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Cholecystokinin Accelerates the Rate of Habituation to a Novel Environment

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CRAWLEY, J. N. *Cholecystokinin accelerates the rate of habituation to a novel environment.* PHARMACOL BIOCHEM BEHAV **20**(1) 23–27, 1984.—Cholecystokinin (CCK_8), an octapeptide present in high concentrations in both gut and brain, has been proposed as a putative satiety signal in a variety of species. Exploratory and social behaviors in particular are inhibited by exogenously administered CCK_8. One hypothesis for the mechanism by which CCK reduces feeding and exploration is that CCK_8 is reducing arousal and attention to environmental stimuli. This possibility was tested by analyzing the rate of habituation to the novelty of objects placed in an unfamiliar arena. A video-tracking computer-assisted animal behavior monitor measured four parameters of exploratory behaviors in rats pretreated with intraperitoneal CCK_8. During the first 30 minutes in the novel environment, CCK_8-treated animals showed a reduced latency to cessation of exploration as compared to saline controls. In repeated five minute sessions on consecutive days, CCK_8 treatment accelerated the decline in exploration over daily sessions. Pentobarbital, a known sedative, induced low levels of exploration throughout the repeated daily sessions. These data suggest that CCK_8 is inducing a more rapid rate of habituation to the novelty of environmental stimuli. An accelerated rate of habituation might underlie some of the satiety-related behavioral effects of this peptide.

Cholecystokinin Habituation Exploration

CHOLECYSTOKININ-sulfated-octapeptide (CCK) inhibits total food intake in fasted rats, mice, sheep, pigs, monkeys, and humans [4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. CCK has been proposed as a signal for satiety, initiating the complete behavioral sequence of satiety in rats [1]. We have shown that CCK induces a constellation of behavioral changes which are consistent with satiety as an underlying behavioral state [2, 3, 4, 5]. These include reduction in exploration, social interactions, and investigation of novel objects. The most characteristic behavioral pattern is the appearance of long pauses of behavioral inactivity. These pauses are not associated with sleep episodes, as EEG recordings are normal [5]. No motor dysfunctions accompany these pauses, as locomotor activity and co-ordination are normal once movements are initiated [3,4]. Rather, the animals appear to be either (A) behaviorally sedated, or (B) more quickly habituated to the novel enviornmental stimuli which normally elicit exploratory behaviors. If the sedation hypothesis is correct, then CCK-treated animals will show consistently lower exploratory tendencies throughout an experiment. If the habituation hypothesis is correct, then exploration should start at a normal level and decline over time, following the same pattern as control animals, but with a more rapid time course. The predicted curves for these two models are illustrated in Fig. 1.

The present study was undertaken to address the alternative hypotheses of general behavioral sedation versus an accelerated rate of habituation. Two paradigms were employed. The time course to cessation of exploration in a single 30-minute session in a novel environment was compared in rats treated with saline or CCK 1, 2.5, or 5 μg/kg. Also, the rate of decline of exploratory parameters was compared over consecutive daily 5-minute sessions in the novel enviornment in rats treated with saline, CCK 2.5 μg/kg, CCK 5 μg/kg, or pentobarbital, a standard sedative. Exploratory parameters were analyzed by a video-tracking computer-assisted animal behavior monitor [6]. The results of these two studies support the habituation rather than the sedation hypothesis as the more likely explanation for the ability of CCK to reduce spontaneous behaviors.

METHOD

Male Sprague-Dawley rats, 250–300 g, housed in a temperature and humidity controlled enviornment on a 6 a.m.-6 p.m. lighting schedule, with food and water constantly available, were divided into groups of ten rats each. CCK_8-sulfate (Bachem, Torrance, CA) was intraperitoneally administered immediately (less than one minute) before testing. Latency to the first period of inactivity of at least five minutes duration was recorded for each rat pretreated with either saline, CCK 1, 2.5 or 5 μg/kg IP. In the second series of experiments, saline, CCK 2.5 or CCK 5 μg/kg IP was administered immediately before testing on five consecutive days.

