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Arecoline-Associated Changes in Open-Field Behavior Following Swim Stress in the Rat. A Possible Relationship to Water Temperature

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JAECKLE, R. S., S. C. DILSAVER, J. HOH AND J. A. PECK. *Arecoline-associated changes in open-field behavior following swim stress in the rat. A possible relationship to water temperature.* PHARMACOL BIOCHEM BEHAV **40**(4) 763–766, 1991.— The rat exhibits a reduction in movement in an open field following a 14-day course of forced swim stress at 12°C. The decrease in movement is greater in rats receiving arecoline relative to those receiving saline prior to placement in the open field. The authors report that when water temperature is increased to 20°, there is a categorical difference in the results. The saline control group exhibits a rise and the arecoline group no change in crossings.

Acetylcholine Arecoline Cholinergic Motor behavior Muscarinic Stress

CHRONIC inescapable stressors activate muscarinic cholinergic mechanisms in the rat. Inescapable footshock (1) and forced swim stress (1–3) enhance the hypothermic response of the rat to oxotremorine. A low dose of arecoline (0.125 mg/kg IP) is intermittently associated with a significant increase in crossings in an open field in drug-naive rats (4,5). The same dose of arecoline consistently produces a dramatic decrease in crossings following a 14-day course of twice daily forced swim stress of 10 minutes duration at 12°C (4). A saline control group simultaneously subjected to forced swim stress under the same conditions exhibits a significant reduction in crossings. However, the magnitude of effect in the saline group is modest compared to that of the arecoline group.

We now report an incidental finding suggesting that water temperature may be a critical variable when using the forced swim stress paradigm. The results are qualitatively and categori-

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[2]Dr. Richard Jaeckle completed the experimental element of this research project at the Ohio State University. His posthumous work is reported by Dr. Steven C. Dilsaver in this article.

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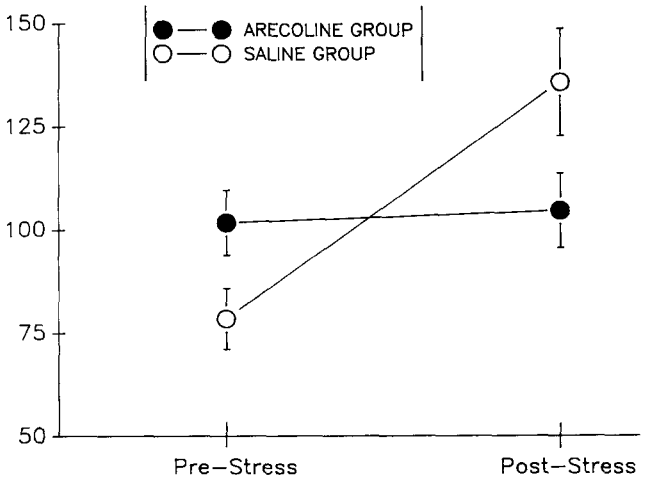

FIG. 1. Crossings were counted for 5 minutes in groups of 20 rats placed in an open field 5 minutes following an IP injection of arecoline (base), 0.125 mg/kg (closed circles) or saline (1 ml/kg) (open circles). Both groups were then subjected to a 14-day course of twice daily swim stress at 20°C for 15 minutes. Prior to swim stress the arecoline group exhibited more crossings than the saline controls. Following swim stress the saline group exhibited an increase in crossings ($p<0.0001$), whereas the arecoline group did not. Mean crossings in the saline group approached being significantly greater than that in the arecoline group poststress ($p=0.06$).

cally different from those previously reported.

METHOD

Dependent Variable

The dependent variable is change in the number of crossings following a 14-day course of twice daily forced swim stress at 20°C in independent groups of rats given intraperitoneal (IP) injections of arecoline and saline prior to placement in an open field. The results are compared with those of a previous study in which the rat was subjected to forced swim stress in water of 12°C.

