THE OPPORTUNITY FOR ALTERNATIVE REINFORCEMENT SHORTENS BOUT LENGTH IN BALB/c AND C57BL/6 MICE

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A Thesis
Submitted to
the Graduate Faculty of
Auburn University
in Partial Fulfillment of the
Requirements for the
Degree of
Master of Science

Auburn, Alabama
August 9, 2008

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THESIS ABSTRACT

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Master of Science, August 9, 2008
(B.S., Allegheny College, 2005)

51 Typed Pages

Directed by M. Christopher Newland

A recent mathematical model has been suggested to partition response rate into two independent components that are estimated through a log survivor analysis of inter-response times. One component is thought to tap motivational and the other motor components of behavior. The robustness of this phenomenon was tested in a new species, with a novel approach to maintaining high rate behavior, and different approaches to manipulated motivational and motor components. BALB/c and C57BL/6 mice were used due to their differences in behavioral measures. A percentile schedule was used to reinforce a high rate of behavior (Mult RI 60, 10:0.5). BALB/c mice nose poked at a higher rate than C57BL/6 mice, but responded similarly to the different behavioral interventions. The addition of a running wheel and food deprivation level significantly decreased total nose pokes. Adding a running wheel increased bout initiation rate, but decreased bout length. Taking the animals off food restriction decreased bout initiation rate. Light cycle changes did not affect nose poking. Pearson correlations support the idea of the behavioral divergence of the two strains.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Christopher Newland for his guidance and time. He would also like to thank his committee members Drs. Alejandro Lazarte and Chris Correia, for their constructive comments and time.

Style manual or journal used: Publication Manual of the American Psychological Association (5th Ed.)

Computer software used: Microsoft Word XP, Microsoft Excel XP, Rs Series for Windows, Med-PC, SigmaPlot 8.0, and Systat 10.2

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CHAPTER 1: INTRODUCTION

Motivational influences over behavior have been discussed since Thorndike (1911) identified the important role that consequences play in behavior through his work with the Law of Effect. Skinner further developed their role by noting a connection between reinforcement rate and probability of responding. Herrnstein (1970) extended this to note that all operant behavior is choice. Here, Herrnstein suggested that researchers should examine the quantitative relation among measurable events rather than qualitative relations. Recently, Shull, Gaynor and Grimes (2001) suggested that overall response rate can be divided into two composite measures controlled independently of one another. In Shull et al's (2001) model, one of the composite measures of response rate is affected by motivational variables while the other is affected by variables that effect motor components of behavior. By systematically altering the parameters of reinforcement we can begin to identify influences of reinforcement through the different measures of response probability, rate, amplitude, latency, and resistance to extinction.

Response bouts

A response bout is a period of engagement on a response device where the responses are temporally similar. Variable interval schedules of reinforcement are one of the earliest sources of the response bout phenomenon (Baum & Rachlin, 1969; Blough, 1963; Shull, 2004). The fixed interval schedule produces relatively long periods of disengagement where the organism is engaged in behaviors other than the reinforced operant, which are followed by periods of bouts as the interval approaches the end (Baum & Rachlin, 1969; Blough, 1963; Shull, 2004). Shull et al. (2001) identified two distinct parameters of a bout; bout initiation rate and within-bout response rate. Bout initiation rate refers to the responses that initiate a bout of responding, and within-bout response rate refers to responses that occur during a bout of responding.

Bout initiation rate

Shull et al. (2001) contended that operant behavior can be viewed as a period of engagement in response bouts and a period of disengagement. Thus, the traditional definition of response rate actually comprises two distinct measures. One measure is bout initiation rate, which is altered by changes in motivational variables. This is a measure of how frequently an animal initiates a bout of responding, or how often an animal changes from a period of disengagement to a period of responding. The idea that periods of disengagement alternate with periods of responding on the reinforced activity is a common way of distinguishing reinforced responding from other behavior (Mechner, 1992; Shull, 1991, Herrnstein, 1970). Several different motivational or incentive-based variables affect the bout initiation rate variable including the rate of reinforcement, the amount or taste quality of the reinforcer, or the availability of alternative reinforcement can affect the bout initiation rate.

Increasing the availability of alternative reinforcement should affect the bout initiation rate if providing opportunities for alternative reinforcement directly affects the motivational aspects of responding by reducing the salience of the original reinforced operant. Time spent responding on the original operant would then be forced to compete with the alternative reinforcement. In the present study a running wheel activity, which is inherently reinforcing if the necessary establishing operations have been imposed, will compete with reinforced nose pokes for operant responding (Belke & Hancock, 2003). Herrnstein (1970) noted that there is constantly reinforcement available in an organism's environment and that the reinforced operant is in constant competition with other reinforcing behaviors. Free access to a running wheel in the rear of the chamber should warrant decreased bout initiation rates. The availability of wheel running will compete with the nose poke response thus decreasing the number of times a mouse will engage in a visit to the photo beam detector.

Within-bout responding

As proposed by Shull, the other composite variable of response rate is within-bout responding. Within-bout responding is controlled by motor variables or reinforcement contingencies that produce high response rates (Shull et al., 2001). A simple way to affect within-bout response rate is to make the operant response more difficult (e.g. increasing the amount of force required to depress a lever) or changing he response device. By making the response more difficult, one would expect a decrease in within-bout responding. Bouts of responding occur as a result of adding a VR or FR component on the end of a VI schedule, for example Shull (2001). Administration of pharmacologically active compounds that affect motor behavior also is considered a variable that affects within-bout responding.

Shull (2001) was able to manipulate within-bout responding and initiation rate variables independently of one another. Thus, while altering an incentive variable initiation rate would increase/decrease while within bout responding remained constant.

Similarly, when a motivational/incentive variable was altered the within bout responding would increase/decrease while the initiation rate remained constant. This suggests that there are two different measures within our traditional measure of response rate that are controlled by two separate types of variables.

