Stress, Behavioral Arousal, and Open Field Activity—A Reexamination of Emotionality in the Rat

KEVIN A. ROTH

School of Medicine, Stanford University, Palo Alto, CA

AND

RICHARD J. KATZ[1]

*Mental Health Research Institute, Department of Psychiatry
University of Michigan Medical Center, Ann Arbor, MI 48109*

ROTH, K. A. AND R. J. KATZ. *Stress, behavioral arousal, and open field activity–A reexamination of emotionality in the rat.* NEUROSCI. BIOBEHAV. REV. 3(4) 247–263, 1979.—The effects of stress upon emotionality, and of emotionality upon the open field activity of rats have now been studied for over four decades. Controversy remains however regarding the degree to which stress alters behavior, and the direction of that change. One reason for this is the absence of an adequate behavioral definition of stress. The present series of experiments demonstrates a standard relatively nontraumatic stress induction procedure which may be used in conjunction with open field testing. Pre-exposure to moderately intense light and white noise facilitated open field activity as measured by initial activity, lowered defecation scores, and supplementary measures (rearing, grooming, center field penetration). Further parametric, psychoendocrine, and pharmacological studies characterized the nature of the facilitation, its time course, and its modification by other manipulations. Our results suggest the initial behavioral response to stress in an open field is activation. Previous studies may have differed in their results relating stress and behavior because of subtle procedural distinctions, some of which may be identified using the present technique.

Activity   Adrenalectomy   Ambulation   Behavioral arousal   Corticosteroids   Defecation
Endorphins   Grooming   Hypophysectomy   Hypothalamic-pituitary-adrenal axis   Naltrexone
Opiates   Open field   Psychoendocrine   Rearing   Stress

OPEN field activity remains the primary unconditioned indicator of emotionality in laboratory investigations of a variety of species. The test involves single or repeated short duration placements into a novel environment of limited complexity [7, 14, 28, 29, 60]. Defecation and various parameters of motor activity have been taken as the major indices of emotional reactivity, with rearing and grooming, and occasionally urination serving as supplementary measures of one or more underlying emotional states. The reliability and validity of defecation and initial degree of ambulation are well established [1, 7, 32, 33] and additionally supported by extensive factor analytic studies [15,51]. These studies have also identified possible determinants of open field behaviors in addition to emotionality.

The simplicity and economy of the test combined with an increasing appreciation of the ecological validity of its dependent variables [7, 44, 51, 60] render it a valuable laboratory procedure, at least in principle, and the potential exists for standardization across species and testing conditions. Despite the potential utility of the test, however, and an extensive literature now spanning four decades, controversy remains regarding a number of findings upon emotionality, stress, and their relation, as measured using typical open field procedures. While a number of reports suggest stress antecedent to, or concurrent with, open field testing is behaviorally inhibitory, this is far from universally true. Examples of behaviorally inhibitory antecedent manipulations include primarily shock [5,39], fear conditioning [5, 8, 9, 16, 30, 41, 44, 45] and isolation [2, 25, 27, 56]. Concomitant stress related manipulations which decrease activity include the above conditions and also the presence of predators [53], excess noise [14, 18, 22, 60], intense illumination [20,22], or a Pavlovian CS⁺ (conditioned stimulus) for shock [44].

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[1]Correspondence to Dr. Katz at above address.

On the other hand, however, isolation has also been reported to decrease rather than increase emotionality, in a closely related design [61]. Also note [13] for a review of some of the theoretical and empirical issues associated with individual housing in rodents. Shock may not always produce inhibition except at traumatic levels [5]; certain forms of noise stress facilitate behavior in open field related settings [10] and predators may have no effect upon open field activity or prey [17].

One possible cause of much discrepancy may be a lack of procedural standardization (see below). In the case of isolation stress, for example, Goldsmith et al. [25] have demonstrated that changes in both behavior and pituitary-adrenal activity are dependent upon time course, with the only evidence of a possible stress effect occurring after 6 months of isolation. Species differences may also account for some degree of variability [7]. A third, and perhaps equally important reason is that stress, while well defined as an endocrine and physiological state (i.e., as supranormal hypothalamic-pituitary-adrenal (HPA) activity, e.g., [55], is at best poorly defined behaviorally). Stress has alternately been equated by different researchers both with emotionality (i.e., a profile of increased defecation and freezing in the open field), or with generalized arousal [7,57]; however, both descriptions have been made with only limited controlled observation. Finally, and closely related to the latter, the evaluation of the acute behavioral effects of stress has often of necessity utilized animals that are debilitated physically.

The present set of experiments was designed with several related aims. At the outset we asked if open field activity could provide a preliminary behavioral definition of one specific aspect of stress. In asking this we utilized a stress procedure which was relatively nondebilitating and non-traumatic in comparison with other stressors such as cold swim, shaker stress, or shock. This was done to minimize confounding exhaustion, trauma, debilitation, or ataxia with observed differences in behavioral performance. A second reason for the utilization of a single nontraumatic stress was to allow a more adequate and factorial study of one particular stressor with potentially wider applicability. Not all stressors are equivalent in their actions or course (e.g. [43]). Given a behavioral definition of acute stress in the open field we further investigated the temporal parameters of the response, and its psychoendocrine and psychopharmacological concomitants. The series of seven experiments, then, offers an overview of one form of stress as both a psychoendocrine and a behavioral response.

GENERAL METHOD

Subjects

A total of 235 adult male Sprague-Dawley rats (Charles River Farms, Portage, MI) 70–80 days old at the start of testing were maintained on ad lib food (Teklad 4.0% fat rodent diet S-0836; Madison, WI) and tap water, and automatically programmed 12 hr/12 hr lighting cycles (lights on from 07:00 to 19:00 hr). Subjects were housed 2 rats/cage in $25 \times 18 \times 17$ cm stainless steel cages.

Apparatus

Testing was carried out in a square white Plexiglas open field each side of which was 1.22 m and the height of which was 45 cm. The apparatus floor was divided into 16 equal squares 30.5 cm/side to allow the assessment of locomotion. The apparatus was cleaned thoroughly between tests by repeated washing with tap water, followed by sponging until dry.

Behavioral Procedure

The subjects were habituated to the presence of an experimenter and typical noises associated with the experiment during the week preceding testing. On three or more occasions one or two laboratory personnel entered the room in which the subjects were housed to perform routine tasks at about the same period in the lighting cycle in which the experiment would later be run. Since security personnel also periodically checked the housing corridor the eventual presence of an experimenter upon the test night was not a novel stimulus for the animals.

All testing began at 21:00 hr (i.e., 2 hr after the onset of the dark cycle). Both subjects were removed from their cage at the same time and placed in $48 \times 27 \times 20$ cm polypropylene cages (Scientific Products series 140) for individual transport. The control (i.e., unstressed) rat was transported approximately six m down an unlit corridor to the test room and immediately (i.e., less than 30 sec) tested. The experimental (stressed) animal was transported a similar distance to a brightly lit room. Lighting was provided by eight 70 W fluorescent lights. The transport cage was set 1.0 m from a speaker which emitted 95 dB white noise. After a 1 hr exposure to the noise-light stress the experimental animal was transported and tested in a manner otherwise identical to its control.

For all subjects the test room was illuminated by six overhead mounted GE F96712R fluorescent lights which provided dim (500 mphot) red (=600–700 nm) illumination which was subliminal for the subjects but allowed experimental observation. A background noise of 20–30 dB was provided by the air circulation system. Subjects were placed in a corner of the open field facing the apparatus wall, and a timer was started.

Depending upon individual experiments subjects were allowed six or twelve minutes prior to removal, with 3 min recording intervals utilized throughout for selected meausres. Each subject was tested once. In order to assure maximal comparability both across experiments and with previously published experiments which generally utilized short exposure periods, data from the initial 3 min interval were used as the primary behavioral indicators for a number of measures. The following measures were obtained: outside squares crossed, inside squares crossed, rearing. Also, latency to leave home square, time spent grooming, and a defecation score based upon boluses/session were recorded for the test interval. Finally, a summary measure of activation, consisting of an algebraic sum of ranks across the above categories was computed to allow an overall comparison of experimental manipulations. For n groups, groups were rank ordered from least (1) to greatest (n) for each category, and an average taken of all ranks across categories. Categories were all ranked numerically except latency and defecation, which were ranked by reciprocals of actual values. It should be noted that this ranking procedure allowed relative comparison within experiments, but did not permit comparison across experiments.

All data are presented as means and standard errors, unless otherwise stated. Analysis was by t-tests and analyses of
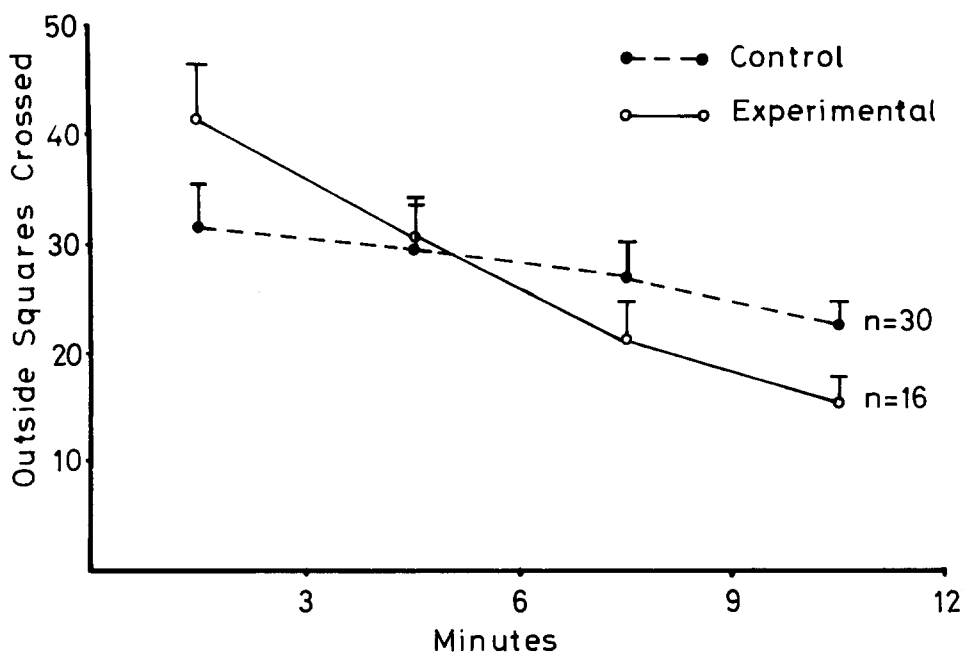
FIG. 1. Stress induced behavioral activation (outside squares). All data as mean and standard error. Experimental subjects were exposed to a noise-light stress for one hour prior to exposure to the open field.

variance using randomized designs and repeated measures designs. All further post hoc comparisons utilized Sheffe-allowances for contrasts. Non-parametric techniques were used to supplement these primary measures when the assumptions necessary for the use of the former were not satisfied. For all experiments three measures most typically employed in other designs (motor activity, latency to move, and defecation score) are presented in graphic form. To simplify the presentation of all additional data, tabular format is consistently utilized throughout.

