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THE INFLUENCE OF FRAGMENTS OF THE LPH CHAINS ON LEARNING, MEMORY AND ATTENTION IN ANIMALS AND MAN

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1. INTRODUCTION

It is apparent that changes in the environment are reflected by chemical and structural changes in the nervous system. Thus, experience may be chemically and subsequently structurally coded. Implicit in this analysis are the dynamic processes of the organism. Not only may various peptides be synthesized and replaced, but it is possible that receptors in the brain proliferate or degenerate in the response to this chemical fluctuation.

The focus of this chapter is the modulation of perception and attention by peptides of the β-lipotrophin (LPH) chain. There are two key points that will be developed. First, the interaction among peptide fragments of the LPH chain may result in modulation of behavior. Second, the effects of peptides of the LPH chain may be best expained as modulation of the perceptual/attentional state of the organism.

2. STUDIES IN ANIMALS

2.1. MSH, ACTH AND THEIR FRAGMENTS

The early work of Mirsky *et al.* (1953); Applezweig and Baudry (1955), and of Miller and his colleagues (Murphy and Miller, 1955; Miller and Ogawa, 1962) anticipated the advances of Ungar (1973). These investigators were among the first to propose that the polypeptide ACTH influenced behavior by exerting an effect on the brain. Since these pioneering efforts, several groups have been exploring the influence of MSH and ACTH on the brain and behavior. The major areas of investigation can best be discussed as emphasizing either pharmacological or behavioral approaches.

2.1.1. *Pharmacological Approaches*

The pharmacological approach, best characterized by the Utrecht group, initially, focused on a single reliable behavior to use as a bioassay of peptide effects. The major questions of interest with this approach were those of dosage, molecular structure, and route of administration. These issues were pursued by the Utrecht group in the early stages of research with aversive paradigms. Typically, rats were trained to either exhibit or inhibit a response in order to avoid receiving a painful electric shock. After learning to avoid the aversive stimulus the animals were given extinction trials. During extinction the shock was turned off and the animals were tested to determine how long they continued to respond in the absence of reinforcement. Results from several laboratories have indicated that animals given MSH, ACTH or their fragments, show prolonged

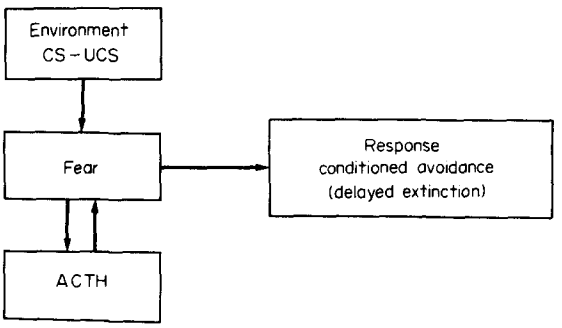

FIG. 1. Classical model of the influence of ACTH on the maintenance of the conditioned avoidance response.

responses during extinction as compared with untreated animals. Two explanations have been suggested.

The earliest explanation of the effects of these peptides was that they influenced the emotional state of the organism and made them more fearful. As illustrated in Fig. 1, this explanation relied heavily on the assumption that avoidance situations elicited a fear or anxiety response. The ACTH secretion in response to the stress of shock was considered a manifestation of the fear drive. The extrapolation that injections of ACTH produced the fear, dominated scientific thinking for about 15 years.

The initial departure from the popular fear hypothesis was proposed by De Wied and Bohus (1966). They interpreted the prolonged extinction observed after treatment with MSH/ACTH as indicating that these peptides enhanced short-term memory processes. Although it was argued that trial-to-trial memory was primarily influenced, their subsequent reports were more consistent with the view illustrated in Fig. 2. Thus, enhancement of memory was interpreted to be a function of the increased general motivational state of the organism. Nevertheless, several experiments have implicated MSH/ACTH fragments in the retrieval processes. Rigter (Rigter and Van Riezen, 1975; Rigter *et al.*, 1976) trained animals to passively avoid shock and then induced amnesia by applying electroconvulsive shock or partial asphyxiation by CO_2. These treatments erase the

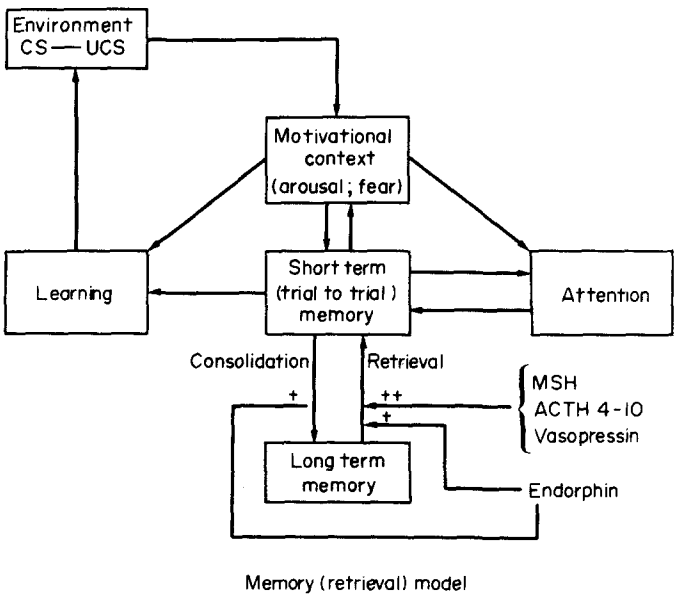

FIG. 2. Departure from the classical model suggesting that peptides influence cognitive abilities, specifically retrieval of memory.

memory of the learning experience. However, treatment of rats with MSH/ACTH 4–10 before the test of retention apparently restores the memory of the experience. These data were interpreted as supporting the proposal that short-term memory processes, especially those processes involved in retrieval of information from long term memory storage, were facilitated by treatment with MSH/ACTH fragments.

However, recent attempts to extend these findings have not generated support for the memory hypothesis. Martinez *et al.* (1979) compared the various times of injections of an ACTH 4–9 analog on performance of the passive avoidance response. Their findings were at variance with those of Rigter since injections before the retention test resulted in less retention than in the saline treated animals. Although different procedures were employed, Martinez *et al.*, concluded that the MSH/ACTH peptides did not influence either memory consolidation or retrieval but rather sensory, attentional or motivational processes.

2.1.2. *Behavioral Approach*

The focus of the behavioral strategy is to elucidate the psychological processes which best explain the influence of peptides. This strategy for investigating peptides uses a variety of behaviors in order to develop a comprehensive analysis of the effects of peptide.

Among the tasks which have been employed are the learning of mazes for the reward of food; active and passive avoidance learning; learning a visual discrimination to avoid shock; reversal shift of the visual discrimination; open field activity; social behavior in the open field; and lever pressing for the reward of food. The constellation of results suggests that MSH/ACTH peptides influence the perceptual/attentional functioning of the organism. The major support for this conclusion was developed with the visual discrimination and reversal shift problem in rats.

In several studies (Sandman *et al.*, 1972; 1973; 1974; 1980), rats were trained with a two choice visual discrimination problem to avoid shock by running to a white door. After the animals acquired the response and successfully avoided shock, the task was reversed so that the simultaneously available black door was the correct response. The initial stage of the experiment measured the animals ability to learn a new response. The reversal stage measured the animals selective attention (MacIntosh, 1965; 1969). An attentive animal solved the reversal problem faster than an unattentive animal because it learned about the dimension of brightness during the original problem and not only that white was correct. Thus, when the problem was changed, the attentive animal tested values on the dimension of brightness (black–white) rather than irrelevant dimensions (e.g. in this case spatial localization).

In the initial studies, treatment of rats with MSH had no appreciable effect on original learning. However, rats treated with MSH required approximately 50 per cent *fewer* trails to solve the reversal learning problem. We have concluded from these data, as well as from data gathered in other paradigms, that the MSH/ACTH peptide enhanced attentional processes.

In a recent, refined analysis, we (Sandman *et al.*, 1980) compared the influence of MSH/ACTH 4–10, α-MSH (1–13), β_p-MSH (1–18), β_h-MSH (1–22) and ACTH 1–24 on discrimination and attention. This study permitted investigation of the putative effects of the redundant chemical information stored in these related peptide chains. Although the prevailing view among investigators studying structure–activity relationships was that behavioral information in these molecules was redundant, Greven and De Wied (1977) recently indicated that the proposed redundancy was specific to extinction of the pole-jumping avoidance response.

