The Effects of Short-term Social and Sensory Isolation upon Behavior, EEG and Averaged **Evoked Potentials in Puppies**

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Fox, M. W. The effects of short-term social and sensory isolation upon behavior, EEG and averaged evoked potentials in puppies. Physiol. BEHAV. 2 (2) 145-151, 1967.—Pups were reared in partial social and sensory isolation for 1 week between the fourth and fifth weeks of age. Emergence from isolation is characterized by intense behavioral arousal, abnormally short latencies of visual evoked potentials (EP) and marked desynchronization of the EEG with paroxysmal or continuous rhythmic 11-16 c/sec activity. Recovery is generally rapid and by 7 days after emergence from isolation, behavior and EEG activity are apparently normal. These findings are discussed in the light of earlier studies on the behavioral and neurophysiological effects of isolation rearing, and the isolation syndrome described in relation to autistic behavior in the human infant.

Abnormal behavior Sensory deprivation

Autism Social isolation Isolation syndrome

Averaged evoked potentials Behavior

EEG Early experience

CONSIDERABLE research in recent years has been focused on the effects of rearing young animals under varying degrees of sensory and social isolation on their later social and emotional development. Social isolation may range from complete social isolation (animals reared singly in sound-proof boxes with no visual or auditory stimulation from their peers), to partial social isolation (animals reared singly without visual access but with auditory and olfactory access, to their peers). Sensory isolation, or deprivation, may vary similarly in intensity, depending on the number of modalities that are experimentally blocked or denied stimulation.

Research in this field has been undertaken by psychologists, notably in the monkey and dog [4, 6, 11, 12, 15, 17, 20, 23, 26, 29, 32, 36, 38, 41].

Berger [3] has shown structural differences in the CNS of dogs and cats deprived of light at an early age. More recently, Wiesel and Hubel [42-44] have demonstrated both structural and electro-physiological deficits in monocularly visually deprived cats, and Klosovskii [19] correlated structural changes in the CNS of dogs with surgical transection of optic and auditory nerves at an early age.

The theoretical basis of such treatment has recently been critically reviewed by Lorenz [22], Thompson and Schaefer [40] and Riesen [30, 31]. Isolation during the behaviorally critical post-natal period, when development of behavior is stimulus-dependent, may result in spontaneous regression of certain innate, species specific behavior patterns. In non-

precocial animals, it is during this time the period of integration [9] that behavioral development can be severely modified. Stereotyped behavior and self-directed activities such as rocking, thumb sucking and tail-chasing, and impaired pain perception and reactions to various stimuli have been described. Behaviorally in both dog and monkey, isolation rearing results in many symptoms resembling the so called autistic behavior of infant schizophrenics [4, 8].

Dogs

On the basis of recent detailed behavioral and preliminary EEG observations on pups reared in partial isolation [11] the present investigation was undertaken on a further series of pups in an attempt to define more precisely the EEG phenomena underlying the behavior manifest on emergence from isolation, and recovery from isolation.

METHODS

Using the split-litter technique, a total of twenty-two pups, Beagles and cross-bred, was studied. Twelve of these were subjected to a week of partial social and sensory isolation between the fourth and fifth week of age, six of this group having chronic electrodes implanted at 3 weeks of age. These were stainless steel tip electrodes implanted beneath the dura in the visual (right and left lateral gyrus) and auditory (left and right estosylvius medius gyrus) using a frontal lead as the indifferent electrode after the aseptic surgical method developed in this laboratory by Myslivecek and Fox [28]; 4

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controls were similarly treated. The electrodes were secured to the skull using gel-foam soaked in cyanoacrylate monomer (Ethicon) and the mid-line incision of the skin over the skull closed with monofilament nylon. The electrode leads were of different color to permit identification and emerged in a tuft at the caudal end of the skin incision at the back of the head. Healing was rapid and tracking of infection rarely occurred, as contrasted by the regular occurrence of infection in the screw-type electrode implantation method where the electrodes emerge directly through the skin over the skull openings. Recordings were taken at 4 weeks, 5 weeks on emergence from isolation, and at daily intervals thereafter. In the remaining twelve pups, recordings were taken over the occipital and temporal regions at 5 weeks of age, using modified Michel wound clip electrodes; attached to the skin after removal of hair and application of electrode paste. This latter group was euthanized at 5 weeks of age, following behavioral and EEG studies, for amino acid analysis of brain parts [1].