EXPERIMENT 1

The first experiment was designed to assess the effects of the stress procedure upon the dependent behavioral variables, i.e., to offer a preliminary behavioral definition of the particular stress procedure using the open field.

Subjects and Apparatus

Forty-six rats identical to those described were randomly divided into experimental (stressed; n=16) and control (basal; n=30) groups, and tested using the standard procedure for a 12 min recording interval. In addition, and to further verify the effectiveness of the stress procedure ten more animals (five for each group) were subjected to identical procedures, but sacrificed by decapitation without the behavioral test. Trunk blood was collected in heparinized containers and centrifuged at 2500 RPM for 20 min. Plasma was removed and frozen at −30°C for later determination of corticosterone by competitive protein binding assay using the method of Murphy [59].

RESULTS

The stress procedure produced an initial state of behav-

ioral arousal, as seen on psychomotor measures, and a reduction in emotionality as seen in a lowered defecation score. Between group comparisons for all measures are presented in Figs. 1 to 3. The effects of stress upon motor activity in outside squares was not significant, F(1,34)=1.9; $p>0.05$, although both a time effect, F(3,144)=7.2; $p<0.001$, and interaction effect, F(3,144)=2.8; $p<0.05$, were significant. A close examination of Fig. 1 indicates the significant interaction of groups and time consisted of initially increased activity in the experimental group ($p<0.05$ Sheffe post hoc analysis) which underwent a rapid decline to a level lower than control over the course of observation. Moreover, initial latency to leave the home square was reduced in the stressed animals although not significantly, Fig. 2; $t(44)=1.0$; $p<0.05$, and defecation was significantly reduced in the stressed group (Fig. 3; $t=3.2$; $p<0.01$ df as above). The remaining scores are presented in Table 1. It is evident that stress did not produce any remarkable changes in rearing. This is confirmed by an absence of significant groups, time or interaction effects (F ratios=1.6, 2.0, 1.3 df as above $p<0.05$). There however was a significant stress induced elevation in inside squares crossed after stress, $t(44)=2.1$; $p<0.05$. Moreover, grooming was significantly elevated by stress ($t=3.1$). Finally, an overall comparison of relative ranks indicates a significant behavioral elevation across categories in the stressed group ($p<0.05$ by randomization test). As an indication of the effectiveness of the stress procedure on a physiological measure, it may be seen that plasma corticosterone was significantly elevated by the experimental manipulation, $t(8)=6.5$, $p<0.001$.

DISCUSSION

The overall profile of animals subjected to stress was of initial activation. The stressed animals appeared less
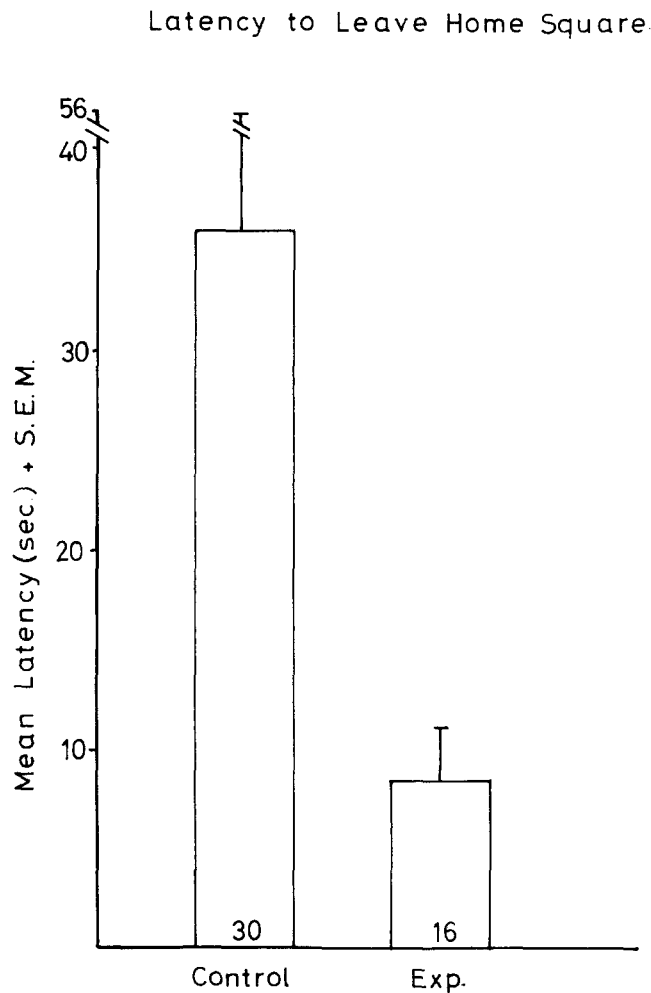
Latency to Leave Home Square

FIG. 2. Stress effects upon initial movement latency. All data as mean and standard error. Experimental subjects were exposed to a noise-light stress for one hour prior to testing in the open field.
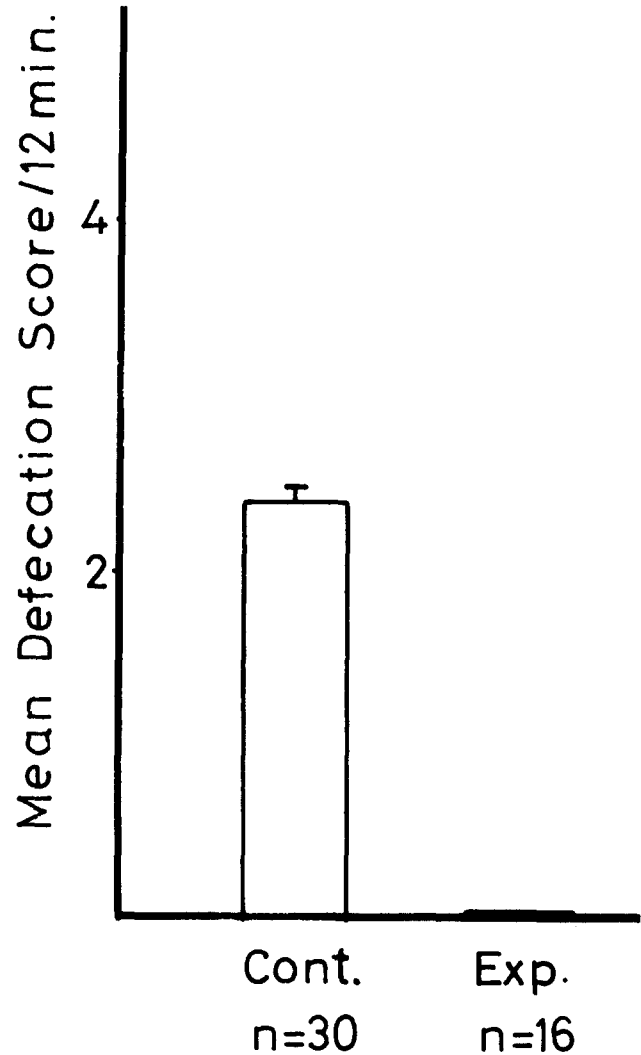

FIG. 3. Stress effects upon defecation. All data as mean and standard error. Experimental subjects were exposed to a noise-light stress for one hour prior to testing in the open field.

emotional as defined by several open field measures including activity, grooming, latency, and center field penetration (e.g. [1, 2, 14, 15, 25, 28, 29]). The stressed animals defecated less in the open field and were initially more active, although the activation response was relatively short and terminal activity levels were in fact lower than normal. It should be noted that the stress procedure did not eliminate the ability to defecate, since experimental rats often defecated just prior to and after placement in the open field while being transported to the apparatus or on being removed from the open field (generally one to two boluses; unpublished observations).

The present results suggest that a mild stress initially is behaviorally activating. They offer several behavioral measures of stress which might prove useful in further psychoendocrine studies. One question which the first experiment raises is whether the observed behavioral activation is a product of antecedent stress, or an effect of removal from a highly stressful environment to a possibly less stressful testing situation. This was further investigated in Experiment 2.

EXPERIMENT 2

We initially observed a behavioral facilitation of open field activity in rats after stress. One interpretation of this rests with the activating effects of stress while a second suggests that removal from a stressful situation produced some degree of relief which then contributed to the observed facilitation effect. By the first interpretation, continued stress through testing should not affect the behavioral outcome. By the second interpretation, testing with continued stressors present should reduce typical experimental activation. We therefore tested animals with and without a continuing stress during the test procedure to examine these two predictions.

