The effects of VMH lesions in Charles River rats

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Questions have arisen concerning the suitability of the Charles River rat in investigating motivation and its physiological correlates. Old male Charles River rats were given VMH lesions and tested on a number of tasks. In all cases, data from Charles River rats were quite comparable to data from other strains of rats.

Jacobs (1969) has suggested that Charles River rats may be "spontaneously occurring obese hyperphagic" rats, and for that reason he questions the value of their use in investigating the effects of ventromedial hypothalamic (VMH) lesions, as well as other motivation-related phenomena. Since a number of other authors investigating VMH-lesion effects have used Charles River rats (e.g., Lipton, 1969; Marks, 1970; Thomas & Mayer, 1968), it appears necessary to evaluate the possibility that this strain of rat may produce unique data.

Kennedy (1950, 1953) has noted that the feeding behavior of older intact rats was similar to that of the hyperphagic rat. Valenstein, Cox, & Kakolewski (1969) have demonstrated that female rats have a higher probability of showing increased weight gain following VMH lesions than do males. Gold (1970) has shown both greater absolute and percentage weight gains for females than for males. Given these previously cited data, it should be more difficult to demonstrate hypothalamic hyperphagia in the older male Charles River rat than in other strains of rats.

Generally, authors have chosen to measure only one or two parameters when investigating the effects of VMH lesions. Sufficient data exist to indicate that the effects of VMH lesions are a cluster of both behavioral and physiological changes (e.g., Kennedy, 1950, 1953; Marks, 1970). Therefore, to investigate adequately the question of the similarity of effects of VMH lesions in Charles River rats of other strains, measures of quinine aversion, reactivity to touch, rate of free fatty acid (FFA) mobilization, and body composition were taken, in addition to a weight-gain measure.

SUBJECTS

The Ss were nine male albino rats of the Charles River CD strain, approximately 185-190 days of age at

the time of surgery. The preoperative weight of the Ss was 664-799 g.

APPARATUS

Nine 100-ml animal drinking tubes were used in the quinine aversion tests. A Bausch and Lomb Spectronic 20 colorimeter was used in the FFA mobilization analysis. A 1-gal Waring Blender and six soxhlet apparatuses were used in the determination of body composition.

SURGICAL PROCEDURE

All Ss were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal, 50 mg/kg). Six Ss were given VMH lesions (coordinates AP = 7.0, L = 0.7, DV = 1.0, above the base of the skull). The lesions were made by passing direct current of 2.0 mA for 20 sec

through a No. 2 insect pin, insulated except at the tip.

HISTOLOGY

After the conclusion of testing, each S was given an overdose of sodium pentobarbital and perfused with 0.9% saline. The brain was removed, fixed with neutral buffered formalin, embedded in celloidin, and sectioned at 30 micra. Every 10th section was stained with thionin.

PROCEDURE

One week following surgery and each week thereafter for 9 weeks, all Ss were weighed. Each week each S was also tested for its response to a pencil thrust into its cage. A 3-point rating scale was used to evaluate their response: 0 = indifference—ignoring the pencil and moving away when it touched them; 1 = curiosity—sniffing and/or lightly gnawing on the pencil; 2 = attack—immediate biting as soon as it was seen or felt.

Water consumption and quinine aversion tests were conducted 10, 30, and 55 days following surgery. All Ss were adapted to the drinking tubes for 2 days prior to taking any measures. Following this adaptation, water intake was measured during the next 24 h. The following day, quinine (0.006% quinine HCl for the first test and 0.0006% quinine HCl for the last two tests) intake was measured for

Fig. 1. Weight gain of control and lesioned Ss. The lesioned Ss presented had major damage in the VMH. Open figures and dotted lines indicate control Ss. Solid figures and solid lines indicate lesioned Ss. The figures to the right of each S number indicate the weight of that S at the time of sacrifice.

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24 h. Aversion scores were calculated by dividing water intake into the difference between water and quinine intake, yielding a percentage suppression score: high scores indicating aversion and low scores indicating little or no aversion.

