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PRIMATE DISPLACEMENT ACTIVITIES AS AN ETHOPHARMACOLOGICAL MODEL OF ANXIETY

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Using a within-subject cross-over, vehicle-controlled design, we investigated the acute effects of benzodiazepine receptor ligands with different mechanisms of action on the displacement activities (scratching, self-grooming, and body shake) of seven male macaques living in social groups. Our aim was to test the discriminative validity of displacement activities as an ethopharmacological model of anxiety. Subjects were given i.m. lorazepam (0.10, 0.20, 0.25 mg/ kg) and FG 7142 (0.1, 0.3, 1.0 mg/kg). The frequency of displacement activities was decreased by the anxiolytic lorazepam and increased by the anxiogenic FG 7142 in a dose-dependent manner. Displacement activities were apparently more sensitive to anxiolytic treatment than other behavior patterns indicative of an anxiety state (i.e., visual scanning of the social environment and fear responses directed to dominant males). These results suggest that primate displacement activities are a valid ethopharmacological model of anxiety. Anxiety 2:186–191 (1996). © 1996 Wiley-Liss, Inc.*

INTRODUCTION

In stressful situations, nonhuman primates frequently display certain behaviors that are apparently out of context and consist of different body care activities such as scratching, self-grooming, and body shaking. These behaviors, which are referred to as "displacement activities" in the ethological literature, have been reported not only in primates but also in a variety of other animal groups (MacFarland, 1966). Classical ethological studies postulated that the occurrence of displacement activities is associated with motivational conflict or frustration (Tinbergen, 1952). However, more recent data suggest that, at least in nonhuman primates, displacement activities may also reflect an emotional state of anxiety (Maestripieri et al., 1992b).

Ethological studies of nonhuman primates have documented an increased frequency of displacement activities in situations of uncertainty, social tension, or impending danger such as spatial proximity to dominant males (Troisi and Schino, 1987), competition at feeding sites (Diezinger and Anderson, 1986), interaction with unfamiliar individuals (Rowell and Hinde, 1963), uncertain dominance relationships (Schino et al., 1990), and risk of aggression (Aureli and van Schaik, 1991). Pharmacological studies of chair-restrained monkeys have demonstrated that increased scratching is one of the symptoms of the behavioral syndrome induced by anxiogenic treatment (Ninan et

al., 1982; Insel et al., 1984; Crawley et al., 1985). However, these studies failed to comment on the specific meaning of the drug effects on displacement behavior. Two studies have explicitly investigated the relationship between displacement activities and anxiety in unrestrained group-living monkeys treated with anxiogenic and/or anxiolytic compounds. Schino et al. (1991) reported that, in adult female macaques, the acute administration of lorazepam caused a selective reduction in the frequency of scratching, this being especially marked in low-ranking animals. Maestripieri et al. (1992a) found that midazolam reduced scratching behavior in 30-week-old macaque infants, while treatment of the same infants with β-CCE, an anxiogenic compound, increased the frequency of scratching and

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was associated with a marked increase in the infant's contact-seeking and contact-maintaining behavior with the mother.

The aim of this study was to provide further pharmacological evidence for the ethological hypothesis that displacement activities are behavioral manifestations of anxiety in nonhuman primates. To do this, we investigated the effects of two benzodiazepine (BDZ) receptor ligands with different mechanisms of action on the displacement behaviors of male macaques living in social groups. The BDZ receptor ligands used in this study were the agonist lorazepam, which in humans, has anxiolytic properties (Greenblatt et al., 1983) and the inverse agonist FG 7142, which exerts anxiogenic effects (Dorow, 1987). We hypothesized that, if displacement activities are behavioral manifestations of anxiety, their frequency of occurrence should decrease after treatment with lorazepam and increase after treatment with FG 7142.

In addition to displacement activities, we recorded other behavior patterns including locomotion, visual scanning of the social environment, fear responses to dominant males, aggression, and social grooming. Locomotion was recorded to obtain a quantitative measure of the sedative effects of drug treatments. Since there is evidence that the level of anxiety influences both visual scanning of the social environment and fear responses to dominant males (Vellucci et al., 1986), we recorded these anxiety-related behaviors to compare the effects of drug treatments on displacement activities with those on referent behavioral measures. The elicitation of displacement activities depends in part on the conditions of the animal's fur and skin, and both aggression and social grooming can alter such conditions. In addition, aggression and social grooming affect the emotional state of the animal (Aureli et al., 1989). Therefore, we recorded aggression and social grooming to exclude that changes in the frequency of displacement activities were secondary to drug-induced changes in these behavior patterns.

