Differential Responding for Brain Stimulation Reward and Sucrose in High–Alcohol-Drinking (HAD) and Low–Alcohol-Drinking (LAD) Rats

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Background: This study examined the associations among selective breeding for alcohol preference, intake of sweet solutions, and responding for brain stimulation reward (BSR), a nonoral reinforcer, in alcohol-preferring high–alcohol-drinking (HAD)-1 and nonpreferring low–alcohol-drinking (LAD)-1 rats.

Methods: Adult male HAD-1 and LAD-1 rats were trained to lever press for medial forebrain bundle stimulation. Current intensity was varied in separate sessions to generate a rate/intensity function. To further examine BSR responding, the animals responded for stimulation at 100 Hz and at a fixed current intensity on an FR1 schedule. In subsequent sessions, the schedule was increased to FR6 and then to FR12. To examine responding for the sucrose solution, we trained a separate group of HAD-1/LAD-1 rats to bar press for sucrose on an FR1 schedule. Similar to the BSR experiment, in following sessions, the schedule was increased to an FR6 and then to an FR12 schedule.

Results: No significant differences were observed between the two rat lines across a range of current intensities. As the reinforcement schedule increased, HAD-1 rats exhibited a dramatic decrease in BSR responding, whereas the LAD-1 rats displayed a more protracted reduction. In contrast to BSR, marked elevations in responding were observed for sucrose as the schedule increased. However, in HAD-1 rats, response rates were similar on the FR6 and FR12 schedules, whereas LAD-1 rats showed a reduction in response rates from the FR6 to FR12 schedule. Furthermore, HAD-1 rats exhibited significantly more responses compared with LAD-1 rats across the three reinforcement schedules. An analysis of the response profile for the three reinforcement schedules suggested that few if any postreinforcement pauses were exhibited when the reinforcer was BSR compared with sucrose in both lines.

Conclusion: Medial forebrain bundle BSR is a powerful reinforcer in both HAD-1 and LAD-1 lines. However, BSR responding was not associated with selective breeding for alcohol preference. In contrast, selective breeding for alcohol preference was associated with sucrose consumption, especially as the amount of work increased. The lack of correspondence between BSR and sweet taste rewards in HAD-1 and LAD-1 lines may suggest important differences yet an overlapping brain reward mechanism in the control of motivated behaviors in these selected lines.

Key Words: Alcohol, Alcohol-Preferring Rats, Brain Stimulation Reward, Self-Stimulation, Sucrose Reward.

AN ANIMAL MODEL in the study of alcoholism is the high–alcohol-drinking (HAD) and low–alcohol-drinking (LAD) rat lines that were bred for alcohol preference (Li et al., 1993; Lumeng et al., 1995; McBride and Li, 1998). Indeed, HAD rats have been shown to consistently drink high levels of alcohol (approximately 9.5 g of alcohol/kg of body weight/day), whereas LAD rats drink very low amounts of alcohol (approximately 0.5 g of alcohol/kg/day)(Murphy et al., 2002). Although it is not clear what factors regulate the disparate alcohol-seeking behaviors in these rat lines, it has been hypothesized that they may be due to genetic differences in reward-related neural circuitry (McBride and Li, 1998).

HAD and LAD rats have been shown to differ in their responsiveness not only to alcohol but also to other palatable, conventional reinforcers. Specifically, in homecage studies, the HAD-1 and the replicate line, HAD-2s, both exhibit a greater preference for a 0.1% (w/v) saccharin solution (Overstreet et al., 1997; Stewart et al., 1998) and a 10% sucrose solution (Badia-Elder et al., 2000). Preliminary homecage studies demonstrate that the HAD x LAD F2 progeny also show a positive correlation between alco-
hol preference and saccharin intake (0.1% w/v) (Stewart et al., 1998). Thus, it seems that compared with the LAD rats, HAD rats are innately more sensitive to the reinforcing properties of alcohol as well as several conventional reinforcers (i.e., sucrose, saccharin).

Similar to the HAD and LAD lines, homecage studies have shown that compared with alcohol nonpreferring (NP) rats, the alcohol-preferring (P) rats exhibit a greater preference for sucrose (2–8% w/v) (Stewart et al., 1994) and saccharin (0.1% w/v) solutions (Bell et al., 1994; Kampov-Polevoy et al., 1995, 1996; Overstreet et al., 1993, 1997; Sinclair et al., 1992). Together, these data from selectively bred alcohol-preferring and nonpreferring rat lines suggest that high oral alcohol preference is positively associated with intake of sweet solutions. Hence, it is possible that the association between high intake of sweet palatable reinforcers and ethanol (EtOH) may have a common genetic basis (Stewart et al., 1994).