The results of our study are illustrated in Figs 3 and 4. The speed of learning the original problem diminished with administration of the same dose of compounds of increasing molecular weight. Learning at the initial stage of learning was enhanced significantly with administration of MSH/ACTH 4–10. Except for ACTH 1–24, all of the

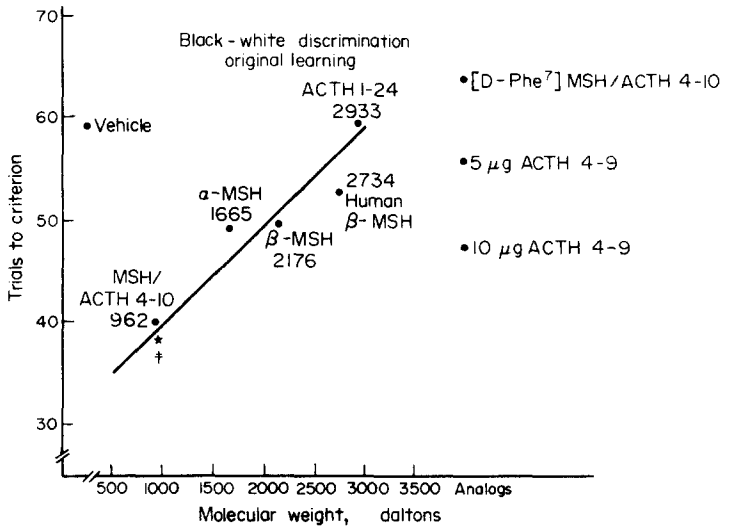

FIG. 3. Linear relationship between molecular weight of related peptides and the number of trials for rats to learn a visual discrimination problem.

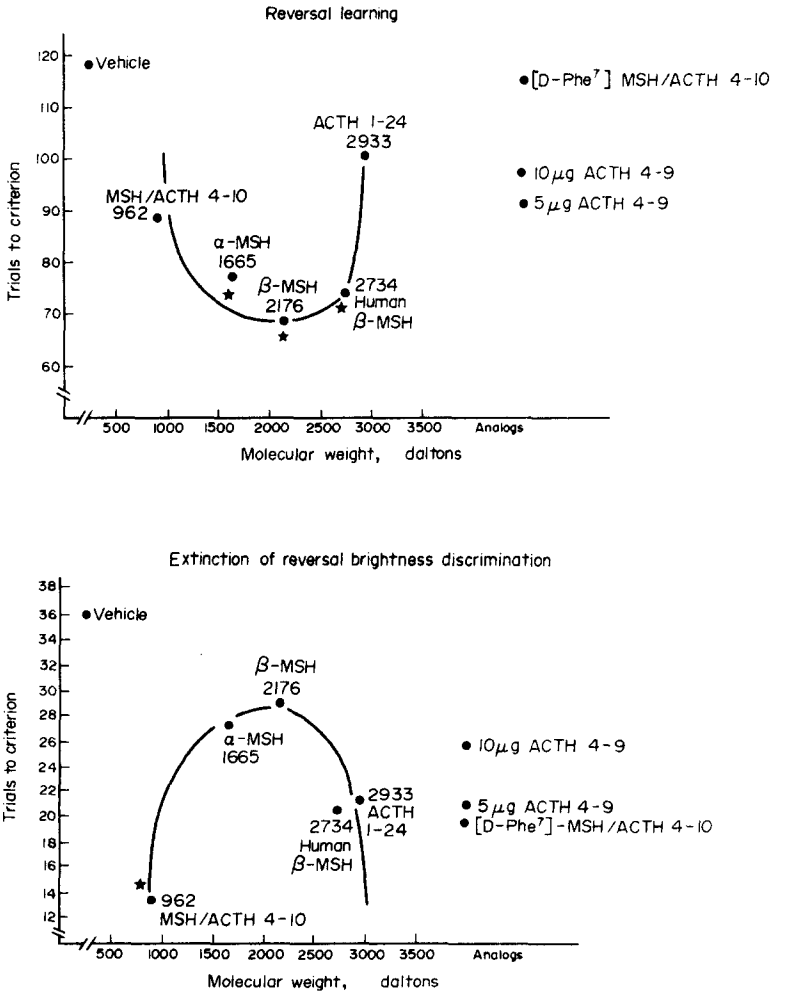

FIG. 4. Quadratic relationship between the molecular weight of peptides and the reversal learning of the visual discrimination problem.

other peptides also improved learning, though not achieving acceptable levels of statistical significance.

From Fig. 4 it is apparent that the structure–activity relationships were much different for reversal learning and extinction. Maximal enhancement of reversal learning (an index of attention) was achieved with administration of α-, β and (human) β-MSH. Thus, when plotted according to molecular weight, a significant quadratic relation with learning (attention) was apparent.

The results of the early phases of the learning process (original learning) are in agreement with the conclusions of De Wied and Bohus (1966). If behavioral information was coded redundantly in these related molecules a monotonic relationship would be predicted between performance and molecular weight. The relationships observed in this study support such a speculation and suggested that 'trial-to-trial' memory may be influenced by the 4–10 fragment.

However, the results of the reversal learning problem indicated that only compounds with MSH-like configurations improved performance. These findings suggested that attentional function may be specific to a particular peptide sequence. Thus, the fit of a molecule with its putative receptor may direct discrete behavioral patterns.

It is clear that neurogenic compounds influence learning and attention. Since these two constructs are related in all theories of learning, it was not surprising that they share biochemical substrates. The findings of the present study illustrated the similarities of the related peptides, as may be expected from their shared structural elements, and the qualitative differences among them, as may be predicted by their different configuration.

2.1.3. *Developmental Studies*

The influence of early treatment with MSH-like peptides on later behavior has been investigated in several studies. The rationale of this approach is that the brain and endocrine system are not fully developed in immature organisms and thus may be extremely pliable. Administration of peptides at early stages of development may result in structural changes which are reflected in permanent alterations in behavior. Although this reasoning has been explored fruitfully with steroids, relatively little developmental work has been done with MSH, ACTH and their fragments.

In a series of studies reviewed elsewhere (Sandman *et al.*, 1977), we reported that MSH treatments between days 2–7 postnatally resulted in significant improvement in the learning of adult rats. Increased efficiency in receiving reinforcement with a difficult operant schedule (DRL–20) characterized adult rats given MSH as infants. Similarly, rats given MSH as infants acquired and extinguished an active avoidance response when tested as adults. This finding was not consistent with reports of delayed extinction after treatment of adult rats with MSH/ACTH in the active avoidance response.

Consistent findings also were generated with the visual discrimination procedure. Rats treated as infants with MSH performed the discrimination and reversal problem more accurately than rats treated with the vehicle solution. However, this result pertained only to the male animals. There was no effect of MSH on the learning performance of female rats (Beckwith *et al.*, 1977b). A second independent study conducted in our laboratory (Champney *et al.*, 1976) with a behaviorally active analog of ACTH 4–9 (Met $(O_2)^4$, D-Lys^8, Phe^9) confirmed the positive findings for male animals and also concluded that the influence of the peptide on the learning of female rats was different from that for male rats.

In a study of social behavior (Beckwith *et al.*, 1977a), infant rats were again treated with MSH between the ages of 2–7 days and then observed in the open field when they were 45 and 120 days old. Pairs of rats of the same sex were placed in the open field for 5 min and the time in contact with one another was measured. The findings indicated that females treated with MSH as infants spent the greatest amount of time in contact with each other. This effect was apparent at 45 but not at 120 days. Treatment of infant male rats with MSH also increased contact time compared to control animals and the

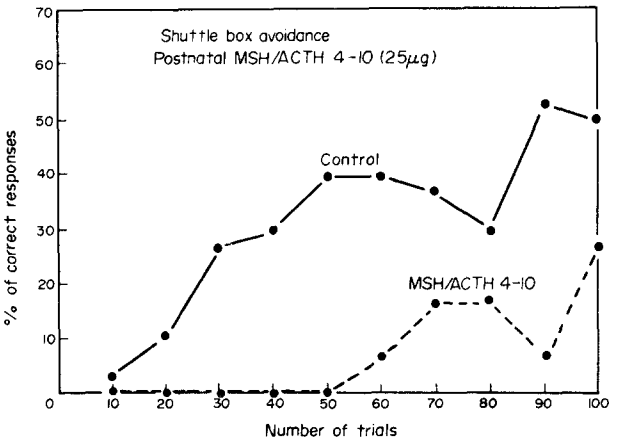

FIG. 5. Neonatal injections of high doses (25 μg/rat) of ACTH 4–10 disrupt avoidance learning in adult rats.

effect persisted for at least 120 days. These complex findings indicated that early treatment with MSH influenced behavior and that the effect was sexually dimorphic.

