Reports

Behavior Disruption in Cebus Monkeys as a Function of Injected Substances

Abstract. *Intraperitoneal injections of blood substances from schizophrenic or stressed normal human donors into primates trained to perform a precision timing task resulted in significant prolongation of time taken to complete the task as compared with injections of blood substances from normal donors.*

The search for biochemical correlates of mental disorder has gained momentum in recent years, but overall findings fail to support conclusively the organic etiology hypothesis (*1*). Conflicting results have been prevalent and many of the data appear confounded by the lack of adequate behavioral or physiological controls. Recently, the problem of the measurement of objective behavior has been more effectively attacked in a number of studies by training animals to perform motor tasks under controlled conditions prior to injection of body fluids from schizophrenic and normal donors. The rate of rope-climbing by rats has been developed as one such bioassay technique and behavior disruption specific to body fluids from schizophrenics has been reported and verified (*2*). It is disturbing to note, however, that these and other studies have failed to include a control for stress variables so commonly allied to acute mental disorder. In a critical review of the field, Horwitt particularly emphasized the potential significance of stress physiology as a contaminating variable in studies assessing differences between schizophrenics and normals (*3*). Also, conclusive evidence exists that the "stress reaction" produces changes in plasma protein fractions, particularly globulins, which were once thought to be indicative of specific diseases (*4*). This is of interest because the proposed "toxic factor" of schizophrenia has been increasingly linked to the globulin plasma protein fractions (*5*). Nevertheless, none of the studies linking injection of schizophrenic body fluid with behavior disruption in organisms ranging from mouse to man have as yet controlled for or measured the effect of the stress variable.

In the present experiment an attempt has been made to combine objective measurement of behavior with more adequate control of physiological variables. In addition, primates were chosen for study to provide a bioassay organism phylogenetically closer to man.

Precision timing behavior, the dependent variable utilized in the present investigation, is a recently developed learning task which yields a number of measures of potential value in work involving physiological variables. The technique requires an organism to respond to a discriminative stimulus by pressing a lever for a specified time interval, that is, a duration greater than x units but less than x plus y units, in order to receive reinforcement. A specified number of daily presentations of a randomly recurring discriminative stimulus constitutes a session. Measures derived from the technique are: response duration, variance of response duration, number of reinforcements per session, number of responses emitted in the absence of the discriminative stimulus, and total time taken to complete a test session. Selection of an appropriate reinforced interval permits measurement of direction, as well as magnitude, of response change to experimental manipulation.

Eight *Cebus apella* monkeys (six experimental, two alternate) were trained in a modified Skinner box, under 22-hour food deprivation, to respond to a discriminative stimulus by holding down a lever for at least 1.0 second, but no longer than 1.6 seconds. Responses falling within this interval were reinforced with ½ ml of sweetened milk. Three hundred trials were given daily and training continued until 70-percent reinforcement for seven consecutive days was attained. Electronic equipment delivered all stimuli and recorded all response measures. Testing as well as training was carried out in sound-deadened isolation chambers. After training, and the completion of a control injection series of isotonic saline and Morgan's Medium (TC-199), the experimental injection series was begun. The effects of injecting human serum or plasma from normals, from acute catatonic schizophrenics, and from stressed normals were compared in a 6 by 6 Latin square design. Each monkey was thus used as his own control and received all experimental treatments, while the order of injection of substances varied from monkey to monkey.

Normal samples were drawn from clinically healthy professional donors and staff members of a local blood bank. Samples from psychotics were drawn from newly admitted patients manifesting catatonic symptoms, and not under medication. Stressed normals were nonmedicated, hospitalized patients scheduled to undergo major surgery within 1 hour after samples were withdrawn. Patients with known metabolic disturbances or with infectious conditions were excluded. All blood samples were 12-hour fasting samples, and were processed and injected within 8 hours after withdrawal from donors. Acid citrate dextrose solution was used as an anticoagulant for plasma samples, and Witebsky substance was used to remove anti-B isoagglutinins from both serum and plasma.

The design provided a minimum interval of 6 days between injections for the individual monkey, and the dosage level was 7.5 ml/kg. Injections were given outside the testing room to minimize conditioned fear responses that might carry over to the testing situation. A 20-minute period between injection and the start of the experimental run permitted systemic uptake of injected substances and minimized the measurement of trauma specific to catching and injection procedures.