Given the association between high–alcohol-drinking behavior and intake of sweet palatable reinforcers, one might hypothesize that high–alcohol-drinking rats may simply have an enhanced sensitivity to rewarding stimuli in general (i.e., sex, drugs, electrical brain stimulation, etc.). Over the past few years, our laboratory has evaluated this hypothesis by using several reinforcement paradigms comparing P and NP rats and HAD and LAD rats. One such paradigm is reinforcement produced by electrical brain stimulation (Olds and Milner, 1954). Currently, no research is available exploring the relationship between alcohol preference and preference for a “nonconventional” reinforcer such as electrical brain stimulation.

As with conventional reinforcers (i.e., food, water, etc.), BSR has been found to reinforce behaviors (e.g., lever pressing). However, several differences emerge in the behavioral patterns sustained by BSR and conventional reinforcers (Bachmanov et al., 1996; Lewis, 1993). First, the most salient difference is the strength/power of the stimuli, as demonstrated by the fact that very high response rates (5,000–10,000/hr) are observed with BSR, even under certain partial reinforcement schedules. Second, behaviors reinforced by BSR extinguish at a faster rate than behaviors reinforced by conventional reinforcers. Third, BSR is a more rapid, instantaneous reinforcer, whereas a greater time delay occurs with conventional reinforcers. Finally, BSR typically produces poor performance under partial schedules of reinforcement, whereas conventional reinforcers produce better performance. Indeed, alterations in the schedule of reinforcement produce striking differences between behavioral patterns produced by conventional reinforcers compared with BSR. For example, response rate (i.e., bar pressing) typically increases in frequency as the reinforcement schedule increases with conventional reinforcers; however, response rate decreases as the schedule increases with BSR (Czachowski and Samson, 1999; Files et al., 1993; Gahtan et al., 1996; Samson et al., 1992; Schaefer and Michael, 1986, 1992a). Given these differential response profiles, it is possible that examination of the qualitative nature of response profiles and time course analyses of both BSR and conventional reinforcers may be useful in illuminating neuroregulatory processes (e.g., onset, termination) and their potential overlapping effects on common neurochemical systems (i.e., dopamine, opioids, etc.) that may regulate the two types of reinforcers.

The purpose of this study was to determine if hereditary influences originating from selective breeding for alcohol preference play a role in sensitivity to brain stimulation reward (BSR) in the HAD-1 and LAD-1 rat lines. A second purpose of the study was to determine if an operant analysis of the reinforcing properties of a highly palatable sucrose solution reinforcer (10% w/v) might reveal additional information about the association between selective breeding for high–alcohol-drinking behavior and sweet rewards not observed in two-bottle choice studies. The use of the operant paradigm allows for control of reinforcement schedule and a better comparison between the sucrose solution and BSR. The final purpose was to compare the rewarding efficacy of medial forebrain bundle (MFB) BSR to sucrose responding across several schedules of reinforcement. The examination of a nonconventional reinforcer with a more conventional reinforcer in the two rat lines may provide information as to whether the HAD-1 rats are more sensitive to reinforcers in general, compared with the LAD-1 rats. In addition, the evaluation of similarities and differences in responding for BSR and a sucrose solution may help delineate overlapping and different neurobiological systems that underlie these two differing types of reinforcers. We hypothesized that sensitivity to BSR and sucrose responding would be positively associated with selective breeding for alcohol preference, independent of the schedule of reinforcement.