Behavioral testing over a five minute session employed a video-tracking computer-assisted animal behavior monitor

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(SRI International, Menlo Park, CA). As previously described [6], the color channels in the videocamera follow the colored tape placed on the back of the animal. The location of the animal is converted to an X-Y co-ordinate which is sampled ten times per second. A VAX computer program then analyses the sequential location of the animal to yield parameters including number, duration, and variability within pauses, proximity to the corners and edges of the open field arena, number, duration, distance, and linearity of movements, and frequency, duration, and proximity of approaches to a labeled object.

In the present experiment, each test animal was placed in a 60×60 cm arena lined with blotter paper, containing a color-labeled 7×7×11 cm wire mesh box. In the first experiment, each animal was allowed to remain in the arena for 30 minutes. Latency to the first period of inactivity of at least 5 minutes duration was recorded for each rat pretreated with either saline, CCK 1, 2.5 or 5 μg/kg IP immediately before testing. In the second experiment, each animal was allowed to remain in the arena for five minutes. Group 1 was administered saline immediately before testing on five consecutive days. Group 2 was administered CCK 2.5 μg/kg IP immediately before testing on five consecutive days. Group 3 was administered CCK 5.0 μg/kg IP immediately before testing on five consecutive days. Group 4 was administered sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL), 25 mg/kg IP; 15 minutes before testing on four consecutive days. To test reversal of habituation, on day five, the wire mesh box was replaced by an open plastic cup, with saline and CCK drug treatment identical to days 1, 2, 3, and 4. Statistical analysis was performed by Analysis of Variance followed by a Newman-Keuls a posteriori test for significant differences of each treatment group on each test day.

RESULTS

Latency to the first pause of at least five minutes duration within a single 30-minute session was reduced in CCK-treated rats (Fig. 2). One-tailed ANOVA for the four treatment groups yielded, $F(3,36)=7.81$, $p<0.01$. Newman-Keuls comparison of group means yielded $p<0.01$ for CCK 2.5 and CCK 5.

CCK 2.5 and 5.0 μg/kg significantly reduced approaches to a novel object, reduced number of movements, reduced total distance traversed, and increased total pause time as compared to saline controls on the first and second day of testing (Figs. 3–6). One-way ANOVA of number of approaches, Day 1, yielded, $F(2,27)=3.69$, $p<0.05$, Newman-Keuls $p<0.05$ for CCK 2.5 and CCK 5; Day 2, $F(2,27)=5.80$, $p<0.01$, Newman-Keuls $p<0.05$ for CCK 2.5 and CCK 5; Day 3, $F(2,27)=2.46$, non-significant; Day 4, $F(2,27)=0.13$, non-significant. Duration of pauses ANOVA for Day 1, $F(2,27)=4.03$, $p<0.05$, Newman-Keuls $p<0.05$ for CCK 2.5 and CCK 5; Day 2, $F(2,27)=5.11$, $p<0.05$, Newman-Keuls $p<0.05$ for CCK 5; Day 3, $F(2,27)=0.28$, non-signficant; Day 4, $F(2,27)=0.04$, nonsignificant. Total number of movements, Day 1, $F(2,27)=5.23$, $p<0.05$, Newman-Keuls $p<0.05$ for CCK 2.5 and CCK 5; Day 2, $F(2,27)=5.88$, $p<0.01$, Newman-Keuls $p<0.05$ for CCK 2.5 and CCK 5; Day 3, $F(2,27)=0.03$, non-signficiant; Day 4, $F(2,27)=0.01$ non-significant. Total distance traversed Day 1, $F(2,27)=6.17$, $p<0.01$, Newman-Keuls $p<0.05$ for CCK 2.5 and CCK 5; Day 2, $F(2,27)=5.71$, $p<0.01$, Newman-Keuls $p<0.05$ for CCK 5; Day 3, $F(2,27)=0.09$, non-significant; Day 4, $F(2,27)=0.01$, non-significant. Separate statistical analysis of exploratory parameters after pentobarbital treatment across the four test days yielded no significant effect of repeated daily testing on response to pentobarbital.