METHODS

SUBJECTS AND HOUSING

Subjects were seven subadult (4–7 years of age) male macaques (*Macaca fascicularis*) born in captivity. Subjects were free from any signs or symptoms of illness, and their mean ± S.E.M. weight was 5.5 ± 0.6 kg. Subjects were housed as members of three social groups consisting of 11, 12, and 38 monkeys, respectively. Three subjects were housed in the largest group, and two each in the two smaller groups. Each social group included 1 or 2 adult males, a variable number of adult females (4 to 17), and several juveniles. The subjects were subordinate to the adult males in their groups. Dominance rank was determined on the basis of the direction of agonistic interactions recorded during pilot observations.

DRUG TREATMENT

Each subject was given i.m. a 0.9% NaCl solution as placebo and three different doses of each drug: lorazepam, 0.10, 0.20, and 0.25 mg/Kg; and FG 7142 (β-carboline-3-carboxylic acid methylamide), 0.1, 0.3, and 1.0 mg/Kg. The lorazepam and FG 7142 dosages were chosen on the basis of pilot observations and previous studies (Schino et al., 1991; Wettstein et al., 1993) showing that these dosages did not cause gross behavioral alterations in monkeys. Lorazepam (Temesta, Wyeth, Basel, Switzerland) was given as commercially available preparation. FG 7142 was ultrasonically dispersed in distilled water with a drop of Tween 20.

STUDY DESIGN

The study used a within-subject cross-over, vehicle-controlled design. Different treatments and dosages were given to each animal in a counterbalanced order. Consecutive sessions using the same drug in the same subject were separated by an interval of 2–3 days, so that a drug cycle was generally completed on each animal in 8 days. Different drug cycles on the same animal were separated by an interval of at least 9 days. Only one animal was treated and sampled on the same test day.

BEHAVIORAL MEASURES

Drugs or placebo were injected 15 (FG 7142) or 30 (lorazepam) minutes before the start of the observation session. Each observation session lasted 120 minutes and was conducted between 12.00 and 14.00 hours. The sampling method was a combination of the "focal animal" technique with either the "complete record" technique (i.e., the observer recorded the timing of every episode of the behaviors under investigation involving the focal subject) or the "instantaneous sampling" technique (i.e., on the instant of the sample point the observer recorded whether or not the focal subject was engaging in the behaviors under investigation; there were 120 sample points per session, one sample point every minute; Martin and Bateson, 1986). The following behaviors were recorded using the "complete record" sampling technique: scratching (a repeated movement of the hand or foot during which the fingertips are drawn across the individual's fur); self-grooming (picking through and/or slow brushing aside one's own fur with one or both hands); body shake (a shaking movement of the entire body similar to that of a wet dog); nonagonistic approach by the alpha male (the alpha male approaches the subject within 0.5 m without showing any overt sign of aggression); fear response to the alpha male's approach (the subject responds to the approach of the alpha male either leaving within 5 seconds or displaying teeth-baring, i.e., retracting the mouth corners and exposing the teeth); aggression done (the subject threatens, chases, or physically assaults another monkey); aggression received (the subject is threatened, chased, or physically assaulted by another monkey);

allogrooming done (the subject inspects and cleans another monkey's fur through rhythmical patterns of fur manipulation); and allogrooming received (the subject's fur is inspected and cleaned by another monkey through rhythmical patterns of fur manipulation).

The following behaviors were recorded using the "instantaneous sampling" technique: locomotion (the subject walks, runs, or climbs); and visual scanning of the social environment (the subject looks at another monkey).

DATA ANALYSIS

In addition to simple measures of behavior patterns, two composite behavioral indices were used in the data analysis. The Displacement Activity Index was obtained by summing the frequencies of the three displacement activities recorded (scratching, self-grooming, and body shake). The Fear Response Index was calculated as the percentage of alpha male's approaches to which the subject responded with fear signals (i.e., [no. of fear responses to the alpha male's approach / no. of nonagonistic approaches by the alpha male] × 100; see De Waal and Luttrell, 1989, for a similar index).