METHODS

Subjects

Male HAD-1 (n = 33) and LAD-1 (n = 31) rats of the S30 generation (Indiana University, Indianapolis, IN) weighing between 305 and 346 g at the initial phase of testing were used as subjects. The animals were individually housed in shoe-box cages in a temperature- and humidity-controlled room on a 12 hr light/dark cycle (lights on at 7:00 AM) with food and water available ad libitum, except during the first 2 days of the training phase, wherein rats were fluid deprived 23 hr daily. All behavioral training and testing took place between 9:00 AM and 3:00 PM. All procedures were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Stereotaxic Surgery and Histology

For BSR experiments, HAD-1 (n = 12) and LAD-1 (n = 12) rats were anesthetized with pentobarbital sodium (45 mg/kg intraperitoneally) and placed in a stereotaxic apparatus. Rats were implanted with platinum bipolar electrodes with a diameter of 0.3 mm (Plastics One, Inc., Roanoke, VA) in the right hemisphere into the MFB. In relation to bregma, the coordinates for the MFB were anteroposterior −2.3, mediolateral +1.6, and dorsoventral −8.8 according to the atlas of Paxinos and Watson (1988). The Teflon-insulated electrode was anchored to the skull with stainless steel screws by dental acrylic.
After the completion of all experimental procedures, the rats were killed via CO₂ inhalation. The brain was immediately lesioned with an A13-65 isolated physiologic stimulator (Coulbourn Instruments, Allentown, PA) by using three 3 sec, 4 mA bursts with 1 sec separation between bursts. After lesioning, the rats were decapitated and the brains were extracted and frozen. Subsequently, histologic evaluations were performed to determine the placement of the electrodes. Once the brains were sliced on a microtome at 50 μm sections, these sections were then stained with cresyl violet acetate (0.5 μl). The sections were examined under a light microscope with lesion placements indicated on drawings adapted from the rat brain atlas of Paxinos and Watson (1998).

**Behavioral Apparatus**

Behavioral training and testing for BSR experiments were conducted in 10 standard operant chambers (Coulbourn Instruments) equipped with a removable lever enclosed in a sound-attenuating cubicle. Each bar press triggered, depending on the schedule of reinforcement (e.g., FR1, FR6, FR12), an electrical pulse of a given current intensity. The stimulation was a 0.2 sec train of 100 Hz biphasic rectangular pulses of 1.0 msec duration provided by an A13-65 isolated physiologic stimulator (Coulbourn Instruments). Responses and reinforcements were controlled and recorded during a 20 min operant session by a Compaq computer using the 4.0 Coulbourn L2T2 operant software package. Sucrose training was conducted in 13 standard operant chambers equipped with two removable levers and two dipper fluid delivery modules enclosed in sound-attenuating cubicles. Depending on the reinforcement schedule (e.g., FR1, FR6, FR12), rats were delivered 0.1 ml of a 10% (w/v) sucrose solution.

**Procedures**

**Experiment 1: Examination of the Effect of Schedule of Reinforcement on Response Rate for BSR in HAD-1 and LAD-1 Rat Lines.** The animals were initially water deprived for 23 hr daily for 2 days before behavioral training, after which food and water were freely available for the remainder of the experiment. During the 2 days of water deprivation, animals were trained to bar press for a 0.1% (w/v) saccharin solution on a continuous schedule of reinforcement (i.e., FR1 schedule) to orient and train the animals to press the bar. Then, all animals were implanted with electrodes and subsequently trained to lever press at the baseline current (i.e., the current range that produced the maximal responding) for the BSR reinforcer under FR1, FR6, and FR12 schedules of reinforcement.

**Experiment 2: Generation and Comparison of a Rate/Intensity Curve for BSR in HAD-1 and LAD-1 Rat Lines.** After the rats were trained to bar press for the BSR current in the operant chamber under an FR1 schedule of reinforcement for constant current over a range of current intensities (10–400 μA) and performance had stabilized, the current intensity was

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**Fig. 1.** Reconstruction of serial coronal sections of the rat brain illustrating the unilateral electrode tips in the MFB for HAD-1 (n = 10) (A and B) and LAD-1 (n = 9) (C and D) rats included in the data depicted in Figs. 3 through 7. Each rat is represented by one solid black circle. Coronal sections are adapted from the rat brain atlas of Paxinos and Watson (1998).
varied in relation to the rat’s baseline or training current. As previously stated, the baseline current (HAD-1 = 350 + 90 μA; LAD = 365 + 45 μA) was arbitrarily defined as the current at which maximal responding was observed. To generate a rate/intensity function in the descending direction, the current was increased by 100 μA and then by 200 μA steps from the baseline current. To generate the ascending component of the rate/intensity curve, the current intensity was lowered four times in 100 μA increments for all animals from the baseline current. The only stipulation imposed was that the current intensity did not fall below 50 μA. In addition, all rats were tested on a 30 μA current to assess the reinforcement value at the minimal level of stimulation.

