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Potentiation of the Locomotor Excitatory Properties of Morphine by Prior Exposure to Inescapable Foot-Shock

Marco Leyton

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montréal, Québec, Canada

September 1986

ISBN 0-315-35565-4

ABSTRACT

Potentiation of the Locomotor Excitatory Properties of Morphine by Prior Exposure to Inescapable Foot-Shock

Marco Leyton

A series of experiments was carried out to determine the possible long-term effects of repeated exposure to shock on the activity inducing effects of morphine. Previously shocked animals were found to be more active than non-shocked animals when tested with morphine or saline under a variety of conditions. These included tests with saline, carried out immediately post-shock in the shock environment, and with morphine, upon re-exposure to that environment in the absence of shock. Animals moved to a neutral environment immediately post-shock were no more active than no-shock control group animals. On the other hand, previously shocked animals, tested 20 hours post-shock in a neutral environment to which they had been habituated, were more active than no-shock animals under both morphine and saline. No differences between previously shocked and no-shock control groups were evident when tests were conducted in a novel environment to which they had not been habituated. When the stress-invoked increases in activity were obtained, they appeared to be cumulative and long-term. They became enhanced over repeated shock sessions and were resistent to both time from the last shock session (up to 19 days), and repeated morphine administra-

tion (up to nine injections). The potential mechanisms underlying these effects are discussed, and their possible adaptive significance are speculated upon. It is concluded that repeated exposure to a stressor mimics some of the effects of repeated exposure to an opiate drug.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Jane Stewart, for her support and encouragement in the midst of my confusion. Without her guidance, this thesis would not have been completed.

Martin Binks, Francine Cote, Jim Dalton, Lisa Jensen, Nicole Milhomme, John Mitchell, Joe Rochford, Paul Vezina and Tom West must all be thanked for their willingness to share their knowledge and experience with me. Their help was timely and invaluable.

To Alexander and Mary Anne, I express my deepest appreciation for putting up with my absence and self-absorption during the period of writing.

This research was supported by a grant (MA6678) to Dr. J. Stewart from the Medical Research Council of Canada.

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What was once a revolutionary concept in psychopharmacology has become an accepted given: many drugs elicit their effects by mimicking endogenous counterparts. The finding of opiate receptors (Pert, Snowman, and Snyder, 1974), followed shortly by the discovery of endogenous opioids (Hughes, Smith, Kosterlitz, Fothergill, and Morgan, 1975; Terenius and Wahlstrom, 1975), ushered in new areas of research that have grown astonishingly in the past decade. The succeeding years have not only found other neuronal systems that are activated by synthetic chemicals (Johnston and Willow, 1982; Richards, Schoch, Mohler, and Haefely, 1986), but have lead to a change in the interpretation of many drug effects. Rather than being pharmacological peculiarities, morphine and other drugs have become models with which to understand more fully the biochemical functioning of the central nervous system.

The commonly used exogenous opiate, morphine, has numerous central and peripheral effects. Acute administration elicits such centrally mediated events as analgesia, altered body temperature and changes in activity levels. Many effects are biphasic. For example, in the rat high doses typically lead to decreased body temperature and motor activity that are followed by hyperthermia and hyperactivity; low doses lead only to hyperthermia and hyperactivity. In the naive rat, medium to high doses typically range from 10-40 mg/kg i.p. while low doses are less than 5 mg/kg i.p. However, various prior experiences can alter this dose response curve. For example, repeated morphine treat-

ment leads to tolerance of the analgesic and the depressant effects with a concurrent sensitization of the excitatory effects: repeated high doses elicit successively less analgesia, hypoactivity, and hypothermia with the ensueing excitatory effects beginning sooner; repeated lower doses elicit greater hyperthermia and hyperactivity.

Opiates and Hyperactivity: Sites of Action

The physiological substrate of the locomotor stimulant properties of opiates has been a source of much investigation. Though still open to debate, the meso-cortico-limbic dopamine (DA) pathway appears to play a major role. The neurons of this system originate in the ventral tegmental area (VTA) with ascending pathways leading to terminal fields in the nucleus accumbens (NAS), olfactory tubercle (OT), and frontal cortex (FC). These areas excite and inhibit each other through ascending and descending pathways. Although the literature describing the functions of each nucleus is often contradictory, it would appear that activation of DA inputs to the NAS and OT leads to an excitation of locomotor behaviour (Kelley, Stinus, and Iversen, 1980; Kalivas, 1985; Cools, 1986). Activation of the DA cells innervating the FC, however, may activate cortical neurons that feedback to inhibit the mesolimbic areas. Disrupting FC input by decortication has been reported to potentiate the hyperactivity from a low dose of the indirect DA agonist methamphetamine (Itoh, Hsiao, and Katsuura, 1985).

In support of the postulate that morphine's excitatory effects are mediated by this meso-cortico-limbic system, the microinjection of opiates into the VTA has been observed to elicit excitatory motor effects and DA release in the terminal fields, particularly the NAS (Broekkamp, Phillips, and Cools, 1979; Kelley et al., 1980; Joyce, Koob, Strecker, Iversen, and Bloom, 1981). This behavioural activation is monophasic without a preceding period of locomotor depression. Importantly, Kalivas and Miller (1985) recently reported a correlation between the behavioural response to and degradation of DA microinjected in the NAS. Between 0 and 30 minutes after microinjection the percentage of radioactive DA recovered declined, while the level of the DA metabolite [^{3}H] DOPAC increased. The motor stimulant effect was also maximal during the first 30 minutes before disappearing by 40-50 minutes. Additionally, it has been reported that intra-VTA morphine increases the single-unit activity of mesolimbic DA cells (Gysling and Wang, 1982). It therefore seems reasonable to nominate the mesolimbic dopaminergic pathway for playing a major role in the mediation of morphine's excitatory effects.

Repeating the administration of opiates leads to a progressively enhanced excitatory effect referred to as sensitization (Joyce and Iversen, 1979; Vezina and Stewart, 1984; Kalivas, Taylor, and Miller, 1985; Kalivas, 1985). Joyce and Iversen (1979) microinjected morphine into the VTA and reported an increase in locomotor activity which was augmented by repeated

administration. This increase was blocked by the specific opiate receptor antagonist naloxone, and the DA receptor antagonist haloperidol. Sensitized locomotor activity is also the response to repeated systemic morphine administration and the development of this enhanced motor response is also blocked by the neuroleptic pimozide (Vezina and Stewart, 1984). In addition, Kalivas et al., (1985) reported a progressively increased locomotor response to the daily microinjection of the enkephalin analog D-Ala-Met-enkephalinamide (DALA) into the VTA. This behavioural sensitization was associated with a greater increase in the DA metabolism in the NAS following an acute administration of DALA (Kalivas, 1985).

Although these various findings consistently re-emphasize the importance of increased mesolimbic DA turnover in the development of sensitization, it is becoming apparent that other mechanisms are involved in the increased locomotor activity seen following the systemic injection of opiates. It appears that opiates may act on a DA-independent system in the NAS. Pert and Sivit (1977) reported increased activity following injections of both morphine and DALA in the NAS. This hyperactivity was reversible by naloxone but not haloperidol. Conversely, apomorphine in the NAS also increased activity but was reversible by haloperidol and not naloxone. This suggests the presence of excitatory DA-independent and -dependent systems within the NAS.

In further support of this view, mesolimbic 6-OHDA lesions

which reduce DA levels, abolish hyperactivity from intra-VTA opiate injections (Kelley et al., 1980; Stinus, Koob, Ling, Bloom, and Le Moal, 1980), but not from intra-NAS injections (Kalivas, Widerlov, Stanley, Breese, and Prange, 1983). Naloxone was also able to block this hyperactivity obtained from opiate injections to the NAS, but the neuroleptic fluphenazine was without effect. Furthermore, DA metabolism in the NAS was unaltered during this hyperactivity (Kalivas, et al., 1983). Similarly, 6-OHDA lesions or treatment with the DA antagonist alpha-fluphenthixol were without effect on systemic heroin activity (Vaccarino, Amalric, Swerdlow, and Koob, 1986). These findings imply that opiates can act on the receptors on non-dopaminergic neurons that originate in the NAS. In a more direct test of this notion, intra-VTA microinjection of either morphine or the specific mu opioid agonist Try-D-Ala-Gly-NMe-Phe-ol (DAGO) resulted in increased locomotor activity that became enhanced over repeated administrations (Vezina, Kalivas, and Stewart, 1986). Conversely, the hyperactivity from intra-NAS application of these agents did not increase over repeated administrations (Vezina et al., 1986). It appears that increased locomotor activity can result from the stimulation of DA-dependent and -independent mesolimbic pathways. The progressively augmented locomotor response observed following repeated systemic opiate administrations, however, might involve only the DA-dependent system.

Stress and Endogenous Opioids: Sites of Action

It is well documented that various stressors lead to the release of endogenous opioids. Evidence in support of this release includes the following reports of increased opioid release immediately post-shock: increased opioid levels in whole brain immediately post-stress (Madden, Akil, Patrick, and, Barchas, 1977); beta-endorphin release from the rat anteriour pituitary (Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale, and Bloom, 1977) with the expected decrease in pituitary beta-endorphin like immunoreactivity levels (Baizman, Cox, Osman, and Goldman, 1979) and increased plasma beta-endorphin levels (Guillemin et al.,1977; Mueller,1981) as well as decreased binding by rat brain homogenate of [^{3}H] N-leu-enkephalin (Chance, White, Krynock, and Rosecrans, 1978).

Analgesia is the behavioural response most frequently monitored as a possible indicator of endogenous opioid activity post-stress. The nature of the stressor appears to determine which of a number of possible pain reducing mechanisms are activated; not all, however, are opioid mediated (Hyson, Ashcraft, Drugan, Grau, and Maier, 1982; Drugan, Ader, and Maier, 1985). Opioid mediation is typically determined by observing the reversal or prevention of analgesia by specific opiate receptor antagonists such as naloxone (Amir and Amit, 1978; Lewis, Cannon, and Liebeskind, 1980; Maier, Davies, Grau, Jackson, Morrison, Moye, Madden, and Barchas, 1980; Grau, Hyson, Maier, Madden, and Barchas, 1981) or the presence of cross-tolerance to the analgesia from previous

morphine injections (Chesher and Chan, 1977; Drugan, Grau, Maier, Madden, and Barchas, 1981; Nabeshima, Yamada, and Kameyama, 1983; Terman, Lewis, and Liebeskind, 1986).

It is well established that stressors initiate an array of parallel and interacting physiological responses. The release of numerous neurotransmitters and neuropeptides have been demonstrated in response to a wide variety of stressors. Much of the early work on the physiological changes induced by stress centred on NE. In a comprehensive review of the neurochemical changes elicited by stress, Anisman, Kokkinidis, and Sklar (1984) concluded that mild acute stress increased NE utilization. More severe stress on the other hand, perhaps due to the greater demands on the NE stores, elicited an increased synthesis of the catecholamine. In a similar fashion the chronic application of stressors appears to progressively increase the organism's neurochemical response to them (Keim and Sigg, 1976). Chronically shocked mice show both increased NE levels and utilization (Tsuda and Tanaka, 1985; Irwin, Ahluwalia, and Anisman, 1986). Supporting this notion is the observation that a mild stress which would not alter amine levels in a naive rat, reduced NE in a rat previously exposed to a more severe stressor (Anisman and Sklar, 1979; Tsuda, Tanaka, Ida, Tsujimaru, Ushijima, and Nagasaki, 1986; Irwin et al., 1986). Further, a two-minute restraint stress followed by a second two-minute restraint up to 90 minutes later yielded augmented catecholamine turnover to the subsequent stress (De Souza and Van Loon, 1986).

Dopamine has received particular attention recently for its role in the stress response. Mild, acute stress appears to selectively increase DA metabolism in the VTA and frontal cortex (Thierry, Tassin, Blanc, and Glowinski, 1976; Lavielle, Tassin, Thierry, Blanc, Herve, Bathelemy, and Glowinski, 1978; Reinhard, Bannon, and Roth, 1982; Miller, Speciale, McMillen, and German, 1984; Deutch, Tam, and Roth, 1985) as reflected by increased metabolite levels. Such mild stress seems not to disturb levels in the mesolimbic terminals. More severe acute stress, however, leads to a more extensive activation of DA metabolism with increased DOPAC levels in the anteromedial and sulcal cortices, NAS, OT, and amygdaloid complex (Fadda, Argiolas, Melis, Tissari, Onali, and Gessa, 1978; Herman, Guillonneau, Dantzer, Scatton, Semerdjian-Rouquier, and Le Moal, 1982). Chronic foot-shock stress leads to parallel changes. Immediately after the tenth daily 20-minute shock session, Herman, Stinus, and Le Moal (1984) noted increased DOPAC levels in mesocortical and mesolimbic areas. This pattern of progressively increasing utilization for mild to severe stress is evident with DA as it was with NE.

A possible function of these changes in catecholamine metabolism may be to enable the organism to cope with the stress. The depletion of these neurotransmitters from acute stress might be due to their involvement in various coping responses. As the severity or frequency of the stressor increases, the demand on the amine stores also increases. The resulting depletion possibly stimulates a compensatory increased synthesis rate. In sup-

port of the importance of catecholamines for coping with stress, it has been demonstrated that depleting an animal's brain NE level prior to the introduction of stress potentiates other responses to stress such as increased ulcer formation and plasma corticosterone release (Glavin, 1985) and escape deficits after inescapable shock (Anisman, Pizzino, and Sklar, 1980). Further, the catecholamine depletor tetrabenazene leads to behavioural deficits reminiscent of acutely stressed animals (Glazer, Weiss, Pohorecky, and Miller, 1975). It would appear that catecholamine release is important for aiding the animals to cope. DA agonists, on the other hand, have been demonstrated to prevent the development of stress-induced gastric ulcers (Hermandez, Adcock, Orlando, Patrick, Nemeroff, and Prange, 1984). As suggested by Fibiger, Zis, and Phillips (1975), DA's function may be in the initiation of action and hence be important for escape and avoidance behaviour.

