When the train of flashes was reinforced by hypothalamic stimulation, the reaction increased if previously present, or appeared if it had been absent. After several sessions the reaction became constant and at times very strong (Fig. 2). On trials in which only a train of flashes were presented the pupillary diameter remained unchanged.

In two cats the rotating object was presented the same number of times as the train of flashes, the rotating object without reinforcement and the train of flashes always reinforced by hypothalamic stimulation. At the beginning of the experiment the rotating object produced a strong reaction, while no pupillary dilation was observed in response to the flashes.

The response to the rotating object decreased gradually, however, and after several sessions became completely or almost completely extinguished (habituation of the orientation reflex). In contrast, conditioned responses to the train of flashes appeared because of systematic reinforcement by hypothalamic stimulation. This experiment shows that the increase or the appearance of a mydriatic response to a train of flashes followed by hypothalamic stimulation cannot be attributed to unspecific activation of the orienting reflex (pseudoconditioning), but represents true conditioning.

Our results show that a healthy pretrigeminal preparation possesses neural structures lying above the transection adequate for classical conditioning and habituation processes. The preparation may be useful for further study of these phenomena. Of course, the experimental possibilities are severely limited by the striking reduction of sensory and motor paths available. However, this preparation has the following advantages in comparison with the intact animal: (i) immobilization simplifies observation and recording of reactions, (ii) the absolute lack of pain permits surgical intervention which would otherwise require anesthesia or interfere in other ways with the conditioned response, and (iii) the isolated cerebrum provides a simpler background for experiments.

J. AFFANNI*
P. L. MARCHIAFAVA†
B. ZERNICKI‡

Istituto di Fisiologia dell'Università di Pisa and *Centro per la Neurofisiologia del Consiglio Nazionale delle Ricerche, Sezione di Pisa, Pisa, Italy*

13 JULY 1962

References and Notes

1. C. Batini, G. Moruzzi, M. Palestini, G. F. Rossi, A. Zanchetti, *Arch. ital. Biol.* **97**, 1 (1959).
2. G. Santibanez-H., R. Tarnecki, B. Zernicki, *Acta Physiol. Polon.* **11**, 881 (1960).

* Fellowship of the Consejo Nacional de Investigaciones Cientificas (Argentina); present address: Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina.
† Fellowship of Consiglio Nazionale delle Ricerche, Italy.
‡ Fellowship of the Ministero Affari Esteri (Italy); present address: Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland.

29 January 1962

Conditions Influencing Vocal Responsiveness of Infant Chimpanzees

Abstract. Infant chimpanzees were tested to determine the effects on distress vocalizations (whimpering, screaming) of stimulus conditions approximating the physical relationship to the mother. Under such conditions spontaneous vocalizations were infrequent, and vocal responsiveness to a painful stimulus was substantially reduced.

The infant mammal's first and probably most important contacts with the external environment occur with the mother. For the newborn chimpanzee, the maintenance of an adequate physical relationship to the mother is essential to survival in the wild and the achievement of this relationship is based on reciprocal adjustments between mother and infant. The initiative in cleaning the neonate, placing it near the breast, and supporting it in this position is taken principally by the mother (*1*), while the infant, by virtue of behavior mechanisms functioning shortly after birth, participates by grasping the mother's coat, by making searching movements which facilitate the location of the nipple, and by sucking. If for any reason an adequate adjustment should fail to occur, the infant usually evidences heightened responsiveness expressed in increased motor activity and whimpering or screaming.

This report is concerned with the general problem of the relationship between infantile responsiveness and conditions of maternal stimulation. Specifically, it examines the effects of various patterns of cutaneous stimulation on distress vocalizations (whimpering, screaming).

The subjects were three female chimpanzees (Bandi, Cari, and Flo) separated from their mothers at birth and housed individually. In the first experiment each animal was tested three times a week during the first 8 weeks of life. Fifteen test conditions were presented in each session, each condition consisting of 30 seconds of continuous stimulation.

Table 1 shows the results for eight test conditions which are particularly relevant to this report. The data in the first two columns were obtained with the subject in the supine position on the test table, and a comparison is made of vocal responses to ventral stimulation produced by light tickling, rhythmic stroking, continuous light pressure (palm of hand), a 5 by 11 inch cylinder loosely covered with terry cloth, and the bare table (no ventral stimulation). The data in the second two columns compare responses when the subjects were in the prone position on the bare table, on the table loosely covered with terry cloth, and on the cylinder (*2*). The results clearly indicate that stimulation by the cylinder, which most closely approximates the subject's normal physical relationship to the mother, was the most effective stimulus in reducing vocalizations (Friedman test, $p < .05$).

Further information on the nature of this effect was provided by two subsequent experiments, each comparing responses to shock to the foot when the infant was held (ventral surface firmly against the experimenter's chest) and when it rested on a bare surface (table, or floor of living cage). The first experiment started when the subjects were between 109 and 115 days of age and was completed in two sessions. Each session began with the shock at 2.00 ma, and the subject received ten shocks (2 seconds duration, 2 seconds apart) in one of the two experimental conditions (held, not-held),

Table 1. Mean duration of vocalization per session (in seconds), and percentage of sessions in which vocalization occurred. Based on twenty-four, 30-second test sessions per subject. The stimuli are given in italic type.