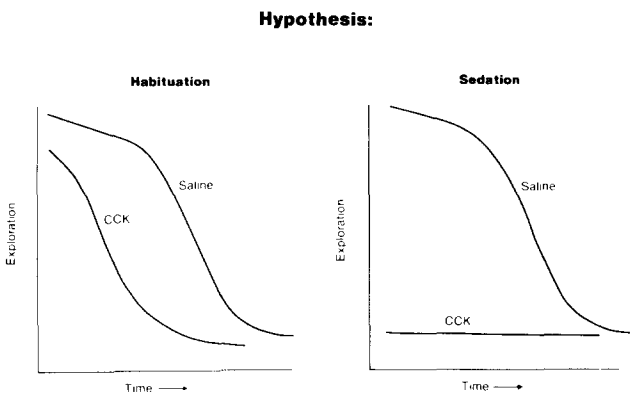

FIG. 1. Alternate hypotheses for the role of cholecystokinin (CCK) in reducing spontaneous exploratory behaviors. A gradual reduction in exploration, at a rate exceeding control values, would support the explanation of an accelerated rate of habituation. A constant low level of exploration would support the explanation of a generalized state of behavioral sedation.

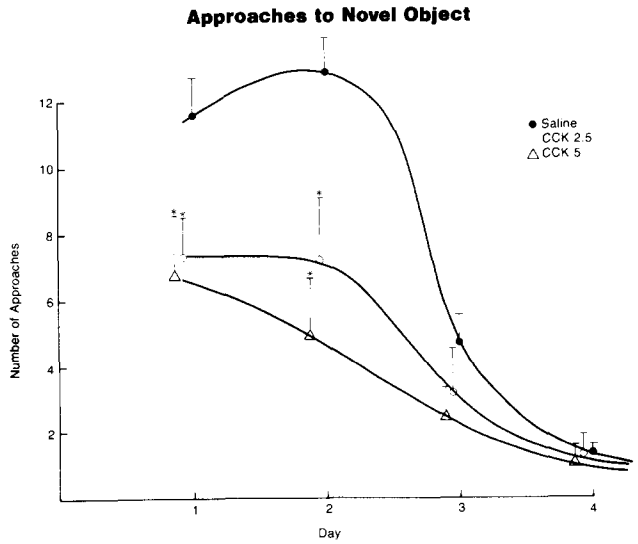

FIG. 2. Latency to cessation of exploration was defined as the number of minutes until the rat paused for at least five minutes. Rats were pretreated with saline, CCK 1 μg/kg, CCK 2.5 μg/kg, or CCK 5 μg/kg IP; immediately before placement in the arena environment, as in Figs. 3–6, but for a single 30 minute session.

Linearity within moves and grooming movements within pauses (Table 1) yielded no statistically significant differences between saline, CCK 2.5, and CCK 5 within any testing day.

Replacement of the wire mesh object with a plastic cup on Day 5 increased exploratory parameters in all three treatment groups (Fig. 7). One-tailed paired t-test statistics yielded a decrease in total pause time on Day 5 as compared

