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Aggression and Brain Serotonergic Responsivity: Response to Slides in Male Macaques

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KYES R. C., M. B. BOTCHIN, J. R. KAPLAN, S. B. MANUCK AND J. J. MANN. *Aggression and brain serotonergic responsivity: Response to slides in male macaques.* PHYSIOL BEHAV **57**(2) 205–208, 1995.—The association between central serotonergic responsivity (measured by prolactin response to acute administration of fenfluramine hydrochloride) and aggressivity was examined in 40 adult male cynomolgus monkeys (*Macaca fascicularis*). Prolactin response to fenfluramine was distributed bimodally with 24 monkeys displaying a "low" prolactin response and 15 showing a "high" prolactin response to the fenfluramine challenge. Behavioral responsivity was assessed by placing the monkeys individually in an open-field enclosure and presenting a series of photographic slides depicting both threatening and nonthreatening images. Monkeys that were low prolactin responders displayed significantly more aggressive gestures in response to a threatening slide of a human being than did the high responders ($p < 0.05$). Insofar as fenfluramine-stimulated prolactin release assesses serotonergic responsivity, these data support related findings in people and nonhuman primates linking reduced serotonergic activity and aggression.

Aggression serotonin 5-hydroxytryptamine Fenfluramine Prolactin *Macaca fascicularis*

INTRODUCTION

A PROPENSITY to exhibit impulsive, aggressive behavior has been linked to reduced levels of central serotonergic (5-HT) activity in both human beings and animal models (2,3,13). In nonhuman primates, for example, pharmacologically induced increases or decreases in central 5-HT activity have been shown to affect inversely levels of aggression (11,12). Other studies have demonstrated a similar inverse relationship between naturally occurring variability in serotonergic activity and indices of aggressive behavior (6). Previous work in our laboratory (1) has shown that, in a group of 75 adult male cynomolgus macaques (*Macaca fascicularis*), animals having a low index of central 5-HT responsivity (measured by prolactin response to fenfluramine challenge) (5,10) engaged in more violent aggression and less affiliation than did their high serotonin counterparts.

Here we report, in a subset of the original 75 monkeys, the association between central 5-HT responsivity and aggressivity as assessed in a nonsocial setting with a standardized behavioral assessment procedure (7). In this procedure, photographic slides containing both threatening and nonthreatening images were presented to monkeys placed individually in a controlled environment (i.e., open-field enclosure).

METHODS

The subjects were 40 adult male cynomolgus macaques (ages 10–14 yr) chosen randomly from a larger group of 75 monkeys involved in a 28-mo experiment evaluating the effects of diet and social stress on progression of coronary artery atherosclerosis (see reference 1). The subjects were imported from Indonesia as adults (ages estimated from dentition) and housed in eight, 5-member social groups.

Fenfluramine Challenge

Prolactin response to acute administration of fenfluramine hydrochloride (an indirect central 5-HT agonist) is considered an index of overall central serotonergic responsivity (5,10). Elevated plasma prolactin levels in response to fenfluramine presumably reflect greater central serotonergic responsivity. Conversely, low prolactin levels in response to fenfluramine would suggest lower serotonergic responsivity. Fenfluramine challenge offers a less invasive technique for assessing serotonergic function than direct measurement of cerebrospinal fluid (CSF) 5-HIAA concentrations. Further, findings with human beings indicate that prolactin response to fenfluramine correlates positively with CSF 5-HIAA concentrations (9).

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The fenfluramine challenge was administered as follows. During month 23 of the main study (approximately 3.5 mo before the behavioral assessment) each monkey was given 4 mg/kg fenfluramine hydrochloride i.m. between 0800–1000 h. The procedure involved placing all the animals of a social group in a squeeze cage, giving each the injection, and returning them to the group home cage. One hour later (0900–1100 h), the group was returned to the squeeze cage and each animal anesthetized with ketamine hydrochloride (10 mg/kg i.m.). Blood samples were collected by venipuncture 10–20 min after the animals were anesthetized. The samples were transferred into tubes containing EDTA and frozen at −20°C. for later determination of plasma prolactin levels. Two weeks before the fenfluramine challenge, baseline prolactin samples were taken. Using the procedure described above, subjects were given a sham injection of sterile saline i.m. One hour later, blood samples were collected and frozen at −20°C. The animals were fasted for 12 h before both the sham and challenge procedures. Plasma prolactin levels were determined by radioimmunoassay (rabbit anti-human prolactin serum purchased from Ian Worsley, Winnipeg, Manitoba, Canada). The lower limit of detection for this assay is 1.0 ng/ml.