METHOD

Subjects and Behavioral Procedure

Ten adult male Sprague-Dawley rats identical in descrip-

TABLE 1

EFFECTS OF ACUTE NOISE STRESS UPON BEHAVIORAL
ACTIVATION IN AN OPEN FIELD (MEAN ± STANDARD ERROR)

Measure	Control score	Experimental score	$p^* =$
Rearing (min 0–3)	9.3 ± 1.0	9.8 ± 1.5	n.s.
Rearing (min 3–6)	8.9 ± 1.0	10.0 ± 1.0	n.s.
Rearing (min 6–9)	9.7 ± 1.0	6.9 ± 1.4	n.s.
Rearing (min 9–12)	8.7 ± 0.8	7.3 ± 1.4	n.s.
Center field penetration	3.9 ± 1.1	8.4 ± 2.0	0.05
Grooming (sec)	17.5 ± 2.5	45.9 ± 11.8	0.01
Mean activation (composite of other scores)	1.0 ± 0.0	2.0 ± 0.0	0.05
Corticosterone (μg/dL)	12.9 ± 5.1	51.9 ± 4.4	0.001

*Probability of across cells difference, statistics in text.
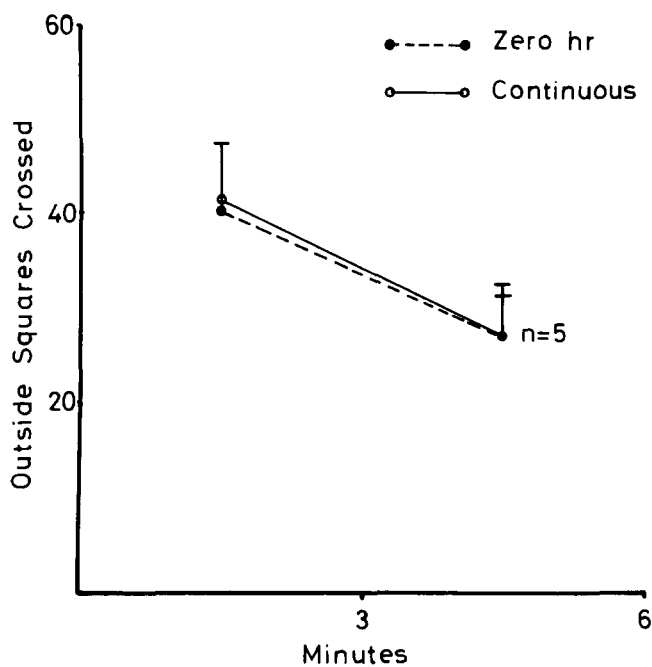
FIG. 4. Effects of continued vs offset stress upon activity (outside squares). All data as mean and standard error. All rats were stressed with stress either continued during the test or terminated upon placement in the open field.

tion and housing to those previously described were subjected to the standard stress procedure (Experiment 1) or a stress procedure identical to the above in which the white noise continued through testing. In the present design a 6 min test (i.e., two 3-min intervals) was employed.
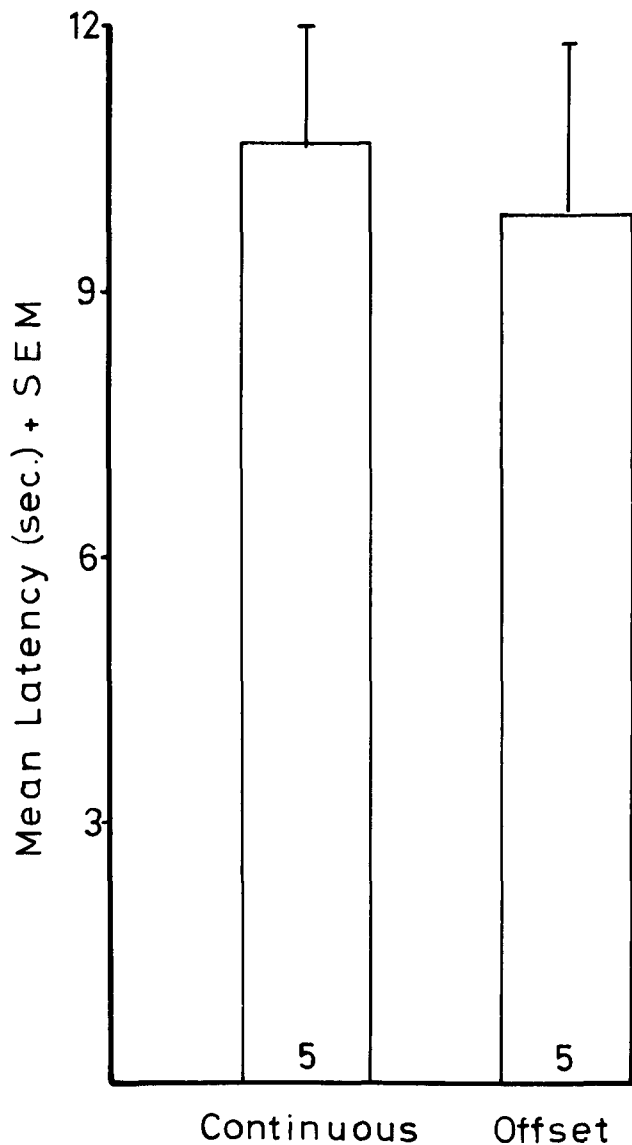

FIG. 5. Effects of continued vs offset stress upon movement latency. All data as mean and standard error. All rats were stressed with stress either continued through the test or terminated with placement in the open field.

RESULTS

The continued stress produced no remarkable alterations in the behavior of the rats in comparison with the initial stress procedure. The absence of effect upon outside squares may be observed in Fig. 4 (F ratios for groups, trials and interaction=0.3, 0.7, 0.1; df in all cases=1, 8 $p>0.05$). Tests upon scores for latency (Fig. 5) and defecation (Fig. 6) are also not significant (t values=0.5, 1.1, respectively; df=8). Scores for rearing, center field penetration and overall activation are presented in Table 2 (F ratios for rearing groups trials and interaction effects=0.3, 0.7, 0.1, df=1.7; $p>0.05$; t value for center field penetration=0.1, $p>0.05$). No rats in either group showed substantial or consistent grooming. Because all scores were less than 10 sec these data are not
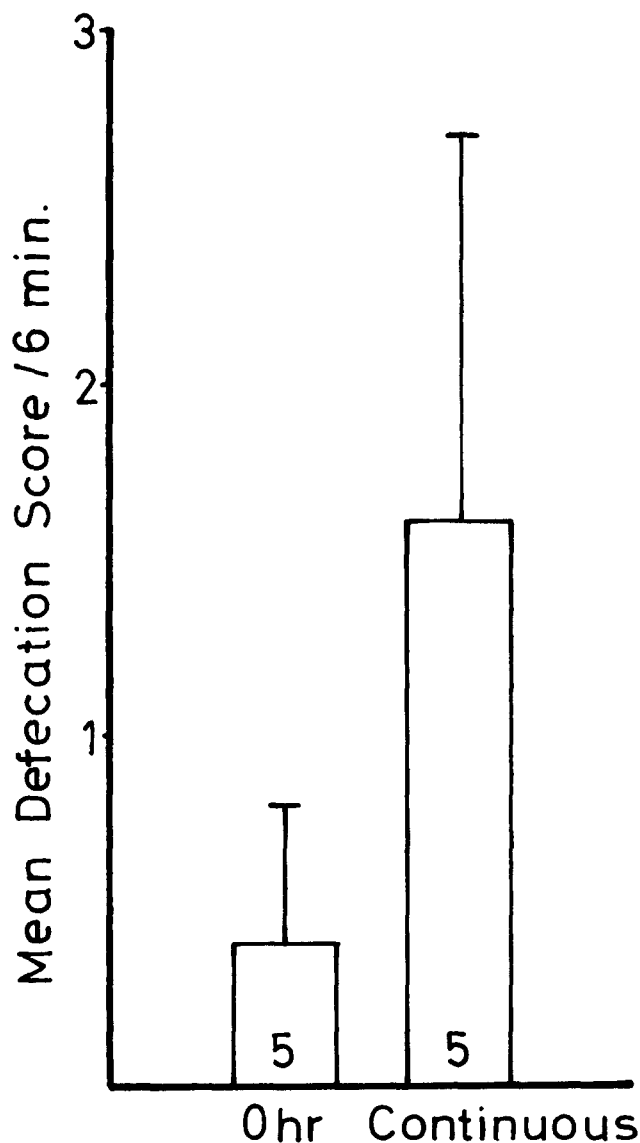
FIG. 6. Effects of continued vs offset stress upon defecation. All data as mean and standard error. All rats were stressed with stress either continued through the test or terminated with placement in the open field.

presented separately. Close inspection of all measures indicated a virtual identity for the two groups. This is confirmed by the summary measure (Table 2, $t=0$).

DISCUSSION

The present results failed to find any unique effect of stress offset upon open field activity in comparison with a group receiving continued stress. While it might be argued that it is difficult to rule out any effect of offset with the presently employed sample size the virtual identity of the two groups also argues against any major stress offset effect. Stress offset therefore may not be a significant determinant of the dependent behavioral variables under the presently defined conditions.

The low level of grooming seen in Experiment 2 is a direct

TABLE 2

EFFECTS OF ACUTE NOISE STRESS UPON OPEN FIELD ACTIVITY (MEAN ± STANDARD ERROR)—STRESS OFFSET AT TIME OF TEST OR CONTINUED THROUGH TEST INTERVAL

Measure	Continuous stress score	Offset stress score	$p^*<$
Rearing (min 0–3)	41.2 ± 6.2	40.4 ± 3.7	n.s.
Rearing (min 3–6)	27.0 ± 5.1	27.0 ± 4.4	n.s.
Center field penetration	3.2 ± 1.6	3.0 ± 0.8	n.s.
Mean activation (composite of other scores)	1.5 ± 0.2	1.5 ± 0.2	n.s.

*Probability of across cells difference, statistics in text.

function of the shorter test interval. Little grooming occurs during the initial 6 min of a given test, and grooming for shorter tests is a less reliable measure. While the behavioral response is established as an immediate consequence of the stress procedure, its course over time is not known. Experiment 3 therefore examined the effects of an interposed delay between stress and testing, and its effect upon stress induced arousal.

EXPERIMENT 3

Experiment 3 systematically varied the interval between stress and test, ranging from a zero delay (i.e., a replication of Experiment 1) to 96 hr.

