

## BRIEF REPORT

### Interaction of Stress and Morphine in the Rat Using a Classical Conditioning Design

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Previous studies have suggested that aversive and stressful stimulation may affect endogenous opioid systems and produce physiological changes (e.g., analgesia, Straub tail response) typically associated with opiate stimulation. The present experiment addressed the effect of stressful but not immediately painful stimulation upon an opiate-mediated syndrome using a novel procedure. Noise stress and morphine administration were factorially varied in the classical conditioning of environmental preference in a two-choice apparatus. In comparison with control subjects which showed no change, morphine produced a preference shift toward the conditioned environment which was further potentiated by a noise stimulation. Thus, stress may potentiate the reinforcing effects of opiate alkaloids.

Foot shock, cold water immersion, and other stressful procedures are known to release endogenous opioid peptides (endorphins) from the central nervous system (Akil, Madden, Patrick, & Barchas, 1976), and to produce naloxone reversible analgesia (e.g., Akil et al., 1976; Amir & Amit, 1978; Bodnar, Kelly, Spiaggia, Ehrenberg, & Glusman, 1978). Other stressors may produce related opioid-mediated syndromes involving grooming, Straub tail, and motor activation (Katz, 1979; Katz & Roth, 1979; Katz, Roth, & Schmaltz, 1979). To date these stress syndromes have been assessed in the context of additional aversive stimulation (i.e., through alterations in pain sensitivity) or in motivationally ambiguous circumstances (i.e., through changes in open-field behavior).

It may be questioned whether stress-induced changes in behavior are restricted to the above testing circumstances or whether they are motivationally and behaviorally more general. This question is of interest because the behavioral functions of endogenous opioid peptides are not yet

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completely delimited. Evidence from other studies indicates endogenous and exogenous opioids are involved in motivationally positive behaviors as well as aversion (see Jaffe, 1975; Belluzzi & Stein, 1976). The present experiment examined the effects of stressful but relatively painless noise exposure upon a conditioned preference response. In a previous study Katz and Gormezano (1979) indicated that increased choice of a non-preferred chamber could be obtained by pairing it with opiates or enkephalin analogs. This choice paradigm may be conceptualized as a classical (Pavlovian) pairing of opioid with specific aspects of an environment with a subsequent test used to test the effectiveness of the pairing operation. A related study by Reid and colleagues (Stapleton, Lind, Merriman, Bozarth, & Reid, 1979) suggests this finding is general with respect to dose, particular choice of endorphin, and apparatus.

The present experiment utilized a factorial design to examine the possible modulation by noise exposure of the conditioning of opiate preference using the above procedure. The hypothesis tested was that noise might interact with opiates in the conditioning process to alter the normal degree of conditioned preference.

*Methods and materials.* *Subjects:* Forty adult (70 days) male Sprague-Dawley rats were group housed with food (Teklad 4.0% fat rodent diet) and tap water continuously available. Twelve hour light/dark cycles (lights on = 0700—1900 hr) were maintained through automatic programming.

*Apparatus:* The apparatus has been described in detail in a separate publication (Katz & Gormezano, 1979). Briefly, the testing apparatus consisted of two highly distinctive interconnected chambers. One chamber was white, cubical (18 cm per side) and had a grid floor made of stainless-steel dowels. The other was black, prismatic (18 cm height) with an isosceles triangular base (18 × 18 × 25.4 cm) and a wire-mesh floor. A 9.5 × 9.5 cm aperture with a sliding door connected the sides. Data were recorded based on an electronically operated timer.

*Behavioral procedure:* Subjects were placed in the apparatus with initial side placement counterbalanced across subjects and sessions. All sessions were 30 min and were separated by a minimum of 24 hr. Three sessions preceded experimental testing. Over this time, side preference developed and stabilized. For all subjects, the black side was preferred. The fourth session was a conditioning session. During this session access to the black chamber was prevented. Subjects were placed in the non-preferred (i.e., white) side and 10 min later were briefly removed and injected with a low dose of morphine (as morphine sulfate 1.5mg/kg intraperitoneally) or 0.9% sodium chloride vehicle. The dose of drug was based upon previously published results (Katz & Gormezano, 1979) and was chosen to permit evaluation upon a threshold drug effect. For half the subjects of each (i.e., drug, vehicle) group a stress of 95 db white noise

was presented from a speaker mounted directly adjacent to the boxes. The noise was administered concurrently with the drug or vehicle and continued throughout the remaining 20 min of the conditioning session. Subjects only received noise in the presence of the nonpreferred side of the box. The fifth session involved free choice and assessment of altered side preference. Again initial placement was counterbalanced across subjects and conditions.