Experiment 3: Examination of the Effect of Schedule of Reinforcement on Response Rate for Sucrose in HAD-1 and LAD-1 Rat Lines. HAD-1 (n = 20) and LAD-1 (n = 19) rats were initially water deprived for 2 days before training. Afterward, water was freely available for the remainder of the experiment. The rats were trained to bar press for sucrose (10% w/v) during a 60 min operant session on an FR1, FR6, and FR12 schedule of reinforcement until stable baselines for each schedule were generated.

Data Analysis
Response rates for brain stimulation and the sucrose solution by HAD-1 and LAD-1 on the three schedules of reinforcement were analyzed by using a 2 x 3 ANOVA with rat line as the between-subjects factor and schedule as the within-subject factor. Separate single-factor ANOVAs were used on HAD-1 and LAD-1 responding data. Subsequent least squared differences post hoc analyses were used to determine whether a significant difference existed between the three schedules of reinforcement on response rate for the HAD-1 and LAD-1 rat lines.

Data generated by the rate/intensity function (i.e., the response rate for each current level) was analyzed by using a 2 x 9 ANOVA with rat line as the between-subjects factor and current intensity as the within-subject factor. Additional one-way ANOVAs were run on HAD-1 and LAD-1 responding data to further delineate differences within the two rat lines at the varying current intensity levels. Subsequent planned least squared differences post hoc analyses were used to explore significant effects between and within the rat lines for both the response rates at each current intensity level.

RESULTS

Histologies

Figure 1 shows a reconstruction of serial coronal sections of the rat brain illustrating the unilateral electrode tips for the HAD-1 (n = 10) and LAD-1 (n = 9) rats. The histologic placements showed that the electrodes were implanted in the anterior to posterior MFB (bregma −0.92 to −4.16 mm). Two HADs and three LADs were excluded from the study due to poor electrode placement or loss of the electrode.

Results for Experiment 1: Examination of the Schedule of Reinforcement on BSR Response Rate in HAD-1 and LAD-1 Rat Lines

Figure 2 illustrates the mean number of bar presses for HAD-1 and LAD-1 rat lines on FR1, FR6, and FR12 schedules of reinforcement. Under the FR1 schedule of reinforcement, the mean number of responses for the HAD-1 and LAD-1 rats was 1378 ± 307 and 1395 ± 377, respectively. The range of responses for HAD-1 and LAD-1 rats was 109 to 3402 and 157 to 3897, respectively. The 2 x 3 mixed ANOVA showed that overall response rates were similar for the HAD-1 and LAD-1 rat lines. As seen in Fig. 2, an increase in the schedule of reinforcement produced a significant overall decrease in responding for brain stimulation [F(2,34) = 8.12, p = 0.001]. However, the analysis did not reveal a significant effect of rat line [F(1,17) = 0.78, p = 0.39] or a significant interaction between the schedule of reinforcement and rat line [F(2,34) = 0.19, p = 0.83]. Even though the comparisons did not demonstrate an interaction, further analyses were believed to be warranted due to a priori planning. Thus, in a separate one-way ANOVA for HAD-1 rats, response rates yielded an overall effect of schedule [F(2,18) = 9.06, p = 0.002]. Post hoc analyses confirmed that the FR6 and FR12 schedules of reinforcement produced significantly lower rates of responding compared with the FR1 schedule (p < 0.05). In contrast, a separate ANOVA for LAD-1 rats did not reveal a significant difference between the schedules of reinforcement [F(2,16) = 1.99, p = 0.17]; however, nonsignificant reductions in responding were observed as the schedule of reinforcement increased.