Behavioral Assessment

The 40 experimental animals were chosen randomly for behavioral testing prior to evaluation of the results from the fenfluramine procedure. Testing took place in an open-field enclosure that measured 3 × 2 × 1.75 m using methodology similar to that developed previously by our group (7). At one end of the enclosure, a rear-projection slide screen (40 × 40 cm) was centered on the wall 20 cm above the floor. The monkeys were observed with the use of a closed-circuit television camera mounted below the slide screen. The enclosure was illuminated with two 40-watt incandescent lights located side by side in the center of the ceiling that permitted clear observation of the monkeys and reduced illumination for slide projection. A computerized system controlled the slide presentation and recorded the behavioral scoring.

Eight 35-mm color slides (Kodak Ektachrome 64 film) were used. The slides contained threatening, nonthreatening, and neutral images intended to elicit a range of behavioral responses. The slides included: (a) adult male monkey with mild open-mouth threat in a face-on orientation; (b) upper torso of a male technician dressed in clinical attire (i.e., mask, bonnet, and gown) and wearing monkey catch gloves; (c) python; (d) infant monkey sitting beside its mother; (e) two monkeys grooming; (f) fruit (variety of small berries from Indonesia); (g) trees (Indonesian jungle scene); and (h) a blank slide with light greenish tint (no stimulus content) that served as a control slide.

During testing, each monkey was placed individually in the enclosure. A 10-min acclimation (baseline) period preceded the slide presentation. Each of the eight slides were then presented sequentially for 30 s with a 2-min interslide interval. During the slide presentation, an observer (blind to the results of the fenfluramine challenge) recorded the duration of attention to the slides and the occurrence of agonistic responses directed toward the slides, including (a) aggression: threat (open-mouth stare), lunge, charge, contact aggression (e.g., slap, push, bite screen), growl; and (b) submission: withdraw from screen, crouch, lipsmack (discrete bouts), grimace, and squeal. After the last slide, there was an additional 10-min (baseline) period followed by the removal of the subject. Throughout testing, white noise was presented to minimize reaction to extraneous sounds.

Each subject was tested with the same slide series for three consecutive days between 0830–1200 h. The order of subject testing and slide presentation was randomized for each session (day). To control for potential order effects of the slides, the slide order was rerandomized at the beginning of a new session if a given slide appeared first or last in consecutive sessions.

Statistical Analysis

All analyses were computed using the BMDP statistical package on a VAX computer. Prolactin data were not obtained for one monkey, leaving data from 39 animals available for analysis. The behavioral data were analyzed with respect to the monkeys' prolactin response to fenfluramine challenge using a one-factor (Prolactin Response low, high) analysis of covariance (ANCOVA). Baseline prolactin concentration was used as the co-