Behavioral data were analyzed using repeated measures analysis of variance (ANOVA) with drug dosage as the within-subjects factor. Percent data were arcsin transformed to ensure normality. Significant ANOVAs were followed by contrasts (Abacus Concepts, 1989) to compare the effect of each dose with the effect of placebo and by Kendall coefficients of concordance to test the similarity of the individual dose-response curves.

RESULTS

EFFECTS OF LORAZEPAM

Lorazepam decreased the frequency of displacement activities in a dose-dependent manner ($F = 3.36$, df = $3/18$, $p < 0.05$). There were wide individual differences in the effects of anxiolytic treatment on displacement activities, as indicated by the lack of correlation among individual dose-response curves ($W = 0.33$, $K = 7$, $N = 4$, n.s.). Lorazepam did not influence either the scanning of the social environment ($F = 0.39$, df = $3/18$, n.s.) or the fear responses to the approaching alpha male ($F = 0.72$, df = $3/12$, n.s.).

Locomotion was not significantly affected by lorazepam, indicating an absence of sedative effects ($F = 1.22$, df = $3/18$, n.s.). The effect of lorazepam on the frequency of aggression by the subjects did not reach statistical significance ($F = 2.40$, df = $3/18$, $p = 0.10$), even though there was a dramatic decrease (77–88%) below the baseline values. Anxiolytic treatment did not modify either the frequency of aggression addressed to the subjects by other group members ($F = 1.30$, df = $3/18$, n.s.) or their participation in allogrooming interactions (allogrooming done: $F = 0.92$, df = $3/18$, n.s.; allogrooming received: $F = 0.65$, df = $3/18$, n.s.) (Figures 1–3).

EFFECTS OF FG 7142

Following the acute administration of FG 7142, no subjects showed convulsion or other signs of motor excitation (twitches, jerks or tremor). Anxiogenic treatment caused a dose-dependent increase in the frequency of displacement activities ($F = 4.58$, df = $3/18$, $p < 0.01$). This effect was consistent across all the subjects, as indicated by the similarity of the individual dose-response curves ($W = 0.44$, $K = 7$, $N = 4$, $p < 0.05$). In addition, FG 7142 caused a dose-dependent increase in both the scanning of the social environment ($F = 10.78$, df = $3/18$, $p < 0.0005$) and the Fear Response Index ($F = 5.22$, df = $3/15$, $p < 0.02$). Individual dose-response curves were significantly correlated in both cases (scanning: $W = 0.53$, $K = 7$, $N = 4$, $p < 0.01$; Fear Response Index: $W = 0.63$, $K = 6$, $N = 4$, $p < 0.05$).

There was a significant decrease in locomotory activity after FG 7142 administration ($F = 3.21$, df = $3/18$, $p < 0.05$). Aggression by the subjects increased significantly ($F = 3.95$, df = $3/18$, $p < 0.05$), but the individual dose-response curves were not significantly correlated ($W = 0.27$, $K = 7$, $N = 4$, n.s.). FG 7142 did not modify either the frequency of aggression addressed to the subjects by other group members ($F = 0.26$, df = $3/18$, n.s.) or their participation in allogrooming interactions (allogrooming done: $F = 2.08$, df = $3/18$, n.s.; allogrooming received: $F = 0.43$, df = $3/18$, n.s.) (Figures 1–3).

DISCUSSION

The effects of the acute administration of different benzodiazepine receptor ligands on displacement activities displayed by male macaques living in social groups support the hypothesis that, in nonhuman primates, these behavior patterns are behavioral manifestations of anxiety. Anxiolytic and anxiogenic treatments exerted opposite effects on displacement activities: lorazepam caused a dose-dependent decrease in the frequency of displacement activities, whereas the β-carboline FG 7142 caused a dose-dependent increase. These changes were not mediated by drug effects on other behaviors that can alter the emotional state of the animal or the cutaneous stimuli eliciting displacement activities. These results confirm and expand those of previous ethopharmacological studies that used displacement activities as a measure of anxiety in adult (Schino et al., 1991) or infant (Maestripieri et al., 1992a) macaques observed during social interactions.

