component which appeared only after 48 hours. It was estimated by dilution studies that this component could represent no more than a maximum of 5 percent of the total protein. Sialic acid analyses on transferrins B_2 and C could detect no significant difference. Further experiments are being conducted to determine the nature of the structural difference between these two components. Although differences in sialic acid content would be sufficient to account for observed variations in electrophoretic mobility of certain transferrins, the variation would be expected to extend to a specific genetically determined amino acid difference in the protein backbone. Although no definite selective advantage is known which could maintain the transferrin polymorphism (*16*), the genetic variation may influence resistance to infectious disease (*17*). The variation in charged groups may also affect the dissociation of the iron-transferrin complex according to the equilibrium postulated by Laurell (*18*) and alter the transfer of iron to sites of storage or synthesis (*19*).

W. CAREY PARKER
ALEXANDER G. BEARN

Rockefeller Institute,
New York, New York

References and Notes

1. V. M. Ingram, *Nature* **178**, 792 (1956).
2. M. D. Poulik, *ibid.* **177**, 982 (1956).
3. H. E. Schultze and G. Schwick, *Behringwerk-Mitt.* **33**, 11 (1957).
4. O. Smithies, *Biochem. J.* **61**, 629 (1955).
5. B. S. Blumberg, in *Conference on Genetic Polymorphisms and Geographic Variations in Disease, Bethesda, Md., 1960* (Government Printing Office, Washington, D.C., 1960), p. 249n; L. Warren and B. S. Blumberg, *Clin. Research* **8**, 372 (1960).
6. O. Smithies, *Biochem. J.* **71**, 585 (1959); E. R. Giblett, C. G. Hickman, O. Smithies, *Nature* **183**, 1589 (1959).
7. H. G. Kunkel, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1954), vol. 1, p. 141.
8. D. A. J. Tyrrell and F. L. Horsfall, Jr., *J. Immunol.* **69**, 563 (1952); P. W. Choppin, S. Osterhout, I. Tamm, *Proc. Soc. Exptl. Biol. Med.* **98**, 513 (1958).
9. I. Werner and L. Odin, *Acta. Soc. Med. Upsaliensis* **57**, 230 (1952). The sialic acid described in the present experiments is the N-acetyl derivative.
10. L. Svennerholm, *Arkiv Kemi* **10**, 577 (1957).
11. H. E. Schultze, R. Schmidtberger, H. Haupt, *Biochem. Z.* **329**, 490 (1958).
12. H. Harris, D. G. Pennington, E. B. Robson, *Biochem. J.* **74**, 44P (1960); S. H. Boyer and W. J. Young, *Nature* **187**, 1035 (1960).
13. M. D. Poulik, *J. Immunol.* **82**, 502 (1959); *Clin. Chim. Acta*, in press.
14. H. E. Schultze, *Deut. med. Wochschr.* **83**, 1742 (1958).
15. J. R. Preer, Jr., *J. Immunol.* **77**, 52 (1956).
16. E. R. Giblett and A. Turnbull, *J. Lab. Clin. Med.*, in press.
17. C. M. Martin and J. H. Jandl, *J. Clin. Invest.* **38**, 1024 (1959).
18. C. B. Laurell, *Blood* **6**, 183 (1951).
19. We are greatly indebted to Dr. E. R. Giblett for providing a sufficiently large sample of transferrin type B_2C serum to perform these experiments. We are also indebted to Dr. H. J. Muller-Eberhard for performing the ultracentrifugal analysis. This research was aided by a grant from the National Foundation.

21 November 1960

Critical Period in the Social Development of Dogs

Abstract. Litters of puppies were isolated, with the bitch, in fenced acre fields from 2 to 14 weeks of age. They were removed indoors at different ages, played with for a week, and returned to the field. The pups manifested an increasing tendency to withdraw from human beings after 5 weeks of age and unless socialization occurred before 14 weeks of age, withdrawal reactions from humans became so intense that normal relationships could not thereafter be established.

The term *imprinting* is generally defined as a capacity of some species of birds to develop a permanent attachment to any species, including man, made available to it during a critical period in its early development. This period of primary socialization usually ends with a mounting tendency to flee from strange species (*1*). The present experiment demonstrates a similar phenomenon in a mammalian species.

This study derived from the observation that purebred cocker spaniels exhibited intense flight responses to humans after they had been raised in an acre field with a minimum of human contact prior to 14 weeks of age. Our unsuccessful attempts to tame or socialize them led us to examine the age when human contact would most effectively reduce the withdrawal response at 14 weeks of age.