**Results.** All data are presented as means and standard errors. Initial inspection indicated considerable (approximately fivefold) variability in errors across cells, and nonparametric techniques were therefore utilized for the assessment of experimental effects. Initial analysis by Friedman two-way analysis of variance (Siegel, 1956) indicated a significant effect of groups ( $\chi^2, r(9) = 19.1, p < .001$ ). It may be seen in Fig. 1 that while groups were matched for initial choice (in all cases session four is represented in column 1) they subsequently differed depending upon experimental treatment. The control group showed no change in preference pattern. The morphine groups with and without stress both showed reduction in preference which are consistent with conditioning to the opposite side. The largest change was in the group receiving both morphine and stress. Post hoc contrasts using Silverstein's *C* procedure (Silverstein, 1978) indicate that significant ( $p < .05$ ) differences exist between the control procedure and the drug procedure, that no unique stress effect is evident with respect to an unstressed control, but that stress significantly increased the amount of conditioning to morphine.

A further preliminary analysis of this effect is possible via the examination of percentage group change in preference, i.e., how many rats of each group of 10 showed altered preference after the control or experimental manipulations. Percentage rats showing increased preference in control,

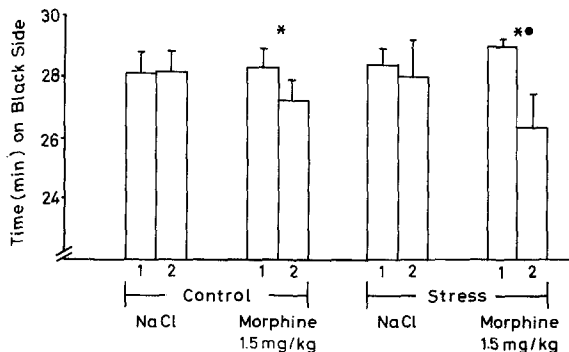


FIG. 1. Effects of morphine sulfate (1.5 mg/kg) and stress upon conditioning of a side preference in the rat ( $n = 10/\text{cell}$ ). 1, Predrug baseline; 2, postdrug choice; morphine 1.5, morphine-treated group; NaCl, vehicle treated group. \*, Significantly reduced from control,  $p < .05$ ; \*\*, significantly reduced from morphine,  $p < .05$ . All comparisons based upon nonparametric multiple comparison procedure of Silverstein.

morphine, stress, and morphine stress groups, respectively, was 20, 50, 30, and 90. These results clearly support the above analysis.

*Discussion.* The present study paired opiate, noise, their combination, or a control involving neither with a nonpreferred environment in a classical conditioning design. The findings replicated and extended previous studies. A modest but significant degree of conditioning was shown to a low dose of morphine after a single injection. This replicated a previous study (Katz & Gormezano, 1979) and procedurally extended studies from other laboratories (Stapleton et al., 1979). In addition, noise, while itself not producing a statistically significant effect, did facilitate the conditioning process to an opiate. This provides evidence of an interaction of stress and opiates using a novel paradigm. The stress procedure has previously been shown to produce a fourfold elevation in plasma corticosterone which is also accompanied by heightened activity (Roth & Katz, 1980). At least on this parameter the stimulation may be said to be stressful and arousing. The stimulation did not appear to be highly aversive, at least based on a choice measure. Examination of column three (stress/noise) indicates a modest increase in preference for a novel environment paired with the stimulation. This is not a significant increase, and the possible existence of more subtle aversive aspects of stimulation not found with the present test cannot be entirely excluded. Nonetheless, a failure to find a decrease in preference may indicate that the stimulation is not highly aversive.

Several interpretations of these findings are possible. It is possible that noise exposure per se produced a motivationally mixed state, and that opiates reduced its aversive aspects while leaving its positive aspects intact. Alternately, stress might have activated some system which could algebraically sum with or possibly act synergistically with the system normally activated by morphine. Examination of Fig. 1 indicates that synergism is more likely than mere summation. Clearly both of these explanations may be true in part. In either case the finding of significant facilitation is of interest, and may in fact conceivably point to a determinant of abuse patterns in human addictions.

A final interpretation is based upon the effects of opiates upon memory. Possibly opiate administration had some memory disruptive effects, and the subsequent trial represented a reacquisition of the dark preference. Opiates may interfere with memory (e.g., Castellano, 1975; Gallagher & Kapp, 1978; Jensen et al. 1978). If preference for dark was based upon learning across trials, which was subject to disruption, then an additional trial may have represented a gradual reestablishment of preference after disruption. It should be noted that opiates do not necessarily produce amnesia, and under selected circumstances they may actually enhance memory (Modadori & Waser, 1978; Stein & Belluzzi, 1978). In a previous study opiates administered outside the box (Katz & Gormezano, 1979) did

not affect preference, moreover opiates administered in the preferred side also do not affect preference. Two additional groups of five rats were confined to the black side of the box and injected with morphine or vehicle. Neither group differed from baseline, nor did they differ from each other (scores: vehicle trial three =  $27.5 \pm 1.1$ ; drug trial four (i.e., pretest) =  $27.1 \pm 1.3$ ; vehicle trial five =  $26.9 \pm 1.4$ ; drug trial five =  $27.3 \pm 1.2$ ). These findings do not support a memory-consolidation based interpretation of the data.

