. We previously found that naloxone hydrochloride at a dose of 0.4 mg/kg was as effective as doses up to 3.2 mg/kg in inducing a significant increase in isolation calling rate (8).

#### Statistical Analysis

Statistical analysis was performed using the paired *t*-test and the Wilcoxon Signed Rank test from the Statview 512+ software program.

#### RESULTS

The effects of the drugs and their combination on isola-

tion call production are shown in Fig. 1A-D. An enhancement of isolation call rate was found in three of the four animals with naloxone but not with yohimbine. Over three separate experiments, using the same dose of naloxone, this increased calling rate was shown to be statistically significant when compared to control levels,  $t(11) = -2.11$ ,  $p = 0.05$ . As previously demonstrated, yohimbine alone at this dose failed to produce a significant change in separation calling rate from control levels. The combined administration of the two drugs, naloxone and yohimbine, produced no synergistic effects on separation call production. In three animals, the combination resulted in no change over control levels, but in the fourth animal the combination produced an increase equivalent to naloxone administered alone. No differences were found when comparing the sonograms of the isolation call in control and experimental subjects.

An unexpected finding was the significant increase in another sonographically distinct vocalization, the twitter, when the drugs were administered in combination over control levels,  $t(11) = -1.954$ ,  $p = 0.038$ , or either drug administered alone [naloxone:  $t(11) = -2.332$ ,  $p = 0.019$ ; yohimbine:  $t(11) = -1.947$ ,  $p = 0.039$ ]. Figure 2A-D shows the results for the various treatments for each subject. The effect is especially dramatic in subject 2, who only made twitters during the combined drug trials. No differences were found when comparing twitters sonographically to twitters produced in social settings without drugs. To test whether the increase in twitters was true evidence of synergism, it was necessary to demonstrate that the increased vocalization resulting from the combined drug trials was greater than the sum of changes

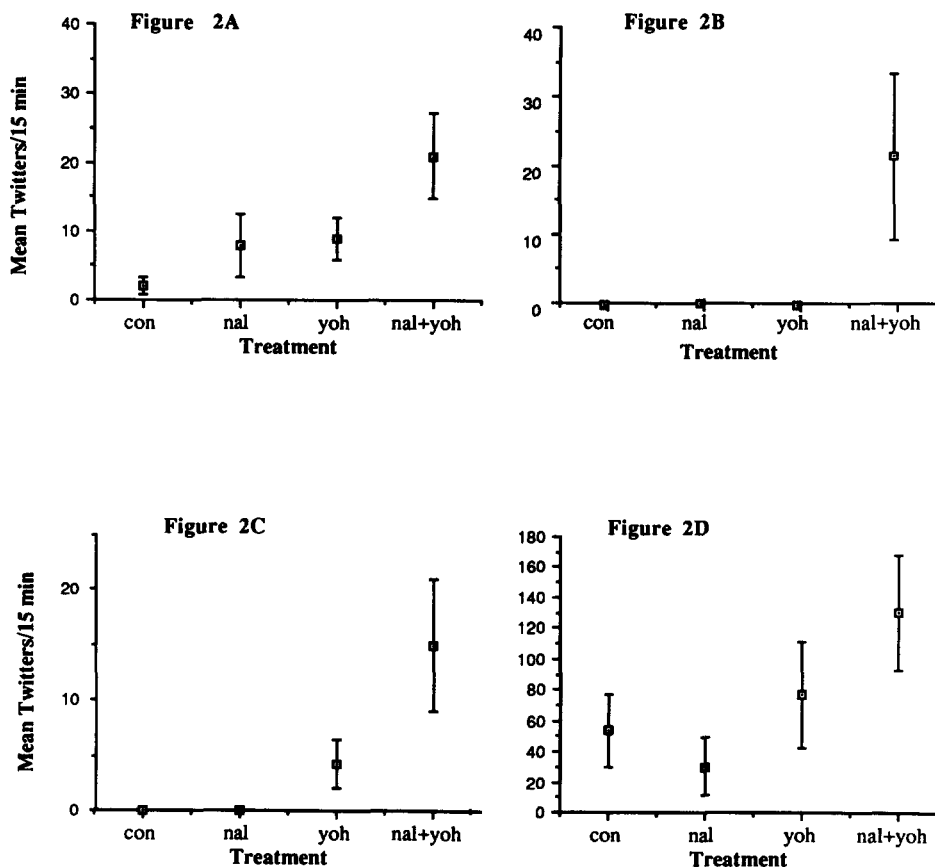


FIG. 2. (A-D) Drug effects on twitter rate of production over three separate experiments. Results for subjects 1-4 are shown in A-D respectively.

in calling rate when the two drugs were administered separately. A significant synergistic effect was demonstrated for two monkeys, subjects 2 and 3 (Wilcoxon Signed Rank Test,  $z=1.753$ ;  $p=0.04$ ). In subjects 1 and 4 the increased twitter production for the combined drug administration was found to be additive rather than synergistic.