Bout length and the problem classifying responses into bouts

Bout length is a third composite variable of response rate that is described by Shull (2002). Bout length is the number of responses that occur during a response bout. One problem that arises with partitioning response rate is that of distinguishing whether an individual response is a visit initiation or a within bout response. One method that has been attempted is to set a cutoff IRT value so if the cutoff value is set at 1 second all responses that occur before 1 second IRT has elapsed are considered within bout responses and all responses that occur after the 1 second IRT has elapsed are considered visit initiations. Responses are easily misclassified; some responses that should be considered within bout responses are recorded as bout initiations and vise-versa. Mellgren and Elsmore (1991) tried to reconcile this problem by varying the IRT cutoff length to prove the main conclusions do not depend on the particular cutoff value. However, this does not fully account for misclassifying individual responses.

Shull (2001) suggested that a log survivor analysis can be used to classify responses as either a visit initiation or a within-bout response. He used computer simulations to test hypotheses about the analysis of behavior into bouts of responding, and nonlinear regression analysis to estimate the average rates for both the visit initiations and number of within bout responses. Shull varied two different variables p(V) is the probability of going from a period of disengagement to a period of responding (visit initiation), and *p*(D) is the probability of a response while engaged will end a visit (within bout responding). Shull attempted to replicate how a rat would respond given similar environmental constraints. One way he accomplished this was running the simulations in real time and having the two components alternating every 50s for 40 cycles. This basically emulated the restraints on responding that a rat would experience. The values of *p*(V) and *p*(D) were determined by using a trial and error method until appropriate values were achieved that were similar to the response rates generated by the rats.

Log survivor analysis

Within-bout response rate and visit-initiation rate can be estimated by employing a log survivor analysis. A log survivor analysis provides a good template for highlighting the two-mode component of responding. An example is provided in figure 1. Equation 1 is used to model the data:

$$Y(t) = (1-p)\, e^{-wt} + pe^{-bt}$$

The log survivor analysis begins with the cumulative proportion of responses plotted as a function of the interresponse time duration (sec). In other words, the log survivor plot shows the proportion of IRTs that are longer than some duration (sec). The y-axis represents the cumulative proportion of IRT's, 1 represents 100% of the IRT's, .8 represents 80% and so on. The x axis is the length of the IRT in seconds. The decay function shows that a small percentage of the IRT's were longer than 1 second. For example, approximately fewer than 5% of IRT's were greater than 10 seconds, and 90% were shorter than 1 second. The event record chart that is inset into figure 1 depicts four examples of response bouts. Each line represents an individual response, and the lines are separated by varying lengths of inter-bout intervals. The open circles in the example represent the different IRT lengths and are plotted according to shortest (highest response rate) to longest (lowest response rate) length in a survival function. For example, approximately 85% of the IRTs were less than 1 sec in figure 1.

Figure 1 also features a line that is derived from the sum of the following two negative exponential functions in equation 1, where $Y(t)$ equals the proportion of IRTs that are greater than some specified duration (t). The term to the left of the plus sign ($(1-p)\, e^{-wt}$) represents the within-bout responses component of the plot, while the term to the right of the plus sign (pe^{-bt}) represents the bout initiation responses component of the plot. For each of the terms e is the natural log base, and t is the unit of time specified by the x-axis. The parameter w *is the* within-bout response rate, and b is the bout initiation rate. The equation provided for figure 1 describes the within-bout response rate as 3.5 responses/sec, and a bout initiation rate of .11/sec, or roughly one bout per 9 seconds. The proportion of all IRTs that are within-bout responses is 91% leaving 9% of the responses as bout initiations. The bout length can be estimated using one divided by p (1/.09) or roughly 11 responses per bout in the example.

Shull (2001) used a log survivor plot to classify responses as either bout initiations or within-bout responses. This proved to be an excellent method for classifying responses as the y-axis clearly represented responses per visit and the slope represented different bout initiation rates. However, Shull used VI and a tandem VI VR schedule of reinforcement. While VI and VR schedules of reinforcement can produce high levels of reinforcement they do not necessarily require a high response rate. Reinforcement is not contingent on the animal responding a predetermined number of times; rather it is delivered for the first response after a designated amount of time or number of responses. A schedule of reinforcement that would reinforce a high response rate, while maintaining a consistent reinforcement rate would be optimal. There is a point with both of these schedules where response rate hits an asymptotic level where it no longer influences reinforcement rate. This phenomenon has been characterized by using a hyperbolic function plotting response rate as a function of reinforcement rate (Herrnstein, 1970).

The log survivor analysis is not without its critics. Several researchers point out what they believe is a better method for modeling data (Langton, Collett, & Silby, 1995; Tolkamp, & Kyriazakis, 1998). Langton et al. (1995) suggested a log frequency analysis entered around a maximum likelihood estimation to reduce the misclassification of responses, the maximum likelihood approach models the mixture of two or more exponential functions. Tolkamp and Kyriazakis (1998) criticize both the log survivor analysis and log frequency analysis solely for its application to feeding behavior citing that the models will not result in biologically meaningful quantitative estimates. None of the critiques of the log survivor analysis are relevant to what is being examined in the present study because the partitioned value of response rate is being studied not feeding behavior, and there is only one exponential function being fit. The present study aims to extend for Shull et al’s (2001) log survival analysis by applying the model to a different species and reinforcement contingencies.

Percentile Schedule

The percentile schedule offers a way to keep reinforcement rate constant while some targeted dimension of behavior varies (Galbicka, Kautz, Jagers, 1993). We have targeted high rate behavior. To do so, we target short interresponse times. Reinforced inter-response times are based on the specific animals' previous distribution of inter-response times. An IRT is eligible for reinforcement if it is shorter than some of the previous IRTs. There are two components of the percentile schedule. The first is the look-back window, which specifies how many previous IRT's are considered when determining whether the current one qualifies for reinforcement. The second component is the percentile criterion, which sets a value for which the current IRT must be shorter than a specific percentage of the previous IRT's. For example, on a percentile 20: 0.75 schedule of reinforcement responses are reinforced if the inter-response time is shorter than 75% of the previous 20 inter-response times.