EEG recordings were taken on an Offner 8 Channel Type R Dynograph, and evoked potentials to visual and auditory stimulation at various frequencies were recorded on a computer of Average Transients (CAT 400B) and monitored on an oscilloscope and Grass 6 channel EEG recorder. A Grass photostimulator was placed 2 in. in front of the animal, the duration of stimulation being 10 µsec, and the source of light being directed straight into the eyes: intensity of illumination was set at 16 (equivalent to 1 million peak ft-c. at 10 in. distance from the photostimulator). EEG recordings on the Offner were taken with the electrodes linked (cascade method), i.e. left visual to right visual, right visual to right auditory (or temporal), right to left auditory and left auditory to left visual. Electrode connections for evoked potentials and EEG recording on the Grass were monopolar, each left and right visual or auditory lead paired with the frontal indifferent electrode.

Behavioral observations were conducted in an arena equipped with one-way windows using a variety of situational tests described in greater detail earlier [11]. The pups were run through these tests at 5 weeks of age for approximately 30 min prior to EEG and EP recordings.

Treatment of Animals

Treatment during isolation. From the fourth to the fifth week of age, pups were placed in a darkened room (illumination approximately 0.5 ft-c.) in separate cages which permitted no visual contact among litter mates. These pups received scheduled daily contact with humans for approximately $1\frac{1}{2}$ min for cage cleaning and feeding. Controls were kept singly in the same type of cage and had similar human contact, but were reared in the animal house with visual contact with other animals and personnel. From 5 weeks onwards, isolation pups were moved to the same environment as the controls.

Treatment during recording. During evoked potential recordings, animals were swaddled in a towel and placed in a light-proof and sound-proof box. This procedure provided adequate restraint, minimized movement artifacts and was not distressing to the pups. Recording sessions were of approximately 30 min duration per animal. EEG recordings were taken while the animals were under identical conditions, in a well lighted recording room, being held on the lap of an assistant.

RESULTS

The behavior of the isolation-reared pups when tested in the arena has been described in greater detail in an earlier series of pups by Fox and Stelzner [11] which received the same treatment. The isolates were hyperactive and would run around the behavior arena after an initial short period (1-2)min) of crouching and crawling when first placed in the arena from their isolation quarters. Compared with their littermate controls, their activity was often random and not directed towards component stimuli of the empty arena such as the walls, door and floor. Controls showed more non-specific exploratory behavior of the arena itself and were more vocal. Before and after 5 min observing the pup alone in the empty arena a small toy, cloth bedding and a mirror were added for 5 min to determine stimulus-specific activity. Isolate pups were non-reactive towards the mirror, and spent significantly more time with the cloth than with the toy, mainly sniffing and rarely chewing, and their frequency of interaction was greater than in the controls. Many isolates constantly ran to and from the cloth and toy while the interaction of controls was more sustained, chewing and pulling the cloth and toy, and approaching the mirror with tail wagging. When these objects were removed, the isolates would aimlessly run around the arena (random, nondirected activity) while the controls would remain by the arena door and cry or explore the empty arena and also frequently vocalize. Isolates rarely vocalized.

Approach and following responses to a human were easily elicited in controls, and irregularly in isolates (Fig. 1(a)), which would begin to follow and then break off and explore the arena or specific stimuli. Finally all the pups were observed together in the arena in which the toy and cloth had been replaced. Isolates had little vocalization, rarely wagged their tails, and would frequently leave the group and explore (non-orally) the inanimate objects in the arena or indulge in self-play, e.g. tail-chasing. Controls would play. chew and lick each other and fight over the toy or cloth after apparently unsuccessful attempts to initiate play in the isolates (Fig. 1(b)). Detailed observations and quantitative data comparing these controls and isolates have been recently reported [11]. In a simple problem-solving situation, isolate pups were unable to negotiate a simple wire-mesh barrier, 5 ft long and 21 ft high placed between it and the observer. Control pups performed significantly better and learned to come around the barrier after only a few trials and showed distress vocalization in this test situation.

EEG recordings in all isolation-reared animals showed the presence of 14-16 c/s rhythmic low amplitude activity which was frequently synchronous in all channels, when first removed from isolation (Figs. 2(a), 2(b) and 2(c)). Also desynchronization with faster components and overall decrease in amplitude was evident. In some pups, this fast rhythmic activity was paroxysmal, recurring at intervals varying from every 4 to every 15 secs and varying in duration from 0.5-2.0 sec. In one animal, this activity was continuous, being most predominant over the occipital regions. All subjects were highly alert during recordings, and prior experience has shown that it is impossible to obtain sleep recordings from these pups on the first day as they are so behaviorally aroused. The rhythmic fast activity decreased in amount and frequency of occurrence of its bursts in some pups on the second day, but in others persisted for over 7 days (Figs. 2(a), 2(b) and 2(c)). This activity was associated with hyperactive and aroused behavior, for in those pups that remained hyperactive and alert (i.e. slow recovery from isolation) it was seen to persist for several days after emergence from isolation.



FIG. 1(a). Control pups (C) approach observer, while isolates (I) have weak approach and following responses.