The mean number of cumulative bar presses in HAD-1 and LAD-1 rat lines on the FR1, FR6, and FR12 schedules of reinforcement is illustrated in Fig. 3. Both rat lines responded in a rapid, continuous, and repetitive manner throughout the entire operant session. There was an absence of postreinforcement pauses (PRPs) at the FR6 and FR12 schedules, thus indicating performance more characteristic of a CRF response schedule (Skinner, 1938), particularly in BSR studies (Schaef er and Michael, 1992). As revealed in Fig. 4, when these cumulative data are plotted, a near vertical line (i.e., slope approaching infinity) approximates the relative absence of PRPs. Analysis of the HAD-1 response profile revealed a slope of 75.29, 41.29, and 19.87 for the FR1, FR6, and FR12 schedules, respectively (see Fig. 4). In the LAD-1 rats, the response profile revealed a slope of 81.65, 60.12, and 43.44 for the FR1, FR6, and FR12 schedules, respectively (see Fig. 5). Thus,
although the repetitive response profiles were exhibited throughout the entire 20 min sessions under the FR1 schedule for both rat lines, a marked difference between the two line was apparent under the FR6 and FR12 schedules. During the initial 5 min of the operant session under the FR1 schedule, response rates were approximately 354 and 301 responses per 5 min for LAD-1 and HAD-1 rats, respectively (Figs. 4 and 5). Under the FR12 schedule, response rates were approximately 78 and 31 responses per 5 min for LAD-1 and HAD-1 rats, respectively (Figs. 4 and 5). Clearly, both lines exhibited more PRPs under the higher schedules. Hence, the ability of the data to fit a linear plot as well as the steepness of slope seemed to decrease as the schedule increased in both HAD and LAD lines.

Results for Experiment 2: Generation and Comparison of a Rate/Intensity Curve for BSR in HAD-1 and LAD-1 Rat Lines.

Figure 6 shows that an inverted-U-shaped curve was produced after evaluation of the nine current levels in the HAD-1 and LAD-1 lines. As the current level increased, responding increased, and thereafter no change was observed, which was then followed by a reduction in responding. Analyses of the current intensities showed an overall effect of current intensity on responding for BSR \([F(8,136) = 10.38, p < 0.001]\). However, analyses did not reveal a line effect at any of the nine current levels between the HAD-1 and LAD-1 rats \([F(1,17) = 0.18, p = 0.68]\) or a current intensity by line interaction \([F(8,136) = 0.67, p = 0.72]\).
Separate ANOVAs revealed an overall effect of current intensity on response rate for HAD-1 rats \( F(8,72) = 6.10, p < 0.001 \). As seen in Fig. 6A, and confirmed by post hoc analysis, the current intensity level ranges of 30 \( \mu \)A, 145 \( \pm 80 \) \( \mu \)A, 250 \( \pm 94 \) \( \mu \)A, and 1055 \( \pm 95 \) \( \mu \)A produced significantly lower levels of responding compared with the baseline current 350 \( \pm 90 \) \( \mu \)A \( (p < 0.05) \). In addition, a separate ANOVA revealed an overall effect of current for the LAD-1 rats \( F(8,64) = 4.99, p < 0.001 \). As seen in Fig. 6B and confirmed by post hoc analyses, the current intensity ranges of 30 \( \mu \)A, 108 \( \pm 24 \) \( \mu \)A, and 1065 \( \pm 43 \) \( \mu \)A produced a significant decrease in response rate in LAD-1 rats compared with the baseline current 365 \( \pm 45 \) \( (p < 0.05) \).

Results for Experiment 3: Examination of the Effect of the Schedule of Reinforcement on Response Rate for Sucrose in the HAD-1 and LAD-1 Rat Lines.

Figure 7 illustrates the mean number of bar presses for HAD-1 and LAD-1 rat lines on the FR1, FR6, and FR12 schedules of reinforcement for sucrose. On the FR1, FR6, and FR12 schedules of reinforcement, the mean number of responses for the HAD rats was 192 \( \pm 30 \), 687 \( \pm 57 \), and 761 \( \pm 111 \) responses, respectively. The mean number of responses for the LAD-1 rats on the FR1, FR6, and FR12 schedules was 118 \( \pm 22 \), 452 \( \pm 65 \), and 321 \( \pm 60 \), respectively.

A 2 \( \times \) 3 mixed ANOVA showed that responding for

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**Fig. 5.** Slope analysis for the cumulative response profile of the LAD-1 rats on the FR1, FR6, and FR12 schedule of reinforcement for brain stimulation reward.