Five litters of cocker spaniels ($N = 18$) and three litters of beagles ($N = 16$) were raised in acre fields bounded by an 8-foot high wooden fence. The mother and her litter were raised alone in the pen and received food and water through drops in the fence. Pups from each litter were taken from the field for a week of socialization at 2 weeks of age ($N = 6$), 3 weeks, of age ($N = 6$), 5 weeks of age ($N = 7$), 7 weeks of age ($N = 7$), and 9 weeks of age ($N = 3$), and then returned to the field. During this week indoors, pups were played with, tested, and cared for throughout three daily half-hour periods (*2*). Controls remained in the field ($N = 5$) until the entire litter was taken indoors for final testing at 14 weeks of age.

At the start of the week of socialization pups removed from the field at 5 weeks of age scored significantly higher on a test of attraction to a handler [Handling Test (*3*)] than those removed at 2, 3, or 9 weeks of age ($p = 0.01$ to 0.05; t, tests). The low scores of 2- and 3-week-olds were due simply to their physical and motor immaturity, while 9-week-olds exhibited low scores because they had a marked tendency to avoid the handler. By the end of the week of socialization, however, all save the still immature 2-week-olds were equally attracted to the handler.

The progressive development of avoidance responses was evident in daily, 10-minute tests of the amount of time a puppy spent in physical contact with a passive, reclining human. Two-week-olds, again, were too immature to do much but sleep, eat, or crawl about randomly; 3-week-olds were immediately attracted to the experimenter and spent most of the 10-minute period pawing, mouthing, and biting him and his garments; 5-week-olds exhibited wariness at first, but they became comparable to 3-week-olds before the end of the first play period; 7-week-old pups, however, were frightened and wary of contacting the experimenter over the first *two* days of socialization, while 9-week-old pups exhibited these reactions over the first three days. No p values are given since this test was administered only to the last three litters; however, notes taken on all animals reveal no exceptions to this pattern of progressive avoidance, and it was persistently observed in the other situations of the socialization period.

After removal from the field at 14 weeks of age a series of tests was administered over a 2-week period. The handling test was administered to all pups at the start and at the end of this period of testing, a period involving daily contact with humans. At 14 weeks the pups socialized in their second week, and the controls scored significantly lower in "attraction to the handler" than did the fifth, seventh, and ninth week groups ($p \leqslant 0.05$, t tests). By the 16th week, however, only the control group remained significantly low, and it scored lower than all other groups ($p \leqslant 0.02$, t tests). These results are illustrated in Fig. 1.

Since the control animals appeared as timid on the final day of testing as

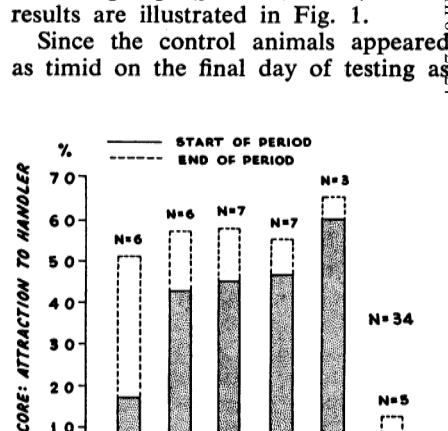

Fig. 1. Performance in the handling test. A measure of attraction to the handler, comparing performance at the start and at the end of the final period of testing (14 and 16 weeks of age).

Table 1. Rank order for tests administered after 14 weeks of age. (The higher numbers rank low.)

Test	Rank at age socialized					Controls
	2 wk	3 wk	5 wk	7 wk	9 wk	
			Handling			
Initial attraction to handler	5	4*	3*	2*	1*	6
			Leash control			
Eating in strange situation	4	3	1.5*	1.5*	5	6
Fewer balks	4	5	3*	1*	2*	6
			Reactivity test			
Total activity	2	4	3	1	6	5
Heart rate	6	3	4	1	2	5
Vocal, panting, and tail wagging	2	3	4	1	5	6

*Distinctly superior rank

on the first, one control animal, selected at random, was petted and fondled each day for a period of 3 months. The handling test was readministered at that time, and the animal showed only a slight positive change in score. It is our impression that any of the control pups would have been similarly resistant to socialization.

The leash-control test (*3*), devised to test an animal's resistance to training to a leash, was administered over a period of 10 days. There was significantly greater ease in the training of animals socialized at 5, 7, and 9 weeks of age than with the two younger groups and the controls ($p \leqq 0.01$, Wilcoxen rank test, and see Table 1). It appears that while socialization experiences during the second and third weeks of age were effective, as judged by the handling test, the stress conditions of leash training favored the scores of animals socialized during the fifth, seventh, or ninth week of age.