A good way to measure motor function is to establish a high rate of behavior. Different DRH schedules of reinforcement are useful tools in examining motor functioning based on the high rate of behavior it establishes. Donlin (2005) used a DRH 9:4 schedule of reinforcement to produce high rates of behavior. This schedule provides reinforcement if a lever is pressed 9 times in 4 seconds, thus producing relatively high response rates. Implementing a DRH schedule of reinforcement provides an excellent model for altering within-bout response rate. One issue that arises when studying high-rate behavior is that it becomes increasingly difficult to separate motivational from motor influences of operant behavior. If response rate can be partitioned into two separate variables that target either motivational influences (bout initiation rate) or motor influences (within-bout response rate) of operant behavior then this becomes a reason for concern when examining these two types of variables. The percentile schedule of reinforcement is useful because it separates the motivational variable from the high rate behavior.

Many studies that use a log survivor analysis employ variable interval schedules of reinforcement. There exist certain advantages to using a percentile schedule of reinforcement versus a variable interval schedule of reinforcement. One advantage to using a percentile schedule of reinforcement versus a variable interval schedule is that the reinforcement rate is held constant across different sessions reducing the effect of reinforcement rate on response rate. One can selectively reinforce short inter-response times similar to that seen in Kuch & Platt (1976) where key pecking in pigeons was reinforced for emitting short or long inter-response times based on their previous distribution of inter-response times. Different inter-response times were reinforced (short or long) without altering reinforcement rate. Kuch and Platt (1976) found that both long and short inter-response times different significantly from the baseline rate of inter-response times. Using the percentile schedule of reinforcement, reinforcement rate can be held constant eliminating is effect on response rate.

Species Differences

Using mice as experimental subjects provides and excellent opportunity to study strain differences given the vast numbers of both inbred and outbred strains. Inbred strains of mice are particularly of interest due to the possibility for eventually incorporating a knockout mouse which is a major advantage for specificity of function studies. C57BL/6 and BALB/c inbred strains were chosen for the present study due to the mass of studies using both behavioral and pharmacological manipulations. These two particular inbred strains differ greatly both behaviorally and physiologically from one another. There are differences in the sensitivity to dopaminergic compounds between the two strains. C57BL/6 mice experience increased levels of activity after administration of amphetamine while BALB/c mice experience inhibition of activity after administration (Kitahana & Valatx, 1979). This is interesting for the present study because given the differences in activity level after administration of amphetamine there is a possibility of observing marked differences between C57BL/6 and BALB/c mice in the distribution of response to visit initiations versus within-bout responses after amphetamine administration.

These two strains of mice differ significantly in their relative responsiveness to environmental stimuli. For example, BALB/c mice show much higher levels of anxiety-like behaviors in novel environments than C57BL/6 mice (Tang et al, 2005; Crawley et al., 1997; Belzung & Griebel, 2001). Several studies have revealed that C57BL/6 mice tend to prefer novel to familiar environments when given a choice paradigm while BALB/c mice are characterized as neophobic (Belzung & Barreau, 1999; Belzung & Berton, 1997; Beuzen & Belzung, 1995). The neophobia found in BALB/c mice has been characterized as “trait” anxiety which refers to the anxiety as a stable and enduring feature of the organism’s behavioral repertoire (Hode et al., 1999). The reasons for difference in fearfulness for the BALB/c mice are unknown but could include such variables as life history, test situation, or housing conditions. It has also been suggested that the difference may be due to neuroanatomical, neurochemical or genetic factors (Belzung & Griebel, 2001).

There exist several physiological differences between C57BL/6 and BALB/c mice. Chapouthier et al (1991) reported that C57BL/6 and BALB/c mice different significantly in the affinity and density of benzodiazepine receptors. BALB/c mice have a greater affinity while C57BL/6 have a greater density of benzodiazepine receptors.

There are differences in susceptibility to the sensitivity of different drugs (Belzung & Barreau, 1999). Specifically, one of these differences is seen in the opiod receptors. Naloxone, an opiod antagonist, eliminates the action of opiods and antianxiety drugs at the receptor do not block the antianxiety effects of benzodiazepines in BALB/c, while it does for C57BL/6 mice (Agmo et al, 1999). Furthermore, naloxone induces analgesic effects blocked by a kappa receptor in BALB/c mice, but is seen in no other strains (Vaccarino, 1988).

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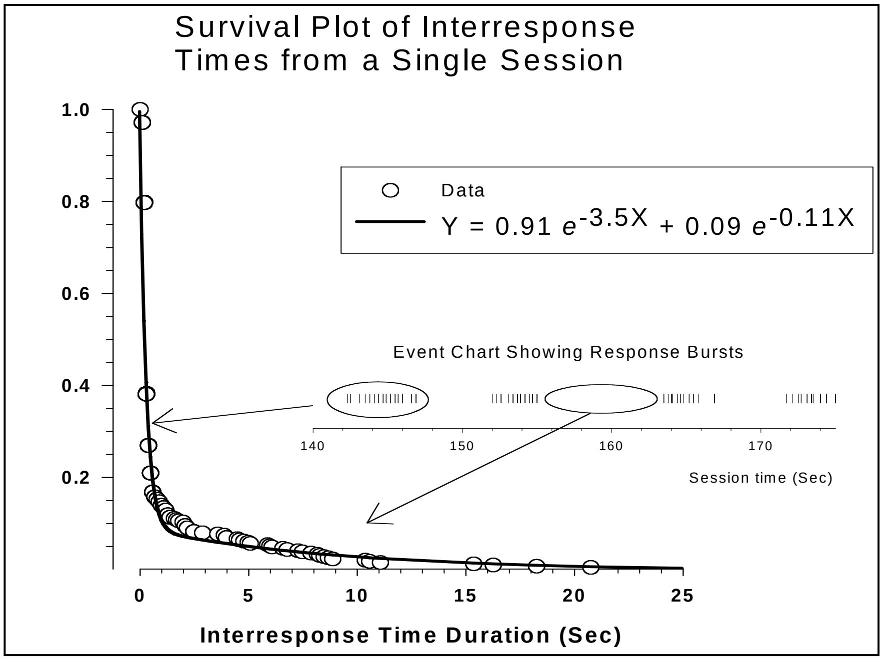