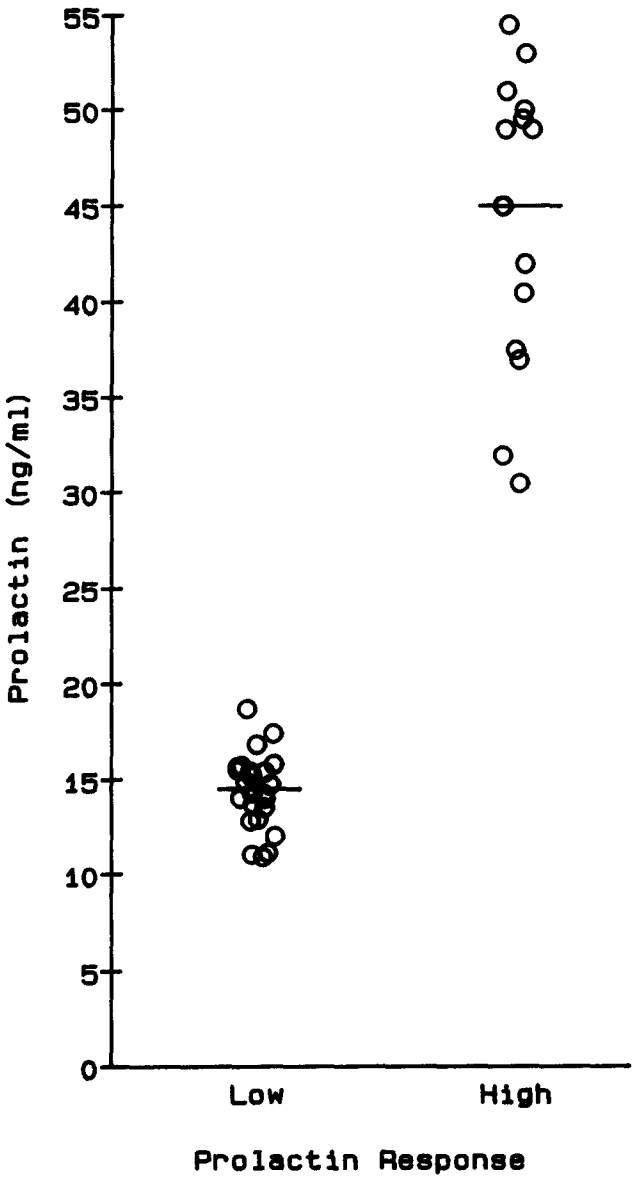

FIG. 1. Scatterplot displaying the bimodal distribution of prolactin response to fenfluramine challenge. The two prolactin response groups are referred to as low responders (n = 24) and high responders (n = 15). The horizontal bar within each data set represents the mean prolactin response.

variate based on its correlation with prolactin response to fenfluramine (discussed below). Logarithmic transformation of the behavioral data and baseline prolactin concentrations was used to reduce skewness and equalize group variances. A chi-square analysis was performed to evaluate potential bias due to dominance rank. For all analyses, differences were considered statistically significant at the $p < 0.05$ level. Data are reported as mean ± standard error of the mean.

RESULTS

Prolactin Response to Fenfluramine

The distribution of prolactin response to fenfluramine was bimodal (Fig. 1); therefore, the subjects were grouped as "low prolactin responders" ($n = 24$) and "high prolactin responders" ($n = 15$) for the purpose of analysis. Baseline prolactin concentrations for all 39 animals were low ($\bar{X} = 1.9 \pm 0.3$ ng/ml) but correlated significantly with the response to fenfluramine ($r = 0.49$, $p < 0.01$). Therefore, baseline prolactin concentration was used as a covariate in all analyses comparing the groups' behavior. A chi-square analysis of dominance rank indicated an equivalent distribution of animals at each rank (1 = dominent to 5 = subordinate) in both response groups ($\chi^2 = 0.74$, df=4, $p = 0.74$).

Behavioral Assessment

Preliminary analysis revealed that only two of the slides engendered any agonistic responses, the technician and the threatening monkey. Therefore, all analyses were limited to these two slides. A range of aggressive (threat, push, slap, bite) and submissive (withdraw, lipsmack, grimace) gestures and vocalizations were directed at both slides. An analysis of covariance demonstrated that the "low prolactin responders" displayed significantly more aggressive gestures in response to the slide of the technician ($F_{1,36} = 4.32$, $p < 0.05$) than did the "high prolactin responders" (Fig. 2). Further, the two groups differed directionally in the same way in their response to the slide of the threatening monkey, but this difference did not attain statistical significance ($F_{1,36} = 0.89$, $p = 0.35$).