Stress and Opiates: Possible Overlapping Sites of Action

Morphine appears to mimic many of the effects of stress. As discussed above, opiates administered either systemically or microiontophoretically increase meso-cortico-limbic DA release. This effect is naloxone reversible (Mathews and German, 1982). Similarly, morphine also enhances the turnover of striatal DA, NE, and GABA (Smith, Conchita, Freeman, Sands, and Lane, 1980). The stress-induced release of neurotransmitters may be a result of the observed associated release of endogenous opioids. These

heightened brain levels of opioids might stimulate the release of various other transmitter substances. In support of this idea is the finding that the increased DA metabolism in FC induced by acute, mild shock was antagonized by naloxone (Miller, et al., 1984). Other indicators of the similarities between stress and exogenous morphine administration include: increased activity before anticipated foot-shock (Imada, Kondo, and Imada, 1985); decreased eating and drinking after shock (Imada et al., 1985); suppression of linear locomotion in mice by naloxone (Ukai and Kameyama, 1985); stress-induced "opioid-like effects on investigatory behavior" (Arnsten, Berridge, and Segal, 1985); and the potentiation of various morphine induced phenomena such as altered body temperature (Stewart and Eikelboom, 1981; Appelbaum and Holtzman, 1984; Appelbaum and Holtzman, 1985; Ushijima, Tanaka, Tsuda, Koga, and Nagasaki, 1985), and analgesia (Appelbaum and Holtzman, 1984; Appelbaum and Holtzman, 1985). Interestingly, administration of the opioid blocker, naloxone, which brings on withdrawal-like symptoms in animals repeatedly exposed to morphine, will also elicit withdrawal-like symptoms in previously stressed animals (Christie and Chesher,1982; Morley and Levine,1980). To further illuminate this phenomenon, Williams, Drugan, and Maier (1984) subjected rats to two daily inescapable shock sessions. Twenty-four hours later, when an injection of morphine was followed by a naloxone challenge, the stressed animals displayed augmented withdrawal-like symptoms as compared to non-stressed controls. This effect was not evident in rats

that were exposed to an identical amount of escapable shock. In a second experiment, the enhanced response was blocked by administering the opiate receptor blocker, naltrexone, prior to each shock session. It would appear that the effect was specific to the release of endogenous opioids and not a result of the stress per se. The elevated endogenous opioids may have summated with the morphine to potentiate the withdrawal-like symptoms that followed the injection of the opiate receptor blocker. These varied findings reinforce the notion that morphine and stress engage many common sites of action.

Like morphine, amphetamine also engages the DA system. The psychomotor stimulant increases DA release, decreases DA reuptake, and blocks its catabolism. Low doses lead to hyperactivity, while high doses elicit repetitive stereotyped behaviour characterized by compulsive sniffing, head movements, and rearing. Chronic administration leads to the sensitization of these activities; repeated low doses lead to augmented hyperactivity (Robinson, 1984), while repeated high doses increase the degree of stereotypy (Robinson and Becker, 1982; Kolta, Shreve, De Souza, and Utretsky, 1985). Interestingly, not all amphetamine-induced behaviours display sensitization suggesting that they are not all mediated by the same mechanism. In general, however, stereotypy appears to be mediated by the striatum (Creese and Iversen,1974) while locomotion is likely mediated by the N.Acc. (Kelly, Seviour, and Iversen, 1975).

As with opiates, stress has been demonstrated to potentiate

some of the behavioural effects of amphetamine. Repeated exposure to stress has been reported to potentiate the activity from low doses of amphetamine (Herman et al., 1984; Robinson, Angus, and Becker, 1985) and the stereotypy from high doses (Antelman, Eicher, Black, and Kocan, 1980; Maclennan and Maier, 1983). Furthermore, repeated amphetamine augments the stereotypy obtained from mild stress (Antelman et al.,1980).

Given the posible common substrates for opiates and amphetamine sensitization, one might hypothesize cross-sensitization between the two. In support, Kalivas (1985) reported a cross-sensitization between DALA and amphetamine. Repeated administration of DALA to the VTA lead to a potentiated activity response to acute systemic amphetamine. Stewart and Vezina (1986) have recently reported the converse experiment. Animals pretreated with amphetamine for several days display an enhanced activity response to either a low dose of systemic morphine or to morphine microinjected directly into the VTA.

The Present Experiments

A wide variety of intense stimuli initiate a multi-faceted stress response that includes the release of endogenous opioids. These stressors have been reported to potentiate a variety of exogenously administered morphine-induced behaviours. As the response both to stress and opiate administration appears to engage the mesolimbic DA system, and the repeated stimulation of this system appears to increase its response to future activation, it was considered reasonable to investigate for a stress-

induced potentiation of morphine's excitatory effects. This possibility was bolstered by the previous demonstrations of cross-sensitization between different pharmacological agents that involve the mesolimbic DA system. Examples include potentiation from opiates to amphetamine, amphetamine to opiates, stress to amphetamine, and amphetamine to stress.

EXPERIMENT 1

Given the convincing body of literature demonstrating a stress-induced release of endogenous opioids, it was proposed that inescapable foot-shock would elicit opiate-like alterations in locomotor activity. Further, it was proposed repeated foot-shock sessions would lead to a sensitized locomotor response. That is, paralleling the effects of repeated opiate administration, successive periods of stress might evoke progressively augmented hyperactivity.

Extending this line of reasoning, it could be hypothesized that if repeated stress shares common substrates with repeated opiate administration, then previously shocked animals might reveal a sensitized response to an acute morphine injection. Repeatedly stressing animals may be equivalent to repeatedly administering opiates.

To investigate for these possibilities, rats were subjected to five days of 20-minute intermittent, inescapable, shock sessions. If, over days, these animals were to show progressively enhanced locomotor activity levels, the finding would support the idea that exposure to a stressor mimics some of the effects of opiate administration. The effect of repeated exposure to stress was studied in both in the stress environment and in a neutral activity box.

To test for the effect of repeated exposure to a stressor on the sensitization of the activity inducing effect of morphine, animals were taken to their respective shock environment without

ensuing shock administration, injected with morphine, and then immediately tested in the activity boxes. It was hypothesized that previously shocked animals would be more active in response to the morphine injection. In addition, all animals were tested after saline injection.

Methods

Subjects

Thirty-two male Wistar rats obtained from Charles River Canada Inc. (St. Constant, Quebec) were individually housed in a controlled environment (temperature of 21±1 degrees C) with food and water available ad libitum. The animals weighed 275-300 grams upon arrival to the laboratory with testing beginning two weeks later. The animals were habituated to the handling and injection procedures during this period. Testing was performed during the light cycle between 12:00 P.M. and 5:00 P.M.

Apparatus

Eight activity boxes equipped with photocell beams were used to record locomotor activity. The box (40 cm x 30 cm x 25 cm) was constructed of a transparent Plexiglas front and rear, pressed wood sides, a removable wire screen ceiling, and 22 stainless steel rods for the floor. Two evenly spaced photocell beams cut across the width of the box while a centred third pair monitored from the sides. The photocells were 4.0 cm off the rod floor. Breaking a beam was recorded as an activity count by a Microcom computer. The red light from the photocells provided sufficient

light for observation. Four of the boxes were wired for inescapable foot-shock produced by a Lafayette shock generator and grid scambler.

A distinctively different set of eight Grason-Stadler shock boxes (23.0 cm x 29.5 cm x 19.0 cm) were located in a second room. Inescapable foot-shock was provided through a floor of 18 stainless steel rods. Each box was sound attenuated and equipped with a miniature light bulb and a fan for ventilation.

Morphine sulphate was mixed with physiologically isotonic saline (0.9%) and injected in a volume of 1 ml/kg.

Design and Procedure

Pre-exposure to Stress: Four groups of eight animals each were tested in Experiment 1, two received shock and two no-shock. Both of the shocked groups were given foot-shock for 20 minutes (0.8 mA, 1 sec./10 sec.), every other day for five sessions. One group was shocked in the activity boxes (Sh-AB), the other in the second set of shock boxes (Sh-SB) placed in a distant room. Immediately following the shock session, the activity level of both groups was recorded for 60 minutes in the activity boxes. The two corresponding no-shock control groups were placed either in the AB for 80 minutes (NS-AB), or for 20 minutes in the shock boxes followed by 60 minutes in the activity boxes (NS-SB). To control for a possible effect of time of day on the measure of activity, only two of the four groups were tested each day. This enabled all groups to be tested at approximately the same time of day. Hence, animals were tested every other day.

Morphine and Saline Test Days: Nineteen days after the last shock session, all animals were given tests in the AB after either saline or morphine injections. In the first test, animals were injected with saline in their respective shock environments, but without shock presentation. Following 20 minutes of re-exposure to the environment, activity levels were monitored in the AB for 60 minutes.

To increase resistence to extinction of the stress response, an additional day of shock followed this test day. The procedure was as described previously.

Two morphine test days, given at two day intervals, followed this final shock session. Given the expectation of more prominent carry-over effects from the 5.0 mg/kg dose of morphine, all subjects were first tested with the lower 0.5 mg/kg dose and with the higher dose on the next test day. The procedure was as described for the saline test day.

Results

Morphine and Saline Test Days

Pre-exposure to Stress: The activity scores recorded each pre-exposure day from animals given shock in the AB and their group control were analyzed separately from those of the animals given shock in the other shock room. It did not appear appropriate to compare directly the scores of animals immediately placed in the AB for activity monitoring with the scores of animals foot-shocked in another environment prior to being placed in the AB.

Activity counts were recorded at five-minute intervals by

the Microcom computer. However, unless otherwise specified, data were analyzed and presented for 20-minute intervals.

In order to monitor the changes that occurred in activity over days as a function of time after stress, the data were subjected to an analysis of variance for stress group x 20-minute time interval x days. As illustrated in Figure 1, animals that were shocked in the activity boxes showed a different pattern of activity during and after stress, as compared to their no-shock control group. The nature of this difference changed over days as reflected in a significant interaction between stress x time x days [$F(12,168)=3.81$, $p<.001$]. As is apparent from Figure 1a, for the first two days, the stressed rats were more active during the 20-minute shock period. However, this difference diminished over the following three days.

It can be seen from Figure 1b that during the 20 minutes following shock, stressed animals were less active than the control group on the first three days. A further analysis of variance for stress x days carried out on the scores in the five minutes immediately following shock, revealed that this difference could be attributed to the lowered activity of the stress group compared to the no-shock control group in this five minutes [$F(1,14)=49.41$, $p<.001$] (See Figure 2a). By the fourth day, activity had begun to increase sufficiently in the following 15 minutes to mask the initial reduction (See Figure 2b).

For the second 20 minute period following shock, the shocked

Figure 1. Successive 20-minute group mean activity counts (± 1 S.E.M.) over foot-shock days 1-5 for animals tested in the stress environment in Experiment 1.

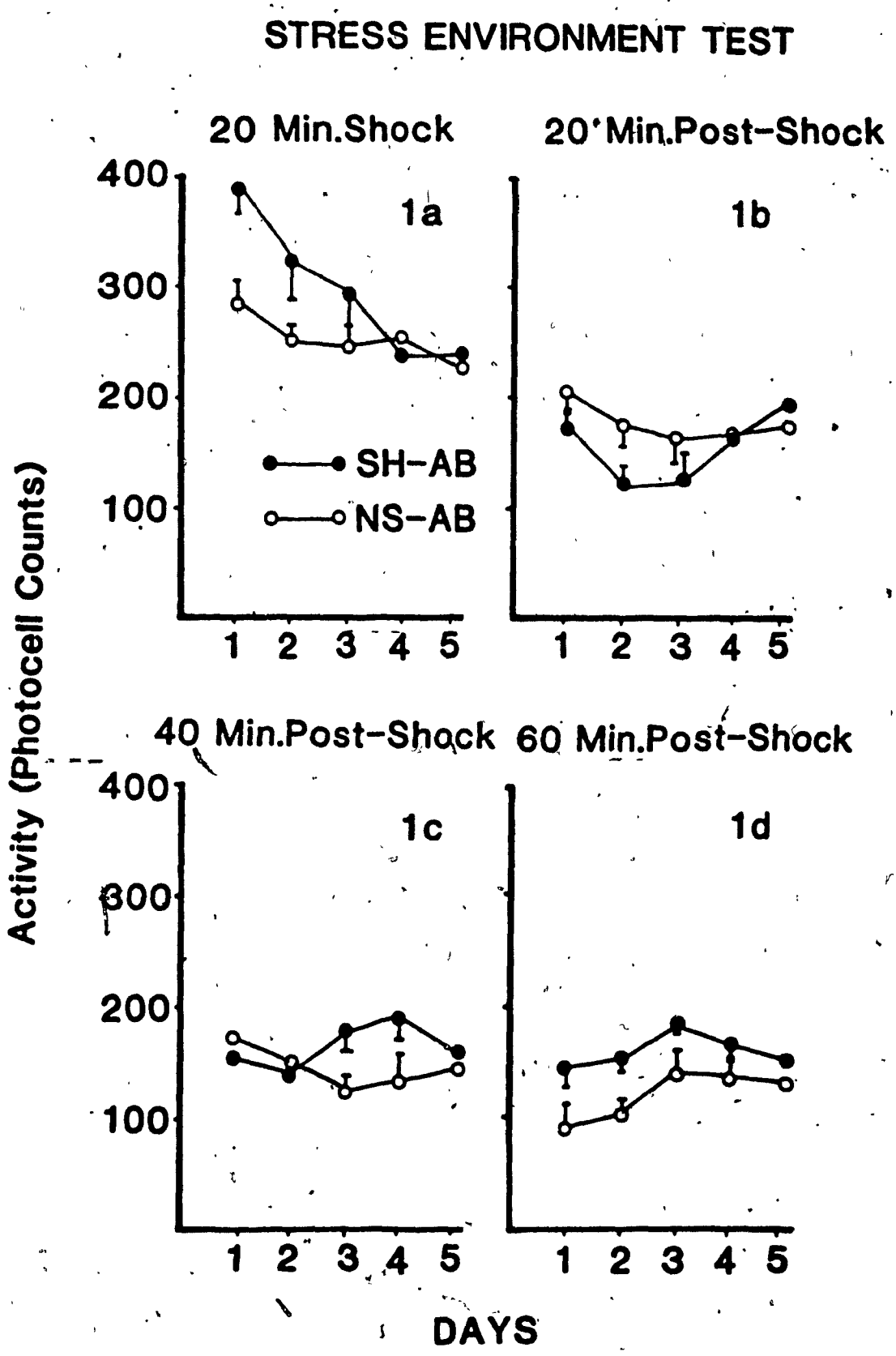
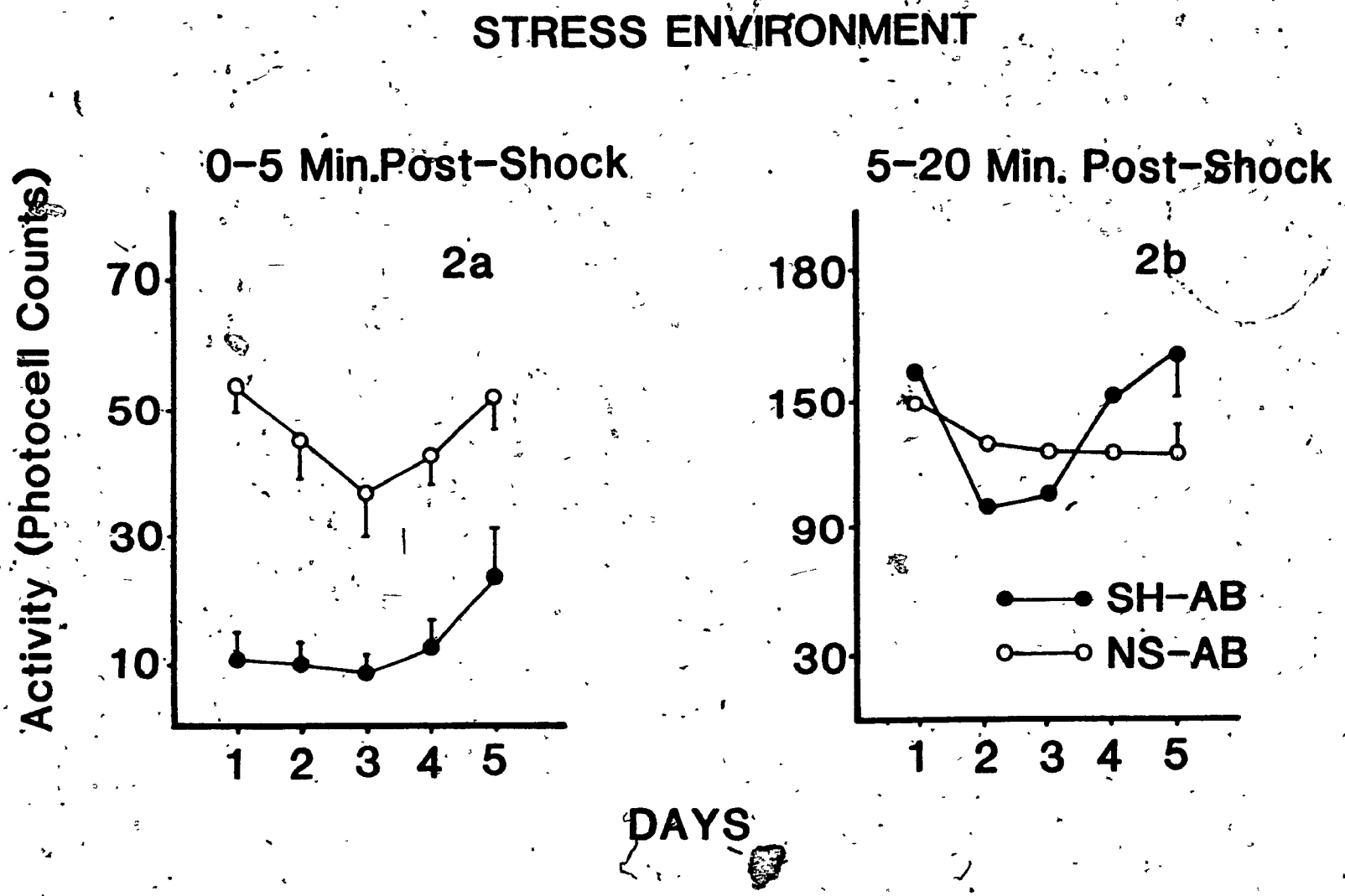