Figure 1. Example of a partition analysis that breaks responding into periods of bouts separated by inter-bout intervals. An event record (inset) shows four individual response bouts, and one stray response following the third bout. Each vertical line shows a lever-press. Open circles show the survival plot of interresponse times and the line shows a fit of Equation 1. Approximately 85% (330 of 386) interresponse times are less than one sec in this example. To generate the survival function, all interresponse times are sorted from shortest (representing high response rates) to longest and plotted as a shown as a survival function (open circles). The equation describes responding as bouts of 3.5 responses/sec and a bout initiation rate of 0.11/sec, or one bout every 9 seconds. The average bout length is 1/0.09 or about 11 responses in this example.

CHAPTER 2: THE OPPORTUNITY FOR ALTERNATIVE REINFORCEMENT SHORTENS BOUT LENGTH IN BALB/c AND C57BL/6 MICE

Shull, Gaynor, and Grimes (2001) developed a model of operant behavior that partitions responding into bouts containing two composite variables, within-bout response rate and bout initiation rate. Shull later extended this model to include bout length as a third variable (Shull, 2004). Qualitatively different variables affect within-bout response rate and bout initiation rate. Within-bout response rate reflect motor variables and bout initiation rate is influenced by motivational variables (Shull et al., 2001, Shull, 2004, Shull, 2005). The model suggests that within-bout response rate and bout initiation rate can increase or decrease independently of one another. Changes in bout length, while sometimes less reliable, tend to covary with bout initiation rate (Shull, 2004). Bout length has yet to achieve the independence in affecting overall response rate that within-bout response rate and bout initiation rate have displayed.

The model has been replicated in rats but has failed in pigeons, a difficulty that has been attributed to the high response rates and relatively low number of long pauses seen in pigeons under the most commonly used experimental procedures (Shull, 2005). To date, the model has not been applied to mouse behavior. Behaviorally divergent mouse strains provide an excellent template for testing this model. C57BL/6 and BALB/c mice differ across a number of behavioral dimensions. The C57BL/6 mice consistently

show high levels of general locomotor activity and low levels of anxiety, as seen on tests said to show ecological validity, like the elevated plus maze or open field activity tasks, while the BALB/c mice show the opposite pattern. (Crawley et al. 1997, Tang, Orchard, & Sanford, 2000, Beuzen & Belzung, 1995). The common behavioral assessments used in these strains include open field activity tasks and elevated plus mazes, but there is a lack of tasks that address operant behavior.

Procedures that generate high-rate behavior can be useful in examining motor deficits but they present a challenge. First, it can be difficult to separate motor from motivational influences (Newland, 1995). Second, conditions that generate high-rate behavior often impose a direct relationship between responding and reinforcement rate, so any condition that impairs responding also lowers reinforcement rate, which further reduces reinforcement rate, resulting in a downward cycle that mixes motivational and motor influences. The independence of motivational and motor determinants is important when applying or testing Shull’s model because otherwise the parameters do not provide distinct information about the variables influencing responding.

The challenge, then, lies in generating high reinforcement rates while disassociating the close relationship between response rate and reinforcement rate. The solution is the percentile schedule of reinforcement applied to the interresponse time (IRT) and a time-based reinforcement schedule that is applied to criterion IRTs. To be eligible for reinforcement an IRT will have to be shorter than 50% of the previous 10 IRTs. By selectively reinforcing short IRTs, the percentile schedule of reinforcement generates high response rates (the equivalence of short IRTs) while continuously adjusting the reinforcement criterion according to an individual animal's most recent performance. This prevents behavior from deteriorating should some other condition impair responding. Criterion IRTs are reinforced under a Random Interval 60” schedule, so reinforcement rate is held constant unless response rate becomes extremely low.

The aim of the present study is two-fold. The first goal is to manufacture the necessary interventions to affect certain bout parameters independently of others. The second goal is to use Shull’s (2001) bout parameters to characterize strain differences on a task that generates high rate behavior.

General methods

Subjects

The present study employed 4 BALB/c and 7 C57BL/6 mice, all with no previous experimentation history at the start of the experiment. Mice were housed individually in a room with 12 hour light/dark cycles. For most conditions the animals were maintained on a reverse light-dark cycle (lights on at 6:00am lights off at 6:00pm). Animals were maintained at 85% free-feeding weight and were allowed free access to water at all times excluding experimental sessions. All experiments were approved by the Auburn University Animal Care and Use Committee.

Apparatus

Four MedPC (St. Albans, VT) rat operant chambers fitted to accommodate mice were situated inside sound attenuating ventilated shells in the present study. Each chamber contained a photo-beam based nose poke device, a lever which was not used in the present study, and a 7” diameter running wheel that was present only for certain conditions. The nose-poke device was located to the left of the food tray. The running wheel was located in the back of the operant chamber during certain sessions. Signal lights are located above both the nose poke device and the lever. A houselight is provided near the top of the chamber directly above the food tray. A pellet dispenser delivered 20 mg sucrose pellets into a food tray. MEDPC was used to program the experiments and collect data with 0.01" resolution.