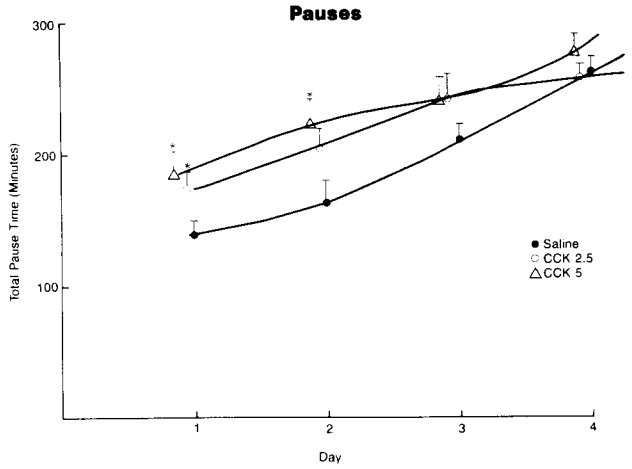

FIG. 3. Number of approaches to a novel object was defined as the total number of physical contacts, of at least 3 second duration, with a wire mesh box placed in the center of a 60×60 cm open field. Rats were pretreated with intraperitoneal saline, CCK 2.5 μg/kg, or CCK 5.0 μg/kg, immediately before, or sodium pentobarbital, 25 mg/kg, 15 minutes before the five-minute test session in the novel arena environment, on four consecutive days. N=10 for each treatment group, $*p<0.05$.

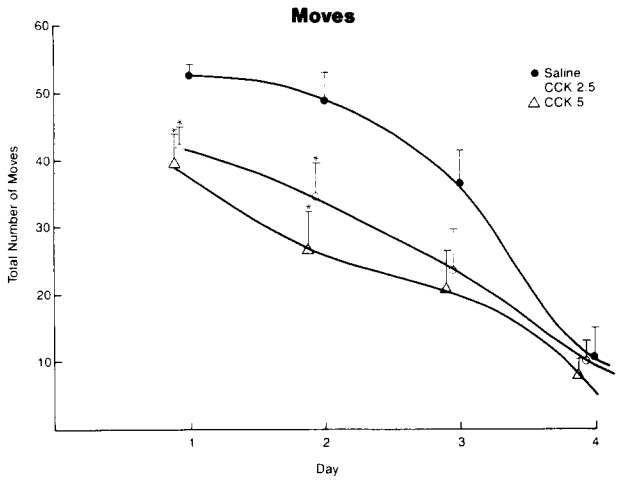

FIG. 4. Total time spent in pauses was defined as the cumulative number of seconds in which the rat did not move outside an area of 5 cm radius for a minimum of 3 seconds. Rats were pretreated with saline, CCK 2.5 μg/kg, CCK 5 μg/kg, or pentobarbital 25 mg/kg, as in Fig. 2. N=10 for each treatment group, $*p<0.05$.

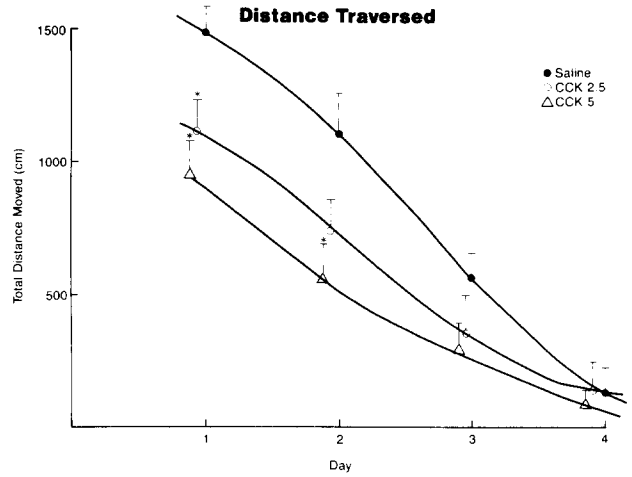

FIG. 5. Total number of moves was defined as the number of times the rat initiated a movement of greater than 5 cm distance. Rats were pretreated with saline, CCK 2.5 μg/kg, CCK 5 μg/kg, or pentobarbital 25 mg/kg IP, as in Fig. 2. N=10 for each treatment group, $*p<0.05$.