In a recent series of studies in which we have increased the dosage 2.5–5 times that of the earlier dosage and have used MSH/ACTH 4–10 instead of MSH, a very different, perhaps opposite, behavioral profile has emerged. A short time (30 sec) after injection of the 2–7 day old rat pups they were observed to writhe and nearly convulse. This effect was not seen with the vehicle, which was matched for pH with the peptide, nor was it observed in the previous studies using smaller doses. As adults these rats have deficits in learning the active avoidance response (Fig. 5) and the reversal shift problem of the visual discrimination task. These data supported the view (Sandman *et al.*, 1980) that many of the effects observed were dose dependent. Thus, the beneficial effects of MSH, ACTH and their fragments hang in a delicate balance. On the one hand, with a relatively broad range of dosage, lies the promise of a treatment for deficits of attention. On the other side of the balance lies a possible cause of deficits of attention.

2.2. ENDORPHINS

Embedded in the C-terminal of the LPH molecule are enkephalin (LPH 61–65) and the endorphins (α 61–78; γ 61–77; β 61–91), although the physiologically active enkephalin may not be derived from the LPH chain. The discovery of the analgesic properties of these related molecules catalyzed enormous interest in the study of peptides. Although we now know that many peptides possess analgesic properties, some with even greater potency than the natural endorphins, the excitement initially generated by the enkephalins and endorphins captivated scientists from divergent disciplines and subsequently accelerated the research of peptide effects on the brain and behavior.

2.2.1. *Analgesia*

Even though it is now considered somewhat of an epiphenomenon, injections of the endorphins into the ventricles or into discrete areas of the brain can produce profound analgesia. The analgesic effects of methionine and leucine enkephalin have been small and short-lived. However, by substituting d-alamine in the second position an analog was produced with very powerful analgesic properties (Walker *et al.*, 1979a).

Similarly, injections into the brain of α, γ, and β-endorphin produced analgesia (Walker *et al.*, 1977b). Among these related molecules β-endorphin is the most potent in producing analgesia. As with the enkephalins, substitution of D-alanine in the second position resulted in profound analgesia persisting for up to six hours. However, periperal administration of enkephalin, endorphins or their potent d-ala-2 analogs even in larger

doses (1–10 mg) did not result in analgesia. Thus, other effects of these peptides have been explored.

2.2.2. *Other Effects*

Although the extra-opiate effects of the enkephalins and endorphins have not been studied extensively, the studies which have been conducted clearly indicated that these peptides are behaviorally active. In addition to the analgesia after central administration, a number of behavioral responses not directly associated with analgesia have been reported. For instance, intracerebral injection of opioid peptides increased grooming behavior (Gispen *et al.*, 1977), decreased electrically induced self stimulation (Stein and Belluzzi, 1978), depressed levels of sexual activity of male rats in the presence of estrous females (Meyerson and Terenius, 1977), stimulated penile erection and spontaneous seminal emission (Walker *et al.*, 1977a) and increased food intake in rats (Belluzzi and Stein, 1978). All of these effects are reversible by naloxone.

Peripheral administration of opioid peptides also results in a number of behavioral responses. In one of the earliest studies of the behavioral effects of enkephalin, Kastin *et al.* (1976) reported that small doses (80 μg/kg) enhanced maze learning of hungry rats. It was unlikely that this effect was due to appetite, general motor activity, or arousal. In a later series of studies De Wied *et al.* (1978) reported that Met-enkephalin was as potent as MSH/ACTH 4–10 in delaying extinction of the avoidance response. They further indicated that the endorphins were even more potent than MSH/ACTH 4–10 and that α-endorphin was the most potent on a molar basis.

Rigter (1978) has reported that Met and Leu enkephalin can attenuate amnesia by CO_2. (This study was identical in design to earlier ones testing MSH/ACTH 4–10). The results presented a puzzling profile suggesting that Met and Leu enkephalin influenced memory in different ways. Met-enkephalin reduced amnesia when given before acquisition, before retrieval, or both times. Leu-enkephalin reversed amnesia only when given before retrieval or if given both before acquisition and retrieval. As depicted in Fig. 2, it could be argued that the endogenous opioids influenced both consolidation and retrieval processes of memory, whereas the MSH/ACTH peptides influenced only retrieval.

Among the most striking reports of the multiple independent actions of peripheral administration of the endorphin is that of Veith *et al.* (1978). The different effects of α, γ, and β-endorphin on the behavior of rats were examined in the open field. Veith *et al.* reported that each of these related peptides exerted specific, non-overlapping effects. Treatment with α-endorphin typically produced penile erection and spontaneous seminal discharge—an effect interpreted as relating to pleasure. The effect of γ-endorphin was to increase defecation and diminish exploratory behavior. Peripheral administration of β-endorphin resulted in increased grooming, an effect reported by other investigations (Gispen *et al.*, 1977). These data suggested that peptides which shared an amino acid core, nevertheless, exerted discrete and specific influences on behavior.

2.2.3. *Developmental Findings*

Several factors converged to suggest that the effects of exposure to β-endorphin early in life would have a profound influence on the organism. First, earlier studies with the MSH/ACTH fragments suggested that neonatal exposure to their related sequence of amino acids exerted a persistent influence. Second, Gautray *et al.* (1977) reported that β-endorphin was elevated in the amniotic fluid during fetal distress, a condition which can result in profound physical and cognitive impairment. Third, it is known (Simon and Hiller, 1978) that the rat is not born with a fully developed complement of opiate receptors. The most rapid proliferation occurs during the first 21 days postnatally and matures by about 140 days of age. These factors suggested our initial experiments.

In the first experiment (Sandman *et al.*, 1979) rats were injected with 50 μg (β-endorphin or a vehicle control), s.c. each day from days 2 through 7 of life. The rats were then

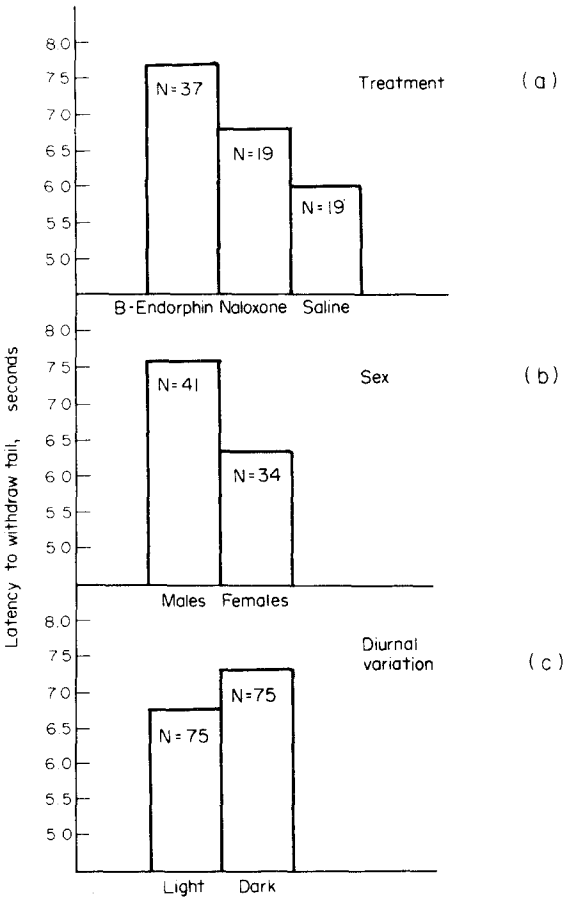

FIG. 6. Neonatal injections of β-endorphin cause a chronic elevation in sensitivity to thermal stimulus.

left undisturbed until day 90 when tests for analgesia were performed. Two tests were employed, the tail-flick and shock-jump measures of sensitivity to pain. The results indicated that early exposure to β-endorphin permanently increased the threshold for thermal (Fig. 6) but not shock-induced pain. An attenuated, but similar, finding was observed and recently replicated (Monder *et al.*, 1979) for the opiate antagonist naloxone. At this time the mechanism of action is unclear; however, alteration of opiate receptors or of levels of β-endorphins are possible factors. The similarity between animals exposed to β-endorphin early in life to those raised in isolation deserves further study.