Another interesting finding of this study is that displacement activities were apparently more sensitive to anxiolytic treatment than other behavior patterns indicative of an anxiety state. The nonsedative low doses of lorazepam that we used in this study did not affect either visual scanning of the social environment or fear responses directed to dominant males, two behav-
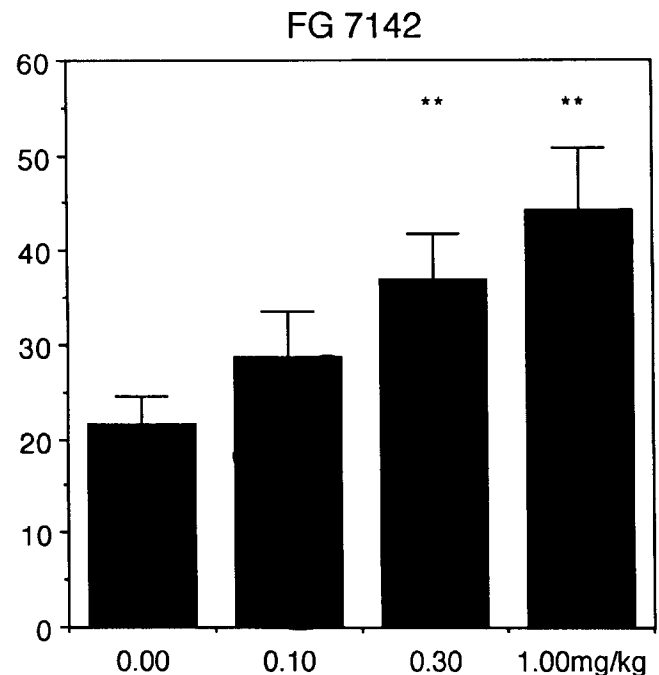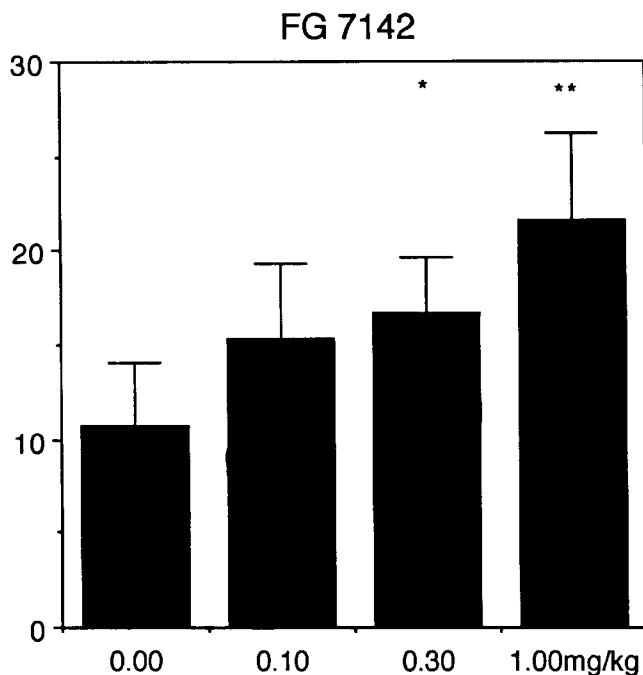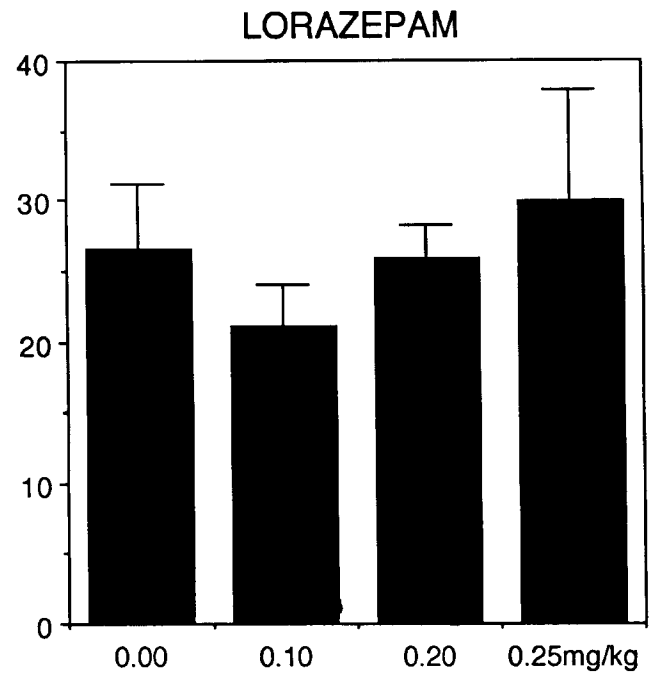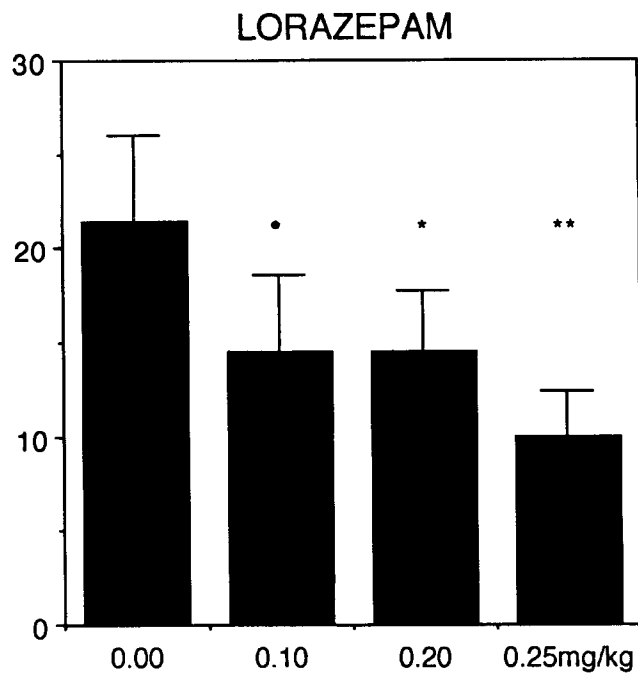
LORAZEPAM