Definition of and Conditions Used While Measuring Crossings

A crossing is defined as entry of an extremity into one of the squares into which the open field was divided. Crossings were measured in a room illuminated by red light. This is equivalent to darkness for the rat. The rat does not perceive the color red (7,8). The animals were allowed 10 minutes to adapt to the red light condition prior to being placed in the open field.

Design of the Open Field

The open field was divided into 16 equal squares of 506.25 cm^2 each. The floor and walls were light gray. Red tape divided the field into squares.

Selection of the Dose of Arecoline

The dose of arecoline was determined in a series of preliminary studies (4). Samples of 8 Sprague-Dawley rats were then randomly assigned to groups given an IP injection of 0 (saline), 0.125, 0.25, 0.5, 1.0, or 2.0 mg/kg of arecoline (base).

The group receiving 0.125 mg/kg of arecoline exhibited significantly more crossings relative to the saline control group at baseline. However, following a 5-day course of twice daily forced swim at 8°C, this low dose of arecoline decreased crossings. This finding was also obtained when the rat was subjected to a 14-day course of twice daily forced swim stress at 12°C (4).

Animals

Adult, male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were housed in The Ohio State University's vivarium. Two groups of 20 rats each were used in this study. The ambient temperature in the room in which they were housed was 21.1 to 22.2°C. The animals were maintained on a 12-hour day/night cycle (lights on at 6:00 a.m. and off at 6:00 p.m.). Rat chow and water were available ad lib.

Pharmaceutical Agents

Arecoline hydrobromide was purchased from Sigma Chemical Co. (St. Louis, MO). The rats in the experimental group received 0.125 mg/kg of arecoline (base) by IP injection 5 minutes before placement in the open field. Control animals received normal saline (1.0 ml/kg of 0.9% NaCl per kg of weight).

Procedure for Forced Swim Stress

The animals were subjected to forced swim stress at 20°C for 15 minutes at 8:00 a.m. and 4:00 p.m. for 14 days. The depth of the water was adjusted so that the animals could not touch bottom. Forced swim stress sessions began the morning following the initial (baseline) challenges with arecoline and saline. The course of swim stress concluded two days before remeasurement of motor behavior.

Statistical Analysis

SAS was used in the analysis of the data. The dependent variable was assessed for normality of distribution both before and after subjection to forced swim stress using the Shapiro-Wilk test (10). Variance between samples both before and after the course of swim stress was formally assessed for homogeneity using PROC TTEST (11). Change in crossings following swim stress within and between groups was assessed for significance using the one-way ANOVA for repeated measures and independent data, respectively. A two-way ANOVA for repeated measures was used to determine the probability of there being an effect of stress or an interaction between stress and treatment (injection of saline or arecoline).

All measures of variance in the text refer to the standard error of the mean (SEM). The critical value of α was set at $p<0.05$, two-tailed.

RESULTS

Distribution of Data

The Shapiro-Wilk test indicated that crossings were normally distributed in both the experimental (w: normal = 0.98, probability $<$ w = 0.95) and control groups (w: normal = 0.95, probability $<$ w = 0.35). The requirement for homogeneity of variance was met (df = 19,19, F = 1.15, probability $>$ F = 0.76). Crossings were once again normally distributed in both groups fol-