FIG. 1(b). Group behavior: control pups (C) playing, isolates (1) interact minimally and show preference for inanimate object.

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EEG RECORDINGS OF ISOLATE & CONTROL DOGS



Occlusion of vision by placing a hand or towel over the eyes of an alert 5-week-old control pup caused synchronization of EEG with the appearance of medium amplitude slow waves of 3-4 c/s. Similar phenomena are observed in older dogs, but not in tense, nervous dogs (i.e. highly aroused) or during nociceptive stimulation (e.g. by pinching the interdigital web). In all controls, this activity was observed after occlusion of vision, but not in the Isolates on the first day after emergence from one week of isolation. The absence of 3-4 c/s activity in Isolates was attributed to intense arousal, causing desynchronization and suppression of 3-4 c/s activity in these extremely alert animals. This activity appeared on down at various times, while the peak of the positive wave had a more definite point in time (Table 1). This difference was approximately 30 msec (p < 0.001, Mann Whitney U 1 tail test); but by 6 weeks, the difference was insignificant, latencies of control and isolates being similar, with few exceptions. No significant differences in amplitude of the evoked potentials were found. These exceptions were the "slow recovery" isolates, in which latencies only 5 msec shorter than controls were found at 6 weeks: these differences were statistically insignificant (Figs. 4 and 5). A slight increase in latencies of auditory evoked potentials in the isolates was observed at 5 weeks (5-10 msec longer than controls).



FIG. 3. Absence of slow wave activity when eyes covered in isolate on first day of emergence from isolation (2), but rapid recovery (3), as contrasted by slow recovery in subject (4).

TABLE 1

Average Latencies of Evoked Responses (of First Positive Wave) In Control and Isolation Reared Pups

Controls (N = 4)			Isolates (N = 6)		
Visual (0.4/sec)			Visual (0.4/sec)		
(Chronic electrodes)			(Chronic electrodes)		
4 wk	5 wk	6 wk	4 wk	5 wk	6 wk
75	75(76)*	74	75	45† 55*	73
55	Auditory 45	45	55	Auditory 55	46

*Surface electrode animals in parenthesis.

 $\dagger (p < 0.001).$

subsequent days, again occurring earlier in the less aroused pups (Fig. 3).

In all isolate animals, average evoked potentials to photic stimulation were consistently of shorter latency than in controls at 5 weeks. The latency of the peak of the first positive wave was measured as the onset was more variable, drifting

VISUAL EVOKED RESPONSES IN CONTROL & ISOLATION-REARED DOGS 5- WEEKS OLD



FIG. 4. Markedly shorter latencies in isolates 1, 2 and 3 as compared with controls: LD and LS = Gyrus Lateralis Dextra et Sinistra. Surface electrodes. Delay of 50 msec means the start of the averaging epoch came 50 msec prior to each stimulus presentation. CI-3 and I 1-3 denote individual control and isolate pups all from the same litter.



FIG. 5. Shorter latencies in Isolates 1s and 2s compared with littermate control, evident at 5 weeks on emergence from isolation, but rapid return to normal 1 week later (only EP from Gyrus Lateralis Dextra shown).

DISCUSSION

Partial social and sensory isolation, if extended over long periods, can produce severe and more permanent alterations in behavior [39]. With isolation for 1 week, from 4 weeks of age, at the onset of the critical period of socialization [33] less permanent changes in behavior were observed by Fox and Stelzner [11]. The pups maintained in partial social and sensory isolation similarly for one week in this present study, typically are hyperactive, randomly hyper-exploratory and suggest a highly aroused state when first removed from isolation. Associated with the high level of behavioral arousal, EEG records show desynchronization and rhythmic low level fast activity of 14-16 c/s, sometimes, instead of continuous rhythmic fast activity, there are interruptions and short interspersed bursts of such activity. In contrast, the controls would frequently show high amplitude slow wave resting and drowsy patterns. In this regard, the effects of occluding the vision did not result in the appearance of slow alpha-like activity in the isolates. This was attributed to intense arousal causing desynchronization and suppression or inhibition of this effect in the highly alert animals. Spindle-like activity was always present in the isolation reared pups and normally is only seen in pups of a similar age following nociceptive stimulation (personal observations). Evoked responses to photic stimulation suggest an increased sensitivity to visual stimulation in the isolates.