STRESS ENVIRONMENT TEST
20 Min.Shock
20'Min.Post-Shock
1a
1b
400
300
200
100
SH-AB
NS-AB
1 2 3 4 5
40 Min.Post-Shock
60 Min.Post-Shock
1c
1d
Activity (Photocell Counts)
DAYS

Figure 2. Group mean activity counts (± 1 S.E.M.) over foot-shock days 1-5 for animals tested in the stress environment in Experiment 1. Figure 2a represents the change over days during the five minutes immediately following shock. Figure 2b represents the 15 minutes after the five minutes immediately following shock.


STRESS ENVIRONMENT
0-5 Min.Post-Shock
5-20 Min. Post-Shock
Activity (Photocell Counts)
2a
2b
70
50
30
10
180
150
90
30
1 2 3 4 5
1 2 3 4 5
SH-AB
NS-AB
DAYS

animals did not differ from the non-shocked over the initial two days, were more active during the third and fourth days, and were equally active again on the fifth day (See Figure 1c).

During the final 20 minutes post-shock, the stressed animals tended to be more active than the control group across all five tested days. However, this difference was largely confined to the first three days (See Figure 1d).

Overall, the pattern of activity in stressed animals's appeared to be characterized by a period of hypermotility that began during the final 20 minutes of testing on day 1, and over days, both began sooner in time, and lengthened in duration.

Animals that had been shocked in the other set of boxes displayed a pattern of activity different from that of animals shocked in the AB during the subsequent 60 minutes in the activity box. As illustrated in figure 3, the stressed and non-stressed animals did not differ.

Morphine and Saline Tests

Figure 4 shows the results from the Sh-AB and NS-AB groups when returned to the AB without shock administration. When the data were collapsed into 20 minute intervals, saline injected animals from the shock and no-shock groups did not differ. Further inspection of the data, however, suggested that a finer analyses of the first 30 minutes in five-minute intervals might be appropriate. Possibly, the effect of previous shock had been

Figure 3. Successive 20-minute group mean activity counts (± 1 S.E.M.) over foot-shock days 1-5 for animals tested immediately following shock in a neutral environment in Experiment 1.

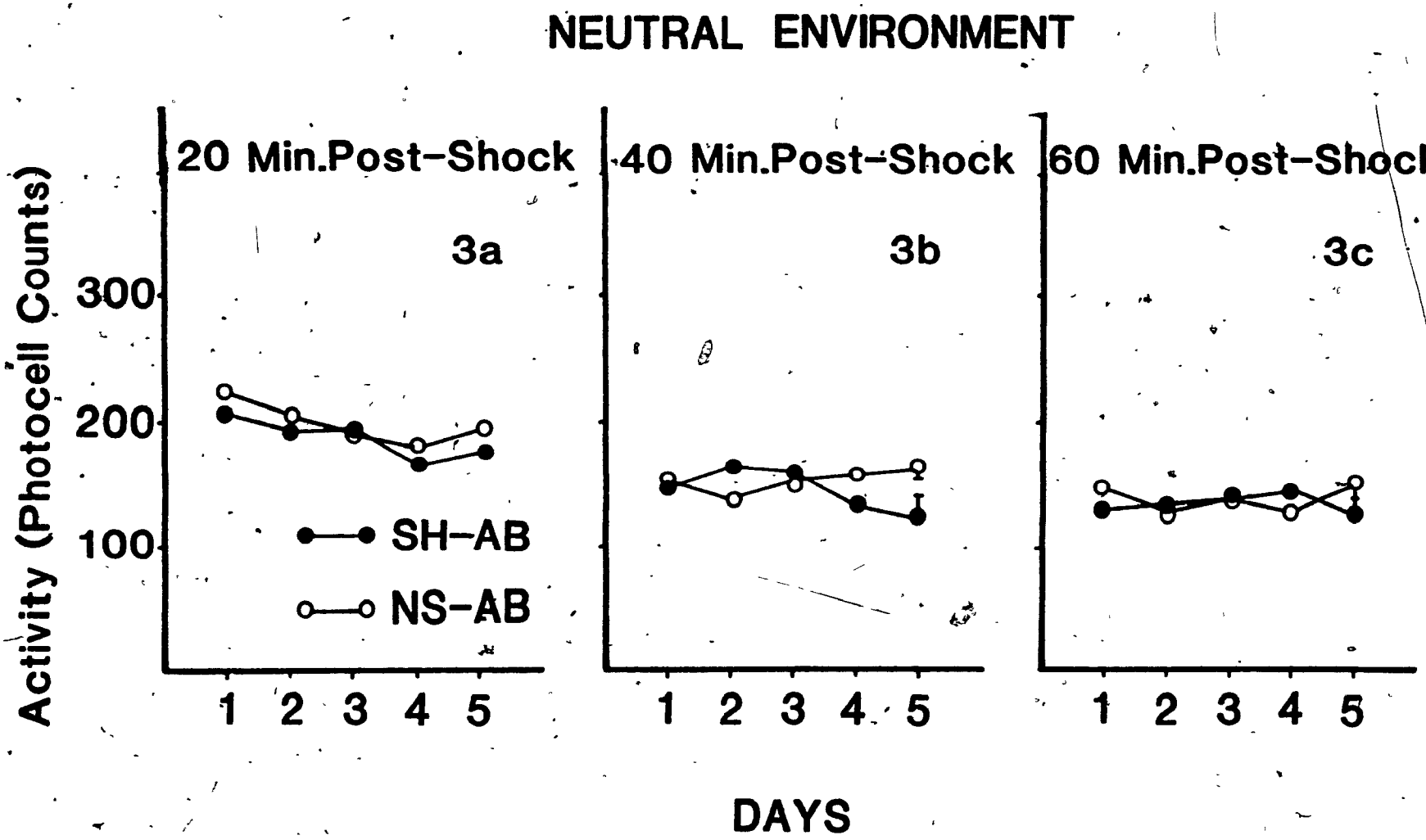
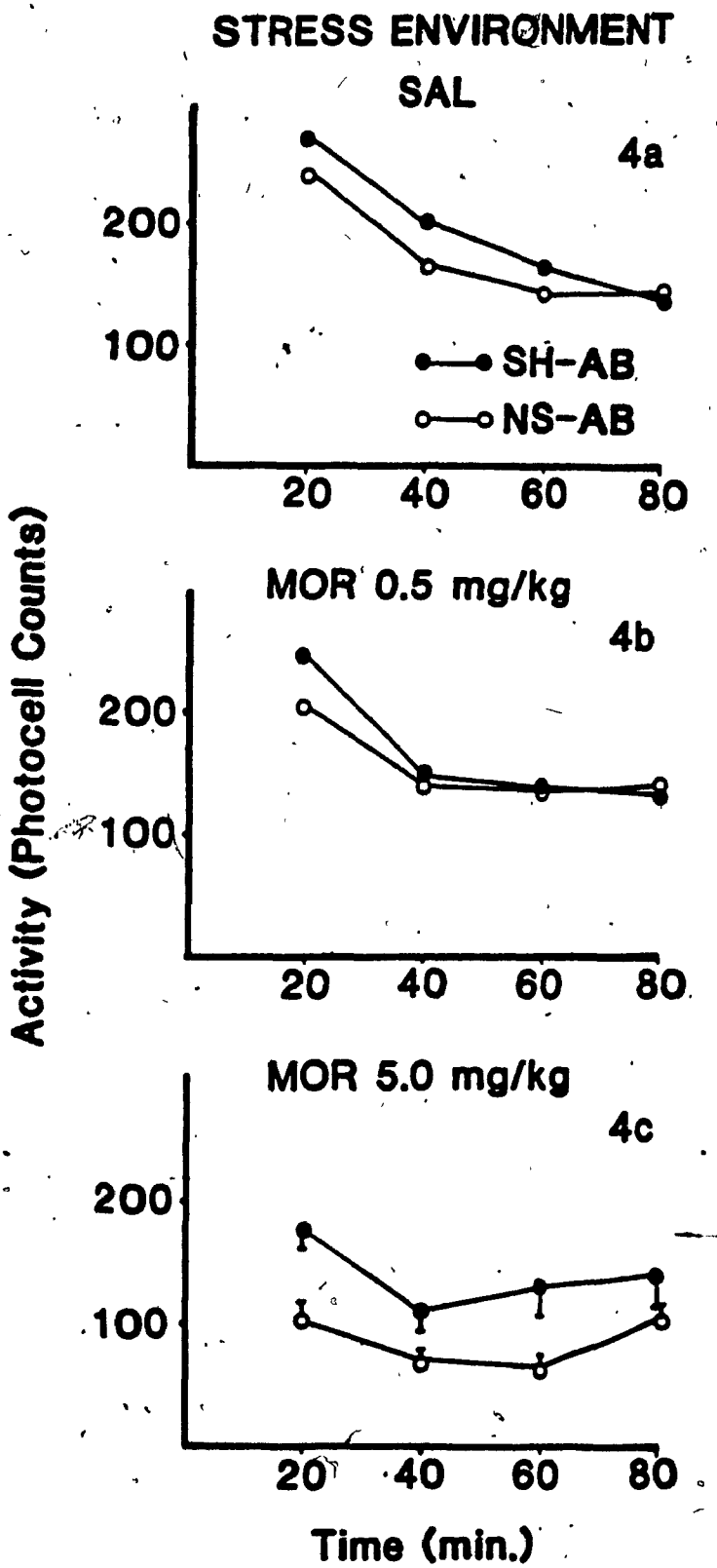

NEUTRAL ENVIRONMENT
20 Min.Post-Shock
40 Min.Post-Shock
60 Min.Post-Shock
3a
3b
3c
Activity (Photocell Counts)
300
200
100
SH-AB
NS-AB
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
DAYS

Figure 4. Group mean activity counts (± 1 S.E.M.) for saline (SAL) (4a), morphine (MOR) (0.5 mg/kg, i.p.) (4b), and MOR (5.0 mg/kg, i.p.) (4c) injected animals in the stress environment on days without foot-shock. Experiment 1.


STRESS ENVIRONMENT
SAL
4a
200
100
SH-AB
NS-AB
20
40
60
80
MOR 0.5 mg/kg
4b
200
100
20
40
60
80
MOR 5.0 mg/kg
4c
200
100
20
40
60
80
Activity (Photocell Counts)
Time (min.)

masked in the 20-minute intervals by a stress x time interaction. This proved to be the case: a significant stress group x five-minute interval interaction was obtained from an analysis of variance of the first 30 minutes [$F(5,70)=17.33$, $p<.001$] (See Figure 5a). During the first five minutes, previously shocked animals were less active than the no-shock control group. Over the following 20 minutes, however, the stressed rats displayed a temporary hyperactivity that subsequently diminished.

When these same animals were systemically administered 0.5 mg/kg of morphine four days later, a less pronounced version of the same stress x five-minute interval interaction was revealed [$F(5,70)=2.33$, $p=.050$] (See Figure 5b). Previously stressed animals were again less active during the initial five minutes and more active during the subsequent 20 minutes.