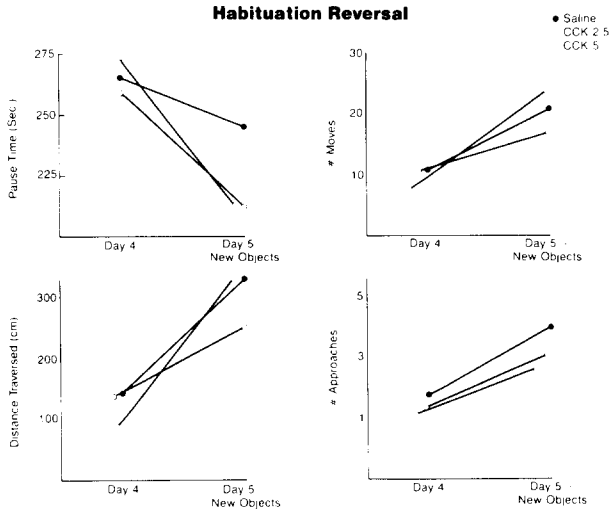

FIG. 6. Total distance traversed was defined as total cumulative cm moved in the five-minute test session. Rats were pretreated with saline, CCK 2.5 μg/kg, CCK 5 μg/kg, or pentobarbital 25 mg/kg, IP, as in Fig. 2. N=10 for each treatment group, $*p<0.05$.

to Day 4 in saline controls, $t(9)=2.58$, $p<0.05$; in the CCK 2.5 group, $t(9)=3.64$, $p<0.05$; and in the CCK 5 group, $t(9)=3.95$, $p<0.05$. Total number of movements increased in all groups, saline, $t(9)=4.13$, $p<0.05$; CCK 2.5, $t(9)=2.48$, $p<0.05$; CCK 5 $t(9)=3.52$, $p<0.05$. Total distance traversed increased in all groups, saline $t(9)=4.81$, $p<0.05$; CCK 2.5, $t(9)=2.33$, $p<0.05$, CCK 5.0, $t(9)=3.21$, $p<0.05$. Number of approaches to the novel object increased in all groups, saline, $t(9)=3.88$, $p<0.05$; CCK 2.5, $t(9)=2.95$, $p<0.05$; CCK 5, $t(9)=3.26$, $p<0.05$.

DISCUSSION

The observed gradual decline in exploratory parameters over repeated daily test sessions in saline controls follows a normal rate of habituation to a novel environment. The data of Figs. 2, 3, 4, 5, and 6 demonstrate that CCK shifts the time course of declining exploration to the left of saline control values. Exploration curves for pentobarbital began at low values on the first day of testing and remained low throughout the four consecutive test days. Trends toward increased

TABLE 1

NORMAL PARAMETERS WITHIN MOVES AND PAUSES

	Linearity Within Moves	Movements Within Pauses
Day 1		
Saline	0.83 ± 0.01	1.23 ± 0.2
CCK 2.5	0.82 ± 0.01	1.05 ± 0.1
CCK 5	0.85 ± 0.02	1.12 ± 0.1
Day 2		
Saline	0.81 ± 0.02	1.23 ± 0.1
CCK 2.5	0.81 ± 0.04	1.13 ± 0.1
CCK 5	0.80 ± 0.2	1.16 ± 0.1
Day 3		
Saline	0.76 ± 0.05	0.98 ± 0.1
CCK 2.5	0.78 ± 0.04	0.98 ± 0.1
CCK 5	0.83 ± 0.06	0.88 ± 0.2
Day 4		
Saline	0.82 ± 0.05	0.81 ± 0.2
CCK 2.5	0.71 ± 0.07	0.84 ± 0.1
CCK 5	0.70 ± 0.12	1.03 ± 0.1
Day 5		
Saline	0.73 ± 0.03	1.17 ± 0.1
CCK 2.5	0.76 ± 0.06	0.94 ± 0.1
CCK 5	0.79 ± 0.02	1.21 ± 0.1

Linearity within moves was defined as the deviation from a straight line between two consecutive locations of the rat. This parameter indicates degree of locomotor co-ordination. Treatments were as in Fig. 2. N=10 for each group, no significant differences by one-way ANOVA on any test day. Movements within pauses was defined as movements within the 5 cm radius. A low score on this parameter indicates extreme tonic immobility. A high score on this parameter indicates excessive grooming. Treatments were as in Fig. 2. N=10 for each group, no significant differences by one-way ANOVA on any test day.