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**Fig. 6.** Current/response curve by HAD-1 (A) and LAD-1 (B) rats for brain stimulation reward. Rats were placed in the operant chamber and allowed to press on varying current levels for a 20 min session. * \( p < 0.05 \) versus baseline (BL) current level values by ANOVA and post hoc analysis least squared difference test. Error bars represent + SEM.
sucrose increased as the reinforcement schedule increased, resulting in a significant effect of schedule \( F(2,72) = 28.95, p < 0.001 \). Post hoc analyses revealed that responding was substantially lower under the FR1 compared with the FR6 and 12 schedules \( p < 0.001 \); however, no significant differences in response rates were observed between the FR6 and 12 schedules \( p > 0.05 \). The ANOVA also revealed a significant effect of rat line, with HAD rats responding significantly greater than the LAD-1 rats for sucrose \( F(1,37) = 21.11, p < 0.001 \). In addition, the analyses further indicated a significant schedule by line interaction \( F(2,72) = 3.82, p < 0.05 \). Subsequent post hoc analysis showed that the HAD-1 rats responded more under all three schedules of reinforcement compared with the LAD-1 rats \( p < 0.05 \).

Figure 8 illustrates the mean number of cumulative bar presses in HAD-1 and LAD-1 rat lines under the FR1, FR6, and FR12 schedules of reinforcement for sucrose. In contrast to BSR, responding under the FR1 schedule produced early termination of responding in the first third of the operant session for both the HAD-1 and LAD-1 lines. In the HAD-1 rats, under the FR6 and FR12 schedules, responding was continuous throughout the 60 min session, resembling the qualitative pattern seen in BSR. However, the repetitive, rapid response pattern displayed in the BSR sessions was not observed for the sucrose reward. Unlike the HAD-1 rats, the LAD-1 rats exhibited a reduction in responding across the operant session in going from the FR6 to FR12 schedule, although rates of responding were similar at the initial 10 min interval. Analysis of the HAD-1
response profile revealed a slope of 2.52, 9.21, and 11.86 for the FR1, FR6, and FR12, schedules, respectively (see Fig. 9). In the LAD-1 rats, the response profile revealed a slope of 1.54, 6.61, and 4.94 for the FR1, FR6, and FR12 schedules, respectively (see Fig. 10). These slope analyses confirm that the continuous rapid accelerating response patterns seen in BSR were not characteristic of responding for the sucrose reward. Furthermore, it is likely that the PRPs were substantially greater for sucrose compared with BSR responding. Nevertheless, the similarity and magnitude of the slopes for the HAD-1 rats under the FR6 and FR12 schedules confirm that the two schedules maintained sucrose responding above and beyond that of the FR1 schedule. Under the FR12 schedule, response rates were approximately 64 and 150 per 5 min for LAD-1 and HAD-1 rats, respectively. However, under the FR1 schedule, response

**Fig. 9.** Slope analysis for the cumulative response profile of the HAD-1 rats on the FR1, FR6, and FR12 schedule of reinforcement for the sucrose reinforcer. Error bars represent ± SEM.

**Fig. 10.** Slope analysis for the cumulative response profile of the LAD-1 rats on the FR1, FR6, and FR12 schedule of reinforcement for the sucrose reinforcer. Error bars represent ± SEM.
rates were approximately 40 and 62 per 5 min for LAD-1 and HAD-1 rats, respectively. These rates were markedly lower than those observed for BSR during the initial 5 min (as previously described).

DISCUSSION

The current data demonstrated that BSR in the MFB is a very powerful reinforcer in both the HAD-1 and LAD-1 rat lines. Both lines averaged 60 to 65 responses per minute and 1346 ± 361 responses per 20 min session. However, no significant differences in responding for BSR were observed between the two rat lines across a range of current intensities (see Fig. 6). These findings suggest that the HAD-1 and LAD-1 rats are equally sensitive to BSR in the MFB. Although it is somewhat difficult to compare across different BSR studies due to differences in BSR apparatus and procedures, when the current data are compared with outbred rats (i.e., Long-Evans versus Sprague Dawley®), it seems that the HAD-1 and LAD-1 rats respond at a similar magnitude for MFB BSR on a CRF schedule (Lynch and Wise, 1985; Ornstein and Huston, 1977). For example, Ornstein and Huston (1977) reported response rates of 58 ± 10 responses per 10 min with current intensities ranging from 24 to 50 μA and a constant frequency of 100 Hz. Lynch and Wise (1985) reported 2000 response per 30 min with current intensities between 18 and 90 μA. It is important to note, however, that optimal response rates in HAD-1 and LAD-1 rats are more readily maintained with higher current intensities (200–400 μA) compared with lower intensities (50–100 μA). At present, it is not clear why the HAD and LAD lines require more current to exhibit a similar sensitivity to BSR. Nevertheless, evaluation of the rate-intensity function in the present study suggests that a differential sensitivity in MFB responding is not associated with selective breeding for EtOH preference in HAD-1 and LAD-1 rats.

