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The Flinders Sensitive Line Exhibits Enhanced Thermic Responsiveness to Nicotine Relative to the Sprague-Dawley Rat

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DILSAVER, S. C., J. A. PECK AND D. H. OVERSTREET. *The Flinders Sensitive Line exhibits enhanced thermic responsiveness to nicotine relative to the Sprague-Dawley rat.* PHARMACOL BIOCHEM BEHAV **41**(1) 23–27, 1992.—The Flinders Sensitive Line (FSL) was derived from the Sprague-Dawley rat by selectively breeding animals with heightened sensitivity to an anticholinesterase. The FSL now consistently exhibits enhanced behavioral and physiological responses to muscarinic agonists relative to its progenitor. The authors now report the FSL exhibits enhanced thermic responsiveness to nicotine relative to the Sprague-Dawley rat. The possible relevance of this finding to investigators interested in the disorders of mood is briefly discussed.

Acetylcholine Cholinergic Depression Flinders Sensitive Line Nicotine Rat Thermoregulation

THE Flinders Sensitive Line (FSL) was derived from the Sprague-Dawley rat by selectively breeding animals with heightened sensitivity to diisopropylfluorophosphonate (DFP) for over 40 generations (16). DFP, a centrally active anticholinesterase, inhibits the hydrolysis of acetylcholine at all peripheral and central muscarinic and nicotinic receptor sites. The selection procedure used to derive the FSL is just as apt to result in a line with unusual physiological and behavioral responses to nicotine as to muscarinic agonists. However, data pertaining to the responsiveness of the FSL to nicotine relative to either its control, the Flinders Resistant Line (the FRL rat), or the Sprague-Dawley rat has yet to be reported. The results of two experiments assessing the thermic response of the FSL to nicotine relative to the Sprague-Dawley are presented in this report.

The FSL and FRL are identical in appearance. However, they are easily distinguished by measuring their thermic responsiveness to oxotremorine. The distribution of the hypothermic responses of the two lines do not overlap. The minimum and maximum hypothermic responses of samples of FSL and FRL rats to oxotremorine do not overlap. The hypothermic response of the FSL always exceeds that of the FRL.

Reasons for interest in the behavioral and physiological responsiveness of the FSL to nicotine relative to its progenitor will be highlighted in the discussion.

METHOD

Dependent Variable

Experiment 1. The dependent variable in Experiment 1 is change in core temperature 20 minutes after the intraperitoneal (IP) injection of saline or nicotine (base). Nicotine (base) was simultaneously given to FSL and Sprague-Dawley rats at intraperitoneal doses of 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, or 2.4 mg/kg. The source of nicotine was the same for both groups. Responsiveness to nicotine was measured at 7-day intervals.

Experiment 2. The dependent variable in Experiment 2 was the mean thermic response of FSL ($n = 11$) and Sprague-Dawley ($n = 104$) rats over a 120-minute period following the intraperitoneal injection of 0.5 mg/kg of nicotine (base).

One hundred and four (104) drug-naive Sprague-Dawley rats have received a dose of 1.0 mg/kg of nicotine (base) by intraperitoneal injection in our laboratory since 1986. All animals ever to have received this dose of nicotine are used in the analysis in order to avoid sample bias. We were concerned that a dose

TABLE 1

CORE TEMPERATURE × MINUTES AFTER INJECTION OF NICOTINE

Rat No. 1.	0	10	20	30	40	50	60	70	80	90	100	110	120
	37.0	37.0	36.8	36.6	36.3	36.0	35.7	35.7	35.9	36.4	36.5	36.8	36.9
change °C		0	−0.2	−0.4	−0.7	−1	−1.3	−1.3	−1.1	−0.6	−0.5	−0.2	−0.1

thermic response rat No. 1 =

$$\frac{0+-0.2+-0.4+-0.7+-1+-1.3+-1.3+-1.1+-0.6+-0.5+-0.2+-0.1}{12}$$

thermic response rat No. 1 = −0.62

$$\text{Thermic response of sample} = \frac{\text{thermic response of rat No. 1, 2, 3 . . . n}}{\text{n}}$$

The method used to calculate the mean thermic response an individual rat is displayed. This parameter is, by definition, the average change in core temperature relative to that of the animal prior to the injection of nicotine. The mean thermic response of the entire sample is the average of these changes.

of nicotine (base) of 1.0 mg/kg IP would be toxic or lethal if given to the FSL. We did not have the luxury of risking the loss of FSL rats. FSL rats are difficult to obtain. We could not justify assuming in the absence of data that the FSL's sensitivity to nicotine relative to the Sprague-Dawley rat would be less dramatic than that to modest doses of oxotremorine. We, therefore, decided to give the FSL rats only 0.5 mg/kg of nicotine (base) by intraperitoneal injection.