In the second study the consequences of injecting pregnant rats with either β-endorphin or a vehicle solution was examined (McGivern *et al.*, 1979). Pregnant rats were injected s.c. every other day, from day 7 through day 21 of pregnancy. The male offspring were crossfostered and studied longitudinally from birth through day 180. Preliminary findings after injection of radioactive β-endorphin into separate rats suggested that approximately 5 per cent of the radioactivity may pass the placental barrier.

The results indicated that early, *in utero* exposure to β-endorphin retarded aspects of development. The rats given the peptide were delayed significantly in eye opening, were less active during early life and failed to exhibit the normal pattern of activity later. By day 40 through maturity, the rats exposed to β-endorphin *in utero* evidenced a significant weight gain. At sacrifice (day 180) their wet brain weights were greater and their tails were longer than matched controls.

Behavioral tests indicated that rats exposed to β-endorphin *in utero* appeared less responsive to environmental stimulation than the animals in the control group. Several measures illustrate this tonic unresponsiveness. The startle response is measured by assessing the animal's somatomuscular reaction to a very loud (60 db) tone. As illustrated

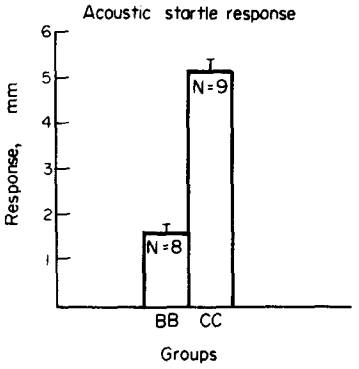

FIG. 7. *In utero* treatment of rats with β-endorphin causes a three fold attenuation in a startle response to an acoustic stimulus.

in Fig. 7, the response of rats exposed to β-endorphin is attenuated by more than a factor of 3 compared with animals given the control solution.

Two different conditions were tested with an open field apparatus. The first condition involved placing a single rat in the center of an open field. Typically, when placed in the center of an open field, rats initially move to a wall and subsequently explore the apparatus. Both of these tendencies were markedly reduced in the animals exposed to β-endorphin (Fig. 8). In a second condition, pairs of animals were observed in the open field. As illustrated in Fig. 9, the rats exposed to β-endorphin spent less time in physical contact and less time in close approximation to each other than the control group. Clearly, *in utero* exposure disrupted the normal open field and social behavior of rats.

Two tests of learning also distinguished the rats exposed *in utero* to β-endorphin from those exposed only to the vehicle solution. In a test of passive avoidance, rats exposed to β-endorphin took significantly longer than control animals to re-enter a chamber in which they had received shock 24 hr earlier (Fig. 10). In a test of visual discrimination there were no differences between groups during initial learning; however, the rats

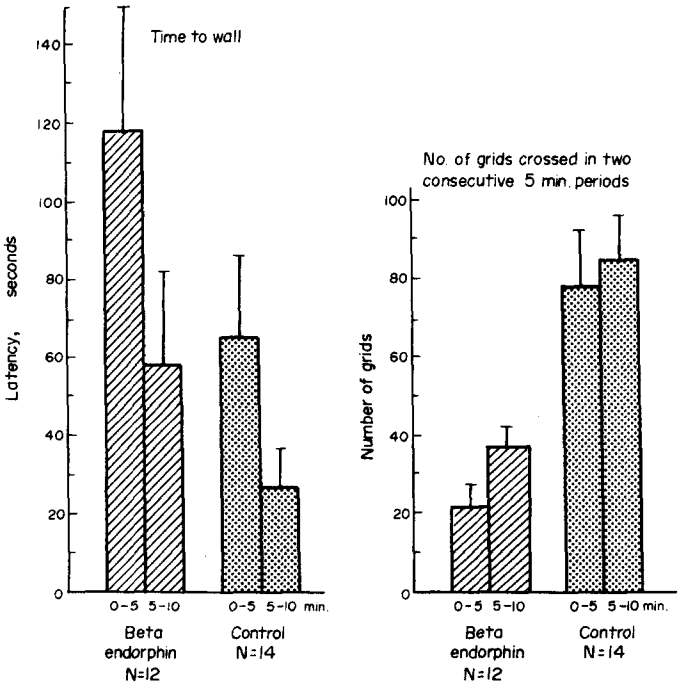

FIG. 8. Left panel: Rats treated *in utero* with β-endorphin remain in the center of an open field longer than controls. Right panel: *in utero* treatments with β-endorphin results in less movement in the open field compared with controls.

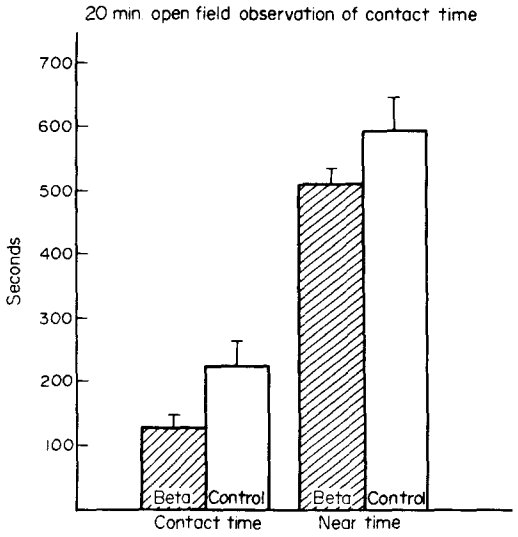

FIG. 9. *In utero* treatment with β-endorphin diminishes two indicies of social behavior (contact and proximal time).

treated with β-endorphin required significantly more trials than controls to learn the reversal shift. This pattern of results suggested impaired attention without a concommitant impairment of memory.

In view of the report of permanent elevation in threshold for a thermal stimulus in rats treated as neonates with β-endorphins, it was of extreme interest to test the animals exposed *in utero* to an identical test. Surprisingly, there were no differences between the rats exposed *in utero* to β-endorphin or vehicle on the tail flick test. However, the rats exposed to β-endorphin were significantly more sensitive to analgesic doses of morphine than the control animals (Fig. 11). Thus, an interesting pattern emerges which suggests critical period effects of the peptides on behavior. Further, the increased sensitivity to

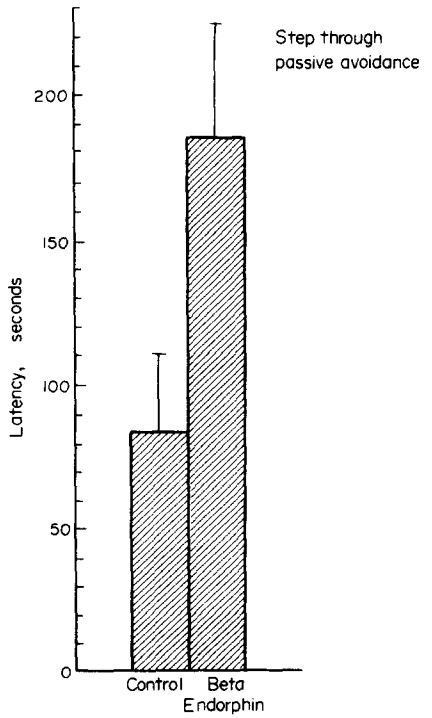

FIG. 10. Increased passive avoidance in rats tested *in utero* with β-endorphin.