BSR has been used extensively to study the neuroanatomical and neurochemical substrates of reward-related behaviors (Kornetsky and Bain, 1990; Lewis, 1993; Wise and Rompre, 1989). Although earlier evidence suggested the importance of multiple systems in mediating BSR (Huston, 1982; Valenstein and Campbell, 1966) and more recent investigators have begin to evaluate novel loci (e.g., ventral pallidum, bed nucleus of the stria terminalis) in regulating BSR (Hardy et al., 2002; Panagis et al., 1998), much interest remains in the MFB as a key system and reward substrate in regulating reward-related behaviors. An evaluation of the histologic placements in the present study reveals that the high-responding animals (e.g., 1200 responses per 20 min) were clearly those with electrodes juxtaposed to the MFB, whereas the low-responding animals (e.g., 500 responses per 20 min) had electrode placements more distal to the MFB (see Fig. 1). These neuroanatomical findings confirm that, as with outbred rat lines (Lewis, 1993), the MFB in alcohol selectively bred HAD-1 and LAD-1 lines represents a pivotal substrate for maintaining BSR.

Although not differentially sensitive to the amount of current required to produce BSR, the two rats lines seem to be differentially sensitive to the amount of work required to obtain BSR. Specifically, HAD-1 rats exhibited a dramatic decrease in responding for brain stimulation as the schedule of reinforcement increased whereas the LAD-1 rats displayed a more protracted reduction in responding. The results seen in the LAD-1 rats are consistent with those reported in outbred rats (Schaefer and Michael, 1986, 1992a). Thus, HAD-1 rats seem to be innately more sensitive to the amount of work required to obtain BSR reward compared with the LAD-1 rats. This subtle difference may reflect a genetic predisposition in the HAD-1 rats that is associated with their avidity for high alcohol consumption. However, despite the line differences in performance under different reinforcement schedules, both rat lines responded at a consistent rate across the entire 20 min operant session for BSR in that responding generally was elevated across the entire session, reflecting little or no satiation for BSR within the MFB (Figs. 4 and 5).

Similar to BSR, the current data suggest that a 10% (w/v) sucrose solution is a powerful reinforcer in both the HAD-1 and LAD-1 rat lines. More important, however, the data clearly reveal a strong association between selective breeding for alcohol preference and intake of a highly palatable sweet solution, with the HAD-1 rats more avidly consuming the sucrose solution relative to the LAD-1 rats. The data of the present study are consistent with studies in outbred rats (Bell et al., 1994; Gahtan et al., 1996; Gosnell and Krahn, 1992; Kampov-Polevoy et al., 1990), mice (Forgie et al., 1988; Ramirez and Sprott, 1978), and rats selectively bred for high and low intake of saccharin solutions (Dess et al., 1998). Specifically, these studies reveal a positive association/correlation between EtOH and saccharin intake. The finding that the HAD-1 rats consume more of a sweet solution compared with their lower alcohol-preferring LAD-1 counterparts also supports previous literature in selectively bred rats (Overstreet et al., 1993, 1997; Sinclair et al., 1992; Stewart et al., 1994, 1998). The data of the present study extend the existing research by demonstrating that even when a highly caloric palatable solution (10% w/v) is used, an association between sweet preference and EtOH intake can be demonstrated in an operant paradigm in HAD-1 and LAD-1 rats. Although previous research has shown an association between sweet preference and EtOH intake in HAD-1 and LAD-1 rats (Stewart et al., 1998), the environment was the homecage in conjunction with a non-nutritive saccharin solution. The present work also extends the existing research by demonstrating that the association between sweet preference and EtOH intake in HAD-1 rats can be observed under continuous reinforcement as well as higher schedules of reinforcement. Interestingly, although sucrose responding was significantly elevated in both lines when moving from the FR1 to FR6 and FR12 schedules,
HAD-1 rats performed similarly under the FR6 and FR12 schedules whereas LAD-1 rats showed a reduction in response rates with increases in the schedule. These qualitative differences in sucrose responding between the HAD-1 and LAD-1 rat lines further support the notion that a genetic basis may underlie the positive association between the avidity for sweet solutions and alcohol intake in various rodent lines and strains and in certain populations of human alcoholics (Kampov-Polevoy et al., 1999; Murphy et al., 2002). It should be noted that a high correlation between saccharin and homecage alcohol consumption has been reported in 15 inbred mouse strains (Belknap et al., 1993). A similar correlation between sucrose and alcohol intake was reported by Bachmanov et al. (1996) in the F2 generation of crosses between the alcohol-prefering C57BL/6ByJ and the alcohol-avoiding 129/J strains of mice. Furthermore, Bachmanov et al. (1996) contended that intakes of sucrose and EtOH are influenced by a few genes. In contrast, several genetic studies in mice have failed to demonstrate overlapping genetic control over intake of sweets and alcohol. Thus, unfortunately, the specific genes controlling intake of sweet solutions and alcohol have yet to be elucidated.