METHOD

Subjects and Procedure

Thirty-seven rats identical to those in Experiment 1 were subjected to the standard stress or control procedure (Experiment 1). In addition to a test at 0 hr (Experiment 1) the following stress-test intervals were employed: 1 hr, 3 hr, 24 hr, 48 hr, 96 hr. During the stress-test intervals subjects were returned to their normal housing which included the presence of a cagemate. At the start of the appropriate interval the rats were then transported and tested, as previously described. A 6 min test (two 3-min intervals) was used. Based upon previous results (Experiments 1 and 2) the first 3-min period was used as an indicator of the initial stress response for outside squares locomotion.

RESULTS

As in Experiment 1, the stress produced an immediate behavioral activation in comparison with the control group. Moreover, the activation had a time course of 1–3 hr, after which the animals showed a behavioral depression which returned to normal over the remainder of testing. This is most evident in Fig. 7, depicting outside squares crossed in min 0–3, $F(6,34)=5.4$; $p<0.005$. Latency to leave home square (Fig. 8) was not significant although the direction of change was consistent with the activation effect and the time course of locomotor activity (F ratio=1.9, df as above). Other significant effects included defecation (Fig. 9) ($F=2.4$;
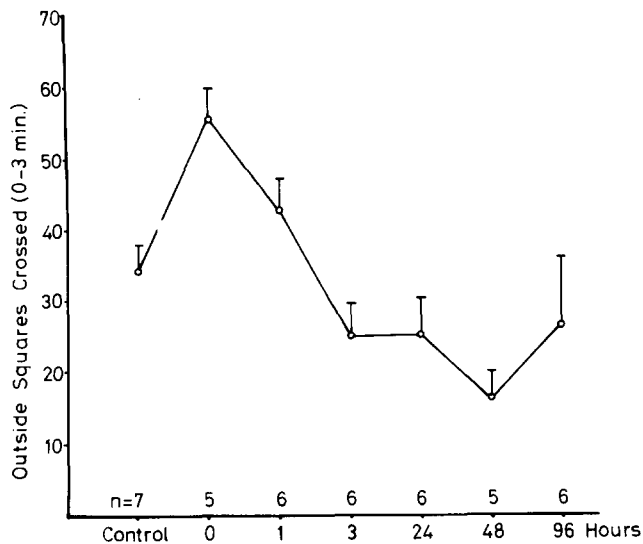
FIG. 7. Time course of stress induced activation (outside squares). All data as mean and standard error. Rats were given no stress or a standard one hour noise light stress, and tested at varying post-stress intervals.
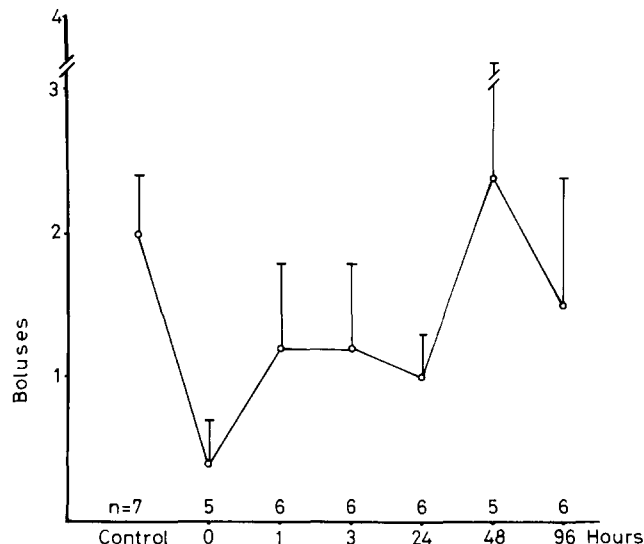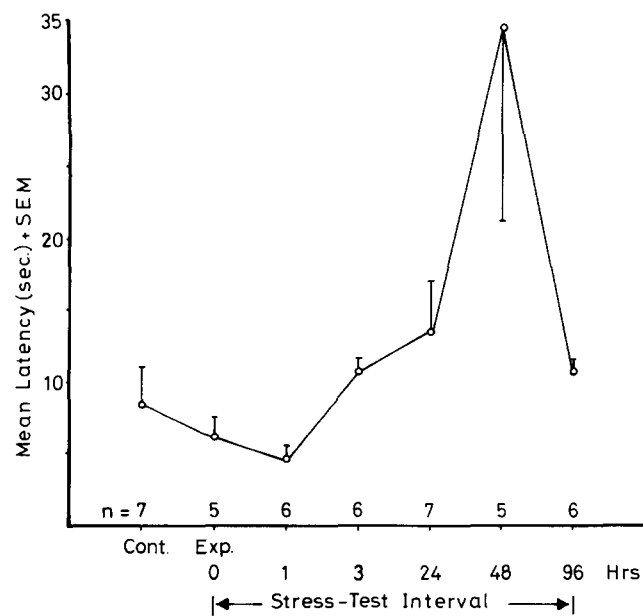

FIG. 9. Time course of stress induced activation (defecation score). All data as mean and standard error. Rats were given no stress or a standard one hour noise-light stress, and tested at varying post-stress intervals.

FIG. 8. Time course of stress induced activation (latency to initial movement). All data as mean and standard error. Rats were given no stress or a standard one hour noise-light stress, and tested at varying post-stress intervals.

df as above $p < 0.05$). Rearing (Table 3) followed the time course as the other variables but was not significant. Center field penetration was significantly altered over time (F=2.4, df as above $p < 0.05$). Grooming scores were too low and inconsistent to measure. No subjects showed more than 10 sec of grooming. A summary measure of activation was significant across time (Fig. 10) ($\chi^2(4) = 28, p < 0.01$ by Friedman two-way analysis of variance [54]).

DISCUSSION

The various stress measures all changed with a roughly similar time course. The activation response was of less than 3 hr duration and was followed by a depression of activity thereafter. Activity recovered to initial levels by the close of testing at 96 hr.

It might be noted that the present time course of activation resembles that of a number of other stress and psychoendocrine syndromes (e.g., stress induced analgesia [3,11] and the incubation of fear after avoidance learning [40]). The established mediation of these other syndromes through the hypothalamic pituitary adrenal axis (HPA) and through endogenous opiate systems might offer insight into the current results. Particularly it suggests that HPA activity or endogenous opiate activity may be involved in the observed facilitation. Having established a time course for the initial behavioral effect, we next asked whether it was stable across tests.

EXPERIMENT 4

It is well established that initial open field activity reflects a different motivation than subsequent activity over repeated tests [7, 15, 19, 32] with repeated testing increasing motor activity which may be related to exploration or territoriality. One question regarding stress induced behavioral arousal then concerns its persistence with repeated testing. Experiment 3 suggested that the effect has a short (3 hr) course following one stress exposure. The present experiment asked a related question. Would the effect be present upon a second (repeated) test? The present experiment examined this question, using a 3-group design.

METHOD

Subjects and Procedures

A total of 24 rats identical in description to those of Ex-

TABLE 3

TIME COURSE OF STRESS INDUCED ACTIVATION (MEAN AND STANDARD ERROR)

| | Group | | | | | | |
	Control (no stress)	Stress (0 hr)	Stress (1 hr)	Stress (3 hr)	Stress (24 hr)	Stress (48 hr)	Stress (96 hr)	$p^* =$
Measure								
Rearing (0–3)	17.1 ± 1.8	21.8 ± 2.8	20.3 ± 1.3	17.7 ± 2.2	12.0 ± 4.4	15.0 ± 2.2	17.0 ± 4.7	n.s.
Rearing (3–6)	15.7 ± 2.3	19.8 ± 2.8	18.8 ± 1.3	14.2 ± 1.8	13.5 ± 2.9	15.6 ± 3.8	14.5 ± 3.7	n.s.
Center field penetration	2.9 ± 1.1	3.6 ± 0.4	5.7 ± 1.1	1.5 ± 0.8	1.3 ± 0.8	1.8 ± 1.6	3.5 ± 2.1	<0.05

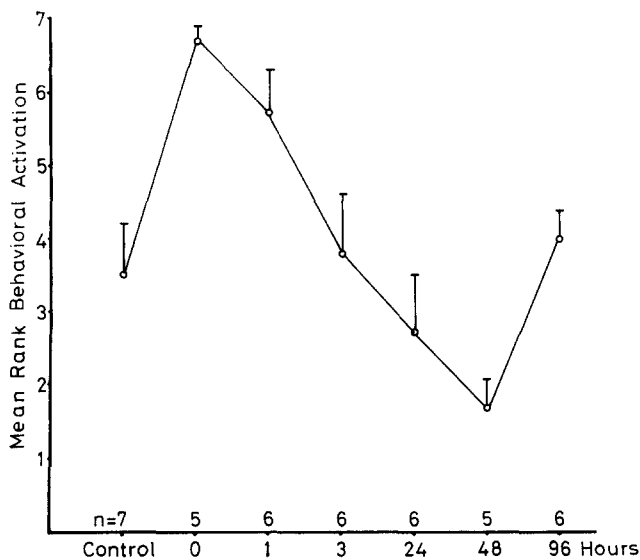*Probability of across cells difference, statistics in text.

FIG. 10. Summary measure of activation based on mean ranks. Mean and standard error. Rats are given no stress or a standard one hour noise-light stress, and tested at varying post-stress intervals.

periment 1 were randomly assigned to one of three groups. Group 1 was an unstressed control group (see Experiment 1). Group 2 was an experimental (stress) group (see Experiment 1) which was re-tested at 24 hr. Group 3 was initially tested 24 hr after stress (see Experiment 3) and was also re-tested 24 hr later. A 12-min test interval was utilized throughout. The present design permitted the evaluation of a repeated tests effect with respect to control performance, initial performance, and performance of a group with equivalent testing delay.