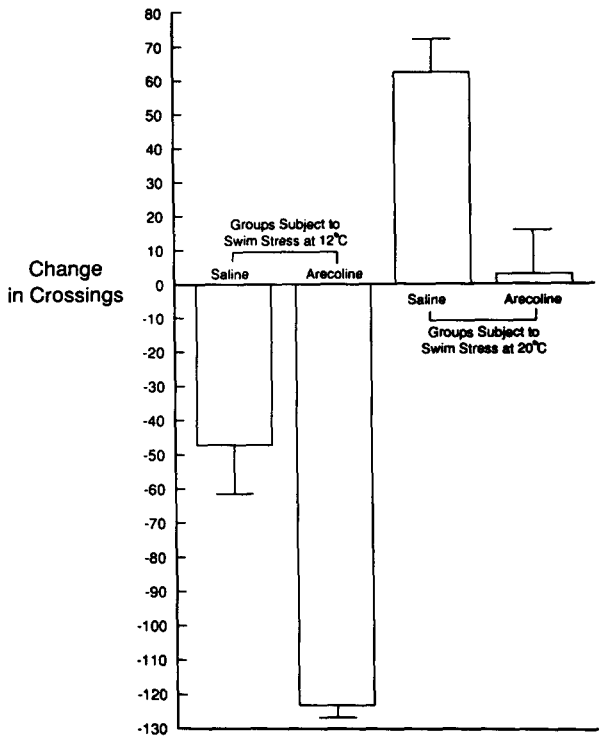

FIG. 2. This illustrates the absolute change in mean number of crossings ± SEM in saline and arecoline groups following a 14-day course of twice daily swim stress at 12 and 20°C. Both groups subjected to forced stress at 12°C exhibited significant reductions in crossings. In contrast, there was a dramatic increase in crossings in the saline group subjected to forced swim stress at 20°C. The arecoline group did not change. The differences between the saline ($p<0.001$) and arecoline ($p<0.001$) groups are highly significant.

lowing the 14-day course of swim stress (arecoline group, w: normal = 0.95, probability < w = 0.35; saline group, w: normal = 0.94, probability < w = 0.25). Variance was statistically similar in both the experimental and control groups ($df = 19,19$, $F = 2.07$, probability > $F = 0.12$).

Change in the Mean Mass of Rats in Each Treatment Group

The mean mass of the control and experimental groups was 175.1 ± 1.7 g (range = 161 to 193 g) and 172.1 ± 2.1 g (range = 160 to 197 g) at the start of the study, $F(1,38) = 0.0004$, $p>0.9$. Following the two-week course of forced swim stress the mean weights of these groups were 243.4 ± 3.0 g (range = 214 to 267 g) and 234.2 ± 4.6 g (range = 176 to 268 g), $F(1,38) = 2.79$, $p>0.10$, respectively.

Mean Crossings Between and Within Groups Before and After Forced Swim Stress

The arecoline group exhibited more crossings (101.8 ± 7.9) than the saline group (78.8 ± 7.8) prior to being subjected to swim stress. This difference was significant, $F(1,38) = 4.58$, $p = 0.038$. However, the reverse was true following the course of forced swim stress. There was a trend for fewer crossings in the arecoline (104.7 ± 8.9) than in the saline (140.9 ± 11.9) group, $F(1,19) = 3.84$, $p = 0.06$.

TABLE 1

Drug	Water Temperature	n	Mean ± SEM
1. Saline	20°C	20	+62.1 ± 9.8
	95% Confidence Interval = +41.5 to +82.6		
2. Arecoline	20°C	20	+2.9 ± 13.1
	95% Confidence Interval = −25.4 to +31.2		
3. Saline	12°C	14	−47.2 ± 13.9
	95% Confidence Interval = −77.2 to −17.2		
4. Arecoline	12°C	14	−123.1 ± 3.1
	95% Confidence Interval = −129.7 to −116.5		

The 95% confidence interval for the saline group subjected to forced swim stress at 20°C exclusively included values >0. That is there was a significant increase in crossings following forced swim stress ($p<0.05$). This result sharply contrasts to that obtained when the rat was subjected to forced swim stress in water at 12°C. The 95% confidence interval exclusively included values <0 indicating that there was a significant reduction in crossings. The mean change in crossings in the arecoline-treated group subjected to forced swim stress at 20°C was not significant. The arecoline-treated group undergoing forced swim at 12°C exhibited a significant reduction in crossings.

The critical value of t for $p<0.05$ (two-tailed) for 13 $df = 2.16$; the critical value of t for $p<0.05$ (two-tailed) for 19 $df = 2.093$.

