Cardiovascular Concomitants of the Conditioned Emotional Response in the Monkey

Abstract. *Flow transducers were surgically implanted on the terminal aorta of five monkeys. A classical conditioning procedure, in which a light preceded a brief shock, was superimposed upon a variable-interval schedule of food reinforcement for key pressing (three monkeys) and alternated with an avoidance schedule of shock reinforcement for key pressing (two monkeys). Suppression of the rate of the response of key pressing and sizable increase in blood flow and heart rate during the light were obtained for all animals.*

There is ample evidence that changes in heart rate can be produced by the use of a classical conditioning procedure with electric shock as the unconditioned stimulus (*1*). As Shearn has pointed out, however, there is still considerable question as to the direction and magnitude of the conditioned heart rate response, and answers to the question of mediation of this response are still largely speculative. Measurement of rate alone yields indefinite information about other events in the cardiovascular system (for example, stroke volume, cardiac output, peripheral flow). One approach to the problem involves the examination of other measures of cardiovascular activity and their relation to heart rate changes.

The recent development of the ultrasonic flowmeter (*2*) has made possible the direct measurement of blood flow in the awake, behaviorally trained animal. By using a classical conditioning procedure with a light as the conditioning stimulus (CS), a brief shock as the nonconditioning, or "unconditioned stimulus" (US), and a CS–US interval of 56 seconds, we have shown that the conditioned response includes an increase in blood flow through the terminal aorta correlated with an increase in heart rate of more than 30 beats per minute (*3*). The study reported here was an extension of this research and represented an attempt to assess the effects of a classical (or Pavlovian) conditioning procedure simultaneously with an autonomic response (blood flow) and with the rate of occurrence of an ongoing conditioned somatic response (depression of a telegraph key). Suppression of the rate of key-pressing in response to a stimulus which signals an unavoidable shock has been used as a measure of an emotional response (*4, 5*). In addition to providing information about the form and development of conditioned cardiovascular responses, our study extends the definition of an emotional response by showing that the suppression of the rate of key pressing is accompanied by a very large response in the autonomic nervous system indicated by changes in the heart rate and blood flow.

The subjects, five adult monkeys (*Macaca mulatta* and *M. nemestrina*), were kept in restraining chairs (*6*) for the duration of the experiment. In each animal was surgically implanted a molded plastic flow section which was placed on the terminal abdominal aorta immediately above the iliac bifurcation. Connecting wires were brought out through the subject's back. Experimental sessions were conducted in a sound-proofed chamber with the flowmeter and associated circuitry outside the chamber.

Three monkeys were conditioned to press a telegraph key for food reinforcement. The reinforcement was scheduled aperiodically such that only occasional responses on the key were effective in obtaining the food. The average spacing between food-reinforced responses was 30 seconds. On such a schedule of reinforcement, the animal will, after some training, respond consistently on the key over fairly long periods of time (*7*). In our experiments, when the response rate had stabilized, a classical conditioning procedure was added. In each such conditioning session, a light was presented for 60 seconds which terminated with a 0.3-second, 10-ma electric shock to the monkey. Three trials were given during every half-hour at intervals of 5, 10, and 15 minutes. The intermittent schedule of food reinforcement for key-pressing, described above, was always in effect, and experimental sessions lasting about 2 hours were conducted every other day. Two other monkeys were trained on a Sidman avoidance schedule (*8*) in which shocks of 0.3 second duration and 10-ma intensity were administered every 20 seconds; however, each response on a telegraph key served to postpone the subsequent shock for 20 seconds; thus, if the key pressing occurred frequently enough the shock could be avoided completely. The classical conditioning procedure, exactly as just described but with a 56-second CS–US interval, was then added. However, instead of being superimposed upon the avoidance schedule, the classical conditioning trials were alternated with the schedule. In other words, the avoidance schedule was not in effect during the classical conditioning trials. Under this procedure, sessions were still conducted on alternate days but lasted from 4 to 6 hours.