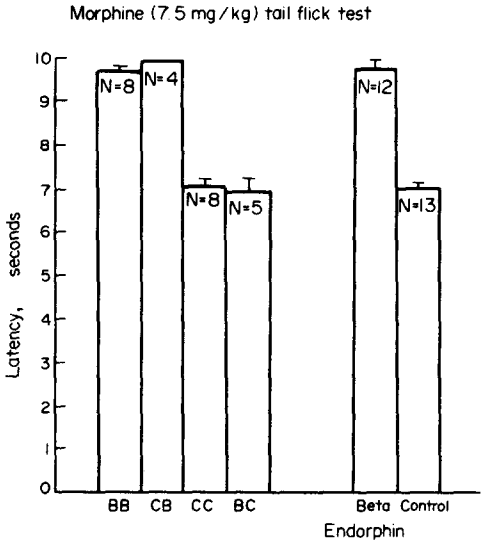

FIG. 11. Increased sensitivity to morphine in rats treated *in utero* with β-endorphin.

morphine after *in utero* exposure to β-endorphin is in direct opposition to the effects reported for *in utero* exposure to morphine (Paul *et al.*, 1978). Prenatal exposure to morphine produces tolerance to subsequent treatment with morphine. It is conceivable that the effects observed after *in utero* exposure to β-endorphin may reflect at least partially, its extra-opiate influences. The dose or type of opiate peptide may influence later behavior when injected during the first week of life. Newborn rats injected with 80 μg/kg (about 1 μg/pup) Met-enkephalin ran a complex, 12-choice maze faster and with fewer errors when tested three months later, an effect greater in females than males (Kastin *et al.*, in press).

The behavioral influences of peptides of the LPH molecule in animals is well established. The effects of fragments of MSH/ACTH on the brain and behavior have been scrutinized for nearly 20 years. Even though the endorphins have had a much shorter scientific life, the amount of data generated with these exciting compounds approximates that with the MSH/ACTH molecules. Although clear effects on the behavior of rats have been reported for these molecules, there is not a consensus of what they do. Studies of the effects of peptides on the behavior of human subjects add considerably to our understanding.

3. STUDIES IN NORMAL HUMAN VOLUNTEERS

A growing number of studies indicate that neuropeptides, especially the MSH/ACTH fragments, influence the physiology and behavior of normal human subjects. Although much of this has been reviewed elsewhere (Sandman *et al.*, 1977), the proliferation of new studies requires a re-analysis of the findings.

3.1. PHYSIOLOGICAL EFFECTS

In the earliest study of the influence of MSH/ACTH fragments on the brain and behavior of normal subjects, Miller *et al.* (1974), examined several central and autonomic nervous system parameters. Male subjects received either MSH/ACTH 4–10 or ACTH 1–24 and were monitored for basal changes in physiological functions as well as during specific tasks. No effect on the EEG was observed in subjects receiving ACTH 1–24. However, spectral analysis of the EEG (0_1 0_2) indicated a decrease in the power output of the 3–7 Hz frequency but an increase in the 8–12 Hz and 12+ Hz frequencies in subjects injected with MSH/ACTH 4–10. Identical findings were reported for the percentage of time in each of these frequency bandwidths. A recent report of findings in

elderly patients after MSH/ACTH 4–10 injections (Branconnier *et al.*, 1979) presented different conclusions. Reduction in the 9–13 Hz range was found with an increase in the slower delta–alpha bands. However, the authors acknowledged this effect was seen primarily in women.

The most striking finding in the study by Miller *et al.* was the delay in the α-blocking EEG response to repetitive stimulation. Typically during the first few trials there is a characteristic increase in EEG frequency to external stimulation. After several trials, supposedly after the subject has habituated to the stimulus, the EEG response diminishes and the predominant frequency is in the alpha range (8–12 Hz). However, treatment with MSH/ACTH 4–10 attenuated the habituation, indicating that the impact of the stimulation remained salient for a significantly longer period of time.

In two separate studies (Miller *et al.*, 1974; Sandman *et al.*, 1977) no effect of the peptide on basal autonomic responses has been observed. However, significant heart rate deceleration (a reliable index of the orienting responses) to novel stimulation has been reported after treatment with MSH/ACTH 4–10 (Sandman *et al.*, 1977). This finding is consistent with the α-blocking response discussed above since both responses reflect increased awareness of the environment.

Perhaps the most elegant means of assessing the impact of the peptides on the electrical activity of the brain is with the averaged evoked potential (AEP). The AEP is a time-locked response of the brain to stimulation which can be detected with computer averaging techniques. Several distinctive waves emerge which relate to the state of the organism, the nature of the stimulus and the response required of the subject. Typically, the earlier components of the response pertain to physiological factors. The response occurring at 100–200 msec reflect perception of the stimulus and the later components are associated with the requirements made of the subject.

Two studies have been conducted to assess the influence of MSH/ACTH fragments on the AEP. In the first study (Miller *et al.*, 1976), the visual AEP was measured while subjects performed a decision making—reaction time task. The subjects given MSH/ACTH 4–10 evidenced a dramatic augmentation of the negative peak about 350 msec after stimulation. Further, MSH/ACTH 4–10 resulted in increased latency and decreased amplitude of the P200 complex. Thus, with a task designed to elicit late components, the peptide enhanced them while diminishing the earlier components.

In a second study, with an uncomplicated task designed to elicit components occurring at 100–200 msec after stimulation, the influence of an orally administered analog of MSH/ACTH 4–9 was examined (Sandman *et al.*, submitted). Five men and five women were given 0, 5, 10 and 20 mg of the analog or d-amphetamine (10 mg) as a positive control in a double blind procedure. The various doses were given one week apart. Testing within each session lasted 5 hr in order to determine the temporal parameters of treatment. Immediately after ingesting the coded capsule, brief, bright flashes of light were projected while EEG's were recorded from the right and left hemisphere of the occipital cortex. The results indicated that dosage, time after ingestion, hemisphere of the brain and sex of the subject all were influential factors determining the effects of the peptide. The P200 complex was enhanced in both hemispheres of women and P100 was augmented in the right hemisphere of men. Area-under-the-curve measures were taken to supplement interpretation of the findings. As Fig. 12 illustrates, several striking results were obtained. The most dramatic effect was the interaction among dosage, hemisphere of the brain and sex of the subject. In men the major effect of the peptide was seen in the right hemisphere of the brain and peaked around 60 min. Both hemispheres in women appeared to be influenced by the peptide but the most obvious effect was in the left hemisphere. In the right hemisphere of both men and women, the influence of d-amphetamine and of 20 mg of the peptide appeared similar.

The fact that sex of the subject and hemisphere of the brain are influenced differently by MSH/ACTH fragments complements earlier studies with these peptides. The enhanced attention in males but not females after treatment with MSH (Beckwith *et al.*, 1977b) or the 4–9 analog (Champney *et al.*, 1976) is in accord with this neurophysiologi-

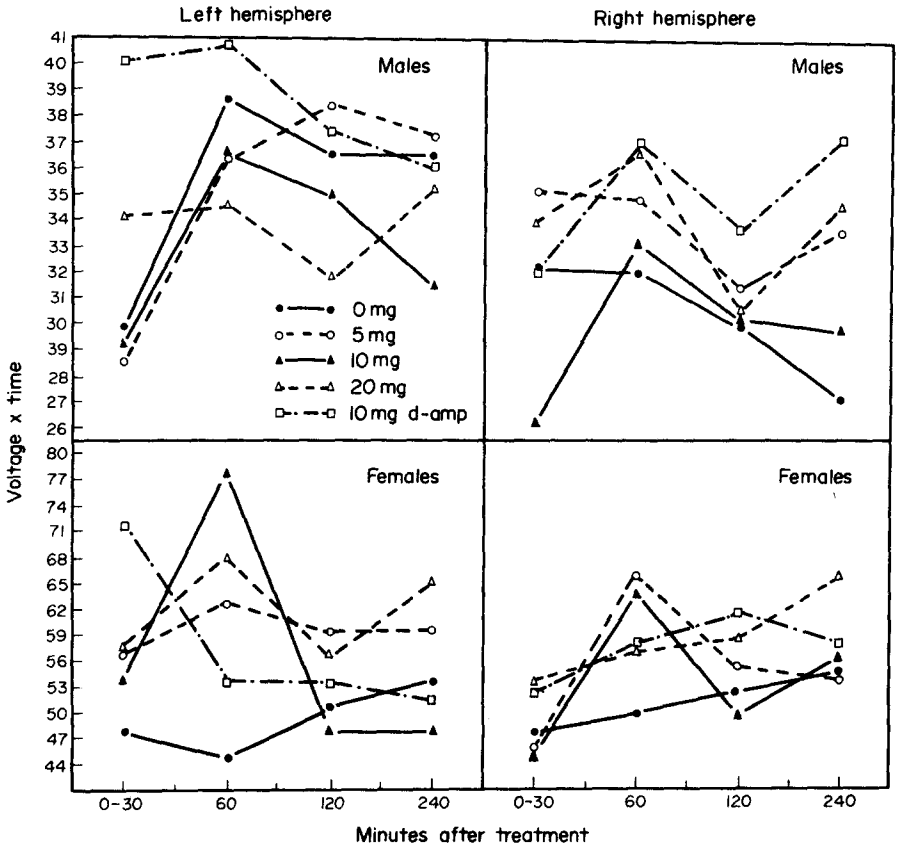

FIG. 12. Influence of oral doses of an analog of MSH/ACTH 4–9 on the cortical visual evoked potential (area-under-the-curve) in the right and left hemisphere of normal men and women.