Two days later, when the rats were injected with the 5.0 mg/kg dose of morphine, previously shocked animals were more active throughout the entire 80 minute session as revealed by a significant main effect of stress [$F(1,14)=8.60$, $p<.02$].

Contrary to these results obtained with animals previously shocked in the activity boxes, animals that were shocked in separate chambers did not differ from their no-shock control group. Their overlapping activity counts are plotted in Figure 6.

Figure 5. Group mean five-minute activity counts (± 1 S.E.M.) for SAL (5a) and morphine (MOR) (0.5 mg/kg, i.p.) (5b) injected animals tested in the stress environment on the days without foot-shock. Experiment 1.

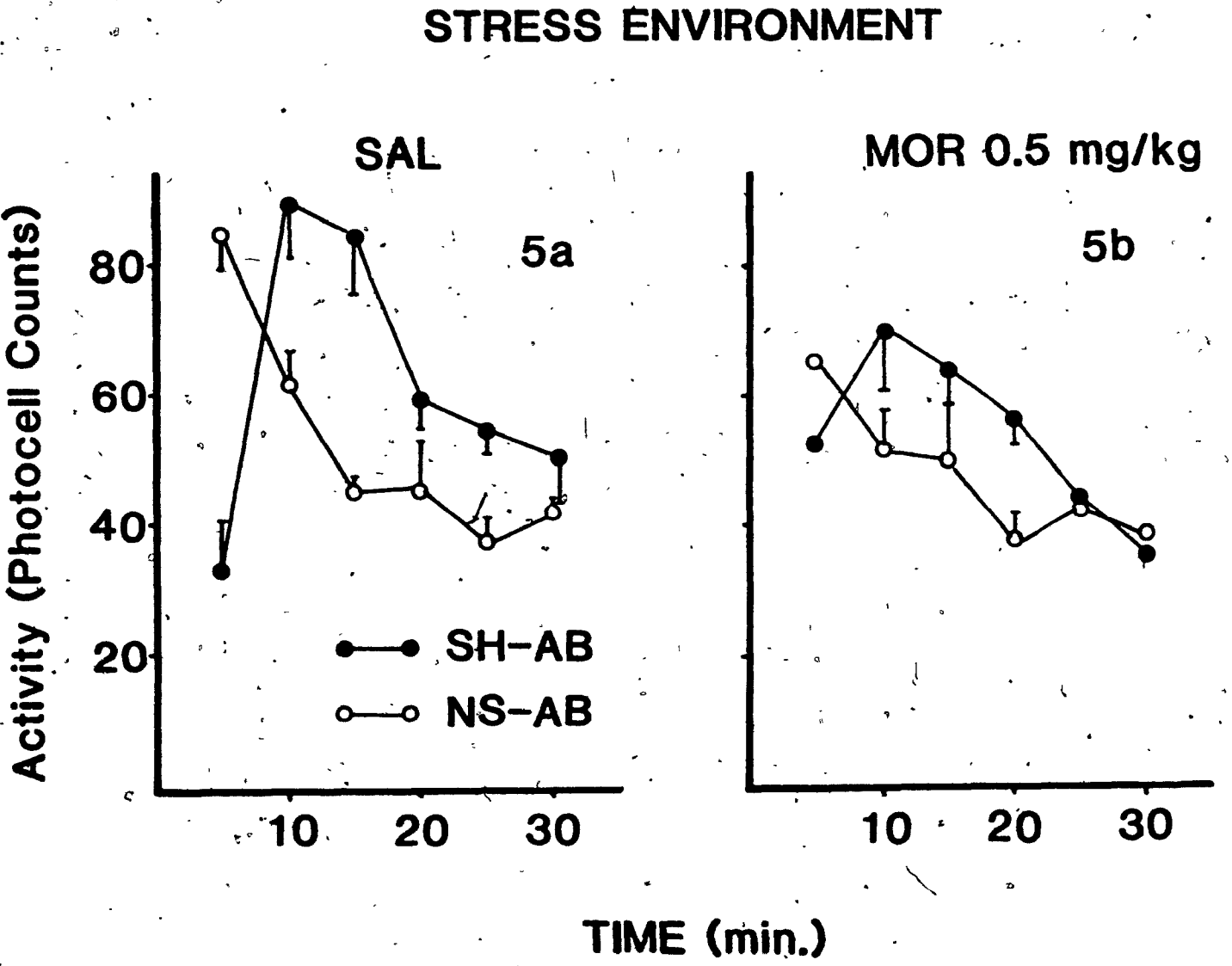
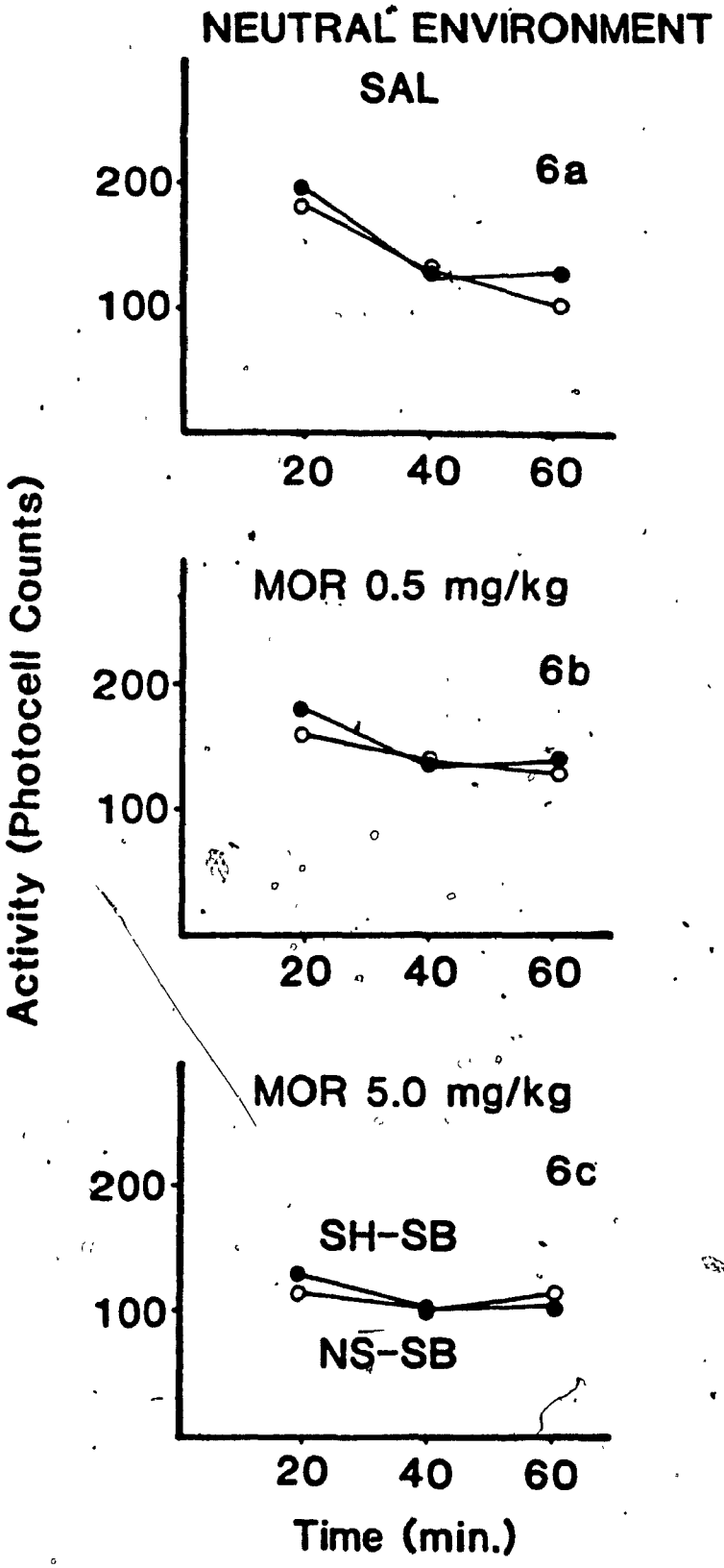

STRESS ENVIRONMENT
SAL
MOR 0.5 mg/kg
5a
5b
Activity (Photocell Counts)
80
60
40
20
SH-AB
NS-AB
10
20
30
10
20
30
TIME (min.)

Figure 6. Group mean activity counts (± 1 S.E.M.) for SAL (6a), MOR (0.5 mg/kg, i.p.) (6b), and MOR (5.0 mg/kg, i.p.) (6c) injected animals tested in a neutral environment immediately following re-exposure to the stress environment. Experiment 1.


NEUTRAL ENVIRONMENT
SAL
6a
200
100
20
40
60
MOR 0.5 mg/kg
6b
200
100
20
40
60
MOR 5.0 mg/kg
6c
200
SH-SB
100
NS-SB
20
40
60
Time (min.)
Activity (Photocell Counts)

Discussion

This experiment clearly demonstrated that inescapable foot-shock can significantly alter motor behaviour. Immediately after stress, animals displayed a short-lived, but profound, freezing response that was followed by a period of increased locomotion. Over days this elevated activity underwent sensitization similar to that seen following repeated opiate administration. Across test sessions, the hyperactivity successively appeared earlier, and covered a larger period of time, reaching a peak length on day four. For reasons that are not clear, the duration of this effect was reduced on day 5 however, the hyperactivity did begin earlier than on any of the previous days. These findings support the hypothesis that acute stress induces hyperactivity, and that repeated stress induces sensitization of this activity. These observations suggest that stressors might engage a physiological substrate that is also involved in morphine sensitization.

Elevated activity levels were also seen in animals re-exposed to the activity boxes, provided they had previously been shocked there. This observation implies that the stress experience can become associated with the environment in which it was given. When tested on the re-exposure no-shock days, the excitatory response to morphine was also enhanced in animals that had been shocked on previous days. In effect, previously foot-shocked rats responded to acute systemic morphine administration in a manner reminiscent of animals having had prior experience with the opiate.

In this experiment, increased locomotor activity was found immediately post-stress (either foot-shock or re-exposure to the environment previously associated with stress) in animals shocked in the activity boxes, but not in animals stressed in another environment. At least two explanations may account for this difference. One is that the endogenous opiate-like effects or neurochemical changes underlying the enhanced locomotor activity obtained from previously stressed animals requires continuous exposure to the stress environment for their full expression. If this were the case then testing in a neutral environment could fail to elicit these changes. Another possibility is that the act of transfering animals from the shock chambers to the neutral activity boxes leads to conflicting effects that counteract the stress-induced activity enhancement. As reported by Nabeshima et al., (1983), and replicated here, the first few minutes following stress are characterized by freezing. We now report that if animals are left in the stress environment, the freezing is followed by hyperactivity. However, once engaged, this multi-staged response may require the continued exposure to the stress environment to allow for the full expression of the ensueing hyperactivity. Therefore, if animals are removed from the stress environment during their freezing stage, the hyperactivity may not develop. Possibly, once freezing has begun, continued exposure to a stressor is required to reveal the potential hyper-motility. It might follow from this that if previously stressed animals were tested for activity without prior re-exposure to the

stress environment, then the potentiated activity response might appear. It is possible that if under these conditions the initial freezing response were not invoked, then an unemcumbered elevated activity level could be expressed in a neutral environment.

As discussed earlier, stress has been reported to potentiate the locomotor activity or stereotypy response from, respectively, a low or high, acute, systemic amphetamine administration (Antelman et al., 1980; MacLennan and Maier, 1983; Herman et al., 1984; Robinson et al., 1985). In each of these studies, the augmented response was obtained in a neutral environmemt. These observations support the possibility that a stress-induced enhancement of locomotor behaviour might be found in a neutral environment with morphine. Considering these reports by other investigators the critical difference between the amphetamine studies and this study might be that our animals were tested immediately following stress as opposed to the amphetamine studies where the potentiated response was recorded at a later time.

The following experiments attempted to address these issues.

EXPERIMENTS 2a and 2b

In the previous experiment increased locomotor activity was found in animals tested immediately post-stress (either footshock or conditioned stress) in the stress environment, but not in animals stressed in another environment. The present experiment attempted to examine more fully the potential for seeing stress-induced hyperactivity in animals stressed in one set of boxes and tested in another. In the present experiment, animals were shocked in a distinctive environment separate from the activity box, but, as in the previously discussed stress-amphetamine interaction studies, they were tested a minimum of 24 hours later. It was proposed that this temporal separation between stress and activity monitoring would allow for the expression of an augmented activity response without a counteracting freezing response.

To address this possibility, groups of animals were shocked for either five or 10 consecutive days. Twenty-four and 48 hours later, their locomotor activity levels were recorded in novel activity boxes immediately following either morphine challenge (0.5 mg/kg i.p.) or saline vehicle injections. If it were found that previously shocked animals displayed increased activity responses, this would support the hypothesis that prior exposure to stress can enhance the excitation of locomotor activity independent of the current presentation of stress. If hyperactivity in previously stressed animals were also displayed under the influence of morphine, it would reinforce the suggestion that the

potentiation of activity by prior exposure to a stressor involves opioid-related sites of action. Conversely, the absence of differences in activity between previously stressed and non-stressed animals would suggest that the stress-induced potentiation of activity seen in Experiment 1 was a situation-specific effect requiring the presentation of stress to be expressed.

Methods

Subjects

Thirty-two male Wistar rats obtained from Charles River Canada Inc. (St. Constant, Quebec) served as subjects. The animals weighed 275-300 grams upon arrival to the laboratory with testing beginning two weeks later. Midway through the study, one animal was dropped from the experiment due to illness.

Apparatus

The apparatus used in the experiment was as described in Experiment 1.

Design and Procedure

Pre-Exposure to Stress: Different groups of animals were exposed to either five (n=7) consecutive days of 30-minute intermittant, inescapable, foot-shock sessions at 0.8 mA or 10 days (n=8) at 0.4 mA. respectively. Shock was delivered 1 sec/10 secs. Appropriate no-shock control groups (n=8) were exposed to the shock chambers without shock delivery.

Morphine and Saline Test Days: Twenty-four and 48 hours following the final shock session, animals were monitored for activity levels for 60 minutes in distinctive ABs located in a room sep-

arate from the shock boxes. Immediately prior to being placed in the AB, the rats were injected with either morphine (0.5 mg/kg i.p.) or saline. Administration was counterbalanced across days. The five-day and 10-day experiments were run successively at a two-month interval. For that reason, the data from each experiment was analyzed separately.