LORAZEPAM

FG 7142

FG 7142

Figure 1.   Effects of lorazepam and FG 7142 on the Displacement Activity Index (mean ± S.E.M. no. of episodes per hour of scratching, self-grooming, and body shake) in seven group-living male macaques. $*p < 0.05$; $**p < 0.01$: these significances refer to the difference to placebo.

Figure 2.   Effects of lorazepam and FG 7142 on visual scanning of the social environment (mean ± S.E.M. no. of episodes per hour) in seven group-living male macaques. $**p < 0.01$: this significance refers to the difference to placebo.

ior patterns that reflect anxiety as demonstrated by their increase after treatment with FG 7142. Nevertheless, the same doses of lorazepam were sufficient for decreasing the frequency of displacement activities. If confirmed by futher research, the high sensitiv-

ity of primate displacement activities to anxiolytic treatment may prove most useful in assessing the relative potency of new anti-anxiety drugs.

A number of features of primate displacement activities suggest that these behaviors can be used as a
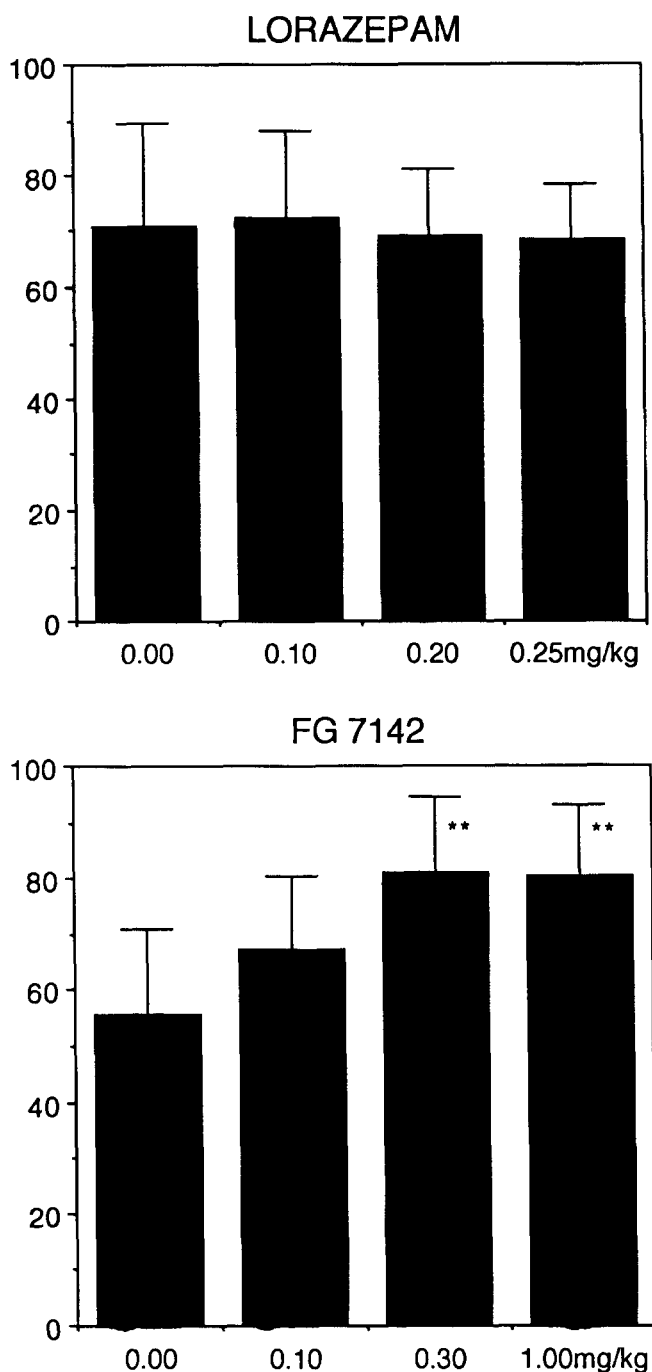
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FG 7142

Figure 3. Effects of lorazepam and FG 7142 on the Fear Response Index (mean ± S.E.M. percentage of alpha male's approaches to which the subject responded with fear signals) in seven group-living male macaques. $**p < 0.01$: this significance refers to the difference to placebo.

valid ethopharmacological model of anxiety. First, as shown by this study, the model discriminates efficiently between drugs that improve anxiety and those agents that make the clinical condition worse, even

though a conclusive judgment about its predictive validity must wait for studies using other benzodiazepine and nonbenzodiazepine anxiolytics including drugs acting on 5-HT receptor systems. Second, the model has face validity because behaviors homologous to primate displacement activities such as scratching and self-grooming have been observed in human subjects experiencing anxiety as measured by self-report questionnaires (Waxer, 1977) and