cal data. Further, studies with human subjects (Sandman *et al.*, 1975; 1977; Veith *et al.*, 1978) have indicated that the MSH/ACTH fragment may elicit sexually dimorphic effects. Clearly, the results of the study of MSH/ACTH peptides on the AEP offer neurophysiological support to buttress this argument.

3.1.1. *Behavioral Effects*

In a series of early studies, the effects of MSH/ACTH 4–10 were studied on a number of behavioral parameters in normal volunteers. Among the most reliable findings were increased visual retention, decreased anxiety, and enhanced visual discrimination (Miller *et al.*, 1974; Sandman *et al.*, 1975). Several parameters were not affected by the peptide, including short term memory for digits, measures of emotionality, reaction time and verbal memory. Gaillard and Sanders *et al.* (1975) and Sanders (1975) suggested from their work that MSH/ACTH 4–10 may act to combat fatigue and to facilitate acquisition of a concept. Even though a semblance of consistency with the animal literature was evident in these early studies, the debate continued concerning the action of these peptides. Several studies were initiated in our laboratory to examine the primary processes affected by the peptide.

The first candidate for study was the influence of MSH/ACTH 4–10 on perceptual threshold (Sandman *et al.*, 1977). Two different perceptual tests were used. One was a detection test in which stimuli were presented for only 6 msec at different levels of brightness. Subjects were required to press one key if they saw a stimulus and another key if they did not. Infusion of MSH/ACTH 4–10 raised the threshold for detection and impaired the subjects ability to accurately report the presence of a stimulus.

The second procedure was a test of discrimination requiring subjects to distinguish between two different sets of stimuli. All of the presentations were above perceptual

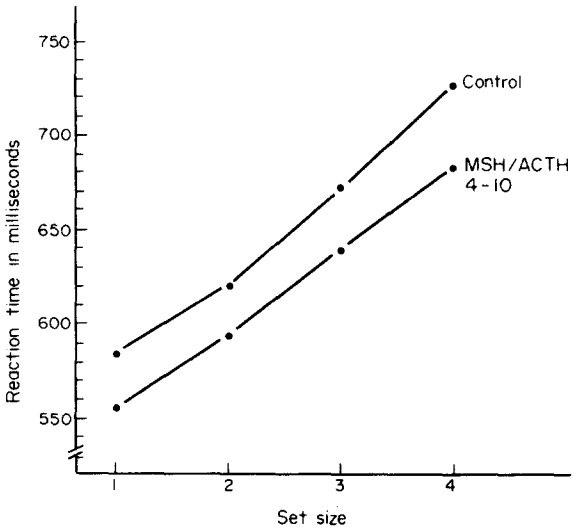

FIG. 13. Treatment of normal human subjects with MSH/ACTH 4–10 alters the intercept but not the slope in the item recognition paradigm.

threshold. When subjects were administered the peptide, their ability to discriminate the two stimuli was improved. These results suggested that MSH/ACTH 4–10 facilitated stimulus processing or selective attention, whereas simple intake or detection of threshold stimuli was impaired. Conceivably the peptide raised the absolute threshold for stimuli and thus functioned as a filtering mechanism to protect the organism from distracting 'perceptual noise'. However, when stimuli were above the threshold, the processing of information was facilitated.

Although the pattern of findings for the study reported above were difficult to interpret as an effect on memory, no clear separation of the effects of MSH/ACTH fragments on attention and memory in the same study had been reported. A recent study in our laboratory (Ward *et al.*, 1979) was designed to test specifically the influence of MSH/ACTH 4–10 on attention and memory. For distinguishing these processes, the item recognition test was employed. In this test, subjects were presented with a memory set consisting of 1, 2, 3, or 4 items. After they memorized the set, probe stimuli were presented. Half of the stimuli were in the set and half were not. The subject depressed one key if the probe was a member of the memory set and a second key if it was not. The data were plotted as a function of reaction time and set size. Changes in memory were reflected by changes in the slope of the function and attentional effects were represented as changes in the intercept. For example, improved memory may be illustrated by faster reaction time for set sizes 3 and 4 but not 1 and 2. Enhanced attention may be inferred by faster reaction time at all set sizes.

Treatment of subjects with MSH/ACTH 4–10 exerted a clear influence on the intercept but no effect on the slope (Fig. 13). In conjunction with other data, the most parsimonious interpretation of the altered intercept function is that MSH/ACTH 4–10 facilitated selective 'encoding' or attention to environmental stimuli.

3.1.2. *Sex Differences*

Although the results of the study by Ward *et al.* were identical for men and women, several other studies have suggested that MSH/ACTH 4–10 influenced men and women differently. As reviewed earlier, data gathered with rats indicated that MSH enhanced visual attention only in males. Conversely, spatial abilities appeared to be augmented by MSH in females. Electrophysiological data have also indicated that men and women were influenced differently by MSH/ACTH fragments. Evoked potentials from the left hemisphere were enhanced in men but potentials from the right hemisphere were affected

in women. A study by Veith *et al.* (1978) was designed to measure in women many of the variables affected by MSH in men.

Women were injected with MSH/ACTH 4–10 either during their menstrual phase (endogenous ACTH is low) or during midcycle (ACTH is high). In addition to indexes of emotion and cognitive state, radioimmunoassays were performed on plasma for FSH, LH, 17β-estradiol and cortisol.

Although there were differences in hormonal levels during the menstrual phase compared with midcycle phase, there was no indication that MSH/ACTH 4–10 influenced any of the hormones. The test of visual memory found to be sensitive to peptide effects in men, was not influenced by the peptide in this study. However, tests of verbal memory (not influenced by the peptide in men) did show significant improvement in women after treatment with MSH/ACTH 4–10. Further, a measure of intra-dimensional shift (an index of visual attention) was impaired in women receiving the peptide. This finding is also different from previous reports with male subjects.

These results are in accordance with behavioral studies of rats and electrophysiological studies of human subjects. The results of this study and of the earlier studies suggested that MSH/ACTH 4–10 augmented verbal abilities in women and visual processes in men. While the precise mechanisms of these effects are not known, the results are consistent with the hemispheric differences discussed earlier.

3.1.3. *Personality Influences*

Although the possibility that personality may be determined by neurochemicals has, at various times in the history of psychology been a dominant theme, few recent studies have examined the interactions between peptides and personality. Except for animal studies of strain differences in response to peptides (Sandman *et al.*, 1973; Stratton *et al.*, 1973) and the sex differences described above, only Miller *et al.* (1976) controlled for possible dispositional differences among subjects. When they selected subjects based upon low anxiety and field dependence scores, they found minimal effects of MSH/ACTH 4–10 on behavior.

In a recent study, Brier *et al.* (1979), reported the interactions between the introversion–extraversion dimension and response to MSH/ACTH 4–10. They found that the dimension of personality determined, to some degree, response to the peptide. The extroverted subjects performed a series of 'mental performance' tasks better after injections of MSH/ACTH 4–10 than during placebo injections. Introverted subjects showed no improvement in performance after receiving the peptide. Further, MSH/ACTH 4–10 decreased forearm blood flow in extroverted subjects but increased flow among introverted subjects. The authors suggest that MSH/ACTH 4–10 may act as a mild central stimulant and thus is more effective in subjects characterized as cortically inhibited (extroverts). These interesting findings deserve further investigation and add refinement to the list of possible effects of peptides.