Procedure

Nosepoking for sucrose pellets was autoshaped. The autoshaping procedure featured a FR1 schedule of reinforcement and was ended by either an animal earning 100 reinforcers or after 12 hours had elapsed. Once the autoshaping component was completed for all mice, animals began responding on a RI t" (Percentile 10:0.5) second-order schedule of reinforcement. The RI parameter, *t* began at 1 sec and was increased until it reached a value of 60 seconds. The Percentile schedule works as follows. A look back window of 10 interresponse times (IRTS) and a 50% criterion value were used for the present study. The look back window and criteria value work together to generate a high rate of responding. When a nose-poke occurred, the IRT that it terminated was compared with the previous 10 IRTs. If the current IRT was shorter than 50% of previous IRTs then it produced a brief tone and was eligible for reinforcement. Thus, to qualify for reinforcement the animal would have to respond faster than it did for half of its previous responses. For example, if the animal's previous IRTs were 1.2", 1.2", 1.0", 0.9", 0.8", 0.8", 0.6", 0.5", 0.4", and 0.3" the criterion for reinforcement for the following response would be an IRT < 0.8".

$$Y(t) = (1-p)\, e^{-wt} + pe^{-bt} \qquad \text{(Equation 1)}$$

Where:
$Y(t)$ = proportion of IRTs > t
p = prop. of responses that are bout initiations)
$(1-p)$ = prop. of responses that are within a bout
w = *within-bout response rate*
b = *bout initiation rate*
$1/p$ = average *bout length* (responses)

Data analysis

All of the analyses excluded post reinforcer pauses. A log survivor analysis was employed to differentiate between bout initiations and within-bout responses (Shull, Gaynor & Grimes, 2001, Shull, 2004, Shull, 2005). To generate the survival function, all interresponse times are sorted from shortest (representing high response rates) to longest and plotted as a survival function. A two-exponential function (Equation 1) is fitted to this survival function of IRTs using nonlinear least squares to estimate bout-initiation rate, within-bout response rate, and bout length. Both sides of the equation were logged (base 10) prior to performing the fit. RS/1 software (Brooks Automation, Chelmsford, MA) was used for data management and to perform the non-linear regressions required to estimate the bout parameters automatically each day when the data was transferred to RS/1.

Pearson's correlations among the different parameters were computed with both strains combined and both C57BL/6 and BALB/c mice exclusively. Total nose pokes, bout initiation rate, within-bout response rate and bout length were averaged across all behavioral interventions

Repeated measures ANOVA's were used to assess statistical significance with strain serving as the between subject variable and the experimental condition serving as the within subject variable. Statistical significance was assessed at an alpha level of 0.05. This was conducted using Systat (San Jose, CA).

Experiment 1: Addition of alternate reinforcement

Once a stable baseline of nose poking appeared, defined by 5 consecutive sessions of responding on a RI 60" with no systematic variation in responding, a running wheel was introduced to compete with nose poking, the final 3 sessions were used for data analysis.

Experiment 2: The role of food deprivation in estimating bout parameters

After the wheel was removed responding was allowed to stabilize. Then, the mice were fed ad libitum for 10 days. Bout parameters were estimated for the ad libitum phase from the last 4 sessions and then the mice were retuned to their initial level of 85% of free feeding weight and bout parameters were estimated again. Responding during the ad libitum phase was low so IRTs from the last 4 sessions of the free-feeding phase were concatenated before conducting the partition analysis.

Experiment 3: Effect of photoperiod on bout parameters

Animals were initially housed in a room under a reverse light/dark cycle so that animals ran experimental sessions during their active cycle. After their body weight returned to 85% and responding stabilized, the light cycle was reversed on a Friday. On the following Tuesday, experimental sessions commenced again during the less active light/dark period. After the period of adjustment, bout parameters were estimated for the opposite light/dark cycle.

Results

All statistical analyses included all cases unless otherwise specified. All error bars are constructed using standard error of the mean.

Experiment 1: Addition of a running wheel.

Figure 2 shows behavioral parameters with and without the wheel. There was a significant main effect of strain (*F* (1, 9) = 8.241, *p* =.018) but not condition (*F* (1, 9) = 3.229, *p* = .109) on total nose pokes. There was one outlier that affected the ANOVA for condition. With that case removed, there was a significant effect of condition (*F* (1, 9) = 10.166, *p* = .013). In both cases there was not a significant interaction. The BALB/c mice nose poked at a significantly higher rate than the C57BL/6 mice, and nose poking occurred at a significantly lower rate while the wheel was present in the chamber during an experimental session.

Each of the three bout parameters showed significant main effects of strain and wheel but no interaction. The upper right panel of figure 2 shows the bout initiation rate for both strains across the three conditions. For bout initiation rate, there was a significant main effect of both strain (*F* (1, 9) = 8.654, *p* =.016) and condition (*F* (1, 9) = 17.718, *p* = .002). There was not a significant interaction (*F* (1, 9) = 0.363, *p* =.562). BALB/c mice initiated significantly more bouts than the C57BL/6 mice, and significantly more bouts were started while the wheel was present in the chamber. The bottom left panel of figure 2 shows within-bout response rate. There was a significant main effect of strain (*F* (1, 9) = 12.708, *p* = .006) but not condition (*F* (1, 9) = 2.576, *p* = .143). The interaction was also not significant (*F* (1, 9) = 0.348, *p* = .570). BALB/c mice had a significantly higher within-bout response rate than the C57BL/6 mice. The bottom right panel of figure 2 shows bout length. Significant main effects were only seen of condition (*F* (1, 9) = 26.879, *p* = .001) on bout length. There was not a significant main effect of strain (*F* (1, 9) = .502, *p* = .497) or a significant interaction (*F* (1, 9) = .909, *p* = .365).

BALB/c mice had higher overall nose-poke rates, faster within-bout response rates, and initiated more bouts. The addition of the running wheel generally increased bout initiation rates, and decreased bout length for both strains. Ultimately, the addition of the wheel decreased overall nose-poke rates.

Experiment 2: The role of food deprivation

For experiment 2, overall response rates were so low during the ad libitum feeding condition that the last four days had to be concatenated in order to have enough responses to perform the partition analysis. One C57BL/6 mouse was excluded from the analysis entirely because response rates were still too low to support an analysis, even after the last 4 days were concatenated. BALB/c mice had significantly more nose poke responses than C57BL/6 mice *(F* (1, 8) = 53.687, *p* < .001). Overall nose pokes were significantly lower during the ad libitum feeding condition *(F* (1, 8) = 30.854, *p* = .001). There was no interaction between strain and condition (*F* (1, 8) = 2.099, *p* = .185).