RESULTS

It should be noted initially that the presence of a differential effect of tests across groups would be most evident as a significant interaction of groups by days. The present design replicated two previous findings, and in addition found a

differential tests effect upon arousal. The two findings which were replicated were an initial stress induced behavioral arousal, and the delayed test effect, which appeared previously and in the present design as a depression of activity with a 24 hr test interval. Figures 11A and B present the effects of the experimental manipulations upon outside squares. A presents the first test, and B the repeated test. Both within and between tests effects were significant as was the groups by tests interaction, F(ratios, respectively = 10.2, 53.2, 10.3; $df=3$, 63; 2, 21; 6, 65; $p<0.005$ in all cases, although a groups effect per se was not significant, $F(2,21)=0.03; p>0.05$. An interaction effect was also significant for latency (Fig. 12) although groups and days main effects were only marginally so (F=4.8, 2.0, 1.5; $df=2$, 21; 2,21; 1,21, respectively, $p<0.05$, <0.05, <0.05). A similar pattern held for defecation (Fig. 13), $F(2,21)=3.6; p<0.05$, although main effects of groups and days were not, F=0.9, 0.2, $df=2$, 21; 1, 21, respectively, $p>0.05$). Rearing scores (Table 4) showed significant trials effects (F=31.5; df as above; $p<0.05$) although neither groups nor interaction effects showed this pattern F=0.6, 0.9; $p>0.05$; df as above). The grooming score (Table 4) was marginally significant for groups (F=2.7, $p<0.09$) and significant for days (F=4.0; df as above, $p<0.05$). The interaction effect however was not significant (F=2.2; df as above, $p=0.1$). The inside squares (Table 4) measure was significant over days (F=30, $p<0.05$) but neither for groups nor interaction (F=0.2; df as above; $p>0.05$). Summary measures for the three groups indicated a significant interaction effect (F=5.3; $p<0.05$) and days effect (F=8.1; $p<0.05$) although groups did not differ (F=1.1; $p>0.05$; df in all cases as above).

DISCUSSION

The present findings, taken as a whole, both replicate earlier findings regarding the arousal effect and its time course at least for such key measures as defecation and activity. They also suggest that a repeated tests design reduced differences across groups. The convergence of all groups in trial two across several measures as measured by significant interaction is consistent with other reports [14, 52, 59, 64]. The failure to observe major differences between Group 2 in its second test and Group 3 in its first test suggest the stress effect is relatively transient across tests and does not affect subsequent tests.
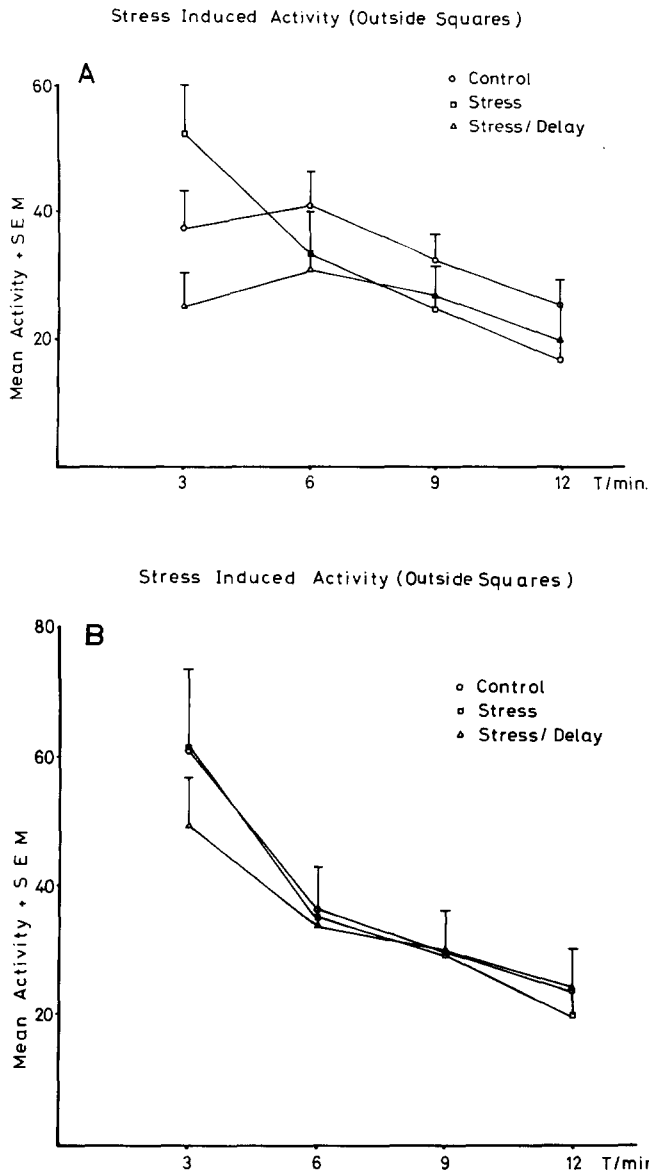
Stress Induced Activity (Outside Squares)
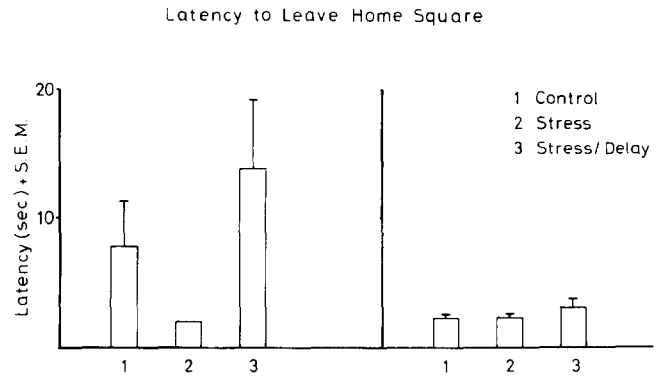
Latency to Leave Home Square

FIG. 12. A and B: Repeated tests effect upon stress induced activation (latency to initial movement). All data as mean and standard error. Subjects received no stress or a standard one hour stress. Two tests were spaced 24 hr apart.

Stress Induced Activity (Outside Squares)

Effect of Stress on Defecation

FIG. 11. A and B: Repeated tests effect upon stress induced activation (outside squares). All data as mean and standard error. First test in A, second test in B. Subjects were given no stress or a standard one hour stress. Two tests were spaced 24 hr apart.
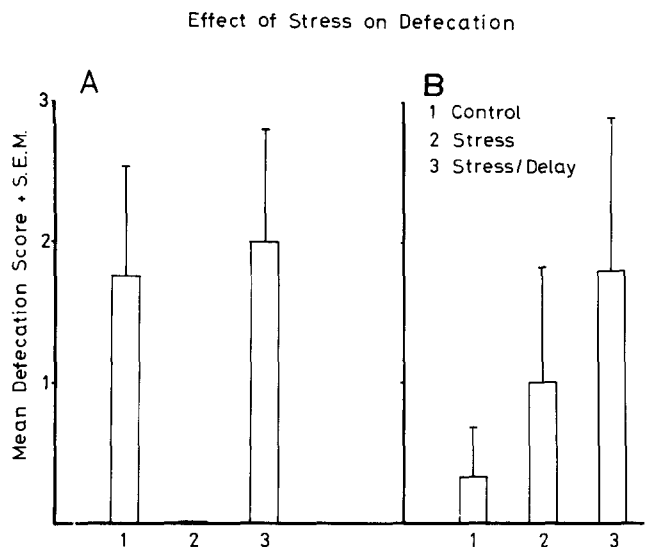
FIG. 13. A and B: Repeated tests effect upon stress induced activation (defecation score). All data as mean and standard error. Subjects received no stress or a standard one hour stress. Two tests were spaced 24 hr apart.

Both based upon the present experiment and Experiment 3, it might well be argued that while stress as employed in the present design is activating, the activation is relatively transient within and across tests. This may suggest that stress delivery across time or tests involves multiple mechanisms rather than a continuum of responding. The precise identification of one or more behavioral, endocrine, or pharmacological correlates of the stress effects remains an issue which will be addressed in subsequent experiments.

Clearly since the physiological stress response is dependent upon hypothalamic-pituitary-adrenal activity [55] one logical strategy involves intervention within this system. The next experiments examined the roles of adrenal and pituitary activity, respectively.

EXPERIMENT 5

In Experiment 5 adrenalectomized animals were subjected to the standard stress procedure (Experiment 1) to examine the contribution of an intact adrenal system upon stress induced activation. If adrenal activity is a necessary component of the activation response then interference with the adrenal response should reduce the behavioral activation.

METHOD

Subjects and Procedure

Ten adult male Sprague-Dawley rats were adrenalectomized under 50 mg/kg sodium pentobarbital anesthesia.

TABLE 4

REPEATED TESTS EFFECTS UPON STRESS ELICITED BEHAVIORS (MEAN AND
STANDARD ERROR)

	Group			
	Control	Stress	Stress-delayed (24 hr)	$p^* =$
Test one Measure				
Rearing (0–3)	10.4 ± 1.2	12.7 ± 2.8	12.0 ± 4.8	n.s.
Center field penetration (0–12)	6.4 ± 2.7	8.2 ± 4.5	4.3 ± 2.1	n.s.
Mean score	1.8 ± 0.2	3.0 ± 0.0	1.2 ± 0.2	0.05
Test two Measure				
Rearing (0–3)	16.8 ± 1.8	18.8 ± 3.7	16.7 ± 4.9	n.s.
Center field penetration (0–12)	13.1 ± 3.9	17.0 ± 6.6	14.8 ± 2.9	n.s.
Mean activation score	2.3 ± 0.3	2.6 ± 0.2	1.2 ± 0.2	n.s.

*Probability of across cells difference, statistics in text.

One week was allowed for recovery, during which time the rats were maintained with a dietary supplement of 0.9% sodium chloride in their drinking water. Other descriptions of procedures were identical to previous reports (Experiment 1). A 12-min test interval was employed.

RESULTS

Adrenalectomy produced no obvious behavioral deficits in the activation response of stressed animals. Results are presented in Figs. 14 through 16 and Table 5. It may be seen that stress produced an increase in outside squares crossed (Fig. 14), F groups (1,8)=6.9; $p<0.05$, F time (2,4)=14.9; $p<0.001$, F interaction (2,24)=2.8; $p<0.06$. Neither latency (Fig. 15) nor defecation (Fig. 16) showed a significant stress effect, although changes were in a predicted direction, $t(9)=$ 1.1, 0.7; $p<0.05$. Rearing scores also showed significant effects of time and interaction (Table 5; F=42.6, 4.1 df as above; $p<0.001$, 0.01, respectively). On the other hand a main effect of groups was not evident (F=2.3, df as above $p>0.05$). Center field penetration was elevated by stress, but was only marginally significant ($t=2.1$; $p<0.06$). The procedure also significantly increased grooming, Fig. 16; $t(9)=10.0$, $p<0.01$. The summary measures for basal vs stressed subjects were significantly different by randomization test (Table 5, $p<0.05$).