The lack of correspondence between BSR and sweet taste rewards in HAD-1 and LAD-1 lines may suggest important differences yet overlapping brain reward mechanisms in the control of motivated behaviors. For example, as suggested by Kampov-Polevoy et al. (1997), the association between sweet consumption and alcohol intake may be regulated by a common mechanism. One such system is the brain opioid reward circuitry. It is now well established that the intake of sweet solutions activates the opioidergic systems in animals (Beczkwksa et al., 1993; Touzani et al., 1991) and humans (Dum et al., 1983; Getto et al., 1984). Alcohol also has been shown to activate the endogenous opioid systems (McBride and Li, 1998).

Unlike the intake of sweet solutions and alcohol, the research on the capacity of opioid agonists and antagonist in modulating MFB stimulation is quite equivocal. Specifically, although opioid agonists/mixed-agonist antagonist can reliably lower BSR reward threshold under some conditions (Kornetsky and Bain, 1990; Wise, 1996), only a few laboratories have been successful in getting even high doses of opioid antagonists (e.g., naltrexone, naloxone) to alter BSR reward threshold (Schaefer, 1988; Schaefer and Michael, 1992b). We recently reported that naltrexone (10–30 mg/kg) was completely ineffective in altering BSR sensitivity in HAD-1 and LAD-1 rats under a CRF schedule (Woods et al., 2001). However, naltrexone was observed to moderately reduce BSR threshold in HAD-1 and LAD-1 rats when the electrode was placed in the dorsolateral bed nucleus of the stria terminalis with high doses (>40 mg/kg) (H. L. June, unpublished data, 2003). Others argue that the capacity of opioid antagonists to modulate BSR reward threshold is dependent on the use of intermittent schedules (Schaefer and Michael, 1992b). Kornetsky and Bain (1990) suggested that the capacity of opioid antagonists to reduce BSR reward threshold may be brain loci/schedule dependent (Kornetsky and Bain, 1990). Thus, several neuronal systems seem to play a significant role in regulating the rewarding properties of sweet solutions and alcohol, which might be common or overlapping with BSR.

**CONCLUSION**

This is the first demonstration that the selectively bred HAD-1 and LAD-1 rats can initiate responding for BSR in a manner similar to outbred rat lines. Unlike sucrose responding, rats respond in a rapid, continuous, and repetitive manner throughout the entire operant session to receive MFB stimulation. However, responding for BSR in the MFB was not associated with selective breeding for alcohol preference. Compared with LAD-1 rats, HAD-1 rats seem innately more sensitive to the amount of work required to obtain BSR, as demonstrated by a dramatic reduction in responding when the reinforcement schedule moved from an FR1 to an FR12. It is possible this difference could be due to factors associated with high-alcohol-drinking behaviors. A similar finding has been observed in HAD-1 and LAD-1 lines with electrodes in the bed nucleus of the stria terminalis (Hardy et al., 2002). In contrast to BSR responding, an association between alcohol preference and a palatable sweet sucrose solution was clearly observed in the HAD-1 rats. Moreover, the association was even more profound as the amount of work required to obtain the sucrose solution was increased to an FR12 schedule. We hypothesize that the lack of correspondence between BSR and sweet taste rewards in HAD-1 and LAD-1 lines may suggest important differences (albeit yet unknown) in brain reward mechanism in the control of motivated behaviors in these selected lines. The overlapping neuronal systems may interact to regulate BSR and highly palatable solutions differentially, with key neurochemical systems (e.g., dopamine and endogenous opioids) differing in the degree of the mediation of preference and reinforcement.

**REFERENCES**


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