There was a significant effect of strain on bout initiation rate (*F* (1, 8) = 6.886, *p* = .03), but the difference between conditions did not reach conventional levels of significance (*F* (1, 8) = 3.895, *p* = .084) until an outlier (mouse 206) was removed from the analysis (*F* (1, 7) = 14.574, *p* = .007). There was not a significant interaction (*F* (1, 8) = 0.456, *p* = .519). BALB/c mice initiated significantly more bouts than C57BL/6 mice.

There were no significant main effects of strain (*F* (1, 8) = 4.041, *p* = .079) or feeding condition (*F* (1, 8) = 0.174, *p* = .688) on within-bout response rate. The interaction was also not significant (*F* (1, 8) = 0.285, *p* = .608). There was a significant main effect of condition (*F* (1, 8) = 8.85, *p* =.018) on bout length but neither the strain (*F* (1, 8) = 1.421, *p* =.267) and interaction (*F* (1, 8) = 2.383, *p* =.1.61) produced a significant difference.

BALB/c mice had more total nose pokes and initiated more bouts than C57BL/6 mice. There was no strain difference in the length or speed of bouts. Taking the animals off food restriction reduced total nose pokes and bout initiation rate. This intervention reduced bout length for C57BL/6 mice, but not BALB/c mice.

Experiment 3: Effect of photoperiod on bout parameters

As shown in figure 4, there was a significant main effect of strain (*F* (1, 8) = 22.722, *p* <.001) on total nose pokes. However, there was not a significant main effect of light cycle (*F* (1, 8) = 0.485, *p* =.496) or a significant interaction (*F* (1, 8) = 0.006, *p* =.942). Photoperiod change did not affect within-bout response rate (*F* (1, 8) = 1.679, *p* =.239) or bout length (*F* (1, 8) = .185, *p* =.678), but it did affect bout initiation rate (*F* (1, 8) = 16.498, *p* =.004). The change in bout initiation rate was not large enough to affect total nose pokes.

Figures 5, 6, and 7 contain correlation matrices for the different bout parameters and total nose pokes. Figure 5 features both strains combined across all experimental conditions. Total nose pokes appear to be moderately correlated with each of the bout parameters (bout initiation rate, within-bout response rate, and bout length). In addition, bout initiation rate was positively correlated with within-bout response rate. All of the aforementioned correlations were significantly different from zero at the 0.05 significance level. Figure 6 features C57BL/6 mice only. Total nose pokes and bout length are the only variables that have Pearson correlations significantly different from zero at the 0.05 significance level. Figure 7 shows BALB/c mice only. Both bout length and bout initiation rate were both moderately correlated with total nose pokes and significantly different from zero at the 0.05 significance level.

The percentile schedule was successful in holding reinforcement rate constant even as response rate was changed drastically. Table 1 showed the consistency of reinforcement rate through all three of the experiments. While there were changes in response rate, sometimes as much as two-fold, changes in reinforcement rate were inconsequential. There was a sizable drop in reinforcement rate for the C57BL/6 mice during the free feeding phase of the food deprivation experiment. This was because the overall response rate was so low (much lower than any other behavioral intervention) that there were too few responses to engender a reinforcement rate similar to those in previous and subsequent interventions.

Discussion

The present experiments lead to two broad conclusions regarding strain differences. First, the baseline rates of the bout parameters differed between the two strains. Within-bout response rates and bout initiation rates were higher for BALB/c mice than for C57BL/6 mice. This led to BALB/c mice having significantly more nose poke responses during a session than C57BL/6 mice. Second, C57BL/6 and BALB/c mice responded similarly to the different behavioral interventions. For example, in experiment 1 both strains showed significant decreases in overall nose pokes and bout length when the wheel was added to the chamber. Although not statistically significant, C57BL/6 mice tended to run further than BALB/c mice. The non-significant result may be due to the small N of the BALB/c group. This may suggest C57BL/6 mice have a greater preference for running, or at least run faster and further, while BALB/c mice nose poke faster and more often. These findings add to others demonstrating strain differences. For example, the BALB/c mice have been characterized as neophobic (Belzung & Barreau, 1999; Belzung & Berton, 1997; Beuzen & Belzung, 1995) and display higher levels of anxiety-like behaviors in novel environments than the C57BL/6 mice (Tang et al, 2005; Crawley et al, 1997; Belzung & Griebel, 2001).

If the three bout parameters describe independent contributions to overall responding, then they should be correlated with overall rate but not with each other. When compared across strains, the different bout parameters were, indeed, correlated with overall rate and, with the exception of within-bout rate and bout initiation rate, only weakly correlated with each other. When Pearson correlations were examined within each strain individually, the correlation between within-bout rate and initiation rate disappeared. The covariation in bout parameters in the data set that included both strains may reflect strain differences in behavior patterns: BALB/c rats had a higher rate of initiating bouts, and these bouts were made up of high response rates. In addition, within-strain correlations between bout parameters and overall rate were weaker for bout initiation rate and absent for within-bout response rate, but were stronger for bout length. The difference between examining the bout parameters with both strains combined versus individually further supports the behavioral divergence of these two strains.

Shull (2001) found that both within-bout response rate and bout initiation rate were selectively sensitive to different parameters and could increase/decrease while the other bout parameter remained constant. However, no study that partitioned response bouts has been able to alter bout length independent of within-bout response rate. The present study showed that the opportunity for alternate reinforcement, in the form of wheel-running, during a behavioral task that engenders high rate behavior increases bout length without affecting within-bout response rate constant. Since the mice ran vigorously in the wheel the entire time it was available, and neither nose-poking nor eating could occur while the animal was wheel-running, this opportunity to run can be viewed as a reinforcer that competes with sucrose-maintained nose-poking (Dunham,1977). Thus a motivational variable, food deprivation, affects bout initiation rate, motor variables affect within-bout response rate, and the availability for alternate reinforcement functions similarly for bout length.