clinician-rated scales (Fairbanks et al., 1982). Third, the model has construct validity because its physiological substrates and precipitating conditions resemble those of clinical anxiety. The physiological changes associated with displacement activities depend on the activation of the autonomic nervous system (Boccia et al., 1989) and include piloerection and cutaneous vasodilatation (MacFarland, 1966). As for etiology, uncertainty and anticipation of unpleasant events are crucial factors in the causation of displacement activities (Aureli and van Schaik, 1991).

Unlike most animal models of anxiety that involve the actual presence of highly aversive stimuli including physical pain (Treit, 1985), displacement activities are elicited by ambiguous situations somehow perceived as threatening, but where no immediate threat is apparent to the animal (Maestripieri et al., 1992b). In this regard, this model is similar to that developed by Kalin and Shelton (1989) and Kalin et al. (1991) in which infant rhesus macaques are briefly separated from their mothers and exposed to a human intruder who stares at them. The fact that uncertainty is a key factor in eliciting displacement activities is interesting in relation to the clinical notion that human anxiety often depends on the meaning attributed to a given stimulus or situation (Uhde and Nemiah, 1989).

Clearly the use of displacement activities as a behavioral model of anxiety requires a greater investment of time and resources than is needed for most of the procedures currently employed in psychopharmacological research (Troisi, 1994). Adopting the taxonomy proposed by Willner (1991), we should consider this model as a simulation (i.e., a model that attempts to use animals to further our understanding of human mental processes) with a range of applicability that, for practical reasons, is inevitably narrower than that of screening tests (i.e., models designed solely to expedite the discovery of new drugs). However, what is lost in time may be gained in validity (Lister, 1990).

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