DISCUSSION

Previous reports upon adrenal involvement in open field activity have been equivocal with some reports suggesting adrenal mediation of emotionality and other reports failing to find effects [1, 4, 12, 22, 23, 45, 46, 50, 57]. Adrenal weights, ascorbate content, or circulating steroids have been taken as indicators of both involvement and relative non-involvement. It is not a major purpose of this discussion to critique the validity of previous approaches, and more detailed discussion may be found in references [1,13] and [57]. Nonetheless, no evidence of adrenal involvement is obvious from the present findings.

In Experiment 1 the emotional activation we observed occurred in conjunction with increased corticosterone, indicating possible HPA involvement. The present results with adrenalectomized subjects suggest that the adrenal glands are not normally necessary for the observed behavioral effect. Overall, the present results are consistent with an extra-adrenal mediation of stress induced arousal.

EXPERIMENT 6

While there is no evidence of a facilitatory adrenal contribution to the observed behavioral arousal it remains possible that the pituitary-adrenal axis or at very least some other hypophyseal system is involved and this may be evident from experimental intervention at the level of the hypophysis. To investigate the possible involvement of the pituitary gland we repeated the stress procedure using both intact and hypophysectomized rats. Should either the HPA, the pituitary-gonadal axis, the posterior pituitary hormones or other pituitary systems be involved in the stress response, this may be apparent by comparing the basal and stress induced behaviors of normal and hypophysectomized rats.
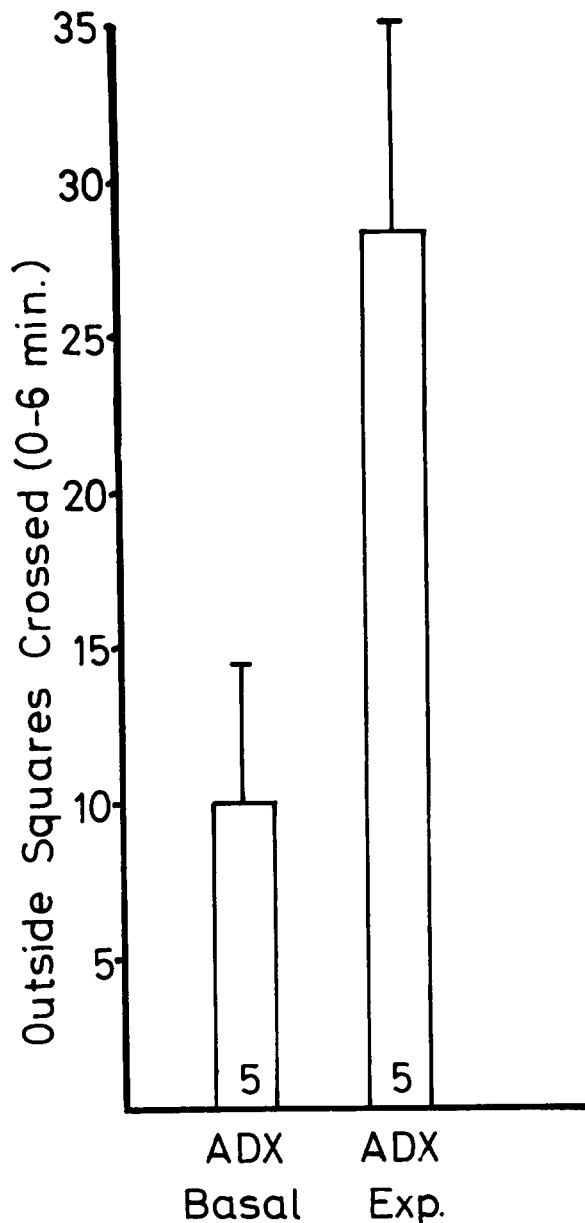
FIG. 14. Effect of adrenalectomy on stress induced activation (outside squares). All data as mean and standard error. Adrenalectomized subjects were given no stress or a standard one hour stress.
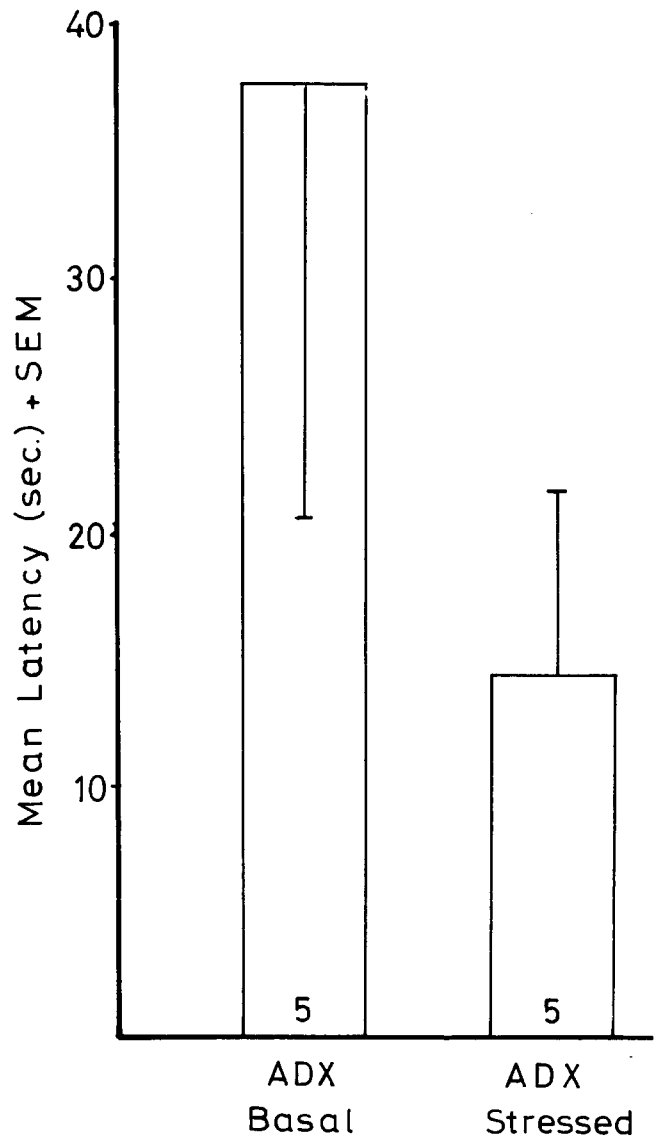
FIG. 15. Effect of adrenalectomy on stress induced activation (latency to initial movement). All data as mean and standard error. Adrenalectomized subjects were given no stress or a standard one hour stress.

METHOD

Subjects and Apparatus

A 2×2 factorial design using six subjects/cell was used to test pituitary involvement in stress induced arousal. The two factors were basal vs stress and hypophysectomy vs control. Rats were identical to those already described. Hypophysectomies or sham operations were carried out by the breeder a minimum of two weeks prior to testing. The operation was carried out under ether anesthesia and utilized a parapharyngeal approach for surgical removal of the hypophysis. To maintain their normal health hypophysectomized subjects were administered a 0.9% sodium chloride solution and a daily supplement of fruit (oranges) in addition to normal laboratory chow. To prevent undue biasing of experimental conditions normal subjects received an equivalent fruit supplement. The present design used a 12 min test interval.

RESULTS

While a stress induced elevation in activity was evident in both experimental groups, they were not different from each other. Hypophysectomy did not measurably alter any aspect of the activation. Hypophysectomy also produced few major alterations in unstressed open field performance. Figure 17 presents data for outside squares. It may be seen that stress equally increased initial activity in both experimental
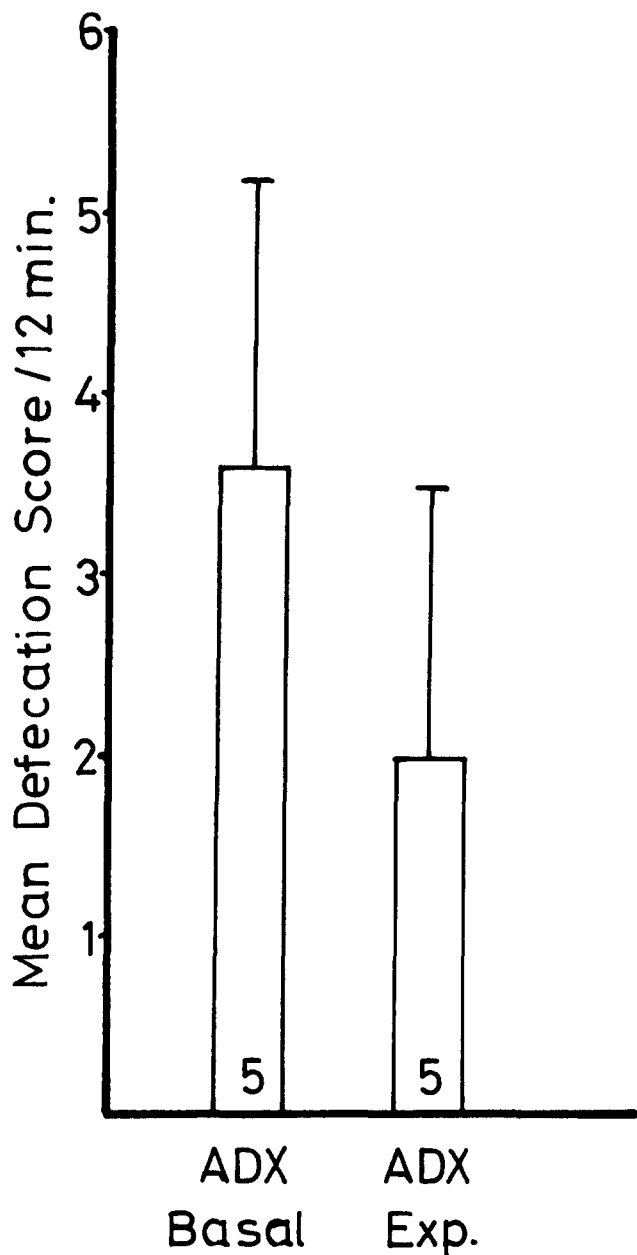
FIG. 16. Effect of adrenalectomy on stress induced activation (defecation score). All data as mean and standard error. Adrenalectomized subjects were given no stress or a standard one hour stress.
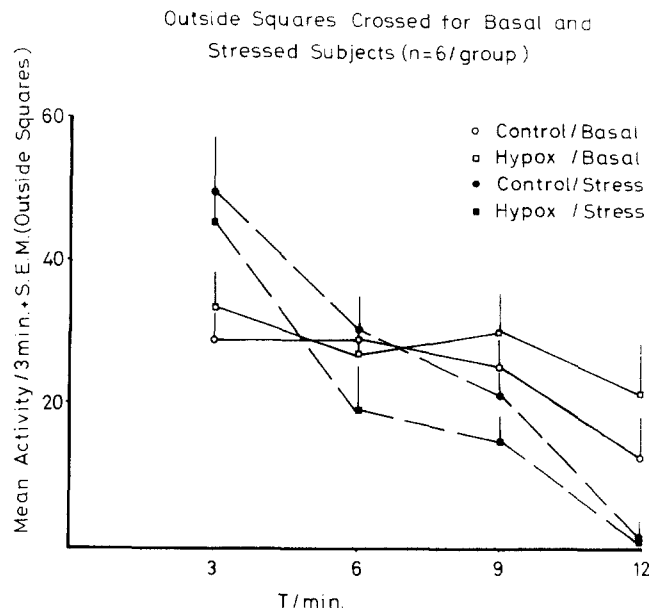