The data were recorded on a Sanborn polygraph. Instantaneous blood flow (velocity) was measured with the ultrasonic flowmeter connected to the previously implanted flow section. A cardiotachometer, triggered from the flowmeter, was used to measure heart rate. Average blood flow (instantaneous flow integrated through a passive resistance-capacitance network with a 2-second time constant) was also recorded. A sensitive strain gauge fastened to the side of the monkey's chair

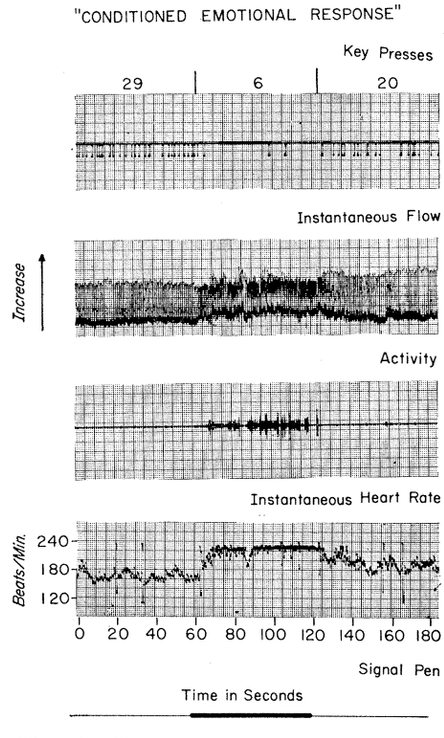

Fig. 1. Simultaneous measures of key pressing, cardiovascular activity, and gross somatic activity prior to, during, and after a classical conditioning trial. The subject was a monkey maintained on a food-reinforcement schedule.

was used to monitor gross motor activity. An additional channel on the polygraph recorded individual key presses, and a signal pen indicated the presence of the light.

Figure 1 shows a typical record from the Sanborn recorder for one food-reinforced animal. The record includes the changes in cardiovascular activity and in the key-pressing response rate immediately prior to, during, and after a classical conditioning trial. The numbers above the key-pressing record indicate the number of responses emitted in equal time intervals before, during, and after the trial. Conditioned suppression of the key-pressing response is evident and has been reported by others (*4*, *5*). In addition, there is an increase in instantaneous flow and an increase in heart rate by more than 40 beats per minute in response to the conditioning stimulus. There is also a considerable amount of gross motor activity during the presentations of the conditioning stimulus. However, the large increase in heart rate precedes the activity, and thus it is unlikely that the rate increase is secondary to the activity. It is possible that the skeletal activity serves to maintain the heart rate at the higher level for the remainder of the time during which the conditioning stimulus is presented.

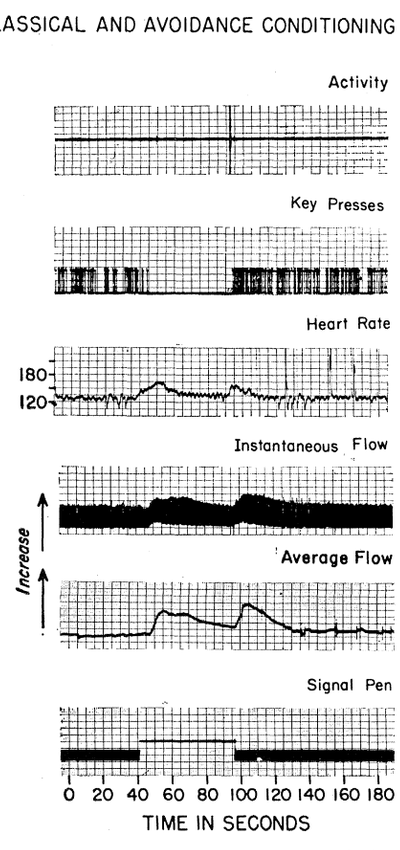

Fig. 2. Simultaneous measures of key pressing, cardiovascular activity, and gross somatic activity prior to, during, and following a classical conditioning trial. The subject was a monkey maintained on a shock-avoidance schedule.

Figure 2 shows a typical record for one of the monkeys on the avoidance schedule. The sequence of events is the same as shown in Fig. 1. In addition, average flow has been measured. Again, suppression of the key pressing response is clearly shown, but the reasons for the suppression are probably more complex. In addition to the unavoidable shock given with termination of the conditioning stimulus, the reinforcement contingency for key pressing was not in effect when the conditioning stimulus was presented. Early in training both of the monkeys on the avoidance schedule increased their rate of key pressing during the light presentation. After several sessions, any responding to the light usually occurred within a few seconds of its onset. Marked increases in both blood flow and heart rate occurred in conjunction with the reduction in key pressing during presentation of the conditioning stimulus.

