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Opiate Effects on Isolation-Induced Hyperthermia[1]

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FROHM, K. D. AND L. B. WALLNAU. *Opiate effects on isolation-induced hyperthermia.* PHARMACOL BIOCHEM BEHAV **19**(2) 163–167, 1983.—The effect of brief separation from companions on temperature was studied in 24-day-old chickens. It was found that socially isolated animals became hyperthermic. Alternatively, control animals maintained in groups larger than 6 animals displayed no differences in temperature between the pre- and post-test. Injections of naloxone produced dose-dependent temperature increases in socially housed animals. Finally, while morphine reversed isolation hyperthermia, it had little or no effect on temperature in animals that remained in social groups throughout the experiment. The findings are discussed in terms of endogenous opioid involvement in separation distress and social bonding.

Chickens Hyperthermia Separation distress Morphine Naloxone Social isolation

SEVERAL recent studies by Panksepp *et al.* [3, 9, 10, 11] have examined neurochemical effects on social bonding mechanisms in a variety of animals using distress vocalization as an indicator of affective response to social isolation. These researchers hypothesize neuorchemical similarities between social bonding and narcotic addiction. In particular, it has been suggested that during proximity to companions, endogenous opioids are released which in turn condition bonding through avoidance of stressful and aversive withdrawal symptoms, i.e., affective separation distress [9,10]. Consistent with this interpretation, these studies have shown that opioid peptides and opiate receptor agonists reliably reduce distress vocalizations [3, 9, 10, 11].

Distress vocalization is a widely used behavioral measure of affective response to separation from companions in studies of social bonding and imprinting [4, 5, 6, 7]. It should be noted that the investigations of Panksepp and coworkers are significant in that they suggest neurochemical mechanisms in social bonding among the other, essentially behavioral models [5,12]. However, there exist several inherent limitations in using distress vocalizations as a dependent variable: (a) its reliability appears questionable at times, in that there is typically a large amount of variability in baseline rates of distress vocalization between individuals and between strains (e.g., [9]). (b) Other stressors, in addition to social isolation, are known to influence rates of distress vocalization, including ambient temperature changes [7] and novelty of the physical environment [6], suggesting high potential for contamination by factors unrelated to social condition. (c) Since rates of distress vocalization for animals in proximity to their companions are essentially zero, it is not possible to test for differential effects between socially maintained controls and isolated animals with manipulations that affect distress vocalizations. In the absence of this baseline, one cannot examine possible interactions between drug effects and social conditions (social vs. separation from companions). This limitation precludes a conclusive statement about whether the neurochemical mechanism in question mediates social bonding in particular or has general behavioral effects independent of social condition.

One alternate measure of separation distress used by researchers [9,11] is body temperature. It has been observed, for example, that birds injected with morphine, endorphins, and enkephalins have lower core temperatures than controls after 15 minutes of isolation [11]. However, the report included neither a pretreatment temperature recording nor temperature data from socially housed animals. In other work, pretreatment and posttreatment temperatures were measured in puppies injected with opiate agonists (morphine and oxymorphone) and then briefly isolated [9]. However, the reported data include only the pre-post differences in temperature for the two highest doses of morphine and the range of differences for oxymorphone. Conspicuously missing are data for control animals and actual mean temperatures for all groups. It should be noted that both sets of data are consistent with some other reports of opiate effects on temperatures [1,2] in that they reflect an opiate-induced hypothermia. However, they fall short of providing any information regarding the effects of social isolation on temperature.

We initially became aware of a separation effect on temperature while studying drug effects on temperature (unpublished data). One interesting outcome was that vehicle control birds were hyperthermic following the brief (15 min) injection-test interval, during which birds were separated from companions. Viewed in the context of other work

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(e.g., [3, 9, 10, 11]), this temperature change could have a number of implications. If separation from companions produces distress that is mediated by reduced endorphin activity [3, 9, 10, 11], then perhaps altered core temperature during separation reflects the same neurochemical mechanism. Temperature measurement may be a useful index of distress in that it is characterized by very low variability and that interactions with drugs can be investigated with both social and isolated animals. In addition, drug effects on socially isolated animals could be extended to a physiological response. This investigation examines the effects of brief separation from conspecifics on temperature, and the effects of opiate agents.