Results

Morphine and Saline Test Days

As illustrated in Figure 7a, five days (Experiment 2a) of intermittent, inescapable, foot-shock increased slightly the activity levels during the initial 20 minutes of testing after morphine injection. Although the main effect for stress was not significant, this initial hyperactivity was reflected in a stress x time interaction [$F(2,26)=3.28$, $p=.052$]. On the other hand, following saline injections, animals from the shock group did not differ from the no-shock control group animals (See Figure 7b).

As shown in Figure 8a, 10 days (Experiment 2b) of shock increased locomotor activity during the 40 minutes following the saline injection. Again, the main effect for stress was not significant. The initial elevation of activity is, however, evident from the significant stress x time interaction [$F(2,28)=3.84$, $p=.043$]. Following morphine challenge, shocked

Figure 7. Group mean activity counts (± 1 S.E.M.) for MOR (0.5 mg/kg, i.p.) (8a) and SAL (8b) injected animals tested in a novel environment in Experiment 2a (five days of shock).

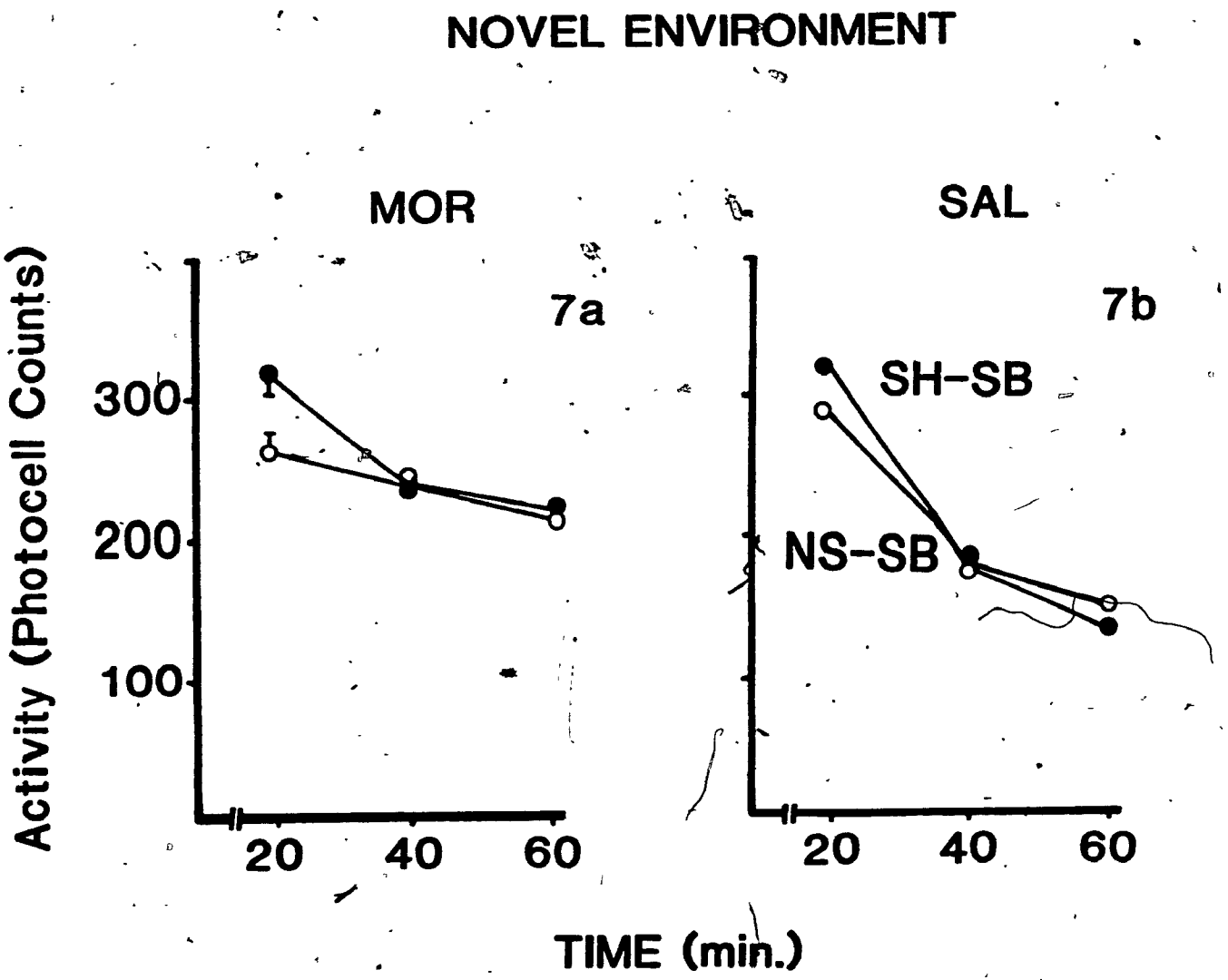
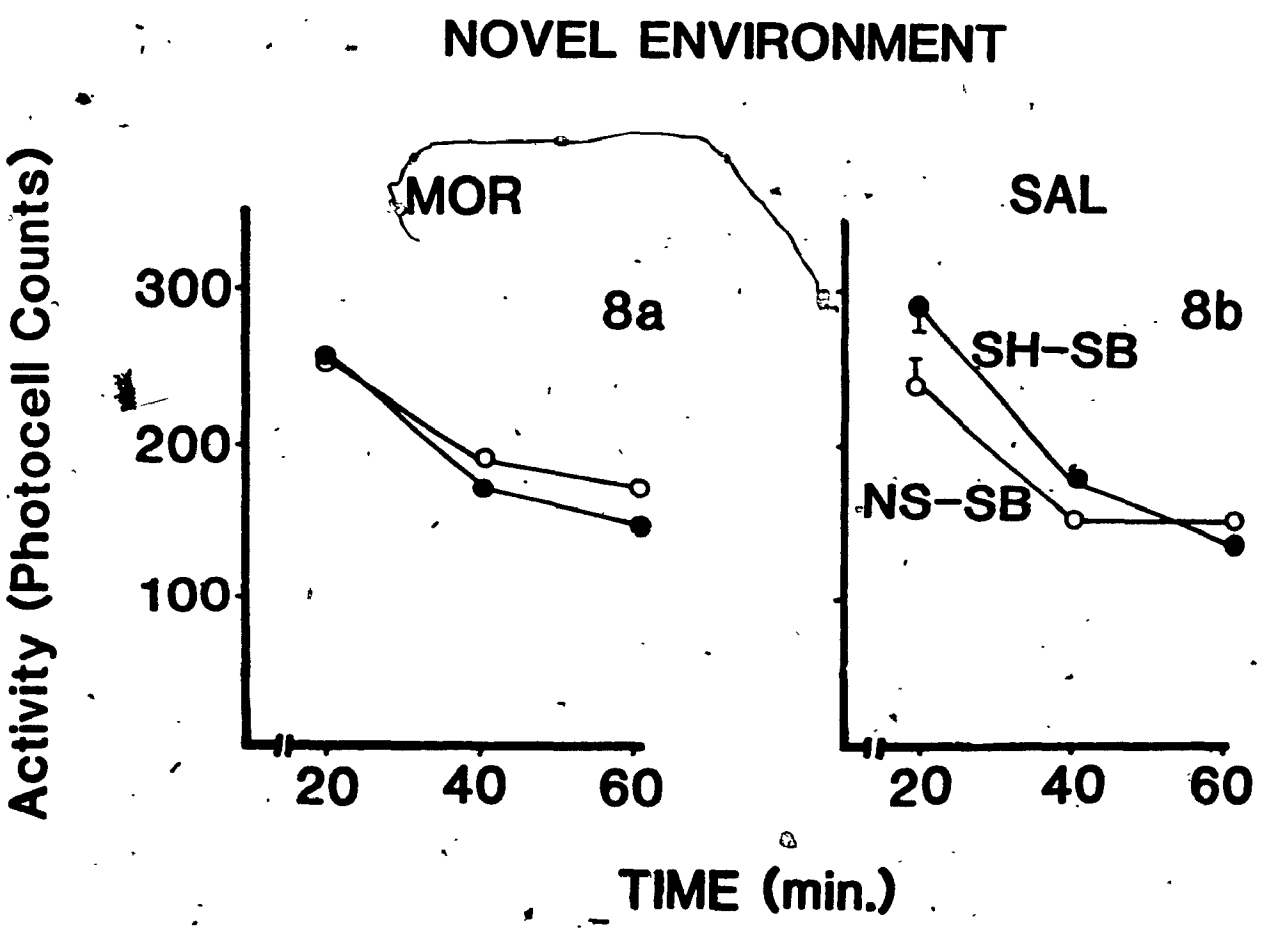

NOVEL ENVIRONMENT
MOR
SAL
7a
7b
SH-SB
NS-SB
Activity (Photocell Counts)
300
200
100
20
40
60
20
40
60
TIME (min.)

Figure 8. Group mean activity counts (± 1 S.E.M.) for MOR (0.5 mg/kg, i.p.) (8a) and SAL (8b) injected animals tested in a novel environment in Experiment 2b (10 days of shock).


NOVEL ENVIRONMENT
MOR
SAL
8a
8b
SH-SB
NS-SB
300
200
100
20
40
60
20
40
60
Activity (Photocell Counts)
TIME (min.)

subjects did not differ from no-shock subjects in this experiment (See Figure 8b).

Discussion

These two experiments were done in an attempt to elucidate the effects of previous shock exposure on activity measured in a neutral environment. The results, however, are inconsistent and equivocal. Five days of prior shock exposure failed to enhance locomotor activity following saline treatment, although evidence for a weak potentiation of the activity response to morphine was present. In the other experiment, animals exposed to 10 days of shock did not differ from control subjects when tested under morphine, but did display a slightly augmented activity level when tested with the saline vehicle. This fragile, inconsistent potentiation in a novel environment was unexpected, and failed to distinguish clearly between the two hypotheses proposed to explain the difference observed in the manifestation of the effect of prior exposure to stress on the activity levels of animals tested either in the stress or non-stress environment. On one hand, the fact that at least weak potentiation of activity was found in these experiments might suggest the expression of sensitization does not require re-exposure to stress: if stress were required, then even a weakly augmented response would not be expected. On the other hand, the novel environment used in the present experiments may not have provided the neutral, non-invasive conditions necessary to test the hypothesis. It is

possible that novelty, itself, induces a stress response that can partially substitute for shock. Testing in a novel environment, even though it was never associated with shock, may have been sufficiently stress-inducing to mimic the effects of re-exposure to the shock environment that occurred in Experiment 1. The fact that it was possible to distinguish in part between the shock and no-shock groups may imply the presence of a previously induced sensitization to the stimulating action of stress.

Although the data from Experiments 2a and 2b are somewhat weak, it would appear that they confirm in part the finding from Experiment 1 that animals pre-exposed to shock tend to be more active than non-shocked animals tested with either morphine or saline. A new finding was that under the conditions of the experiment stress-induced hyperactivity was transferable to a novel environment and not specific to the conditioned stress situation. The fact that it did transfer, however, may have been due to novelty-stress substituting for the shock-stress. Unfortunately, in this experiment, it was not possible to determine whether differences between previously shocked and non-shocked animals would have been found in a truly neutral environment.

EXPERIMENT 3

The present experiment attempted to address more adequately the question of whether stress-induced activity enhancement was specific to a stressful environment. The effect of testing in a neutral environment was again examined, but with an appropriate control for novelty. As in the previous two studies, animals were exposed to daily foot-shock. Unlike the previous experiments, the five shock-exposure days were followed by five days of habituation to the activity box. Activity levels were monitored throughout this period. Following the five habituation days there were two additional test days with morphine and saline injections. All animals received morphine counterbalanced with saline for the two days. It was hypothesized that if exposure to stress on the test day was necessary for the expression of elevated activity, then over habituation days, as the stress induced by the novel test box diminished, the previously shocked subjects should become progressively iso-active to the no-shock control group. If, however, current stress were not necessary, and the novelty stress were a confounding variable, then habituation to the AB should augment the difference between the previously shocked and no-shock animals. During the morphine test, it is expected that activity will change due to the same reasoning; should habituation to the AB neutralize the confounding nature of a novel test environment, then the previously shocked animals will be more active than the no-shock group following morphine injection.

Methods

Subjects

Sixteen male Wistar rats obtained from Charles River Canada Inc. (St. Constant, Quebec) served as subjects. The animals weighed from 275-300 grams upon arriving with testing beginning two weeks later.

Apparatus

The apparatus used in this experiment was as described in Experiment 1.

Design and Procedure

Pre-Exposure to Stress: Once a day for five consecutive days, a group of animals (Sh-SB) was exposed to intermittent, inescapable, foot-shock for 30 minutes. The shock parameters were set at 0.4 mA shock, 1 sec/10 secs. A no-shock control group of animals (NS-SB) was placed in the shock boxes for 30 minutes without foot-shock.

Post-Stress Test Days: Twenty-four hours after the last shock session, all animals were monitored for activity for 45 minutes in distinctive ABs located in a room separate from the SBs. This procedure was repeated for a total of five consecutive days. On the subsequent two days, a morphine test and a final saline test were performed following the same basic procedure as above. However, all animals were now tested for 60 minutes and received either a morphine (0.5 mg/kg i.p.) or vehicle injection counterbalanced for days.

Results

As illustrated in Figure 9, previously shocked animals did not differ from the no-shock control group during their initial exposure to the activity boxes, but, on the subsequent four days, previously stressed animals appeared to be more active than non-stressed animals. The analysis of variance carried out on the total session scores for all five days revealed a significant stress x day interaction [$F(4,56)=5.71$, $p<.001$], but only a weak effect for stress [$F(1,14)=3.76$, $p=.07$]. An analysis of variance that included only the four days following the initial test day showed the stressed animals to be clearly more active than non-stressed animals [$F(1,14)=6.26$, $p<.025$]. Both groups showed a gradual decline in activity over days [$F(3,42)=4.83$, $p<.006$].

Morphine and Saline Test Days

Figure 10 shows the results for the two 60-minute test sessions with either morphine or saline. On these test days the previously shocked animals did not differ from the no-shock group.

Discussion

The present experiment further confirms the finding that spontaneous locomotor activity is increased in animals previously exposed to repeated shock as compared to non-shocked animals. More specifically, this experiment demonstrates that stress-induced locomotor hyperactivity does not require a stressful

Figure 9. Group mean 45-minute activity counts (± 1 S.E.M.) over test days 1-5 in a neutral environment following five previous shock sessions in Experiment 3.