Measurement of the Thermic Response to Nicotine in Experiment 2

Core temperature was measured immediately prior to (t=0) and every 10 minutes for 120 minutes following the injection of nicotine. The core temperature of the undisturbed rat prior to the injection of nicotine (t=0) is defined as its baseline. The thermic response of an animal is the mean difference between core temperature at baseline (t=0) and at each of the 12 points following the injection of nicotine (6). The mean thermic response of a group of rats is defined as the mean of the thermic response of every animal in that group.

The procedure for calculating the mean thermic response of individual rats and that of an entire sample is presented in Table 1.

Method of measuring temperature. Core temperature was measured telemetrically with the Model VM Mini-Mitter (Mini-Mitter Corp., Sun River, OR). These devices are hearing-aid battery powered radio transmitters which emit amplitude modulated (AM) waves at a rate directly proportional to temperature. The instruments are calibrated by measuring their rate of emission at various temperatures in a temperature controlled water bath (Precision Instruments, Model 50). The rate of emission is measured using a digital frequency counter (Universal Instruments, Model 5001). The Mini-Mitter yields reliable and valid findings in psychopharmacological studies of the type reported in this article (7).

Pharmaceutical agent. Nicotine (base) was purchased from Sigma Chemical Co. (St. Louis, MO).

Animals. Adult, male Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN). FSL rats were obtained from the Alcohol Research Center at the University of North Carolina in Chapel Hill. The animals were housed in a vivarium maintained by The Ohio State University with a 12-hour light/dark cycle (lights on at 6:00 a.m. and off at 6:00 p.m.).

Statistical Analysis

Experiment 1. The mean thermic response 20 minutes following the injection of nicotine was entered into a two-way ANOVA using line and dose as factors. An interaction between line and thermic response would be interpreted as indicating a difference in the sensitivity of the FSL and Sprague-Dawley rat to nicotine.

Experiment 2. Significance of the difference in the sample means in Experiment 2 was determined by the calculation of confidence intervals. The confidence interval is "the mean ± (the critical value of t at a given level of significance for n − 1 *df* where n = sample size) (SEM). An analyses using Student's two-sample *t*-test and calculation of confidence intervals result in the same conclusions. Confidence intervals provide the advantage of conveying information about the magnitude of the difference between samples.

All measures of variance in the text refer to the standard error of the mean (SEM).

RESULTS

Experiment 1

Eight (8) FSL and 8 Sprague-Dawley rats were used in this experiment. The mean mass of the former and latter groups were 459 ± 16.0 g (99.9% confidence interval = 407 to 501 g) and 376 ± 6.8 g (99.9% confidence interval = 353.9 to 398.1 g), respectively. These confidence intervals are separated by 8.9 g ($p<0.001$).

Mean core temperatures of the FSL and Sprague-Dawley groups at baseline over the course of the 10 nicotine challenges were 37.4 ± 0.06 (95% confidence interval = 37.26 to 37.54°C) and 37.6 ± 0.1°C (95% confidence interval = 37.37 to 37.83°C), respectively. These intervals overlap by a 0.11°C. Thus, the mean core temperatures of the two groups over the course of the 10 challenges did not differ significantly.

However, the main effect of dose, $F(9,126) = 30.49$, $p<0.0001$, was highly significant. The main effect of line was not. However, the interaction between dose and line, $F(9,126) = 3.84$, $p = 0.0003$, was significant.

Table 2 summarizes the results of Experiment 1.

Experiment 2

The mean baseline temperatures of the FSL and Sprague-Dawley rats were 36.9 ± 0.09 (n = 11) and 37.2 ± 0.08°C (n =

TABLE 2

SUMMARY OF THE RESULTS OF EXPERIMENT 1

	FSL Rats n=8	Sprague-Dawley Rats n=8
1. Mass	459±16.0 g	376±6.8 g
	These means differ at the 0.001 level 99.9% confidence intervals separated by 8.9 g	
2. Mean baseline temperature		
	37.4±0.06°C	37.6±0.1°C
	These are the mean baseline temperatures of all 8 rats in each group prior to the injection of each of the 10 doses of nicotine. These means do not differ	
3. Main effect of dose		
	Highly significant: $F(9,126)=30.49$, $p<0.0001$	
4. Interaction between dose and line		
	Highly significant: $F(9,126)=3.84$, $p=0.0003$	

The results of Experiment 1 are summarized. The main effect of line was significant. The FSL rat was more sensitive to the hypothermic effect of nicotine than the Sprague-Dawley rat.