GENERAL METHOD

Subjects

Production Red chickens were acquired at one day after hatching from Welp, Inc. Birds were housed communally (25 animals per enclosure) in commercial brooders (Brower Mfg. Model 6401). Artificial lighting was provided with overhead fluorescent fixtures on a 14/10 hr light/dark cycle. Access to food (Purina Chick Starter) and water was continuous. Each bird was identified by an aluminum wing band (National Band and Tag Co. Model 4-893) affixed at seven days of age. All birds were tested at 24 days of age.

Equipment

Pre-test and post-test temperature readings were taken with a Yellow Springs Instrument, Inc. Tele-thermometer (Model 43TF) and probe (Model 402). Temperature readings were determined by inserting the probe 3.8 cm into the cloaca and recording the maximum reading over 120 seconds. Measurements were recorded from the Fahrenheit scale, which on this instrument has greater resolution, and then converted to Celsius. The experimenter taking temperature readings was unaware of drug group designation and of pre-test temperature when taking post-test measurements.

Procedure

Subjects were randomly assigned to experimental groups. Each animal was removed from its communal brooder, weighed, and measured for pre-test temperature. In studies involving drugs, injection volumes were then calculated according to weight and the animal was injected intraperitoneally with either the drug or an equivalent volume of saline solution. Birds were then placed in the appropriate social housing condition for the designated treatment duration and finally post-test temperature was measured. During the sampling of subjects, animals were no longer selected from a brooder when fewer than seven animals remained, in order to insure that animals had ample social contact prior to baseline measurements. All testing occurred between 10 a.m. and 2 p.m. and groups were equally represented across time of day.

EXPERIMENT 1

In order to determine if separation from companions produces the apparent hyperthermia observed in other work (unpublished data), this experiment compared birds that were briefly isolated from conspecifics with control animals that were housed socially during the pre- to post-test interval. If social condition is an important variable, then the social controls would be expected to maintain constant temperature and the isolated birds should show the temperature change.

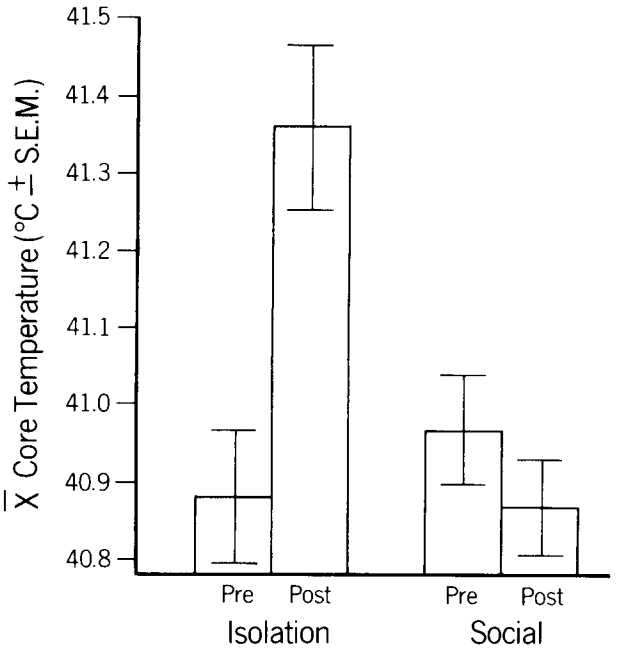

FIG. 1. The effect of brief isolation and social housing on core temperature (°C). Pre-test is recorded just before placement in the social condition and post-test measures are taken 25 min later.

Procedure

Eighteen birds were taken from the brooder, weighed, and pretested for temperature. Those designated as socially housed birds were then placed in a large wire cage (61×61×61 cm) with a minimum of 7 companions that were placed in the cage prior to the start of the experiment. Isolated animals were placed in wire mesh pigeon cages (33×23×38 cm) following the pretest. It should be noted that this condition consisted of physical separation and visual isolation from compansions. After 25 minutes in either treatment condition, birds were removed and once again their temperatures were recorded.

Results

The results are depicted in Fig. 1. Analysis of variance revealed a significant trial effect, $F(1,16)=8.55$, $p<0.01$, and a significant social condition by trial interaction, $F(1,16)=19.66$, $p<0.001$. Simple effects analyses, performed to clarify the interaction [8], determined that isolated birds exhibited an increase in temperature from pre-test to post-test, $F(1,8)=16.96$, $p<0.005$. On the other hand, social condition birds displayed no significant pre- to post-test change in temperature, $F(1,8)=1.99$. Thus, when removed briefly from their companions, these animals develop an increase in body temperature. It should be noted that, preliminary work revealed that the hyperthermia is evident after only 10 minutes of isolation and is independent of the type of holding enclosure (wire cage vs. closed box).