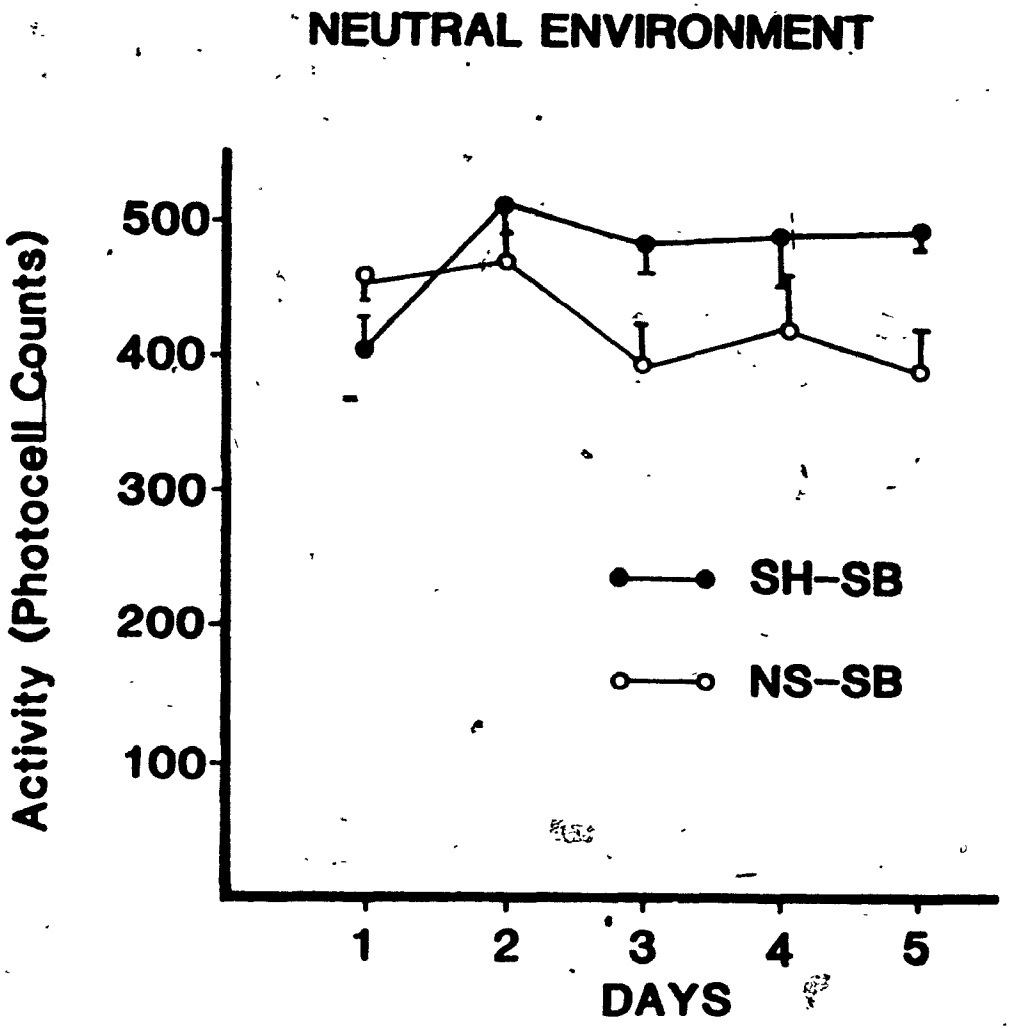
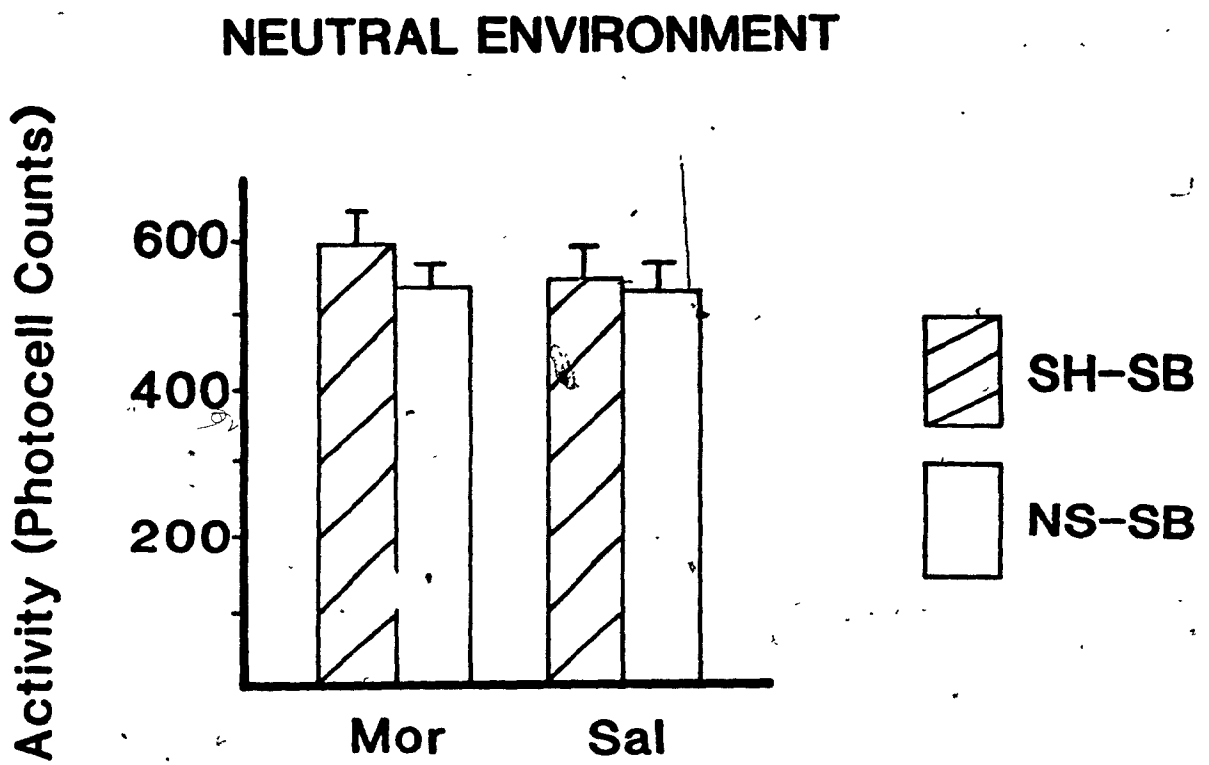

NEUTRAL ENVIRONMENT
Activity (Photocell Counts)
500
400
300
200
100
SH–SB
NS–SB
1
2
3
4
5
DAYS

Figure 10. Group mean 60-minute activity counts (+ 1 S.E.M.) for MOR (0.5 mg/kg, i.p.) or SAL injected animals tested in a neutral environment following five previous shock sessions in Experiment 3.


NEUTRAL ENVIRONMENT
Activity (Photocell Counts)
600
400
200
Mor
Sal
SH–SB
NS–SB

environment to express itself, but can be seen in a relatively neutral environment.

One possible explanation of the positive findings of the present experiment that has not been considered and that could be offered is that previously stressed animals might be more active in the neutral environment because they could have learned that the activity boxes were safe from shock. However, if this were the case, it would be expected that the magnitude of the obtained effect might diminish over habituation days. As fear of the possibility of being shocked extinguished over successive days in the activity box, the hyperactivity might have been expected to decline in a parallel manner. This was not found to be the case; rather the augmented activity persisted over the four habituation days.

The absence of an experimental effect on the subsequent two test days with morphine and saline is perplexing. At this point, an explanation is not readily apparent. Possibly, the lack of an effect on these days is an unfortunate anomaly. The following experiment attempted to more extensively study the interaction between morphine and previous shock on activity levels in a neutral test environment.

EXPERIMENT 4

As was observed in Experiment 1, the enhanced activity obtained immediately post-shock progressively increases or sensitizes over successive shock days.

The present experiment examined whether stressed animals would reveal increased locomotor activity to repeated administrations of low dose systemic morphine as compared to a non-stressed control group. This was tested directly by repeatedly injecting animals with a low dose of morphine (0.5 mg/kg, i.p.) during the days following shock exposure. If prior stress experience sensitizes animals to the opiate's stimulant properties, then previously shocked rats should reveal an augmented activity response to repeated morphine administration. Behavioural monitoring was carried out in activity boxes separate and distinctive from the shock chambers.

Methods

Subjects

Sixteen male Wistar rats obtained from Charles River Inc., (St. Constant, Quebec) served as subjects. The animals weighed 275-300 grams upon arrival with testing beginning two weeks later. Midway through the procedure, one animal was dropped from the experiment due to illness.

Apparatus

The apparatus used in this experiment was as described in Experiment 1.

Design and Procedure

Pre-Exposure to Stress: In the A.M., all animals were monitored for activity for 45 minutes in the AB. In the P.M., one group of animals (Sh-SB) was exposed to 30 minutes of intermittent, inescapable, foot-shock. The shock parameters were set at 0.4 mA shock, 1sec/10secs. A no-shock control group (NS-SB) was carried to the SB for 30 minutes without foot-shock. The distinctive SB were located in a room separate from the AB.

Morphine and Saline Test Days: Twenty-four and 48 hours following the final shock session, animals were returned to the AB for 60 minutes. Subjects were counter-balanced for either morphine- or saline-injection on the two days. Two days later, daily morphine administration in the AB continued for seven further days. This was followed by a day without testing, and then one final morphine test. Five days after this last morphine day, animals were tested with a second saline day.

Results

Data from the first 45-minute test session, that occurred before any animals had been shocked, were subjected to an analysis of variance for stress group x 15-minute interval. Activity scores from the next four 45-minute test sessions, following the beginning of shock sessions, were subjected to an analysis of variance for stress group x days x 15-minute interval. The data from the subsequent 60-minute morphine and saline test sessions were collapsed into 20-minute intervals for analyses.

As plotted in Figure 11, the designated stress group did not

differ from the control group prior to a session of shock. This was reflected in the analysis of variance where the main effect for stress group was not significant [$F(1,14)=0.48$]. On the four days following the initial shock period, however, the stressed animals were more active than the no-shock animals, as reflected by a significant main effect for stress [$F(1,14)=4.83$, $p=.043$]. During the first 60-minute test day, when animals were injected with saline the activity level of previously shocked animals was also significantly greater than that of non-shocked animals [$F(1,13)=5.78$, $p<.04$]. Furthermore, the difference was also apparent following an acute, systemic morphine injection [$F(1,13)=4.94$, $p<.05$] (See Figure 12).

The activity scores from the repeated morphine tests were subjected to an analysis of variance. This included the first exposure to morphine, that was counterbalanced with saline over two days, and the following eight daily morphine administrations. As illustrated in Figure 13, and reflected by a significant stress effect [$F(1,13)=6.92$, $p<.02$], previously shocked animals displayed an increased locomotor activity level that persisted over the repeated morphine injection days. Additionally, over days, both previously shocked and non-shocked animals displayed a progressively enhanced locomotor activity response to repeated

Figure 11. Group mean 45-minute activity counts (± 1 S.E.M.) over test days 1-5 in a neutral environment in Experiment 4. Test Day 1 was before animals had been shocked; Test Days 2-5 were after shock sessions had begun.

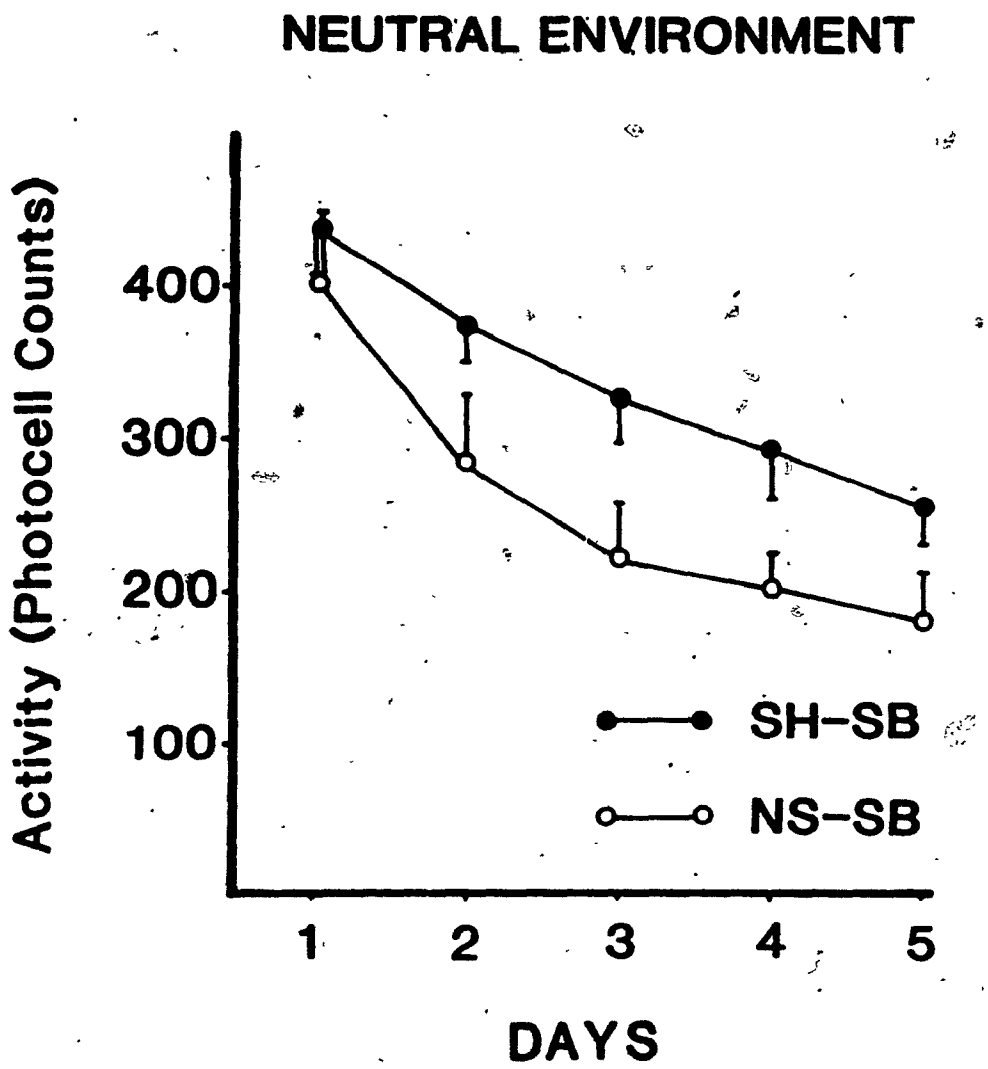

NEUTRAL ENVIRONMENT
Activity (Photocell Counts)
400
300
200
100
1
2
3
4
5
DAYS
SH-SB
NS-SB

Figure 12. Group mean 60-minute activity counts (+ 1 S.E.M.) for the animals' first MOR (0.5 mg/kg, i.p.) and SAL injections. Testing was in a neutral environment. Experiment 4.