The absence of any large or sustained bodily movement during the light presentation is shown in the top record of Fig. 2 and was a characteristic feature of the behavior of both of the avoidance-trained animals. The conditioned cardiovascular response for both animals was similar to that obtained in a prior experiment in which only the classical conditioning procedure was instituted (*3*). This response, which continued for the duration of the conditioning stimulus, consisted of a relatively rapid increase in both rate and flow followed by a much slower decrease. The response to the nonconditioning stimulus (US) is distinct and is similar in magnitude, form, and duration to the conditioned response. These data are in contrast to the data shown for one monkey (Fig. 1) on the food reinforcement schedule. It is suggested that the extended gross motor activity of this animal prevented the decline in heart rate and blood flow during the presentation of the conditioning stimulus and obscured the distinct response to the US.

This study, in confirming the results of our earlier experiment, has shown that the comparatively large conditioned increases in heart rate are reflected, at least in part, in increased blood flow to the tissues served by the terminal aorta. It is unlikely that these cardiovascular changes are secondary to skeletal muscle activity. In addition, from measures of respiration taken in our earlier experiment (*3*), we found evidence that the cardiovascular conditioned response is not dependent upon respiration.

Marked reduction in the rate of the conditioned somatic response of key pressing during the light was concomitant with the conditioned cardiovascular response for all animals. This finding extends and strengthens the definition of a "conditioned emotional response" (*5*). For the food-reinforced animals, suppression of key pressing was simply a function of the superimposed classical conditioning procedure. However, reduced responding during the light for the avoidance-trained animals may have been a consequence of simple extinction due to the removal of the reinforcement contingencies for the avoidance response when the light was on. In the absence of the classical conditioning trials, stable responding (key pressing) for the food-reinforced animals and for both of the avoidance-trained animals was correlated with a comparatively stable, low level of cardiovascular activity. Both of the avoidance-trained animals received few avoidable shocks following training. In spite of the fact that their behavior was under aversive control (shock avoidance), the only major changes in blood flow and heart rate were those induced by the electric shock, itself, and by the conditioning stimulus. The degree of cardiovascular activity while the avoidance schedule was in effect was comparable to that measured prior to conditioning.

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Institute of Neurological Diseases and Blindness in the Department of Physiology and Biophysics at the University of Washington School of Medicine. The work was supported in part by U.S. Public Health Service grant H4741. We gratefully acknowledge the advice of, and equipment loans by, R. F. Rushmer, and the assistance of Marc A. Nathan.
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9 March 1964

Serotonin Deficiency in Infancy as One Cause of a Mental Defect in Phenylketonuria

Abstract. *Serotonin deficiency induced in newborn mice and maintained to adulthood resulted in reduced ability to learn a maze. The serotonin deficiency was produced by overloading with phenylalanine plus tyrosine (to cause phenylketonuria), by feeding of reserpine, and by feeding of chlorpromazine from birth to maturity.*

The accumulation of evidence to show that mental aberrations can be produced in adults by changes in the serotonin contents of their brains (*1*) has led us to inquire whether some of the inherited idiocies also might arise from a defect in the serotonin metabolism and, that this defect, because it is imposed early in infancy, might be peculiarly harmful to the development of the mind. With this in view, our attention has been drawn particularly to the disease, phenylketonuria. This is an inherited metabolic defect in which the enzyme for hydroxylation of phenylalanine to tyrosine is lacking because of a single-gene mutation. As a result, the phenylalanine which is ingested in the food accumulates in the tissues and is converted to metabolic products such as phenylpyruvic acid and phenyllactic acid, which also accumulate. Infants suffering from this defect develop normally physically, but grow into morons or idiots. If the phenylalanine intake is severely restricted, the mental failure seems not to develop. The mental defect is thus attributable to the excess of phenylalanine and its metabolic products. Why should these substances cause idiocy?