EXPERIMENT 2

If the isolation-induced hyperthermia is a physiological response to reduced release of endorphins, then injection of

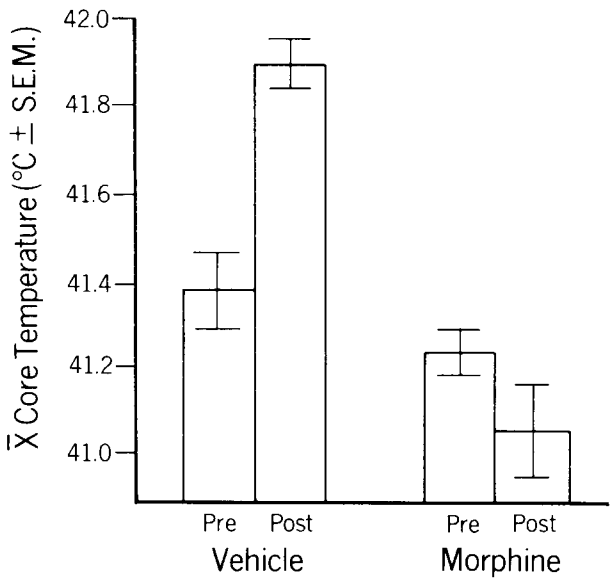

FIG. 2. The effect of morphine (10 mg/kg) and saline vehicle on the isolation-induced hyperthermia. Pre- and post-test are separated by 30 min.

an opiate receptor agonist should reverse the hyperthermia. For example, morphine was shown [3, 9, 10, 11] to reduce distress vocalizations produced by separation from companions. Therefore, the second experiment examined the effectiveness of morphine in reducing hyperthermia in briefly isolated animals.

Procedure

Twenty chickens were weighed and pretested for temperature. Half then received 10 mg/kg of morphine sulphate (Lilly). Control animals were injected with an equivalent volume (0.67 ml/kg) of saline solution. Each bird was placed alone in a small wire cage and temperature was measured again after 30 minutes.

Results

The data are summarized in Fig. 2. Analysis of variance revealed a significant main effect of drug condition, $F(1,18)=40.23$, $p<0.001$, reflecting reduced temperatures for the morphine treatment. However, there was a significant drug by trial interaction, $F(1,18)=15.85$, $p<0.001$, reflecting the fact that hyperthermia occurred only in the vehicle condition. Simple effects analyses across the repeated measures [8] confirmed this conclusion. The pre- to post-test change for the saline group was significant, $F(1,9)=22.98$, $p<0.001$, but no difference was found for the morphine group, $F(1,9)=1.61$. These data replicate the occurrence of hyperthermia in isolated animals which was reported in the first experiment. Furthermore, the increase in temperature was reversed by morphine, a result consistent with the reduction of distress vocalizations by morphine (e.g., [3,11]). Although the morphine effect is consistent with the endogenous opioid model of social bonding, note that it is also compatible with general morphine-induced temperature reductions and therefore may not necessarily depend on social condition [1, 2, 9, 11].

TABLE 1

THE EFFECT OF NALOXONE ON TEMPERATURE (°C) IN SOCIALLY HOUSED CHICKENS

	Dose of Naloxone (mg/kg)		
	0	5.0	10.0
Pre-test			
Mean	41.23	41.24	41.20
Standard Error	0.09	0.08	0.09
Post-test			
Mean	41.49	41.63	41.82
Standard Error	0.09	0.06	0.08

EXPERIMENT 3

If an opiate agonist reverses the isolation hyperthermia by stimulating endogenous opioid receptors, then accordingly, blockade of such receptors in the brain should increase temperature. In our first attempt, chickens were injected with naloxone, a relatively pure opiate receptor antagonist, and then isolated from their companions. No increase in temperature was observed above and beyond the expected hyperthermia (unpublished data). It is possible that a ceiling effect on temperature had occurred, and opiate receptor blockade could not increase temperature beyond the hyperthermia already produced by isolation because little or no endorphins were being released in the isolated animals. Herman and Panksepp [3] describe a similar ceiling effect in studying the influence of naloxone on distress vocalization in isolated infant guinea pigs.