4. STUDIES OF PATIENT GROUPS

4.1. MSH/ACTH Fragments

One of the earliest reports of the behavioral actions of MSH and the first clinical study was conducted in amenorrheic women (Kastin *et al.*, 1968). Slowing of the EEG, increased heart rate, menstrual bleeding and increased feelings of nervousness and anxiety appeared to be related to infusion of MSH. The second clinical study of the effects of MSH was done with endocrine patients and controls (Kastin *et al.*, 1971). The major finding was enhanced somatosensory evoked potential after infusion of MSH. Recently, there has been an intense interest in the ameliorative effects on behavior of the MSH/ACTH fragments and the endorphins. The following is a selective review of current findings.

4.1.1. *Mentally Retarded Patients*

Among the most dramatic effects of MSH/ACTH fragments have been those on the behavior of mentally retarded individuals. To date, three studies have been completed. In the first study (Sandman *et al.*, 1976), 20 mentally retarded men were injected with 15 mg of MSH/ACTH 4–10 and then given tests similar to those administered to normal volunteers. Treatment with the peptide resulted in significant deceleration of heart rate, (an index of the orienting response) to novel stimulation. Essentially, the peptide 'produced' an orienting response since the control subjects gave no evidence of awareness to a change in their environment. In addition, treatment with the peptide improved learning of intradimensional and extradimensional shifts, visual retention, spatial localization and matching auditory patterns.

In a second experiment (Walker and Sandman, 1979), the influence of an orally administered analog of MSH/ACTH 4–9 was examined in a group of retarded adults. Three doses (0, 5 and 20 mg) were tested. The results indicated that although significant improvement in measures of attention were observed, the effects were not as dramatic or pervasive as in the initial study. A number of factors may account for the attenuated effects including reduced potency of the analog, route of administration, etc.; however, a subsequent study suggested that the choice of doses may have been unfortunate.

In the third study four doses (0, 5, 10 and 20 mg) of the ACTH 4–9 analog were examined in retarded clients while they performed their day-to-day activities (Sandman *et al.*, 1980). The clients were paid a wage to bend electrical leads to fit a mold. There were four steps in the process which varied in difficulty from the bending of resistors to quality control inspection. During the course of the study the clients performed the same task each day. The peptides were administered in the morning every day for two weeks. Placebo weeks preceeded and succeeded the treatment weeks. Observations of productivity and of social behavior were done at regular intervals during the morning hours.

The influence of the peptide on productivity is illustrated in Fig. 14. Clearly, the dose of the peptide interacted with the difficulty of the task to produce distinctive curves. The high dose, 20 mg, interfered with productivity in each step. Five mg had mixed effects, enhancing performance only for the more complex tasks. Ten mg improved productivity in all but the first step.

These data provided indirect support for the hypothesis that the MSH/ACTH 4–9

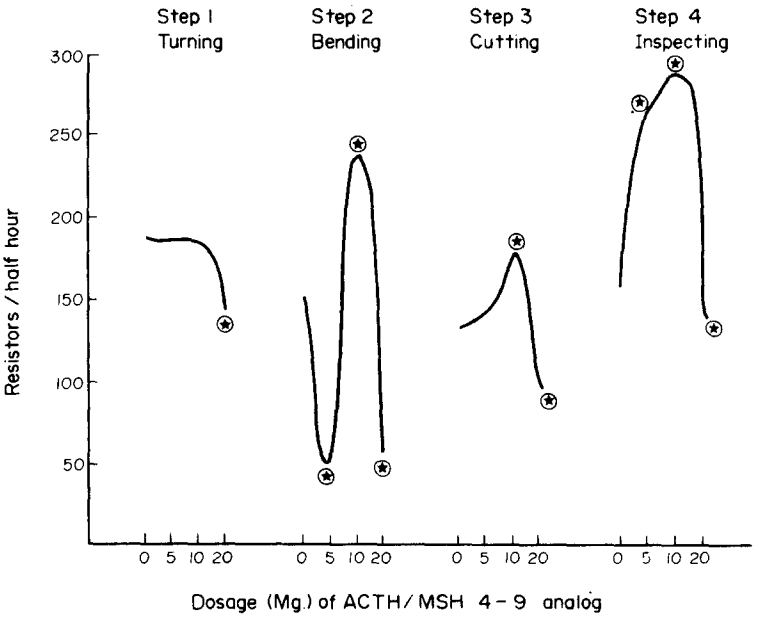

FIG. 14. Influence of oral doses of an analog of MSH/ACTH 4–9 on productivity of mentally retarded patients in a sheltered workshop.

analog exerted an influence on perceptual/attentional mechanisms. Since performance was disrupted by extraneous movement, lack of coordination or inattention to detail, these data may be construed as ecological validation that MSH/ACTH 4–9 influenced attention.

Other data collected in this study indicated that the peptide might also influence social behavior in a dose dependent way. The evidence suggested that patient–patient and patient–supervisor contact increased during treatment, especially with 10 and 20 mg. Self-stimulation also increased during treatment with the peptide. These data are in agreement with the reports of Beckwith *et al.* (1977a), in which rats increased contact time after injections with MSH.

A curious pattern emerged from this study. Increased productivity coupled with greater interpersonal awareness and self-stimulation was evident. Apparently, when the clients worked after treatment with the peptide, they did so with greater concentration and intensity. These data can be reconciled with earlier findings that normal and retarded subjects evidenced enhanced orienting responses to novel stimuli and also retained the ability to discriminate relevant from irrelevant information after treatment with MSH/ACTH fragments.

2. *Elderly Patients*

There is marginal evidence that MSH/ACTH fragments ameliorate the behavior of elderly subjects (Ferris *et al.*, 1976; Will *et al.*, 1978; Branconnier *et al.*, 1979; Miller *et al.*, 1980). Among these studies the study of Branconnier *et al.* is especially compelling. Eighteen mildly senile, organically impaired subjects, displayed reduced depression and confusion and increased vigor after treatment with MSH/ACTH 4–10. In addition, and consistent with Gaillard and Varey's (1979) report, the peptide delayed fatigue associated with a reaction time task. Further, the peptide produced a shift in the EEG to lower frequencies (3.5–4.5 Hz and 7.5–9.0 Hz). Although the authors indicated that these data are in partial disagreement with Miller *et al.* (1974), they note that these effects were a function of responses in women and were also related to order of treatment. The authors suggested that the effects observed were evidence of a non-specific arousing effect even though the changes in EEG are not in accord with such reasoning. More recently, Miller *et al.* (1980), reported improvement in visual retention after receiving MSH/ACTH 4–10 in the elderly. The effect was greater in men than in women.

4.2. Endorphins

The initial speculation that β-endorphin may underly schizophrenia was based upon two reports in rats (Bloom *et al.*, 1976; Jacquet and Marks, 1976). These studies described 'waxy flexibility' and rigidity in rats after large doses of centrally administered β-endorphin. Even though similar observations were made in both studies, the authors offered very different conclusions. Bloom *et al.*, suggested that the 'catatonic-like' states observed in rats and paralleled in psychiatric patients may result from excessive amounts of endorphin. Thus, the 'treatment' for schizophrenia may be an anti-endorphin. Conversely, Jacquet and Marks suggested that β-endorphin itself may be an antipsychotogen. The literature with clinical groups reflects this confusing dilemma as there are studies using either naloxone or endorphin to treat psychotic states.

Perhaps the first issue to be resolved was whether or not endorphin levels are altered in schizophrenia. At least two studies (Domschke *et al.*, 1979; Lindstrom *et al.*, 1978) reported significantly elevated basal levels of β-endorphin in the CFS compared to control subjects. However, Domschke *et al.*, found that the elevation in β-endorphin was only significant for acute schizophrenic states and that chronic states were associated with significantly lower levels. Conversely, Terenius *et al.* (1976) reported that CSF endorphins are elevated in chronic schizophrenia. It appears that both elevated and

depressed levels of endorphin may reflect psychological disorders and careful diagnosis is a prerequisite for effective treatment.