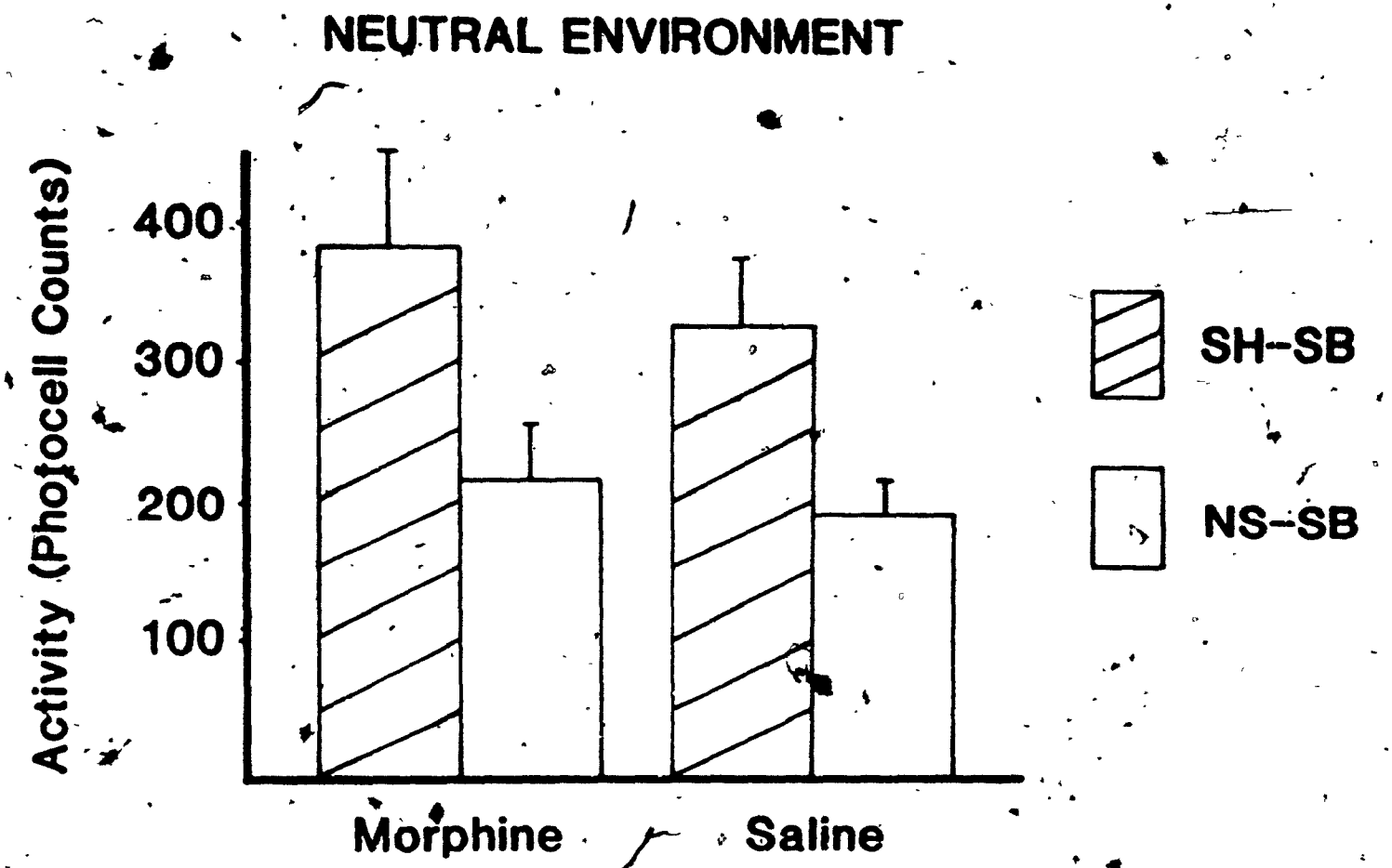
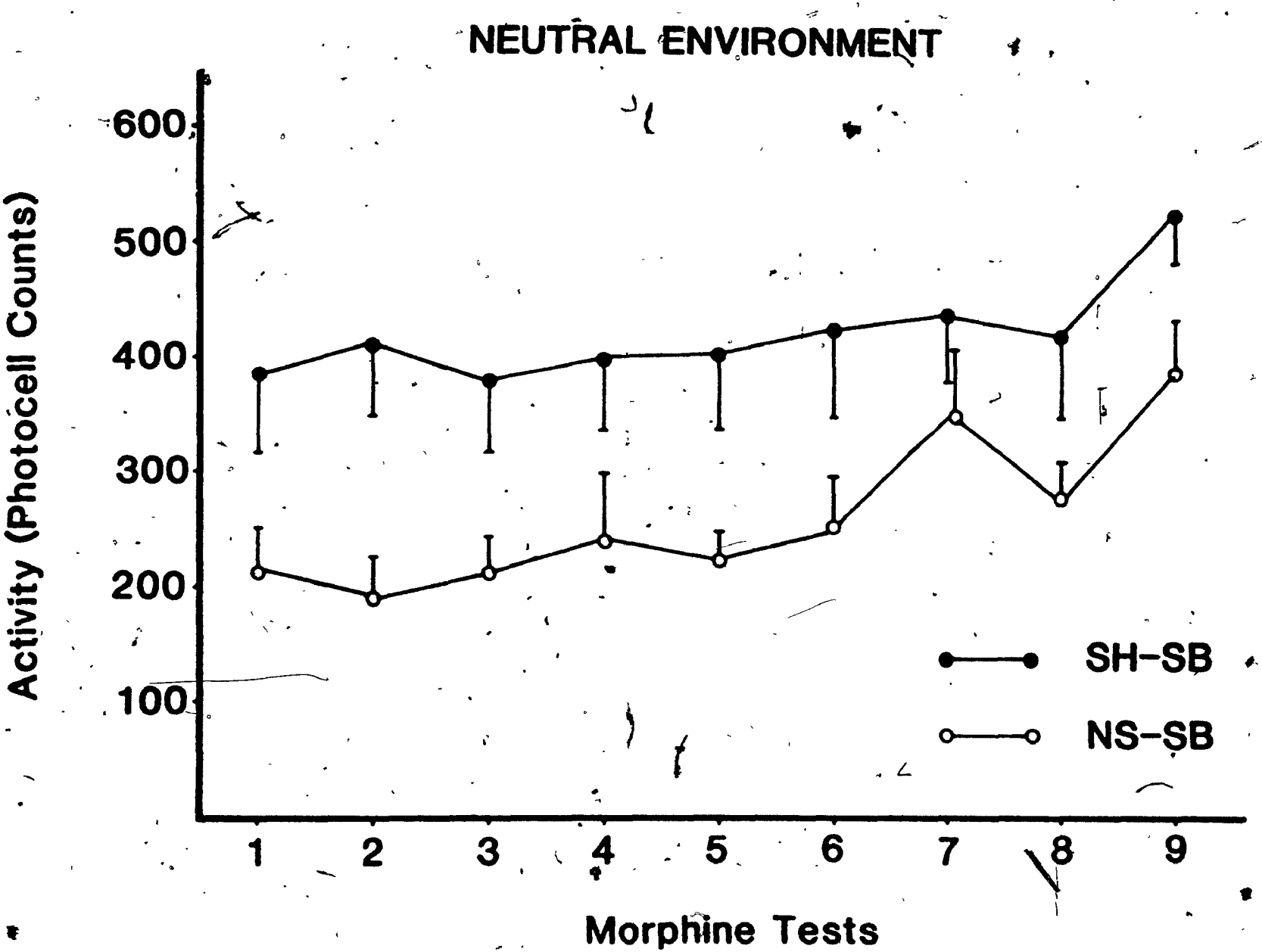

NEUTRAL ENVIRONMENT
Activity (Photocell Counts)
400
300
200
100
Morphine
Saline
SH–SB
NS–SB

Figure 13. Group mean 60-minute activity counts (± 1 S.E.M.) for the repeated MOR (0.5 mg/kg, i.p.) tests in a neutral environment. Experiment 4.


NEUTRAL ENVIRONMENT
Activity (Photocell Counts)
600
500
400
300
200
100
1
2
3
4
5
6
7
8
9
Morphine Tests
SH-SB
NS-SB

morphine administration. This trend to become more active over sessions is indicated by a significant effect of day [$F(8,104)=5.01$, $p<.001$].

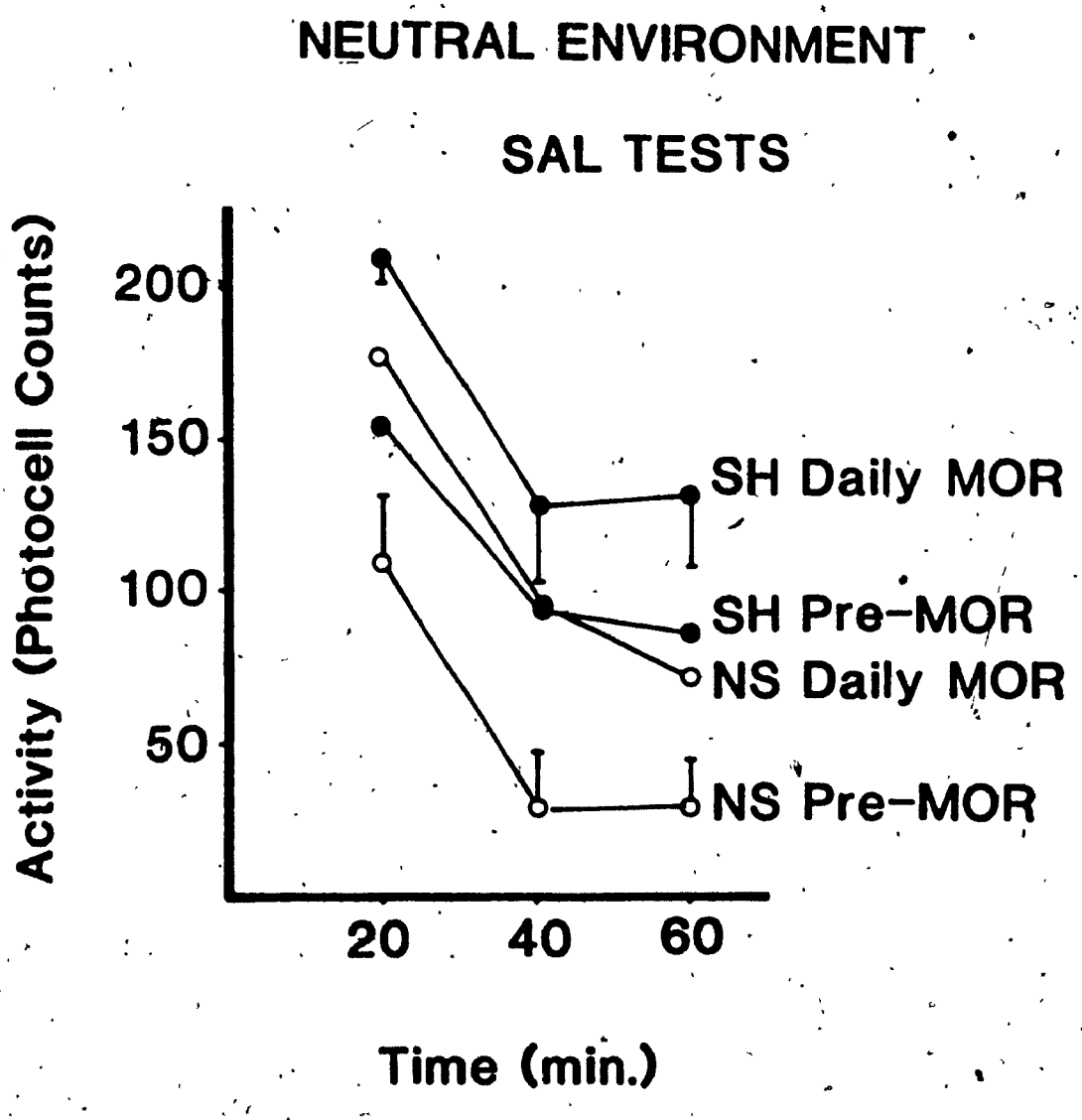
Following the morphine days, there was a second saline test day during which the previously shocked group continued to be more active than non-shocked animals [$F(1,14)=5.78$, $p<.03$]. In order to test whether repeated exposure to morphine increased spontaneous activity levels with saline, data from the first saline test day, carried out prior to morphine experience, and from the second second saline test day, post-morphine tests, were subjected to an analysis of variance for stress x two saline days x 20-minute interval. As illustrated in Figure 14, all animals were more active on this second saline test day than they had been on the first saline test [$F(1,13)=10.06$, $p<.008$].

Discussion

This study supports the suggestion that previous exposure to stress is capable of increasing spontaneous and morphine-induced activity in a neutral environment. Over successive shock days, the stressed animals became progressively more active relative to the no-shock subjects. Following the five days of shock sessions, these animals also displayed a sensitized response to the excitatory effects of morphine.

This stress-induced potentiation appears to be relatively long-lasting. Eleven days after the last shock session, an

Figure 14. Group mean activity counts (± 1 S.E.M.) for SAL Test Day 1 (Before repeated MOR tests) and SAL Test Day 2 (After repeated MOR tests) in a neutral environment Experiment 4.


NEUTRAL ENVIRONMENT
SAL TESTS
Activity (Photocell Counts)
200
150
100
50
20
40
60
Time (min.)
SH Daily MOR
SH Pre-MOR
NS Daily MOR
NS Pre-MOR

augmented response to morphine was still evident. This response to repeated morphine increases over days, thereby implying the development of a sensitized or conditioned response to the drug by both the stressed and non-stressed animals. Furthermore, with a saline injection 17 days post-shock, previously stressed animals were still hyperactive relative to the no-shock group. It is noteworthy that both stressed and non-stressed animals were more active on this second saline test day. This finding suggests that the rats have come to associate the activity box with morphine administration and therefore reveal a conditioned enhancement of morphine-induced locomotion.