In contrast, opiate receptor blockade by naloxone should cancel the effects of endorphins released in response to proximity to companions, inducing an isolation-like hyperthermia in animals that are socially housed. This would preclude a ceiling effect in that socially grouped animals ordinarily do not become hyperthermic. The present experiment compared two doses of naloxone to a saline control in their effects on temperature in socially housed chickens.

Procedure

Thirty chickens were weighed, pretested for temperature, and injected with either 5 mg/kg or 10 mg/kg of naloxone HCl (Endo) or an equivalent volume (1.0 ml/kg) of saline solution. All birds were then placed in the small cages used in isolation conditions above, but in the company of two conspecifics. Post-test temperatures were measured 30 minutes later.

Results

Means and standard errors are depicted in Table 1. Body temperature increased across the pre- to post-test interval, as indicated by a significant trial effect, $F(1,27)=58.76$, $p<0.001$. There was also a significant dose by trial interaction, $F(2,27)=3.65$, $p<0.04$. Simple effects analyses [8] revealed a significant effect for dose of naloxone at the posttest level, $F(2,27)=4.76$, $p<0.025$, indicating that greater doses of naloxone produced greater increase in core temperature. It should be noted that simple effects analysis also revealed

TABLE 2

THE EFFECT OF MORPHINE AND SOCIAL CONDITION ON TEMPERATURE (°C) IN CHICKENS

	Isolation-Saline	Isolation-Morphine	Social-Saline	Social-Morphine
Pre-test				
Mean	41.21	41.27	41.19	41.18
Standard Error	0.05	0.05	0.08	0.07
Post-test				
Mean	41.64	41.08	41.23	41.25
Standard Error	0.08	0.09	0.09	0.05

that even controls showed some temperature increase, $F(1,9)=5.3$, $p<0.05$.

Although the results indicate a dose-dependent naloxone effect on temperature in the predicted direction, it was surpising that a slight temperature increase was observed for controls, because they were socially housed. In Experiment 1, socially housed chickens showed no significant change in temperature from pre-test to post-test. The social condition for that experiment differed in that a large cage with a minimum of 7 companions was used, compared with the small pigeon cage and only 2 companions for this experiment. Perhaps the number of companions used here was inadequate to produce the amount of social contact necessary to replicate the previous finding. Anecdotal observations noted that several of the birds in the present experiment exhibited distress vocalizing, even though they were maintained in a social condition. Despite the control data, the naloxone effect on temperature is consistent with the effects of naloxone on distress vocalization [3,10].

EXPERIMENT 4

The data of the previous experiments may not reflect anything more than a general opiate effect on body temperature. It is important to determine whether the effects of opiate drugs on temperature operate differentially across social conditions or merely produce temperature changes independently of social housing. This experiment used a 2×2×2 factorial design consisting of morphine vs. saline injections, brief separation vs. social housing, and pre- and post-treatment measurement of temperature. If the morphine effect on temperature is specific to a social condition, then morphine should block the isolation-induced hyperthermia but have little or no effect on socially housed animals. This would be manifested in a three-way interaction. It should be noted that all treatment conditions, with the exception of the morphine/social cell, are replications of parts of the previous experiments. In this regard, it is important to examine the socially housed/saline injected controls, because of the discrepant increase in temperature observed for the similar group in Experiment 3.

Procedure

Chickens were weighed, pretested for temperature, and injected with either 10 mg/kg of morphine sulfate or an equivalent volume of saline solution. Following injection, each bird was placed either alone in a small wire cage or in the company of at least 6 conspecifics in a large cage. The four treatment cells comprised 10 animals each. After 30 minutes in either social condition, post-test temperatures were recorded.

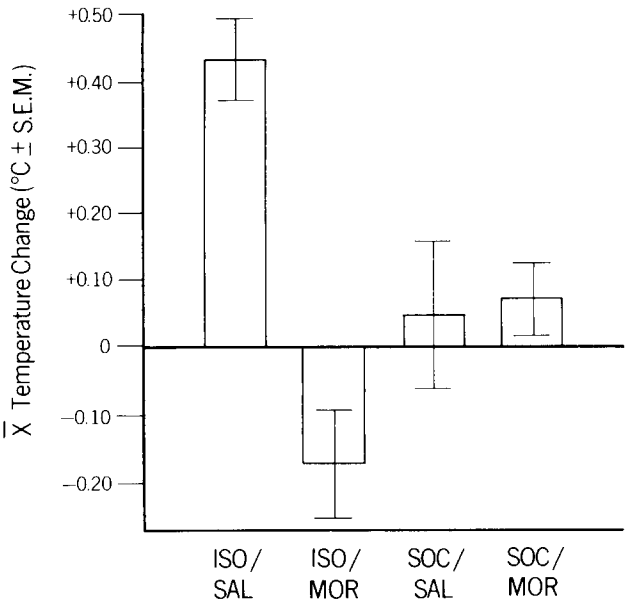

FIG. 3. Mean temperature change (°C) following social and drug treatments. (ISO=isolation, SOC=social, SAL=saline, MOR=morphine.)