The 95% confidence interval for a mean = the mean ± (SEM) (the critical value of t for $p = 0.05$).

The saline group exhibited significantly more crossings following the course of forced swim stress, $F(1,19) = 42.38$, $p<0.0001$. Crossings in the arecoline group did not change, $F(1,19) = 0.053$, $p>0.8$.

Figure 1 illustrates the change in number of crossings in both the arecoline and saline groups.

Effect of Stress and Drug on Crossing

The two-way ANOVA for repeated measures disclosed a significant effect of stress, $F(1,38) = 12.54$, $p<0.0001$, and interaction between stress and drug group, $F(1,38) = 10.30$, $p = 0.0027$.

DISCUSSION

Crossings increased significantly in the saline control group subjected to forced swim stress at 20°C from an average of 78.8 ± 7.8 prior to and 140.9 ± 11.9 following the 14-day course of swim stress ($p<0.0001$). This is exactly opposite to results consistently observed when the Sprague-Dawley rat is subjected to forced swim stress at 12°C. The arecoline group exhibited significantly more crossings at baseline than did the saline group ($p<0.038$). Other groups of drug-naive Sprague-Dawley rats receiving 0.125 mg of arecoline (base) IP have also exhibited more crossings than a saline control group (4,5).

The arecoline group did not exhibit a change in crossings following the course of forced swim stress. This finding is also contrary to that obtained when the Sprague-Dawley rat is subjected to forced swim stress at either 8 (4) or 12 °C (5).

The results obtained were contrary to all expectations. A significant effect of drug group was expected. However, based on previous experiments in which the rat was subjected to forced

swim stress at 12°C, we expected a reduction in crossings in the arecoline-treated sample relative to the control group. Both groups subjected to forced swim stress at 12°C exhibited significant decreases in crossings. The decrease was profound in the arecoline group relative to the saline control group, $F(1,30) = 13.81$, $p = 0.001$. In contrast, swim stress at 20°C did not have an effect in crossings in the arecoline group and was associated with a robust increase in crossings in the saline group.

The results suggest that there may be a significant effect of water temperature on the rat's response to saline and the muscarinic agonist.

Figure 2 illustrates the difference between forced swim stress at 12 and 20°C.

Forced swim at 20°C did not alter the motor behavior of the arecoline group, whereas the saline group exhibited an increase in crossings. This is consistent with the result of the two-way ANOVA. The effect of drug group (saline vs. arecoline) was significant. Further, the analysis indicates that the interaction between the drug and stress is significant. This supports the idea that the increase in crossings in the saline group and absence of a change in this parameter in the arecoline sample are related to the course of chronic stress.

Table 1 summarizes the results of forced swim stress of the Sprague-Dawley rat at 12 and 20°C.

Many events can account for the contrasting results of forced swim at the two temperatures. One mechanism is related to the site of arecoline's action. At low doses arecoline's predominant effect may be on presynaptic muscarinic autoreceptors in the naive rat. Cold water forced swim may shift the site of the predominant effect of this agonist to the postsynaptic muscarinic receptor (9, 12, 13). Similarly, low doses of some muscarinic agonists may augment the release of amines prior to subjecting the rat to forced swim stress. Cold water swim may result in these agonists inhibiting the release of these neurotransmitters [(1–3), see (14,15) for reviews of the effects of forced swim stress on aminergic systems].

Saline-treated animals may become conditioned in the course of chronic swim stress under the conditions used in this study. This would be akin to athletic training enhancing the capacity for aerobic exercise.

CONCLUSION

Following chronic forced swim stress at 20°C the saline-treated group exhibited a substantial increase in crossings. The arecoline-treated group did not exhibit a change. These results do not simply differ quantitatively from those obtained when the rat is subjected to chronic swim stress at 12°C. The phenomena are fundamentally different. There is a qualitative and categorical difference in the results.

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