It must be noted that the system activated by stress remains unidentified. It is tempting to speculate that it is an endorphin system; however, the findings of Bodnar, Kelly, Steiner, and Glusman (1978) and Hayes, Bennett, Newlon, and Mayer (1978) as well as findings from our laboratory (Katz et al., 1979) indicate that nonopioid systems may also be involved in a variety of syndromes which closely resemble those produced by opioids. Thus this issue at present remains unresolved. The present findings are of interest both in their suggestions of an environmental mediator of morphine efficacy, and in their preliminary identification of some potential motivational properties of stress.

## REFERENCES

- Akil, H., Madden, J., Patrick III, R. L., & Barchas, J. D. (1976). Stress induced increase in endogenous opioid peptides: Concurrent analgesia and its partial reversal by naloxone. In H. W. Kosterlitz (Ed.), *Opiates and Endogenous Opiate Peptides*. Amsterdam: North-Holland.
- Amir, S., & Amit, Z. (1978). Endogenous opioid ligands may mediate stress induced changes in the affective properties of pain related behavior in rats. *Life Sciences*, **28**, 1143-1152.
- Belluzzi, J., & Stein, L. (1976). Enkephalin may mediate euphoria and drive reduction reward. *Nature (London)* **260**, 625-626.
- Bodnar, R. J., Kelly, D. D., Spiaggia, A., Ehrenberg, C., & Glusman, M. (1978). Dose dependent reductions by naloxone of analgesia induced by cold water stress. *Pharmacology Biochemistry Behavior*, **8**, 667-672.
- Bodnar, R. J., Kelly, D. D., Steiner, S. S., & Glusman, M. (1978). Stress produced analgesia and morphine produced analgesia. Lack of cross-tolerance. *Pharmacology Biochemistry Behavior*, **8**, 661-666.
- Castellano, C. (1975). Effects of morphine and heroin on discrimination learning and consolidation in mice. *Psychopharmacologia*, **42**, 235-242.
- Gallagher, M., & Kapp B. S. (1978). Manipulation of opiate activity in amygdala alters memory processes. *Life Science*, **23**, 1973-1978.
- Hayes, R. L., Bennett, G. J., Newlon, P. G., & Mayer, D. J. (1978). Behavioral and physiological studies of non-narcotic analgesia in the rat elicited by certain environmental stimuli. *Brain Research*, **155**, 69-90.
- Jaffee, J. H. (1975). Drug addiction and drug abuse. In L. S. Goodman & A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*, 5th ed. New York: Macmillan.
- Jensen, R. A., Martinez, J. R. Jr., Messing, R. B., Spiehler, V., Vasquez, B. J., Soumireu-Mourat, B., Liang, K. C., & McGaugh, J. L. (1978). Morphine and naloxone alter memory in the rat. *Society for Neuroscience Abstracts*, **4**, 260.
- Katz, R. J. (1979). Stress induced Straub tail elevation—further behavioral evidence for the involvement of endorphins in stress. *Neuroscience Letters*, **13**, 249-253.

- Katz, R. J., & Gormezano, G. (1979). A rapid and inexpensive technique for assessing the reinforcing effects of opiate drugs. *Pharmacology Biochemistry Behavior*, **11**, 231-233.
- Katz, R. J., & Roth, K. A. (1979). Stress induced grooming—an endorphin mediated syndrome? *Neuroscience Letters*, **13**, 209-212.
- Katz, R. J., Roth, K. A., & Schmaltz, K. Endogenous opiates as mediators of activation and coping. In *Proceedings of the International Narcotic Research Conference*. Pergamon, Long Island City, N.Y. In press.
- Modadori, C., & Waser, P. G. (1978). Facilitation of memory processing by posttrial morphine: Possible involvement of reinforcement mechanisms. *Psychopharmacology*, **63**, 297-300.
- Roth, K. A., & Katz, R. J. (1979). Stress, behavioral arousal, and open field activity an examination of emotionality in the rat. *Neuroscience and Biobehavioral Reviews*, in press.
- Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.
- Silverstein, A. B. (1978). Critical values for non parametric multiple comparisons. *Psychological Reports*, **43**, 44-46.
- Stapleton, J. M., Lind, M. D., Merriman, V. J., Bozarth, M. A., & Reid, L. (1979). Affective consequences and subsequent effects on morphine self administration of D-ala-2-methionine enkephalin. *Physiological Psychology*, **7**, 146-152.
- Stein, L., & Belluzzi, J. D. (1978). Endorphins and the sense of well being: A psychobiological approach. In E. Costa, & M. Trabucchi (Eds.), *The Endorphins*, Vol. 18, pp. 299-312. Raven: New York.