Results

Means and standard errors are depicted in Table 2. Analysis of variance revealed main effects for drug condition, $F(1,36)=4.15$, $p<0.05$, and trials, $F(1,36)=5.31$, $p<0.03$. In addition, there were significant social treatment by drug, $F(1,36)=4.34$, $p<0.05$, and drug by trials, $F(1,36)=13.86$, $p<0.001$, interactions. As predicted, there was a three-way interaction of social treatment, drug condition, and trials, $F(1,36)=16.63$, $p<0.001$. In order to clarify the nature of this interaction, simple effects analyses were performed [8] for the temperature change from pre-test to post-test in each group (see Fig. 3). As expected, the isolation/saline group showed hyperthermia, $F(1,9)=47.27$, $p<0.001$, and temperature was unchanged in both social groups (social/saline, $F<1$: social/morphine, $F=1.38$). There appeared to be a small decrease in temperature for the isolation/morphine group, but it failed to achieve statistical significance, $F(1,9)=4.97$, $p>0.05$. At the very least, the interaction was obtained because morphine reversed the isolation-induced hyperthermia, but produced no temperature changes in socially housed animals.

GENERAL DISCUSSION

Animals that were briefly isolated from companions became hyperthermic, while untreated birds housed with an adequate number of companions (i.e., $n>6$) maintained stable temperatures. The suggestion that the slight gain in temperature among socially housed controls in Experiment 3 resulted from the small number of companions is supported by findings of Experiments 1 and 4. Morphine blocked the hyperthermia in isolated animals but had no effect on tem-

perature in animals maintained in social housing. Naloxone produced a dose-dependent temperature elevation in socially housed animals.

The consistency of these findings and the demonstrated interaction between drug effect and social condition support the involvement of an endogenous opioid neurochemical system in separation distress and social bonding, in accord with the findings of Panksepp, *et al.* [3, 9, 10, 11]. Core temperature appears to be a reliable and valid indicator of separation distress, as evidenced by the convergence between the present set of findings and those that use distress vocalization as a dependent variable. Furthermore, the use of temperature as a dependent variable facilitated the study of the interaction between social condition and opiate manipulation. In contrast, the zero baseline level for distress vocalization in socially maintained animals precludes the observation of such an interaction.

It should be noted that additional support for an opioid model of social bonding comes from direct biochemical evidence implicating changes in opiate receptors with differential rearing conditions (e.g., [13]). It was found that rats raised in isolation have relatively fewer opiate receptors than those raised socially. Thus, one might infer that normal development of opiate receptors during early life depends on the release of endorphins as a result of social contact.

One might question whether the isolation-induced hyperthermia and distress vocalizations reflect a social bonding process specifically, or a general stress mechanism of which social isolation is but one type of stressor. As noted above, distress vocalizations can be influenced by stressors unrelated to social condition [6,7], and the same could be true for temperature. Therefore, it is conceivable that the ability of morphine to reverse the isolation hyperthermia reflects a more general opiate effect (e.g., sedation) on stress. Panksepp, Meeker and Bean [10] ruled out a sedative effect of morphine in reducing distress vocalization, based on behavioral observations and the finding that other sedative drugs are not as effective in reducing distress vocalization. To further pursue this question, it may be crucial to test for temperature increases in response to a variety of stressors (e.g., electric shock) that are unrelated to social contact. If a similar pattern of hyperthermia and drug effects are observed, then general stress reduction might be a more appropriate interpretation.

It is significant in this regard that other forms of stress are capable of producing hyperthermia in rats (e.g., [14]). However, naloxone reverses these effects [14], supporting an interpretation that a stressor elicits compensatory release of endorphins. These findings contrast with those of the present study in that naloxone produced and morphine reversed isolation-induced hyperthermia. The present findings, as well as those of other investigators [3, 9, 10, 11] suggest that separation distress is associated with reduced opioid activity.

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