Supine		Prone	
Duration	% of sessions	Duration	% of sessions
Tickle			
6.9	69.4		
Stroke			
3.1	37.5		
Pressure			
4.0	44.4		
Cloth			
		1.0	29.2
Bare table			
2.0	38.9	5.7	63.9
Cylinder			
0.6	12.5	0.5	9.7

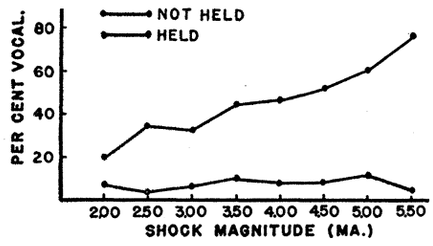

Fig. 1. Distress vocalizations to shock while subject was held and while it was not held and resting on a bare surface.

and then ten shocks in the other condition. This procedure was repeated at progressively higher levels of shock, with intensity increasing in 0.50-ma steps to 5.50 ma. A comparable descending series of shocks terminated the session.

Figure 1 shows the percentage of total shocks that elicited vocalization as a function of intensity and holding conditions. The curves show that vocalization was uniformly low at all shock levels when the subject was held, whereas, when the subject was not held, vocal responses increased with increasing shock. The total percentage of vocalization was 8.1 and 46.0 for the held and not-held conditions, respectively, and for each subject the difference between conditions was significant ($p < .01$) as determined by Wilcoxon tests comparing total vocalizations at each shock level.

The second experiment began when the subjects were between 110 and 123 days of age; it sought to determine the effects of holding on the threshold for initial vocalization to shock. A further purpose was to investigate the relationship of hunger to vocal responsiveness and to holding. Thresholds were established by the method of limits. In each test session the shock was set initially at a level which did not elicit vocalization and increased in 0.50-ma steps until vocalization occurred on two successive presentations. A descending series was then performed which terminated when vocalization failed to occur on two successive presentations. Shock duration was 2 seconds unless vocalization occurred. Four threshold determinations, each including an ascending and a descending series, were made in each session, two while the subject was held, and two while it was on a bare surface. There was a total of eight testing sessions; half of these occurred before the morning feeding when the subjects had been deprived of formula for approximately 10 hours, and half occurred within 1 hour after the regular morning feeding. The order of the holding and the food deprivation conditions was balanced over sessions.

All animals exhibited higher thresholds when held; on 62.5 percent of the determinations, the threshold was arbitrarily recorded at 6.50 ma, the upper limit of shock, because this level was reached without eliciting vocalization. Vocalization failed to occur on only five determinations under the not-held condition, and this failure occurred entirely with one subject. Mean thresholds for the held and not-held conditions (combined over deprivation conditions) were 6.03 ma and 3.51 ma, respectively. The difference between conditions was statistically significant ($p = .01$, Wilcoxon tests) for two infants, and results for the third animal were consistent with this outcome, but could not be established statistically. Hunger had no apparent effect on the threshold for vocalization when the infants were held. For the not-held condition, however, thresholds were lower for all subjects when they were hungry than when they were fed (mean thresholds: hungry, 2.88 ma; fed, 4.13 ma), although the overall effect of deprivation conditions was not statistically significant.

These results indicate that the stimulus conditions provided by holding inhibit vocal responses to aversive stimuli. Although our findings do not rule out the possibility that this effect is specific to vocal activity, we believe it more likely that there is a general decrease in arousal associated with holding which will be reflected in other response measures, for example, muscle tension. According to this interpretation, the arousal-reducing characteristics of physical contact that are present in the neonate should constitute an important factor in the tendency of older infants to seek the mother or an appropriate substitute (*3*) in moments of distress (*4*).

WILLIAM A. MASON
GERSHON BERKSON*
Yerkes Laboratories of Primate Biology, Orange Park, Florida

References and Notes

1. R. M. Yerkes and M. I. Tomilin, *J. Comp. Psychol.* **20**, 321 (1935).
2. It should be noted that conditions involving terry cloth permitted grasping.
3. T. L. McCulloch, *J. Psychol.* **7**, 305 (1939).
4. Support for this research was provided through funds received from grant M-4100 from the National Institutes of Health.
* USPHS postdoctoral fellow.

7 February 1962

Generation by Visible Light of Labile Free Radicals in the Melanin Granules of the Eye

Abstract. The melanin granules of the eye generate a free radical when irradiated with visible light under approximately physiological conditions. The rapid generation in light and decay in dark of the free radical as indicated by electron spin resonance studies, coupled with the anatomical proximity of the melanin granules to the rods and cones of the eye, suggest that these granules may play an important role in the visual process. The investigation of this free radical offers a possible approach to connect the photochemical events in the eye with the subsequent electrophysiological events in the nerve.

Electron spin resonance (ESR) offers a powerful approach to the study of molecules in short-lived excited states (*1*). In both the free radical and triplet states, molecules have an extra degree of reactivity which allows functions forbidden to molecules in the ground state. Conceivably the photochemical events in the eye could be translated into a nerve signal via the pathway of photoactive molecules converted to the free radical state. With this in mind, we inaugurated ESR investigations of the visual process. An ESR signal was found to be generated by visible light in the melanin granules of the eye under approximately physiological conditions. Some aspects of this reaction are detailed below.

Beef eyes were obtained immediately after slaughter and transported to the laboratory in a light-tight, iced container. The eyes were opened under dim red light and the retinas were excised. The choroid layer, Bruch's membrane, and the pigment epithelial layer of the retina were removed intact, and were shaken vigorously with successive washes of isotonic sucrose to bring the melanin granules into suspension. After the fibrous material was removed by filtration through fine silk screening, the filtrate containing the melanin was homogenized in isotonic sucrose with a Potter homogenizer and centrifuged at 1000*g* for 10 minutes. The melanin granules that precipitated at this speed were washed by repeating the suspension and centrifugation procedures. The material then was suspended in isotonic sucrose, layered over 2.0*M* sucrose, and centrifuged at 25,000*g* for a period of 30 minutes.

The precipitate from this treatment, when resuspended in various aqueous solutions, was used as source material