Interestingly, following the nine test days of repeated exposure to morphine, no-shock animals tested under saline were as active as the shocked animals had been in the saline test prior to repeated morphine. These data suggest that repeated morphine administration mimicked the effect of exposure to shock on subsequent spontaneous activity.

General Discussion

A growing body of research has shown that endogenous opioids are released in response to a wide variety of intense stress-inducing stimuli. The evidence includes direct measures of brain opioid levels immediately post-stress (Madden et al., 1977), as well as indirect measures such as changes in stress-induced behaviours that have been shown to be reversed by specific opioid receptor blockers (Amir and Amit, 1978; Williams et al., 1984; Ushijima et al., 1985). Because it is widely accepted that exogenous opiates exert their effects by acting upon the same receptor mechanisms as those occupied by the endogenous opioids, it is not surprising that many investigators have searched for parallels in the behaviours affected by morphine administration. For example, as does morphine, stress produces a form of analgesia that is reversible and preventable by specific opiate receptor antagonists (Amir and Amit, 1978; Lewis et al., 1980; Maier et al., 1980; Grau et al.,1981). This stress-induced analgesia (SIA) summates with analgesia from morphine administered either centrally (Appelbaum and Holtzman, 1985) or systemically (Appelbaum and Holtzman, 1984). Interestingly, SIA also shows a cross-tolerance to previous morphine injections (Chesher and Chan, 1977; Drugan et al., 1981; Nabeshima et al., 1983; Terman et al., 1986). That is, animals previously injected with morphine display a reduced analgesic response following forms of stress that induce opioid mediated analgesia. This cross-tolerance is not evident with non-opioid mediated SIA. Cross-

tolerance is also apparent in the reverse experiment; repeatedly shocked animals display a reduced analgesic response to subsequent morphine injections (Nabeshima, et al., 1983).

Parallel interactions between stress and morphine-induced temperature changes have also been reported. Stress appears to produce an opioid mediated hyperthermia (Stewart and Eikelboom, 1979) that can summate with concurrent morphine administration to either augment the elicited hyperthermia from low doses or the hypothermia from high doses (Stewart and Eikelboom, 1981; Appelbaum and Holtzman, 1984; Appelbaum and Holtzman, 1985; Zelman, Tiffany, and Baker, 1985).

Testing in the Stress Environment

In the present experiments, an attempt was made to demonstrate an interaction between stress and the excitatory effect of morphine on locomotor activity. In Experiment 1, in which the behaviour of animals was monitored immediately post-shock in the shock environment, acute stress elicited a biphasic activity response; profound motor suppression was followed by increased activity that exceeded that of the no-shock control group. Five days of repeated shock produced a progressively augmented locomotor level that began earlier and earlier in time and lengthened in duration. It should be noted, however, that the initial freezing response remained intact over days; it neither increased nor decreased. The enhanced locomotor response from repeated stress is reminiscent of the increased or sensitized activity

response obtained from repeated morphine administration (Joyce and Iversen, 1979; Vezina and Stewart, 1984; Kalivas, 1985; Kalivas et al., 1985). Furthermore, when the animals from Experiment 1 were returned to the shock environment without shock, previously stressed animals remained more active than non-stressed animals, an effect that appears to parallel the greater activity observed in saline-injected animals returned to an environment previously associated with morphine (Vezina and Stewart, 1984). This increased activity was observed as long as 19 days after the last stress session, suggesting that it is a relatively permanent effect and not a lingering carry-over from the shock itself.

When previously stressed animals were returned to the shock environment without shock following a morphine injection, they displayed an enhanced or sensitized locomotor response to the opiate compared to non-shocked control group animals. This stress-induced potentiation was evident with a low (0.5 mg/kg i.p.) or a medium dose of morphine (5.0 mg/kg i.p.).

Overall, these results appear to support the idea that exposing animals to stressors, can activate physiological substrates common to those engaged by the action of morphine at sites that promote hyperactivity. In light of these results, it seems plausible to consider that the overlapping sites of action involve opioid activated pathways that, as discussed above, are normally activated by endogenous opioids released during exposure to a stressful event. Supporting this suggestion is a recent

report by Schnur, Martinez, and Hang (1986). These researchers observed that a low dose of systemic morphine elicited hyperactivity immediately following mild stress, but hypoactivity after acute severe stress. These findings support the contention that stress induces opioid release that can summate with an exogenous administration to potentiate the locomotor response. When combined with the results from Experiment 1, it might be further suggested that repeated stress sufficiently replicates actions of repeated morphine administration to produce an increased or sensitized locomotor response to an acute morphine injection.

Testing in a Neutral Environment

In Experiment 1, stress-induced potentiation of spontaneous and morphine-activated locomotor activity was demonstrated when testing was performed in the environment associated with shock. It was suggested that this effect was due to a release of endogenous opioids elicited by stress (either shock or re-exposure to the environment previously associated with shock) that resulted both in spontaneous hyperactivity and summated with morphine injections to potentiate their influences on locomotor activity. Furthermore, the repeated stress-induced release of opioids might have effected an enhanced reponse to the excitatory locomotor actions of subsequent morphine injections.

Under certain conditions in the present studies, as well as in the stress and amphetamine cross-sensitization literature, the locomotor activating effects of prior stress experiences were expressed in a neutral environment. As discussed in the

Introduction, previous exposure to stress has been reported to potentiate the motor activity effects of amphetamine when testing was performed 24 hours later in a neutral environment (Antelman et al., 1980; MacLennan and Maier, 1983; Herman et al., 1984; Robinson et al., 1985). In Experiment 4 these effects were replicated and extended with morphine. When tested in a neutral environment to which the animals had previously been habituated, shocked animals displayed a progressively increasing spontaneous activity response, relative to the no-shock control group, 20 hours after each successive stress session. On a subsequent morphine test day, previously shocked animals displayed an enhanced or sensitized locomotor response to the opiate. This difference in activity between groups of previously shocked and non-shocked animals was maintained following repeated exposure of both groups to an opiate drug. After receiving morphine injections for a period of nine days in the neutral AB, previously shocked animals remained hyperactive relative to the no-shock control group.

Another observation of interest was that following the nine days of repeated exposure to morphine, no-shock animals tested under saline were as active as the shocked animals had been in the saline test prior to repeated morphine. These data suggest that repeated morphine administration mimicked the effect of exposure to shock on subsequent spontaneous activity.

Implications for Underlying Substrates

As suggested above, the stress-induced hyperactivity obtained in Experiment 1, when animals were tested in the stress environment either immediately post-shock or on subsequent days without shock presentation, might have been due to a stress-induced release of endogenous opioids that mimicked the locomotor excitatory effects of a low dose of morphine. One explanation of the changes observed after repeated shock could be that over successive days of shock, this opioid release was elicited earlier in time and to a greater degree. A result of such a potentiated opioid release could have been the observed progressively augmented locomotor response by previously stressed animals as compared to the non-stressed control group. The only evidence there is to support such a suggestion is the anticipatory or conditioned release of opioids that has been reported to occur in environments previously associated with stressors (Madden et al., 1977). Another explanation of the observed effects arises from the growing body of evidence implicating the mesolimbic DA system in sensitization to the activity effects of opiates and stimulants. Repeated administration of opiates (Joyce and Iversen, 1979; Vezina and Stewart, 1984; Kalivas, 1985; Kalivas et al., 1985), amphetamine (Robinson and Becker, 1982; Robinson, 1984; Kolta et al., 1985), or apomorphine (Kinon, Merson, and Kane, 1984) has been shown to lead to a progressively increased locomotor activity response. Additionally, cross-sensitization between these various drugs that all interact with the DA system has

also been reported. The observations include cross-potentiation between DALA and amphetamine (Kalivas, 1985), amphetamine and morphine (Stewart and Vezina, 1986), morphine and apomorphine (Martin and Takemori, 1985), apomorphine and amphetamine (Riffee and Wilcox, 1985), and methamphetamine and apomorphine (Watanabe and Taniguchi, 1986). Thus it appears that the sensitization of activity observed may be due not so much to an increased amount of opioid, or to an increase in sensitivity of opioid receptors, but rather to a change in the output of the DA neuron in response to repeated activation. The response to severe stress includes increased mesolimbic DA turnover (Fadda et al., 1978; Herman et al., 1978; Herman et al., 1984) that is also followed by a potentiated motor activity response to amphetamine (Antelman et al., 1980; MacLennan and Maier, 1983; Herman et al., 1984; Robinson et al., 1985). Considering these varied reports, it would appear reasonable to suggest that engaging the mesolimbic DA system via either pharmacological or environmental events leads to an elevated or sensitized response to future activation. In further support of this notion, the recent study by Vezina et al., (1986) reported that intra-VTA injection of either morphine or the specific mu opioid agonist DAGO resulted in increased locomotor activity that became enhanced over repeated administrations. As noted by these investigators, in light of previous reports that suggest a DA-independent, opioid activated, excitatory system in the NAS, it was of interest to observe that a progressively increased response to the intra-NAS application of these

agents did not result.

It would seem likely that the present experiments have also engaged stress-induced opioid release and the plausible ensuing increased responsiveness of mesolimbic dopaminergic neuronal activity. In support of this suggestion, Kalivas, Richardson-Carlson, and Van Orden (1986) have reported very recently a study similar to the present one. They found that the excitatory effect of DALA microinjected directly into the VTA, was potentiated by previous repeated shock treatment. Furthermore, previous daily intra-VTA DALA was shown to increase DA metabolism in the NAS in response to an acute, mild, shock session. These findings clearly implicate the mesolimbic DA system in the cross-sensitization observed between opiates and stress.

To summarize, consideration of the present experiments in the light of the above evidence makes it seem likely that the stress-induced potentiated spontaneous and morphine activated locomotor activity responses observed in the neutral test environment involve a mesolimbic DA system that is functionally more responsive to activating events following the application of stressors to the organism. The increased locomotor responses displayed in the environment associated with stress by previously shocked animals is also likely to involve changes in the mesolimbic DA system. In addition, however, it is probable that in the stress environment the Sh-AB group animals are also affected by the environment induced release of endogenous opioids.

It would be of interest to test this hypothesis that stress-

induced hyperactivity in a neutral environment is predominantly due to an increased action of the mesolimbic system whereas when testing takes place in the stress environment, additional opioid release may be involved. One experiment that might ferret out this distinction would be the application of DA receptor blockers prior to each exposure to stress. It might be expected that DA antagonists would completely block the stress-induced hyperactivity observed in a neutral environment but only partially block its subsequent expression in the environment associated with stress.

A possible important consequence of this opioid release in the stress environment is suggested by the noteworthy difference in the behaviour of animals tested in a neutral environment to which they had been previously habituated and of animals tested in the same environment in which they had experienced stressful stimuli: a profound freezing response was displayed by animals previously shocked in the test environment when they were re-exposed to that test environment without ensuing shock. Evidence presented by Nabeshima et al., (1983) suggests that the motor suppression obtained immediately post-shock may be mediated by kappa or delta opioid receptor agonists. Conversely, a recent study by Latimer, Duffy, and Kalivas (1986), implicates the mu opioid receptor agonists in the activation of locomotor excitation. One might speculate, therefore, that the biphasic response of freezing followed by hyperactivity that is observed immediately post-stress in the stress environment, is a function

of released endogenous kappa or delta receptor agonists followed by the release of mu receptor agonists. There is no evidence at this point to suggest whether the differential activation of these binding sites is due to a separate release of distinctive ligands that preferentially engage each type of receptor, or whether it is due to the release of non-specific opioids immediately post-stress. Possibly, a less sensitive kappa or delta receptor mediating motor suppression might be activated during an initial surge of opioid release. Over time, a decreasing opioid level might engage the mu receptor mediating locomotor excitation.

Another possibility that can be considered is that the mesocortical dopaminergic pathway is involved in the observed motor suppression immediately following stress. As discussed in the introduction, it has been suggested that this system inhibits the mesolimbic DA pathway's locomotor activating influences (Itoh et al., 1985). It is possible that the motor suppression displayed following stress might involve kappa or delta opioid receptor agonists that engage the mesocortical DA system.

Alternatively, motor suppression might be mediated by systems either unrelated to, or in conjunction with, the mesocortical DA system. In particular, points near, and including, the nucleus raphe pontis, the posteriour hypothalamus and the substantia nigra pars reticulata (Broekkamp, Lepichon, and Lloyd, 1984; Tseng, Wei, Loh, and Li, 1980; Turski, Havemann, and Kuschinsky, 1983) have been suggested to be involved in opioid-induced akin-

sia.

There is a further implication of the dual mechanism presented here. It might help explain the absence of difference between shocked and no-shock animals tested either in a novel environment or immediately after stress in a neutral environment. It seems plausible to suggest that the initial opioid mediated response to a stressful situation in rodents is freezing. With further exposure to stressors, an activity-increasing mu mechanism may become involved. This secondary activation may be short-lived and dependent upon the continuous exposure to stress. Should the stress-inducing stimuli be removed, such as when the animal is removed from the stress environment or placed in a mildly stressful or novel environment, the shorter-lived excitatory mu receptor mediated response might be reduced, terminated, or masked by a longer-acting freezing response.

Bolles and Fanselow (1980) have suggested that stressful stimuli evoke a fear response that helps the organism cope with potential dangers. The analgesia accompanying stress might enable the animal to respond to the danger without interference from pain signals. According to their theory, fear is followed by pain that encourages recuperative behaviours. Conceptually related to their idea is the suggestion presented now that a biphasic motor response to stressful stimuli may have adaptive significance for the organism. When first exposed to a stressor, the rodent might be expected to engage in its species-specific response of freezing. If this behaviour were ineffective in

avoiding the stressor, an active response would permit an attempt to escape. In the continued presence of the stressor, this escape aiding motor activation might become elevated. Over repeated experiences with the stressor, the escape aiding motor activation might become enhanced; occurring sooner, to a greater magnitude, and lasting longer. If, however, the escape response were successful in eliminating the stressor, there might very well be a rapid termination of the mechanisms mediating these responses. Successfully escaping a stress-inducing event may trigger a rapid return to baseline behaviour. Might such mechanisms underly the beneficial effects of "coping" responses?

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