The rate of FFA mobilization from adipose tissue was based on the procedure of Starr, Crawford, & Haessler (1966). Two samples of epididymal fat were taken from the S, rinsed in iced saline, blotted, and weighed. The samples, each weighing 92-110 mg, were placed into glass-stoppered test tubes with 2 ml of Krebs Ringer bicarbonate solution containing 4% fatty-acid-poor bovine albumin. Just prior to incubation, 0.5 microgram/ml of epinephrine (Adrenaline 1:1000, Parke-Davis) was added to one of each pair of tubes. The medium was incubated at 37°C for 3 h without agitation in a normal oxygen-nitrogen atmosphere. Following incubation and the removal of the fat tissue from the medium, FFA concentrations were determined by the colorimetric method of Walker (1968). The difference between the unstimulated release rate and the epinephrine-stimulated release rate was attributed to the action of epinephrine on the fat pad and was termed FFA mobilization rate.

The whole-body composition procedure was adapted from Han (1967). Each decapitated carcass was eviscerated. The viscera were cleaned of all food and fecal matter and returned to the carcass. The carcass and viscera were then towel dried, placed into a Mason jar, and autoclaved for 15 min at 15 psi. The carcass was homogenized in a Waring Blender with an equal amount of distilled water (w/v). A 5%-6% aliquot was oven dried to a constant weight at 75°-80°C. The dried fat and residue were placed in a cellulose extraction thimble and placed in a Soxhlet apparatus containing purified ether. The fat extraction process continued until a constant thimble weight was obtained.

The difference between the pre- and postdrying weights was taken as the aliquot water content. The difference between the pre- and postextraction thimble weights was taken as the aliquot fat content. The remainder of the aliquot was called dry residue. The percentage composition of these three fractions was determined for the aliquot. These percentages were used to calculate the grams of each fraction in the total carcass.

RESULTS

Four of the six lesioned Ss demonstrated the increased weight gain associated with hypothalamic hyperphagia. Three of the lesioned Ss

Table 1
Reactivity Ratings for Hyperphagic and Control Ss to Touch*

Group	Attack	Curiosity	Indifference
Control	3	22	2
Hyperphagic	22	0	5

*Ratings are the sum of the number of times each behavior was scored over the 9-week testing period. The highest possible score for a category was 27.

had damage in and around the ventromedial nucleus (VMN): in two Ss, the VMN and immediately surrounding area were completely destroyed; in one S, the VMN and arcuate nucleus were damaged, but the damage was much less extensive than in the first two Ss. The fourth S, while demonstrating a somewhat greater weight gain than control Ss, was found to have a lesion which invaded the anterior third of the posterior hypothalamic area, and this S was eliminated from the statistical analyses. The analysis of variance confirmed that lesioned Ss gained reliably more weight than control Ss ($p < .001$; Fig. 1).

Lesioned Ss showed consistently more attack behavior than control Ss, while control Ss showed more curiosity than lesioned Ss ($p < .05$; Table 1).

The concentration of the quinine solution used in the first test, although lower than that used in a previous investigation (Brown, 1970), produced

large suppression scores for both lesioned and intact Ss. The concentration of the solution was, therefore, reduced for the last two tests. Using this 0.0006% concentration, reliable differences were found between groups, with lesioned Ss producing moderately high suppression scores, while little suppression was found with control Ss ($p < .05$; Table 2).

The physiological measures (see Table 3) indicated that the FFA mobilization rate was reliably lower for lesioned than for control Ss. While percentage body fat was reliably higher and percentage body water was reliably lower in lesioned than in control Ss ($p < .05$), when these percentages were transformed to weight, the only reliable difference between the groups was due to a greater amount of fat in the lesioned Ss ($p < .005$).

DISCUSSION

Jacob's (1969) statement, that Charles River rats are "spontaneously occurring obese hyperphagic," can only be evaluated in the context of some comparison. If this were a weight comparison among strains of rats, then Charles River rats are heavier than most commercially available strains, although both the Dublin DR and Simonson strains are quite heavy. If this were a comparison based on taste preference, it would appear that the Charles River rat may be more "finicky" than other strains.

Regardless of these comparisons, the results of this study would

Table 2
Water and Quinine Consumption and Percentage Suppression Scores for Each of Three 2-Day Sessions

Test		Hyperphagic	Control	P Value
1	Water (in ml)	207	52	.05
	Quinine (in ml)*	55	9	.05
	Percent Suppression**	79.4	83.6	—
2	Water	90	40	.05
	Quinine***	39	32	—
	Percent Suppression	55.3	7.6	.01
3	Water	92	49	—
	Quinine***	41	35	—
	Percent Suppression	46.7	14.4	.05

*Quinine HCl concentration in the first test was 0.006% (w/v).