The final test was the reactivity test (*3*). Each pup was placed in a physiological harness and subjected to various stimuli such as cajoling and threat from a human, acoustic-startle, and electric shock. Heart rate, depth of respiration, and muscle tension were concurrently recorded in an electrocardiographic apparatus, while an observer made note of other aspects of the animal's reactions. This test appears to contrast ease of response (high score) with the tendency to "freeze" (low score). The controls were more inhibited than the seventh-week group on each of three subscores ($p = 0.05, 0.01, 0.01$, Mann-Whitney). In Table 1 we see that the seventh-week group ranked highest on all three subscores, indicating primacy in activity level, heart rate, vocal behavior, and tail wagging.

Although further work is necessary, the net results suggest that the seventh week of age was the period in which the pups were most receptive to socialization, and that 2½ to 9–13 weeks of age approximates a critical period for socialization to human beings. These figures are in accord with the findings of Pfaffenberger and Scott (*4*) and Scott and Marston (*5*). It is important to note that within this period motor and perceptual development reach maturity while, on the other hand, motivation to flee from strange species is not yet strong. We suspect that such a balance of factors is characteristic of a period that is critical for the formation of primary social relationships (*6*).

DANIEL G. FREEDMAN*
JOHN A. KING
ORVILLE ELLIOT†

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

References and Notes

1. E. H. Hess, *Science* **130**, 133 (1959).
2. A detailed description of the socialization procedure may be obtained from us.
3. J. P. Scott and J. L. Fuller, *Manual of Dog Testing Techniques* (Jackson Memorial Laboratory, Bar Harbor, Maine, 1950).
4. C. J. Pfaffenberger and J. P. Scott, *J. Genet. Psychol.* **95**, 145 (1959).
5. J. P. Scott and M. V. Marston, *ibid.* **77**, 25 (1950).
6. The support of the Rockefeller Foundation and the Ford Foundation is gratefully acknowledged.
* Present address: Langley Porter Neuropsychiatric Institute, San Francisco, Calif.
† Present address: Department of Anthropology, Harvard University, Cambridge, Mass.

21 November 1960

Physiological Limits for "Subliminal" Perception

Abstract. Cortical, subcortical, and peripheral sensory nerve potentials were studied in cats to determine comparative thresholds of response. Stimuli capable of eliciting responses in peripheral nerve also invariably elicited responses from thalamus and cortex. Together with relevant data from human studies, the results indicate that stimuli subthreshold for perception do not affect the nervous system. They also have implications for studies of "subliminal" perception.

In recent years much investigative and popular interest has been directed toward the problem of "subliminal" perception. Such perception is said to occur when stimuli which are presumably below threshold, because they are not reported, nevertheless exert some influence upon behavior.

Although the psychological aspects of the problem have received careful scrutiny (*1*), little attention has been paid to physiological mechanisms which might mediate "subliminal" perception. Knowledge of such mechanisms could be crucial in clarifying the nature of the problem. For example, if it were known that stimuli of intensity lower than the sensory threshold can elicit brain responses, this would provide a definite physiological basis for "subliminal" perception. On the other hand, if it were shown that brain responses are produced only by stimuli at or above threshold, it would be clear that "subliminal" perception, in the strict meaning of the term, is not physiologically possible.

The probability that subthreshold stimuli do not produce brain responses has been suggested by three studies of cortical potentials evoked by sensory stimulation in man. Because the cortical potential evoked is not ordinarily visible in the scalp electroencephalogram, averaging methods were employed in these studies to bring out the responses. Shagass and Schwartz (*2*) found that the first evidence of cortical response to ulnar nerve stimulation coincided with the sensory threshold and that stimuli which were not perceived produced no response. Geisler, Frishkopf, and Rosenblith (*3*) obtained similar results with auditory stimulation. Dawson (*4*) simultaneously recorded cortical responses and action potentials in median and ulnar nerve and found no fibers in either nerve with thresholds lower than that for producing cortical responses. Taken together, the findings of these studies suggest that subthreshold sensory stimuli produce neither peripheral nerve responses nor cortical responses. However, because these investigations relied upon skin electrodes and averaging techniques, it seemed desirable to test this conclusion in a study employing direct recording methods.

In our experiments, simultaneous recordings were made from exposed cortex and either medial lemniscus, thalamus (ventral posterolateral nucleus), or peripheral nerve in the cat. In a total of 13 animals, cortex was compared with medial lemniscus in three cats, to ventral posterolateral nucleus in three, and to superficial radial nerve in ten. Stimulation was applied to superficial radial nerve by a Grass S4G stimulator through a Grass SIU4B isolation unit. Stimulus intensities were varied systematically in steps of 0.1 volt from low levels, producing no responses, to suprathreshold values. Re-