104), respectively. The 95% confidence intervals for the mean baseline temperatures of the FSL and Sprague-Dawley rats were 36.9±0.20 (95% confidence interval=36.70 to 37.10°C) and 37.2±0.16°C (95% confidence interval=37.04 to 37.38). The 95% confidence intervals for these mean overlap ($p>0.05$).

The mean mass of the 11 FSL rats was 408.7±5.7 (99.9% confidence interval=408.7±30.8 g=377.9 to 439.5 g). The mean mass of the Sprague-Dawley rats was 297.6±4.0 g (99.9% confidence interval=276.0 to 319.9 g), respectively. These confidence intervals are separated by 58 g ($p<0.001$).

The possibility that the mean mass of the rat may be associated with an increase in its thermic response to nicotine was assessed. This was required since the mean mass of the FSL animals differed greatly from that of the Sprague-Dawley rats. The Sprague-Dawley rats at or above the 75th percentile (top quartile) with respect to mass were identified. The mean thermic response of this group was compared to that of those Sprague-Dawley rats with a maximum mass ≥ to two standard deviations below the lower limit of the top quartile.

Those Sprague-Dawley rats in the top quartile with respect to mass weighed ≥ 325.0 g. The mean mass of these 26 rats was 347.1±5.8 g. The 99.9% confidence interval for this mean is 347.1±21.6 g. Those rats weighing ≥ two standard deviations less than this group had a maximum mass of 288 g. The mean mass of these animals was 264.8±2.46 g. The 99.9% confidence interval for this mean is 264.8±8.6 g.

The lower limit of the 99.9% confidence interval for the group in the upper quartile was 325.5 g. The upper limit of the 99.9% of the confidence interval for the group of Sprague-Dawley rats ≥ two standard deviations removed from the top quartile was 273.4 g. The difference between these limits is 52.1 g ($p<0.001$).

The mean thermic response of the rats at or above the 75th percentile with respect to mass was −1.17±0.13°C. The 90% confidence interval for this mean is −1.17±0.22°C (−1.39 to −0.95°C). The mean thermic response of those Sprague-Dawley rats with mass ≥ two standard deviations below the lower limit of the upper quartile was −0.81±0.1°C. The 90% confidence interval for this mean is −0.81±0.17°C (−0.98 to −0.64°C). The 90% confidence intervals for the mean hypothermic response of these groups overlap. Thus the difference in means does not constitute a trend ($p>0.1$).

The mean thermic response of the 11 FSL rats was −1.6±0.10°C. The 99.5% confidence interval for this mean is −1.6±0.39°C (−1.99 to −1.21°C). The mean hypothermic response of the 104 Sprague-Dawley rats receiving an intraperitoneal dose of 1.0 mg/kg of nicotine (base) was −0.93±0.066°C. The 99.5% confidence interval for this mean is −0.93±0.22°C (−1.15± −0.71°C). The 99.5% confidence intervals for the two means do not overlap. Thus the means differ at $p<0.005$.

The results of Experiment 2 are summarized in Table 3.

DISCUSSION

The curve describing the hypothermic response of the FSL rats to nicotine was shifted to the left of that describing the response of the Sprague-Dawley rats in Experiment 1. Though the thermic response of the FSL rats did not differ at any given dose of nicotine, the interaction between line and thermic response was significant.

It is difficult to perform Experiment 1 using the ideal design due to the rarity of the FSL rat. The dose-effect study should be repeated using independent samples of FSL and Sprague-Dawley rats at each dose of nicotine. This is evident from the development of tolerance in the course of the ten week study. Twenty-seven days following administration of the tenth dose of nicotine (2.4 mg/kg IP), the animals in each group were injected with 1.0 mg/kg of nicotine. Hypothermia did not develop. This suggests that tolerance developed in the course of repeating nicotine challenges at 7 day intervals.

Experiment 1 is useful to the extent that a significant interaction between line and thermic response was detected, and documentation that performing a high quality dose-effect study will require independent groups.