**Percent suppression = water - quinine/water.

***Quinine HCl concentration in the second and third tests was 0.0006% (w/v).

Table 3
Physiological Measures

Group	Mobilization Rate	Percent		Weight (g)		
		Fat	Water	Dry Residue	Fat	Water
Hyperphagic	-1.93 ueg/g/h	48.7	37.2	13.8	513	394
Control	+1.68 ueg/g/h	22.6	54.7	22.6	166	394
p Value	.05	.05	.05	—	.005	—

appear to support the idea that Charles River rats do tend to produce both physiological and behavioral data that are in close agreement with much of the published data in the area of hypothalamic hyperphagia. The hyperreactivity to taste stimuli found with lesioned Charles River rats appears to be the same as that reported by Corbit (1965) and Corbit & Stellar (1964). The fact that both lesioned and intact Ss showed high percentage suppression scores on the first test may be a function of a difference in quinine taste thresholds (or preference thresholds) for Charles River rats. Thus, while the absolute values found with Charles River rats may not correspond to those found by other authors, the ordinal relationship between lesioned and intact Ss appears identical.

No data appear to be available concerning the reactivity of the VMH-lesioned rat to touch. Data from a shock threshold study (Turner, Sechzer, & Liebelt, 1966) and several studies reporting reactivity to handling (e.g., Grossman, 1966; Marks, 1970) indicate that the VMH-lesioned S is probably more reactive to this class of stimuli than are intact Ss. These data were replicated with the Charles River rats.

The physiological data indicate that changes in body composition are almost exclusively in the form of additional fat in the lesioned S. While intact Ss of the Charles River strain have greater percentages of body fat than rats of other strains, the relationship between amounts of fat in lesioned and intact rats is the same as that reported by Han (1967) and Bates, Nauss, Hagman, & Mayer (1955). The FFA mobilization data appear to be the same in Charles River rats as those reported by Starr et al (1966) for Sprague-Dawley rats.

All the data collected in this study appear to demonstrate quite clearly that Charles River rats produce the same type of data following VMH lesions as do other strains of rats. In addition, a greater ease of handling, combined with a greater resistance to surgical stress, makes these Ss potentially more useful in some experimental situations than other strains. While it cannot be denied that there may be some differences between the Charles River and other strains of rats, this study has found differences only of degree and not of kind, suggesting that Jacobs's conclusions may not be correct.

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Reflex "training" in frogs*

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It has been reported that repeated stimulation of a point on the dorsal skin of the frog will result in a specific enhancement of the wiping reflex of that zone. Tests of the specificity of this enhancement demonstrate that all areas of the skin and all wiping reflexes are sensitized when the animal is given reflex "training." Handling controls indicate that the increased nonspecific response rate may be due to factors other than learning.

When a point on the dorsal skin of intact or spinal frogs is stimulated, a wiping reflex is occasionally elicited. This reflex is specific to the stimulated zone: a point anterior to the scapula (anterior zone) results in a wiping by the ipsilateral front leg; stimulation of the skin posterior to the scapula (major zone) results in a wiping by the ipsilateral hind leg; and stimulation of the hind regions (caudal zone) elicits a wiping by the heel. It has been reported that repetitive stimulation of a specific point will result in an increased elicitation of the wiping reflex, an enhancement which is specific to the point stimulated and which is retained for over 20 days (Franzisket, 1963). These findings were reported for both intact and spinal animals (*Rana esculenta*) and have created considerable interest in

the frog as a "simple system" in which to do research on learning and memory mechanisms (Bullock & Quarton, 1966; Corning, 1968). We attempted to replicate this reflex training in *Rana pipiens*, paying particular attention to the specificity of the enhancement. Additionally, we included handling controls that would provide a clearer interpretation of the reflex modification.

METHOD

Stimulation procedures followed those detailed by Franzisket (1951, 1963). In 10 intact frogs, using the skin markings as a guide, a specific point on the dorsal surface of the major zone was stimulated 100 times per day for 11 days, with a 10-sec interval between stimulations. The specificity of the response modification was assessed by testing the reactivity of other sites on the first day of training and on the day after completion of training. These sites consisted of the contralateral homotopic point and two points in the ipsilateral and contralateral anterior zone. We refer to these tests as "reflex

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