Additional analyses showed that the two prolactin response groups did not differ significantly in the number of submissive responses directed toward either the slide of the technician ($F_{1,36} = 0.86$, $p = 0.35$) or the monkey ($F_{1,36} = 0.00$, $p = 0.97$). Similarly, the two groups did not differ significantly in the duration of attention to either slide (Technician: $F_{1,36} = 0.12$, $p = 0.72$; Monkey: $F_{1,36} = 0.27$, $p = 0.60$).

DISCUSSION

The results of this investigation extend previous work by showing that animals characterized by an index of low central 5-HT responsivity exhibit increased aggression in nonsocial as well as social settings. Importantly, aggressive response was the only behavioral measure to differentiate the "low prolactin responders" from the "high prolactin responders" in the present study. Further, although high and low prolactin responders differed significantly in aggressive responses to only one slide, there was consistency in the direction of responses to both of the provocative slides (technician and monkey). In contrast, submissive responses and, more importantly, duration of attention, did not differ significantly or consistently between the two groups. This latter finding indicates that differences in aggressive response between the low and high responders were not related to differences in attention to the slides.

Recent evidence suggests that low central 5-HT activity actually may be associated with the more subtle or underlying aspects of aggression, namely a "tendency" (or impulsiveness or "irritability") toward aggressive behavior (3,8). As previously noted, ". . . reduced central 5-HT appears to be associated more specifically with a 'tendency to be aggressive' (given adverse or provocative stimuli) rather than with 'overt aggressive behavior itself' " (4). The data reported here indicate that the standardized behavioral assessment procedure may provide an appropriately sensitive index of aggressivity in monkeys. As such, it is an important adjunct to observations made in the context of a social group. This is because the confounds or constraints imposed by the presence of other animals may make it difficult to use social observation as the sole index of an individual's independent or "inherent" behavioral responsivity.

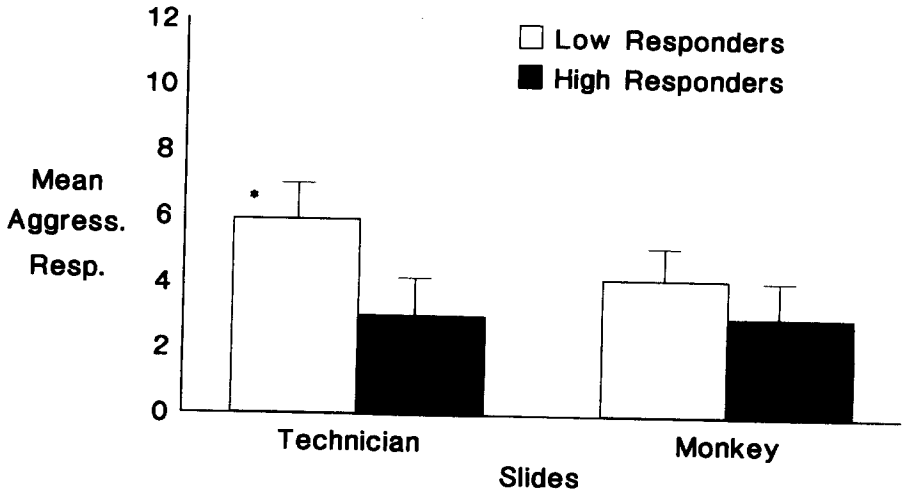

FIG. 2. Mean (± SE) aggressive responses for low (open bar) vs. high (black bar) prolactin responders to Technician and Monkey slides. Mean based on animals' aggressive responses for 3 sessions. Results represent untransformed data. *$F_{1,36} = 4.37$, $p < 0.05$ after logarithmic transformation of aggressive responses and covariate (baseline prolactin).

In summary, in adult male cynomolgus macaques, a lower prolactin response to fenfluramine (indicative of reduced central 5-HT responsivity) was associated with a significantly greater number of aggressive responses directed toward a slide of a threatening image. Insofar as fenfluramine-stimulated prolactin release assesses 5-HT responsivity, these data support related findings in people and nonhuman primates linking reduced 5-HT activity and increased aggression.

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