Experiment 2 strongly suggests that the difference in the sensitivity of the FSL and Sprague-Dawley rats is greater than the results of Experiment 1 indicate. The mean hypothermic response of the FSL rats given an intraperitoneal injection of 0.5 mg/kg of nicotine (−1.6±0.1°C) exceeds that exhibited by 104 Sprague-Dawley rats (−0.93±0.066°C) given twice this dose of nicotine by 172% ($p<0.005$).

The FSL rats weighed significantly more than the Sprague-

TABLE 3

SUMMARY OF THE RESULTS OF EXPERIMENT 2

	FSL Rats n = 11	Sprague-Dawley Rats n = 104
1. Mass	408.7 ± 5.7 g	297.6 ± 4.0 g
	These means differ at $p<0.001$	
	The 99.9% confidence intervals are separated by 58.0 g	
2. Mean baseline temperature		
	36.9 ± 0.09°C	37.2 ± 0.08°C
	These means do not differ	
3. Mean hypothermic response		
	−1.60 ± 0.1°C	−0.93 ± 0.066°C
	These means differ at $p<0.005$	
	Lower limit of the 99.5% confidence interval for −1.60°C is −1.21°C	
	Upper limit of the 99.5% confidence interval for −0.93°C is −1.15°C.	
	The 99.5% confidence intervals are separated by 0.06°C	

The results of Experiment 2 indicated that the FSL rat is more sensitive to the hypothermic effect of nicotine than the Sprague-Dawley rat. The mean hypothermic response of the former exceeded that of the latter by 172% despite having received half the dose of nicotine.

Dawley rats. However, mass of the rat does not appear to be related to thermic response ($p>0.1$). The mean baseline core temperatures of the FSL and Sprague-Dawley rats were essentially identical. Thus, a difference in this parameter does not provide a plausible explanation for the results of Experiment 2.

The sample of Sprague-Dawley rats used as a comparison group presents definite advantages. The mean hypothermic response of 104 Sprague-Dawley rats obtained at random from Harlan Laboratories over a period of four years to a fixed dose of nicotine almost certainly provides an accurate estimate of the mean response of the entire universe (population) of Sprague-Dawley rats. That is, it is highly improbable that 104 animals selected at random over a period of four years from a line as homogeneous as the Sprague-Dawley rat would constitute a biased sample.

Patients with disorders of mood exhibit enhanced behavioral and physiological responsiveness to muscarinic agonists and the centrally active anticholinesterase, physostigmine (2, 14, 15). These (and other) findings have led clinical researchers to propose that the activation of central muscarinic cholinergic mechanisms is related to the pathophysiology of depression. This state of activation could either be a trait or state-dependent phenomenon. The FSL has been advanced as an animal model of depression by investigators interested in the dysfunction of muscarinic cholinergic mechanism in the disorders of mood. The FSL (16–22), like patients with histories of depression (2, 14, 15), exhibits exaggerated behavioral, and physiological responses to agents activating muscarinic cholinergic mechanisms.

Recent findings suggest that nicotinic mechanisms may become of interest to clinical investigators interested in the psychobiology affective disorders. Smokers with histories of depression have greater difficulty in successfully completing a smoking cessation program (13). All treatments for the affective disorders studied to date (3–5, 8–11) and chronic stressors (12,23), including one commonly used to produce behavioral and biochemical changes theoretically related to the neurobiology of depression (12, 23, 24) alter the thermic response to nicotine. It is now necessary to assess the effects of forced stressors on additional behavioral (e.g., motor behavior) and select physiological and biochemical variables.

Nicotine may facilitate the release of amines by activating central nicotinic mechanisms (1,25). This renders the finding that those drugs used to treat affective illness which have been studied to date alter the thermic response to nicotine all the more intriguing (3, 4, 8–11). These drugs either enhance (8,11) or decrease (2, 4, 9, 10) the thermic response to nicotine. The former may increase nicotinic receptor-mediated release of amines and the latter may be a compensatory response drug-induced activation of aminergic mechanisms.

SUMMARY

The two experiments presented suggest that the FSL rat is more sensitive to the temperature lowering effects of nicotine than is its progenitor, the Sprague-Dawley rat. There is now a suggestion that the nicotinic cholinergic system may be involved in the neurobiology of depression. The FSL may exceed the expectation that it is useful in studying dysfunction of muscarinic cholinergic systems in the pathophysiology of depression.

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