FIG. 17. Effect of hypophysectomy and stress upon behavioral activation (outside squares). All data as mean and standard error. Control or hypophysectomized rats were given no stress or a standard one hour stress in a factorial design.

groups, and that this increase declined to below normal levels by the end of the test period. Both groups, time and interaction effects were significant (F=14.1, 9.6, 7.7, respectively, df=3,20; 3,60; 9,60; $p<0.001$ in all cases). Other significant effects across groups include latency, Fig. 13; $F_{(3,20)}$=3.1, $p<0.05$, and defecation, Fig. 19; F=3.4, df as above, $p<0.05$). A groups effect was also significant for rearing activity (Table 6: F=3.21, df as above, $p<0.05$) although neither time nor interaction effects were significant (F=.8, 1.3 df as above $p<0.05$). Center field penetration followed a pattern similar to the above and was significant (Table 6: F=3.4, df as above, $p<0.05$). Grooming was affected in the predicted manner but was not significant (F=1.4, df as

above, $p<0.05$). Summary measures of emotionality for sham and hypophysectomized controls were significant in the predicted ordering of effects (Table 6: $\chi^2 r$=9.3, $p<0.05$). Necropsies were performed upon five of the twelve hypophysectomized subjects, and these indicated complete absence of pituitary tissue. The completeness of the operation was further confirmed by the substantially lowered body weights of hypophysectomized subjects. The 110–160 g, as opposed to a weight range of 160–200 g for sham operated rats.

DISCUSSION

While a stress effect was demonstrated, pituitary involvement in the latter was not. One previous report [24] examined the activity of hypophysectomized rats and found evidence of hypophysectomy induced increases in exploration, particularly rearing. In fact close examination of Table 5 confirms this. This is also reflected in a slightly elevated summary measure of activation. As in this earlier report, however, no other major changes were noted. While the methods of observation and testing differ considerably across studies, the present results may be taken as a confirmation and extension of prior studies.

EXPERIMENT 7

One further class of candidates for examination is the endogeneous opiates. These compounds are believed to be involved in stress [3,11] and are related to endocrine hormones based upon patterns of biosynthesis and release [24, 26, 42, 62]. The final experiment therefore examined the effects of opiate blockade upon stress induced arousal. Experiments upon the immunohistochemical localization of adrenocorticotrophic hormone (ACTH) and upon its normal synthesis and release suggest endorphins may also be in-

TABLE 5

EFFECTS OF ADRENALECTOMY UPON STRESS INDUCED ACTIVATION
(MEAN AND STANDARD ERROR)

	Adrenalectomized control	Group Adrenalectomized stressed	$p^* =$
Measure			
Rearing			
(0–3)	12.4 ± 2.5	20.4 ± 1.8	n.s.
Rearing			
(3–6)	6.2 ± 1.7	7.8 ± 2.0	n.s.
Center field			
penetration	0.6 ± 0.7	3.0 ± 1.7	n.s.
Grooming	35.0 ± 8.6	40.6 ± 8.4	n.s.
Mean activation			
score	1.0 ± 0.0	2.0 ± 0.0	<0.01

*Probability of across cells difference, statistics in text.

volved in stress—we have previously suggested a role for endorphins in behavioral activation, e.g., [36, 37, 38]. We therefore investigated the effects of narcotic blockade upon stress induced behavioral activation.

METHOD

Subjects and Experimental Design

The experiment used 22 adult male Sprague-Dawley rats maintained in a manner identical to Experiment 1. A 2×2 factorial experimental design was employed, in which basal or stressed rats were subjected to either vehicle or narcotic blockade. The procedures were identical to Experiment 1, except that all subjects received a 1 ml/kg injection of vehicle

or narcotic antagonist 1 hr prior to the behavioral test. The antagonist was naltrexone, a specific and long lasting antagonist, with a standard low 2 mg/kg dosage based upon previous experiments [34,37].

RESULTS

As in previous experiments the stress procedure was effective in producing behavioral activation. Moreover naltrexone, while often without effect itself, nonetheless reversed the activated state to baseline. The experimental effects are shown in Figs. 20 to 22. There was a marginally significant groups effect of stress upon outside squares, Fig. 20; $F(3,18)=44$, $p<0.09$, and both interaction and time were also significant ($F=9.6$, 2.1, $df=3$, 54; 9, 54 $p<0.001$; <0.05)
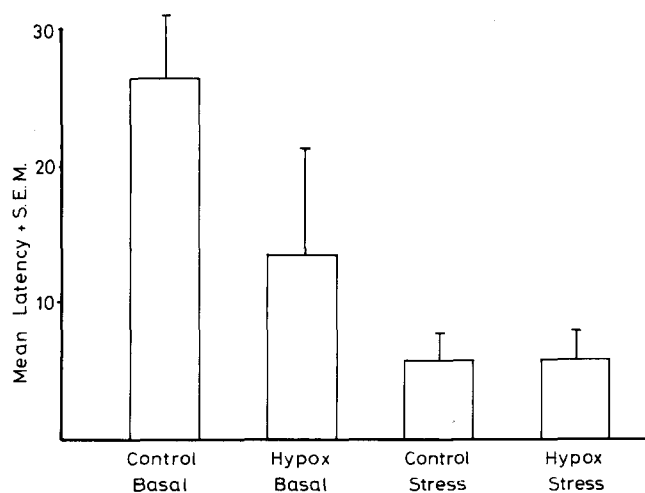

FIG. 18. Effect of hypophysectomy and stress upon latency to initial movement. All data as mean and standard error. Control or hypophysectomized rats were given no stress or a standard one hour stress in a factorial design.
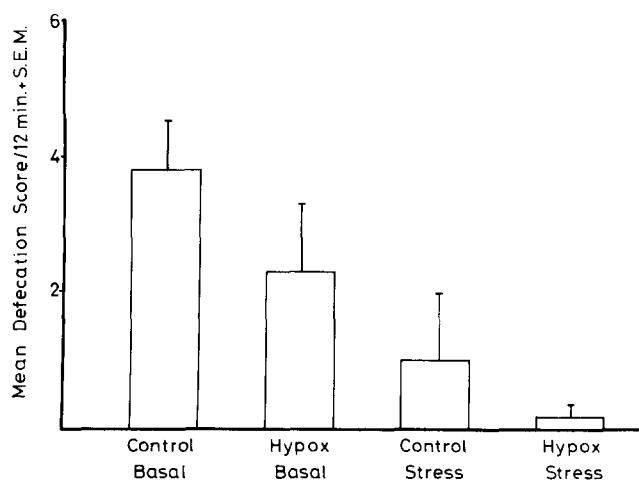

FIG. 19. Effect of hypophysectomy and stress upon defecation. All data as mean and standard error. Control or hypophysectomized rats were given no stress or a standard one hour stress in a factorial design.

TABLE 6
EFFECTS OF HYPOPHYSECTOMY AND STRESS UPON OPEN FIELD ACTIVITY
(MEAN AND STANDARD ERROR)

	Sham basal	Sham stressed	Group Hypox basal	Hypox stressed	$p^* =$
Measure					
Rearing (min 0–3)	11.0 ± 1.0	12.5 ± 1.5	14.0 ± 2.0	15.0 ± 2.0	n.s.
Rearing (min 3–6)	10.0 ± 2.0	11.0 ± 1.0	12.5 ± 3.5	12.0 ± 2.0	n.s.
Center field penetration	0.2 ± 0.2	4.2 ± 1.5	5.0 ± 2.0	3.6 ± 1.4	<0.05
Grooming (sec)	82.0 ± 40.0	106.0 ± 12.0	55.0 ± 10.0	100.0 ± 15.0	<0.05
Mean activation score	1.1 ± 0.2	3.1 ± 0.03	2.4 ± 0.04	3.4 ± 0.03	<0.05