It is now well established [a few of the studies are in (*1–3*)] that phenylketonuric human beings and laboratory animals suffer a deficiency of serotonin and catechol amines. These deficiencies seem to arise (at least in part) from the fact that the enzyme which synthesizes these hormones from their amino acid precursors is inhibited by phenylpyruvic acid and phenyllactic acid (*4*, *5*). Normal animals can be made phenylketonuric by continuous ingestion of very large amounts of phenylalanine or of phenylalanine plus tyrosine (*6*, *7*).

Woolley (*1*) has suggested that the mental defect might arise from the serotonin deficiency. In particular, it was suggested that the deficiency imposed early in infancy with consequent permanent damage to the developing intellect, might be the cause of the mental failure. Others, too, had implied (*2*, *5*), but without direct evidence, that the mental defect might be related to the abnormalities of the metabolism of serotonin. However, they had not stressed the importance of the deficiency in early infancy. The reasons for thinking that the deficiency must be established early in infancy are two. It is well known clinically that such deficiencies imposed in adult life do not cause permanent damage to the mind. It is also well known that to succeed in the control of the idiocy of phenylketonuria, one must start the phenylalanine-low diet early in infancy. The agreement with the idea just mentioned has by no means been general, and many other hypotheses to explain why excess phenylalanine damages the mind have been put forward (*1*).

The purpose of the present work was to produce phenylketonuria in an experimental animal, and to show that it caused a mental failure. If this could be accomplished, the way would be open to show whether, by correction of the serotonin deficiency, the mental failure could be prevented, and thereby, the proof of the idea could be made. It is impossible to do such an experiment in human beings.

Several earlier attempts have been made to show that experimental phenylketonuria induced in rats or mice will cause a mental change. In all of these cases the phenylketonuria was established at weaning time and not in early infancy. The attempts have met with varying success and in only one instance has any evidence been offered to show that the intellectual change was related to the deficiency of serotonin. Yuwiler and Louttit (*7*) for example, concluded that a slight defect in maze-learning ability which they found in phenylketonuric rats was not the result of serotonin deficiency. Polidora *et al.* (*8*) reported a slight decrease in the rate at which phenylketonuric rats swam through a water maze, but made no effort to relate this behavioral change to serotonin. Woolley and van der Hoeven (*9*) and Woolley (*10*) showed that phenylketonuric adult mice exhibited an increase in learning ability in a maze, and related this increase specifically to the deficiency of serotonin.

To produce the mental defect of phenylketonuria in mice, it was necessary to begin with newborn animals, and to maintain in them an excessive amount of phenylalanine. Excess tyrosine was also given because of the claim of Auerbach *et al.* (*6*) that, in rats, this amino acid was needed to suppress the biosynthesis of the hydroxylase enzyme and thus to intensify the disease. DL-Phenylalanine, rather than L-, was used because these same authors had found it to be effective in causing excretion of phenylpyruvic acid in rats, possibly because of the longer persistence of the D-isomer.

Within 24 hours of birth, infant mice were given by stomach tube, daily, nine times each day, 0.01 ml of a fine suspension of DL-phenylalanine (20 mg/ml) and L-tyrosine (10 mg/ml). A curved, 24-gauge needle with a ball tip was used for the intubation. As the mice grew and the capacities of their stomachs increased, the hourly dose was increased until, when they were 2 weeks old, they were receiving about 0.1 ml per hour. During all this time they were allowed to nurse their mothers, which were fed stock ration (Purina chow). At 2 weeks of age, DL-phenylalanine and L-tyrosine (35 g each per kilogram of food) were added to the food and the intubation was stopped. After weaning, the young were continued on this diet until they were 7 to 8 weeks old. They were then fed normal ration for at least 3 days before they were tested for learning ability. This was done in order to clear the tissues of phenylalanine and thus to allow repletion of the serotonin. We had shown earlier (*9*) that, unless this was done, the serotonin deficiency in the mature mice would be reflected by an increase in learning ability. Two kinds of controls were run. In one, the mice were dosed hourly, merely with water, until weaned, and then fed normal ration. In the other, untreated, normal animals were used.

The learning ability of the adult animals was measured in a T-maze as described earlier (*9*). A score of 10 in this test meant that the animals had learned their lesson perfectly, and a score of 5 meant that they had learned nothing at all. It has already been dem-