In view of the findings in human subjects of increased autonomic symptoms when naloxone and yohimbine were given in combination, observations were made and videotapes were reviewed for possible evidence of autonomic changes in the monkeys. As shown in Table 1, autonomic and somatic changes included sneezing, rapid hand rubbing, diarrhea, and excessive salivation. Penile erection was noted in one subject. There was a relative increase in autonomic signs with both yohimbine and the combined drugs; however, when present, the autonomic effects were more pronounced with the combined treatment.

In view of a reported increase in nervousness in the clinical study (2) following combined naloxone/yohimbine administration, we determined whether locomotor activity increased under any of the treatment conditions. Mean activity scores recorded over 15 minutes were as follows: vehicle control (mean  $\pm$  SEM;  $112 \pm 113$ ); naloxone (mean  $\pm$  SEM;  $232 \pm 126$ ); yohimbine (mean  $\pm$  SEM;  $39 \pm 50$ ); naloxone and yohimbine (mean  $\pm$  SEM;  $65 \pm 59$ ). There was no evidence for an increase in locomotor activity during the drug combina-

tion trials. However, activity during the naloxone trials was significantly elevated over control levels,  $t(7)=-2.584$ ,  $p=0.036$ . Thus, the increase in locomotor activity occurred during the treatment in which isolation peep calling rate was also highest.

#### DISCUSSION

This study presents the first evidence that we are aware of for an interaction of noradrenergic and opiate systems on primate vocal behavior. Our findings with regard to the twitter call provide evidence for a synergistic effect of opiate and adrenergic blockade in 2 subjects and for an additive effect in 2 subjects. We view the unexpected enhancement in twitter production with the joint administration of naloxone and yohimbine as expanding the scope of behavioral effects of this drug combination. The failure to demonstrate a synergistic effect in 2 of the 4 subjects suggests that there might be differences either in the density or distribution of specific receptor subtypes in the brains of different individuals, or there might be individual differences in drug metabolism. Differential drug effects in patient or subject subpopulations are not uncommon in behavioral pharmacological studies, and have been shown to provide a useful method for categorizing patient subpopulations in clinical investigations [e.g., (10)]. A synergistic effect on separation anxiety as measured by increased separation calling was not demon-

TABLE 1  
SUMMARY OF AUTONOMIC EFFECTS

Subject	Treatment	Sneezing	Hand Rubbing	Diarrhea	Salivation	Erection/Masturbate
1	Control	0	0	0	0	0
2	Control	0	0	0	0	0
3	Control	0	0	0	0	0
4	Control	0	0	0	0	0
1	Naloxone	0	0	+	0	0
2	Naloxone	0	0	+	0	0
3	Naloxone	+	+	0	0	0
4	Naloxone	0	+	0	0	0
1	Yohimbine	0	0	+	+	0
2	Yohimbine	0	0	0	+	0
3	Yohimbine	+	+	0	+	0
4	Yohimbine	0	+	0	0	+
1	Nal/YOH	0	0	+	+	0
2	Nal/YOH	0	0	+	+	+
3	Nal/YOH	+	+	0	+	0
4	Nal/YOH	0	+	0	0	+

strated. Instead, at doses employed, we found no significant differences for the combined drugs when compared to vehicle or naloxone alone.

While we found autonomic signs similar to those reported in humans by Charney and Heninger (2), they were less consistent across all subjects. Because we used only a single dose of naloxone and a single dose of yohimbine in these studies, a more complete picture of these 2 neurochemical systems on behavior requires further experiment with a broader range of doses.

Findings by other investigators on the interaction between these 2 neurotransmitter systems in laboratory

animals at the neuronal level leave unclear the basis for these behavioral findings. While such studies have demonstrated an interaction between these 2 systems (1,4), the failure to report evidence of synergism at the neuronal level leaves unresolved the mechanism by which these 2 systems synergistically interact to produce changes in behavior.

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