The present study not only extends previous findings by including an environmental contingency that independently affects bout length, but it also supports previous research conducted on partitioning response bout (Shull, Gaynor & Grimes, 2001; Shull, 2004; Shull, 2005). Two behavioral manipulations, availability of alternate reinforcement and food deprivation level, that can be considered motivational interventions that affected the motivational aspects of behavior, and both interventions supported Shull’s (2001) claim that bout initiation rate can change while bout-initiation rate remains constant.

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Figure 1: Distribution of IRT’s

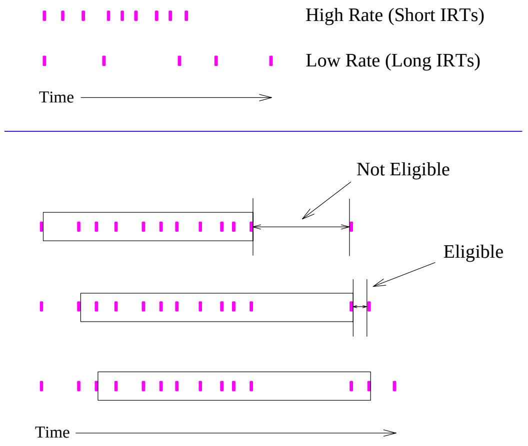

Figure 1. The top portion of figure 1 graphically depicts the difference between low rate (long IRTs) and high rate (short IRTs) behavior. Every hash mark represents a response and the space between hashes represents the interresponse times. The bottom portion of figure 1 shows two examples of 10 response sequences. The top series of responses is not eligible because the current IRT is not shorter than 50% of the previous 10 IRTs The middle series of responses is eligible because the current IRT is shorter than 50% of the previous 10 IRTs.

Figure 2: Effect of in-chamber running wheel.

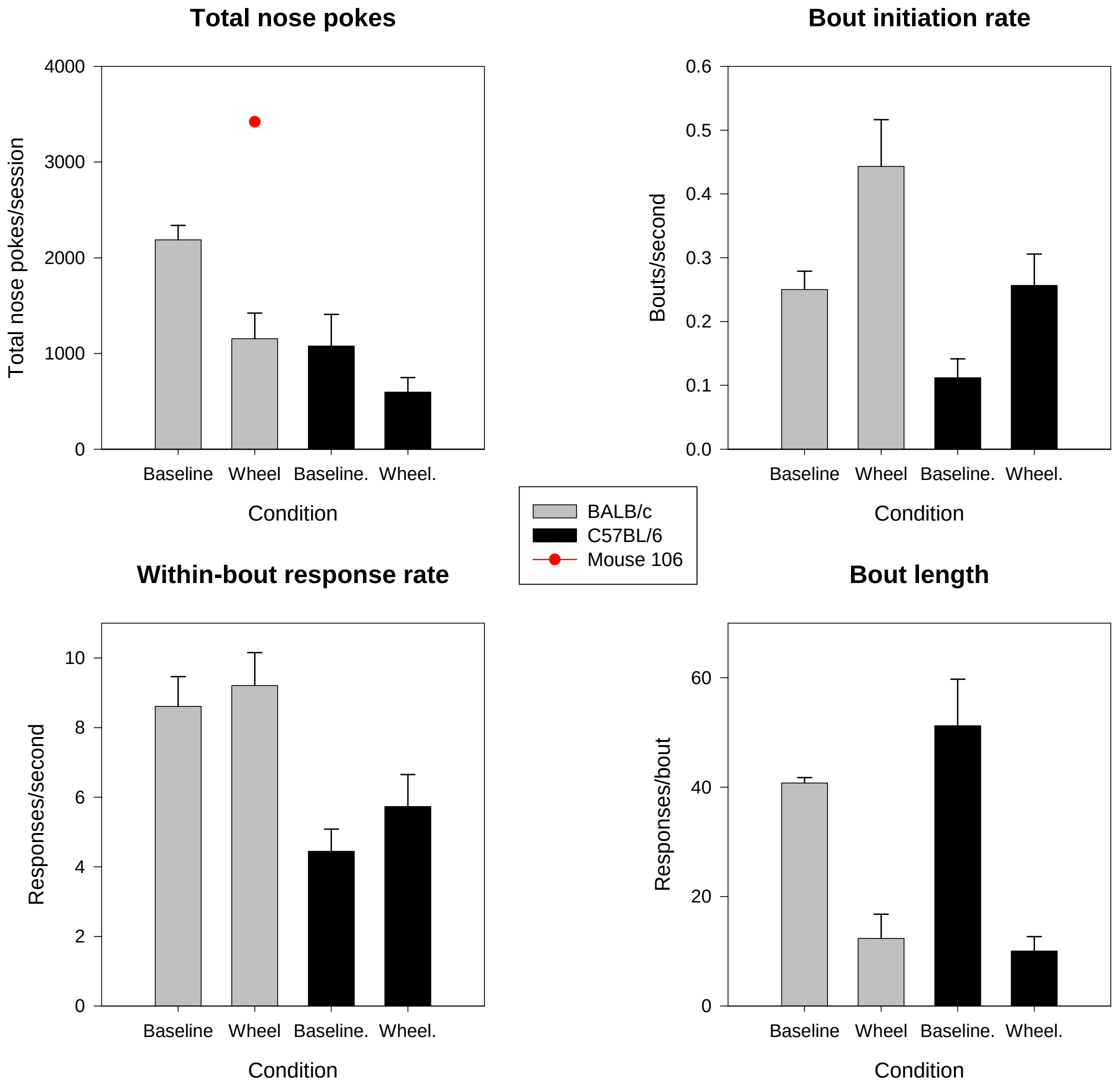

Figure 2. Effect of adding a wheel to the experimental chamber on total nose pokes and the three bout parameters (bout length, bout initiation rate, within-bout response rate). The gray bars represent BALB/c mice while the black bars represent C57BL/6 mice.

Figure 3: Effect of food deprivation.