Approaches testing the possibility that schizophrenia may involve an excess of endorphin have used opiate antagonists such as naloxone as treatment. The results are equivocal; some (Gunne *et al.*, 1977) reporting improvement in schizophrenia and others (Janowsky *et al.*, 1977; Volavka *et al.*, 1977) reporting no effect. However, all studies used mixed diagnosis and included both acute and chronic schizophrenics without separate analyses. Perhaps further controlled tests are required before definitive conclusions can be reached.

The hypothesis that endorphin deficiency may underlie schizophrenia also presents a confusing picture. The initial study of Kline *et al.* (1977), suggested that symptoms of schizophrenia and depression were ameliorated by β-endorphin injections. More recently, Verhoeven *et al.* (1978), reported a dramatic improvement in psychotic symptoms after treatment with an endorphin analog (des-Tyr)-γ-endorphin. Apparently this molecule has no opiate activity and produces no side effects. The possible benefits of endorphin administration await further research.

Although the role of endorphins in the modulation of behavior appears significant, there is an absence of controlled studies in human subjects. Acceptance of the initial and somewhat tenuous assumption that the endorphins form the biochemical basis of schizophrenia may have misguided research efforts. The results of studies in animals suggests that examination of the influence of endorphin on learning and perception will generate more definitive information concerning the physiological significance of these interesting peptides.

5. THEORETICAL ANALYSIS

There are two major conclusions concerning the LPH molecule to be derived from this review. The first conclusion is that peptides of the LPH chains modulate attentional/perceptual functions. The second related conclusion is that there may be a reciprocal relationship among fragments of the LPH chain.

5.1. ATTENTION/PERCEPTION

Most of the behavioral influences described for MSH/ACTH and endorphin can be viewed as a modulation of attentional/perceptual function. A proposed model for understanding the influence of LPH fragments on attention is presented in Fig. 15. Although the model suggests that MSH/ACTH fragments improve, and the endorphins impair selective attention, the model should be viewed as heuristic. The evidence reviewed above clearly implicated MSH/ACTH fragments as modulators of the component of selective attention described in the model. To date, the results indicate that MSH/ACTH molecules raise perceptual threshold to act as a filter and appear to increase the probability of detecting stimuli which are interesting and important. However, there are data which do not fit neatly into this framework. The findings of Rigter *et al.* (1975; 1976), for instance, do not adhere to this model. From the model it could be argued that enhanced short term memory is a secondary result of improved selective attention. Thus, only elements which pass the scrutiny of the attentional filter can enter short or long term memory. However, it could also be argued that an initial perceptual apparatus is essential for activation of the attentional mechanism.

As reviewed, the majority of the research of the effects of C-terminal peptides in the LPH chain (the endorphins) have pursued the possibility that these molecules underlie pathological states. Distressingly few studies have examined the behavioral significance of the endorphins. The few studies which do exist provide little consistency. Some studies suggest that the endorphins exert identical effects as the MSH/ACTH fragments while others suggest these molecules have different effects. The developmental study of the behavioral effects of the endorphins which measured a large number of variables indi-

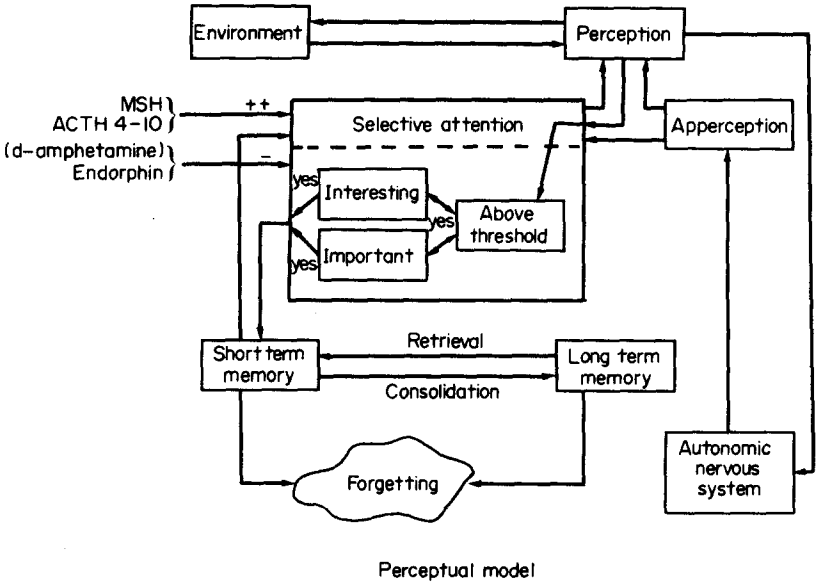

FIG. 15. Perceptual/attentional model of the influences of MSH/ACTH fragments and endorphin. The MSH/ACTH fragments improve elements of attention, whereas the endorphins appear to impair perceptual sensitivity.

cated an overall depression of the organism's reseponse to the environment (McGivern *et al.*, 1979). Further, the pervasive finding that endorphins induce analgesia is quite consistent with this possibility. Thus, for heuristic purposes, it may be useful to consider the possibility that the endorphins and MSH fragments may have opposite effects on perceptual/attentional modulation.

5.2. FUNCTIONAL RECIPROCITY

Although there is evidence that the opiate and MSH parts of the LPH chain both enhance learning, a compelling case can be made that a functional reciprocity exists between the MSH/ACTH fragments and endorphins. Of central interest is the finding that MSH/ACTH and the endorphins are stored and released by the anterior hypothalamus. Further, the provocative findings of O'Donohue *et al.* (1979) and of Watson (in press) that both MSH and β-endorphin are present in the *same* cells of the dorsal thalamus underscores the probability of a functional relationship between these molecules.

Among the most direct evidence of reciprocity is the attenuation of opioid induced analgesia by MSH/ACTH fragments (Krivoy *et al.*, 1977). Szekely *et al.* (1979) reported that α-MSH administered concommitantly with morphine attenuates tolerance and dependence on morphine. The analgesic potency of opioids have related to decreased levels of cyclic AMP in the brain. Thus, Gispen *et al.* (1977) reasoned that the effects of MSH/ACTH fragments on opioid induced analgesia may be mediated by adenylate cyclase activity. Indeed, increases in cyclic AMP in the diencephalon and mesencephalon (Gispen *et al.*, 1977) and in the cortex (Christensen *et al.*, 1976) are reported after treatment with MSH/ACTH fragments. Therefore, it is conceivable that the attenuation of analgesia by MSH/ACTH may relate to the reciprocal influence of MSH/ACTH and endorphin on cyclic AMP activity.

Recent evidence from our laboratory indicates further reciprocity between these two parts of β-LPH. Ten μg of β-endorphin injected into the lateral ventricle of rats produces a marked attenuation of the P1 component of the auditory evoked potential. Subsequent injections of 50 μg of MSH/ACTH 4–10 not only reverses the attenuation but may even enhance the P1 component.

In a related study (preliminary findings) β-endorphin (10 μg) injected into the periaque-

ductal gray (PAG) of rats produced reliable and long lasting (2 hr) 'eliptogenic' spiking in the area of the nucleus gigantocellularis. Subsequent injections of MSH/ACTH 4–10 (20 μg) or of naloxone into the PAG immediately blocked the spiking. The ameliorative effect of MSH/ACTH 4–10 persisted for up to 30 min before the spiking returned.

Inferences drawn from the behavioral evidence also support the hypothesis of a reciprocal relationship between the two parts of LPH. For instance, MSH/ACTH 4–10 antagonizes the morphine induced behavioral arousal of the mouse (Katz, 1979). Increases in social behavior have been reported for the MSH/ACTH fragments (Beckwith *et al.*, 1977; Sandman *et al.*, 1980) but treatment with opioids results in decreased social contact (Panksepp *et al.*, 1978; McGivern *et al.*, 1979). The MSH/ACTH fragments have reliably resulted in enhanced reversal learning (Sandman *et al.*, 1972; 1973; Beckwith *et al.*, 1977) but rats treated *in utero* with β-endorphin evidence a significant deficit in reversal learning (McGivern *et al.*, 1979).

Thus, although there are contraindications in the literature, an emerging array of evidence supports the possibility that the opioid and MSH/ACTH peptides share a reciprocal and modulating influence on the brain and behavior. Further, as illustrated in Fig. 15, the nature of the reciprocity may be viewed as a modulation of attentional/perceptual processes.

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