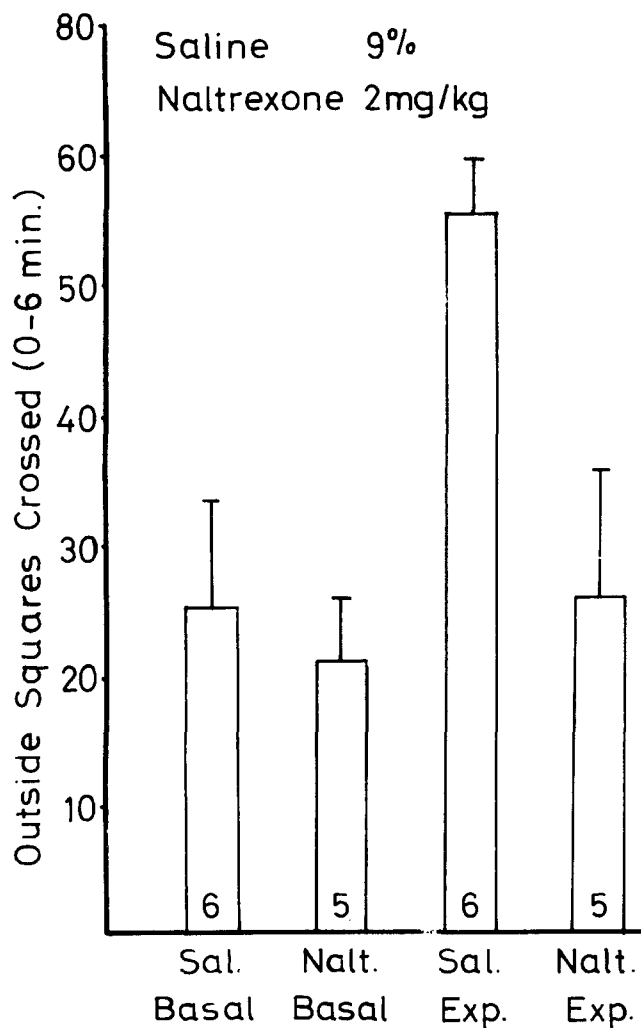*Probability of across cells difference, statistics in text.

FIG. 20. Effect of opiate blockade by Naltrexone (2 mg/kg) and stress upon behavioral activation (outside squares). All data as mean and standard error. Drug or vehicle injected rats were given no stress or a standard one hour stress in a factorial design.
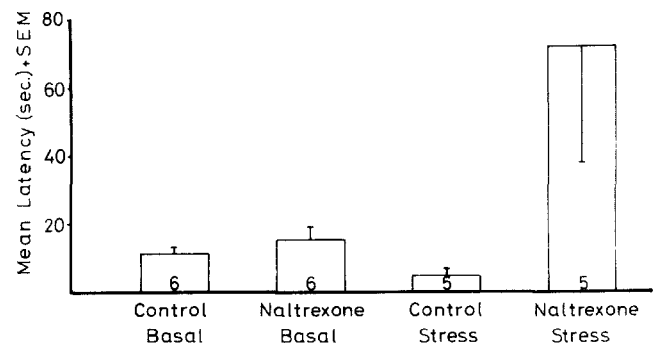

FIG. 21. Effect of opiate blockade by Naltrexone (2 mg/kg) and stress upon latency to initial movement. All data as mean and standard error. Drug or vehicle injected rats were given no stress or a standard one hour stress in a factorial design.

suggesting a differential effect had occurred. A converse pattern was found for latency; however, it was only marginally significant, Fig. 21, $F(3,18)=2.2$; $p<0.01$. Defecation scores showed a similar and statistically significant pattern; Fig. 22, $F(3,18)=3.1$, $p<0.05$. A marginally significant groups effect also occurred for rearing, Table 7; $F(3,18)=2.5$, $p<0.09$, and again time was a significant factor, $F(3,18)=18.7$, $p<0.001$, while there was no effect of interaction, $F(3,18)=1.7$, $p<0.1$. Inside square showed a different non-significant pattern (Table 7, $F=0.7$; $p<0.05$). There was also significant group effects for grooming, Table 7; $F(3,18)=4.2$, $p<0.02$. The four groups were significantly different upon the summary measure ($\chi^2 r=14.3$, $p<0.05$). As may be seen this represents an effect of stress and opiate blockade of stress (Table 7).

DISCUSSION

The present findings suggest a possible role for endogenous opiates in behavioral arousal. Looking at the major behavioral measures it appears that opiate blockade reversed the normal activation response. This is a specific effect since no obvious effects of naltrexone may be seen in unstressed

TABLE 7

EFFECTS OF OPIATE BLOCKADE BY NALTREXONE (2 mg/kg) AND STRESS UPON OPEN FIELD ACTIVITY

	Vehicle basal	Vehicle stressed	Group Naltrexone basal	Naltrexone stressed	$p^* =$
Measure					
Rearing (min 0–3)	17.8 ± 1.6	22.0 ± 3.0	12.3 ± 1.4	13.2 ± 2.0	<0.05
Rearing (min 3–6)	15.8 ± 2.8	19.9 ± 2.6	10.5 ± 1.4	12.0 ± 2.1	
Center field penetration (0–12)	8.0 ± 5.8	8.4 ± 2.9	3.5 ± 2.4	2.8 ± 1.2	<0.05
Grooming	15.0 ± 3.3	39.6 ± 14.7	8.3 ± 2.5	7.8 ± 3.3	<0.05
Mean activation score	2.4 ± 0.3	4.0 ± 0.0	2.0 ± 0.3	1.6 ± 0.3	<0.05

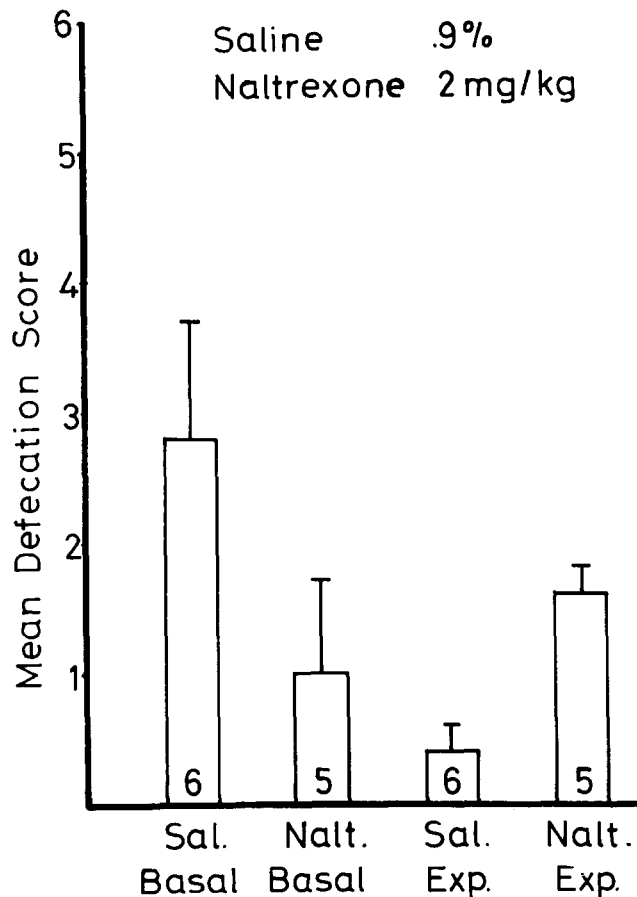*Probability of across cells difference, statistics in text.

FIG. 22. Effect of opiate blockade by Naltrexone (2 mg/kg) and stress upon defecation. All data as mean and standard error. Drug or vehicle injected rats were given no stress or a standard one hour stress in a factorial design.

animals. A pharmacologically specific system therefore appears to mediate one aspect of stress (i.e., behavioral coping and activation). This extends the range of effects of endogenous opiates and offers a possible mediator for the observed effects.

GENERAL DISCUSSION

The present series of seven experiments directly addressed the behavioral definition of one aspect of stress. Clearly it would be premature to advance a claim of generality past the present model. Other stress procedures and behavioral measures may yield quite different results. Nonetheless, the findings of the seven reported experiments might be useful as a model system with potentially greater generality and heuristic value.

Experiment 1 suggested that a non-traumatic procedure produced a behaviorally activated state. This state was remarkably (if also somewhat counterintuitively) similar to the state of non-emotionality as defined in previous open field studies, e.g. [1, 7, 14, 19, 29]. As previous studies have noted, fear may decrease open field activity [44] and more emotional rats tend to show a profile of reduced activity [7]. Additional experiments defined the determinants and parameters of the activated state, and in addition replicated the initial finding on three subsequent occasions.

The present findings suggest that stress and the HPA are amenable to behavioral study, just as sexual behavior and the hypothalamic pituitary gonadal system have been. While adrenalectomy and hypophysectomy are both singularly ineffective, other approaches, making use of opiate blockade, suggest at least one possible mediator of the syndrome. This last finding is consistent with other reports [3,26]. It is possible to view endogenous opiates as mediators of emotional states. Under highly arousing circumstances they appear to facilitate activity, possibly through a direct activating effect or alternately as inhibitors of the emotionality, freezing, and other forms of inhibition. This last suggestion finds support in other paradigms [35].

It would appear from the present set of findings that at least within the confines of open field activity, stress may be conceptualized as several parallel but distinctive systems of

coping. The HPA system is clearly involved in stress and its physiological regulation. A second system apparently depends upon one or more endogenous opiates. This system clearly controls several aspects of behavioral coping. Physiological and psychological coping may be differentially mediated, at least under the present circumstances. It should be noted that although no evidence for HPA involvement in stress related behaviors was found, a role for central hypophyseal-related peptides still remains possible in the present syndrome. Since physiological stress syndromes are thought to be peripherally mediated, this nonetheless implies a degree of dissociation. Whether yet other systems are active, and the roles of these systems remain to be determined. The existence of a biphasic time course and complex interactions upon repeated testing suggest the present effects may be circumscribed, transient, and in fact aspects of yet more complex behavioral processes.

While the present results suggest opiate involvement other recent reports suggest certain behaviorally effective stressors may depend upon brain catecholamines [6]. These findings are not necessarily inimical although the precise procedural and neuropharmacological interrelations of the present and other, e.g. [6] findings is as yet not understood.

We suggest extreme care is necessary in the housing, transport and general treatment of subjects, especially since the magnitude of stimulation necessary to produce changes in the emotionality is not established and may conceivably be small. In recent preliminary observations we have noted stress induced arousal after noise stress exposures as short as five minutes. (In preparation).

As we and others, e.g. [13] have noted, at least some of the controversy upon emotionality and open field may be a function of laboratory procedures and stressors.

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