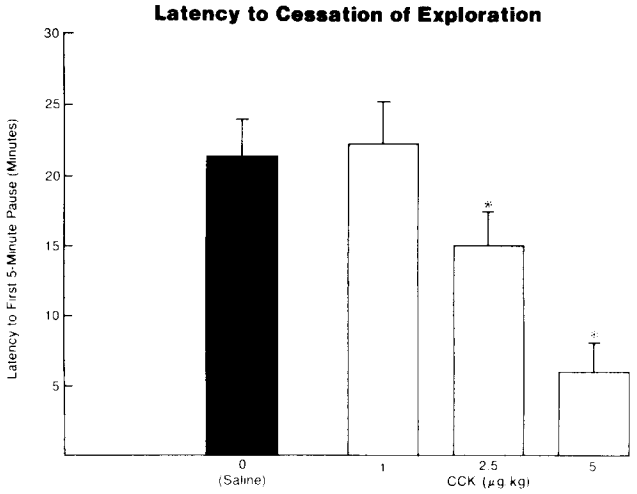

FIG. 7. Habituation reversal was defined as an increase in the four exploratory parameters when the novel object was changed from a wire mesh box to a plastic cup on the fifth consecutive day of testing for 5 minutes in the arena environment. All parameters were significantly changed in all treatment groups on Day 5 at the $p<0.05$ level by one-tailed paired t-test statistical analysis.

exploration over time with this standard sedative are contrary to the CCK effect, and may reflect developing tolerance to repeated pentobarbital treatments. These results suggest that CCK is accelerating the rate of habituation to the novelty of the arena environment. The data of Day 5, showing that replacement of a familiar object by a new object increases exploration in both saline and CCK-treated rats, indicates that CCK-treated animals can respond to a novelty challenge as well as saline-treated animals. These results support the first hypothesis illustrated in Fig. 1. CCK does not appear to induce a sustained behavioral sedation. Rather, the general exploratory tendencies of rats and mice appear to be reduced, [1, 2, 4] allowing a more rapid rate of habituation to a novel environment.

These data do not address the issue of possible aversive internal sensations induced by intraperitoneal injections of CCK. The doses used in this study were within the range which inhibits total food consumption in fasted rats. Therefore, an accelerated rate of habituation might underlie some of the satiety-related behavioral effects of CCK. Alternatively, unpleasant gastrointestinal sensations could reduce an animal's exploratory tendencies in a novel environment. Experiments are in progress to compare the effects of known aversive stimuli to CCK on the exploratory parameters employed in our system. Although the distinction between feelings of satiation and feelings of aversion remains controversial with respect to CCK, the present study serves to eliminate the possibility that CCK is inhibiting spontaneous appetitive behaviors through a non-specific behavioral sedation.

It is interesting to speculate on the causes and consequences of an accelerated rate of habituation to novelty after treatment with a peptide such as CCK. One mechanism involved may be a reduced level of arousal, or of attention to enviornmental stimuli. Several hypophyseal peptides, such as ACTH, vasopressin, oxytocin, and α-melanocyte stimulating hormone, have been implicated in learning and memory processes. CCK might similarly induce changes in rate of learning or extinction, based on its ability to alter some substrate of the habituation process.

In conclusion the present study demonstrates that CCK induces a gradual decrease in exploratory behavior whose rate of decline is greater than the gradual decline seen in saline-treated controls. This finding supports a role for CCK in accelerated habituation to novelty rather than a more global state of behavioral sedation.

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