Analysis of the data revealed no significant difference between experi-

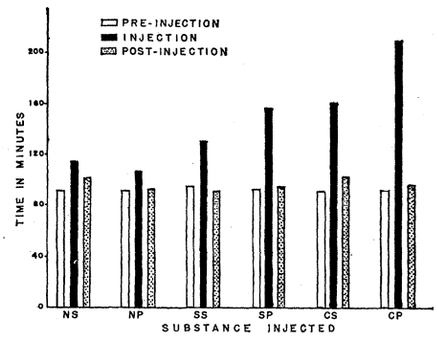

Fig. 1. Mean total time in minutes to complete the task on the day of injection of blood substances, and on the day before and after injection. Abbreviations: *NS* normal serum; *NP*, normal plasma; *SS*, stressed normal serum; *SP*, stressed normal plasma; *CS*, catatonic serum; *CP*, catatonic plasma.

mental treatments (donor category) for total number of reinforcements or for any response-duration measure. Total time taken to complete the 300-trial session, however, varied strikingly as a function of experimental treatment (see Fig. 1). Injections of serum or plasma from catatonics significantly prolonged total session time as compared with serum or plasma from normals ($p < .01$). Serum and plasma from stressed normal donors also significantly prolonged session duration ($p < .05$). Differences between the effects of blood samples from stressed normals and catatonics were nonsignificant. It is of interest, however, that three of the 12 samples from catatonics did not increase session duration at all, while two samples from catatonics produced an effect well beyond the range found for samples from stressed normals. In these two instances the monkeys required over 5 hours to complete 300 trials. The average daily time required to complete the task was 90 minutes.

The observed prolongation of session time found after injection of most samples from stressed normals and catatonics correlated with periods of apparent motor arrest. Animals would squat in fixed positions, often facing the discriminative stimulus panel but failing to respond to it. A number of other investigators have noticed a lack of responsiveness in animals injected with substances from schizophrenics. Workers utilizing the rope-climbing task described earlier, however, have not included a measure of response latency and have applied aversive stimulation to force a response. Our data indicate that the initiation of a response can be the most severely affected dimension of behavior, and that it is a pertinent variable.

The results suggest two alternate hypotheses. First, that all disruptive effects measured are allied to general stress physiology, and differences in degree of disruption correlate with the degree or stage of the stress process. Research with stressed primate donors is being instigated to provide relevant data.

An alternate hypothesis is that two separate factors are responsible for disruptive effects: one, a general factor related to stress physiology; the other, a factor specific to a limited percentage of schizophrenic cases with a common etiology (potentially represented by the two donors whose blood samples produced extreme disruption). The probability is high that a number of discrete and qualitatively different mechanisms underlie the common symptomatology of many psychiatric diagnostic categories. The dearth of biochemical breakthroughs in this field may well relate to an underlying heterogeneity among the cases grouped for study. Behavioral bioassay techniques such as the one utilized in the present study have potential value as selectors of a clinical subgroup worthy of intense biochemical study.

Finally, the data suggested the necessity for control or measurement of the stress variable in any assessment of the disruptive effects of body fluids from psychotics. The question of the specificity to psychosis of the toxic factor or factors implicated in previous studies should be more thoroughly investigated (*6*).

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References and Notes

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Beta-Alanine Utilization of Ebony and Non-ebony Drosophila melanogaster

Abstract. *Carbon-14–labeled β-alanine was injected into newly formed* Drosophila melanogaster *female pupae. Homozygous ebony deposited less* C^{14} *in pupal sheaths, deposited more* C^{14} *in adult body extracts and wings, and decarboxylated and oxidized β-alanine to excrete* $C^{14}O_2$ *faster than did non-ebony homozygotes. Heterozygotes were intermediate in all these activities.*

Early studies by L'Heritier, Neefs, and Teissier (*1*) and others indicate that the population of ebony *Drosophila melanogaster,* in competition with the non-ebony, stabilizes at a low frequency after an initially high frequency. These results are best explained in terms of low fitness of the ebony homozygote, which lowers the frequency of that genotype, and superior fitness of the heterozygote over either homozygote to prevent ebony from showing complete negative selection from the population. Elens (*2*) and Jacobs (*3*) have shown that heterozygous males are more vigorous in mating than homozygous males, especially homozygous ebony. This study was conducted to discover metabolic differences among these genotypes.

In preliminary work, samples of 16 different amino acids (C^{14} labeled) were injected into newly formed female pupae of the three genotypes, and C^{14} activity counts of pupal sheaths, wings, and body extracts as well as excreted CO_2 were made. Of the injected amino acids, only β-alanine showed appreciable differences among the genotypes. More extensive studies of this amino acid were then made and are reported here.

The flies used were ebony and a light tan wild type; all were collected at Beaufort, North Carolina (*4*). They were cultured; crosses were repeatedly made between them, and offspring were selected for homozygosity according to a previously described method (*3*). By means of an automatic micropipette, β-alanine-1-C^{14} and -2-C^{14} (100 μc, 1 mc/mmole of saturated aqueous solution at room temperature) were injected into females when they had become immobile for entering the pupal stage. Each female of each genotype was injected with about 0.005 μl in the dorsal blood sinus just posterior to the heart. In an attempt to treat the