The general picture, therefore, of a pup emerging from isolation, is one of an organism reacting maximally to a relatively enriched environment and having heightened sensitivity to extraneous stimuli. It might be said that such animals are reacting without their afferent filtering systems operating, or in the Pavlovian sense, without internal inhibition. Hutt *et al.* [18] have emphasized in their telemetered EEG studies of autistic children, showing almost identical behavior patterns to isolation-reared animals, that the EEG manifestations of constant desynchronization suggest a state

of chronic reticular arousal. In the autistic child, either socioemotional or neurological (e.g. deafness) factors are involved in the genesis of the syndrome. Stereotypy, impairment or absence of speech and impaired pain perception are symptomatic. Isolation-reared dogs show stereotypy, minimal or no vocalization and impaired pain perception [11, 26]. In the dog, more prolonged isolation results in greater sensory and motor deficits and more severe and persistent behavioral anomalies, including emotional hypo- and hyper-activity and fear of the unfamiliar. Genetic factors are also involved, as some breeds are more susceptible to isolation rearing than are others [12, 20]. Murphree et al. [27] and Dykman et al. [5] have shown that there is a genetic basis for timidity in dogs reared under normal conditions and the observations of Krushinskii [20] and Fuller and Clark [13, 14] show that genetic factors influence the animal's susceptibility to isolation rearing. A genotype-environment interaction is evident, Fox [8] and such overfearful animals, having an inherited trait or timidity will develop abnormally in a "geneticallyinduced" or "familialy imposed" state of isolation. Fox and Stelzner [11] have stressed the unexpectedly marked effects that short-term isolation has on behavior, pups showing similar behavioral anomalies as others reared for much longer periods in isolation with the exception that mass fear responses and avoidance of novel stimuli were not seen. Avoidance behavior normally develops at a later age in the dog, around 6-8 weeks of age [10, 35]. The hypothesis of chronic reticular arousal being a possible underlying factor in autism, as advanced by Hutt et al. [18] is supported by the observations of Lindsley et al. [21]. In long term dark-reared monkeys, Lindsley and co-workers observed "definite reversal or paradoxical effects from light stimulation or absence of stimulation, in which the cortical electrical activity, which normally reflects changes induced in the reticular activating system, was made to block not at the presence of light but at the absence of light, and to show synchronized rhythms not when it was dark but when there was light". They suggested that these phenomena are due to malfunction of regulating and adapting mechanisms in the hippocampus and reticular activating system. Photic stimulation produced markedly enhanced evoked potentials, possibly due to deprivation causing increased sensitization. Similarly Scherrer and Fourment [32] observed hypersensitivity of the unspecific system as reflected in the intense and sustained arousal reactions to light, sound and tactile stimuli. Melzack and Burns [25] noted marked and sustained desynchronization of EEG with a significantly greater proportion of high frequencies in isolation-reared dogs released from isolation. Averaged evoked visual and auditory responses were of lower amplitude in these subjects when placed in an unfamiliar environment. In contrast, Baxter [2] failed to show any detectable EEG abnormalities in the spontaneous EEG of dark-raised cats, but found reduction in flicker-induced potentials at all frequencies of visual stimulation utilized.

These earlier findings are in part confirmed in the present investigation and lend support to the general concepts of environmental adaptation and stimulus-dependent homeostasis of the CNS. Without adequate stimulation for a prolonged period, when suddenly confronted with an enriched environment and stimulation of many modalities, such isolation-reared animals are unable to adapt to what constitutes a "normal environment". A state of acute reticular arousal occurs, characterized by increased sensitivity to visual stimulation and marked desynchronization of EEG and rhythmic fast activity and accompanied by intense behavioral arousal. The views of Melzack and Burns [25] support this general hypothesis, for they postulate that the isolation-reared animal

resembles a neural model in which failure to filter out irrelevant information on the basis of prior experience leads to excessive arousal, which in turn interferes with the mechanisms that normally act in the selection of cues for adaptive response. With short-term isolation, rapid behavioral adaptation occurs with the gradual recovery of normal EEG and EP. Permanent behavioral abnormalities have been reported by earlier workers in long-term, isolation-reared animals. If isolation extends beyond the period of integration [9], which extends from 1-3months of age, during which time the CNS matures and sensory-motor mechanisms are organized, adaptive behavioral processes are no longer plastic, but become rigid and stereotyped and are overshadowed by mass fear and avoidance responses to novel stimuli. This period of integration underlies the critical period of socialization [33], which is an important time for the establishment of primary social relationships, in both canine and human infants [34]. Restricted experience during this period may cause severe and permanent deficits in social behavior [7]. Partial sensory and social isolation rearing, therefore, causes both social and perceptual deficits in behavior. Fuller and Clark [13, 14] studied the effects of long-term social isolation (for 12 weeks, commencing at 3 weeks of age) with pups reared singly in illuminated cages through which they could not see. They concluded that this partial isolation resulted in a blocking of approach and contactual responses by preemptive aversive reactions to unfamiliar stimuli and not a perceptual deficit induced by experiential deprivation. Recovery in the "normal" environment is then protracted, and complete adaptation may never occur as a state of chronic arousal ensues.

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