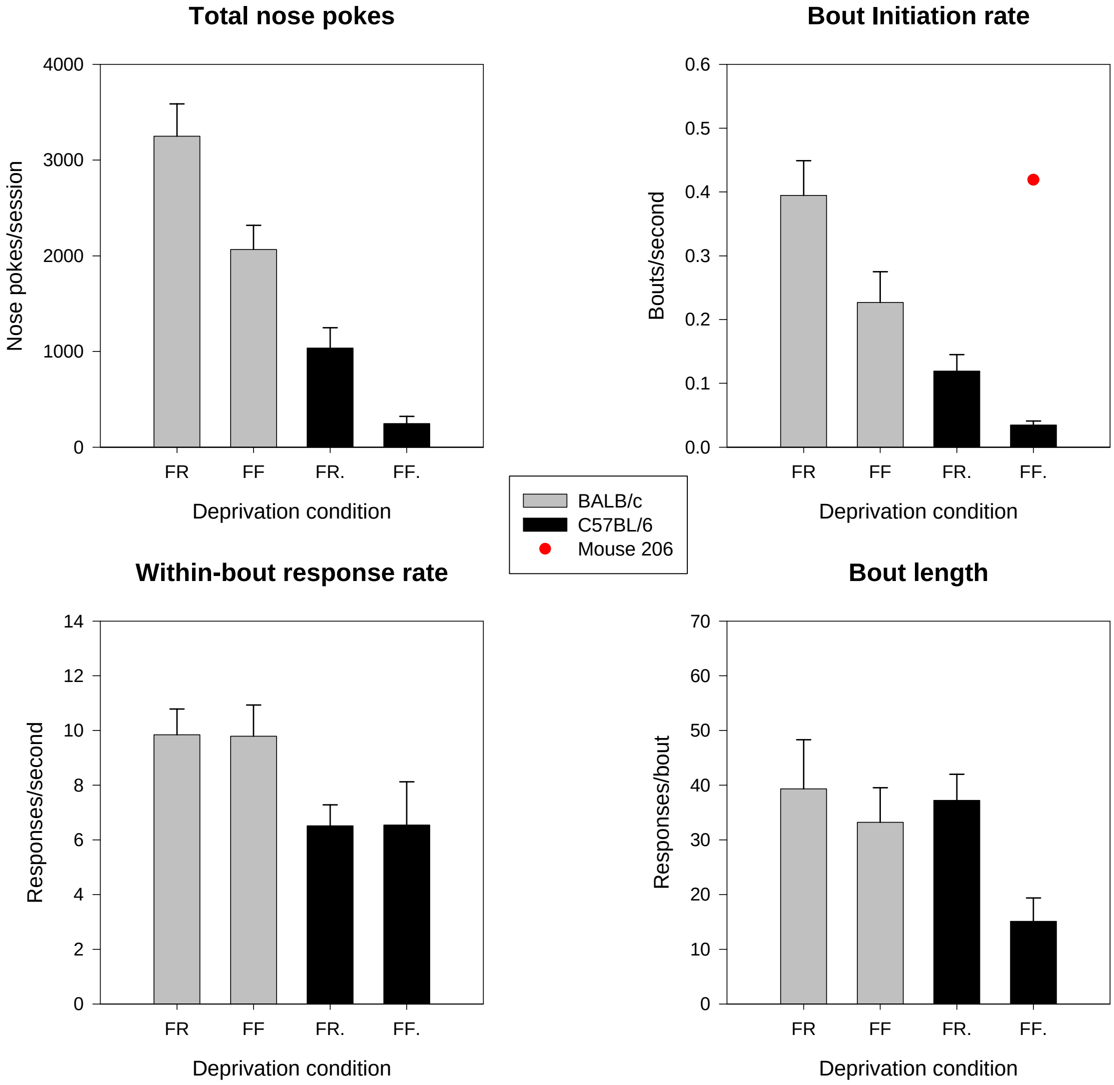

Figure 3. Effect of food deprivation on total nose pokes and the three bout parameters (bout length, bout initiation rate, within-bout response rate). The gray bars represent BALB/c mice while the black bars represent C57BL/6 mice. Mouse 206 is shown separately for the Free feeding condition because it was an extreme outlier.

Figure 4: Effect of photoperiod change.

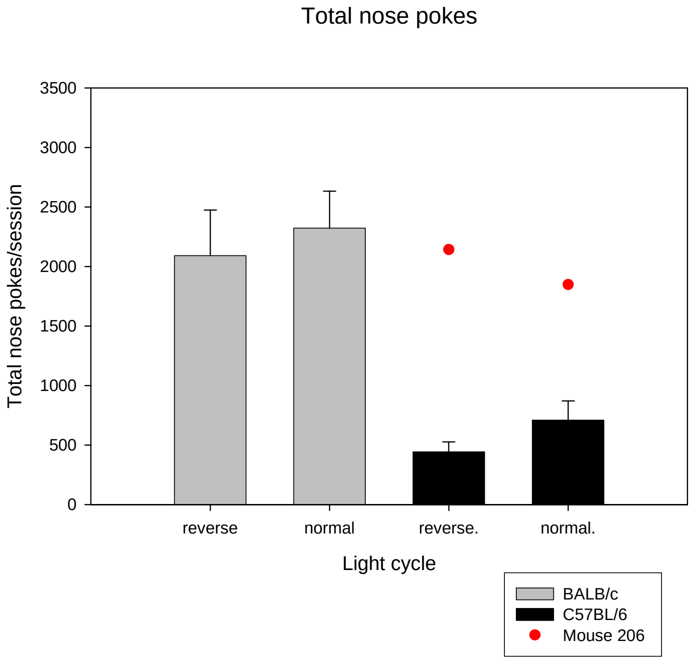

Figure 4. Nose poke responses emitted during both the natural and reverse light cycles. The reverse represents session run during their opposite light cycle (lights on 6am-6pm), and normal represents session run during their normal light cycle (lights on 6pm-6am). Mouse 206 is plotted separately because it was an extreme outlier.

Figure 5: Correlation matrix C57BL/6 and BALB/c mice combined.

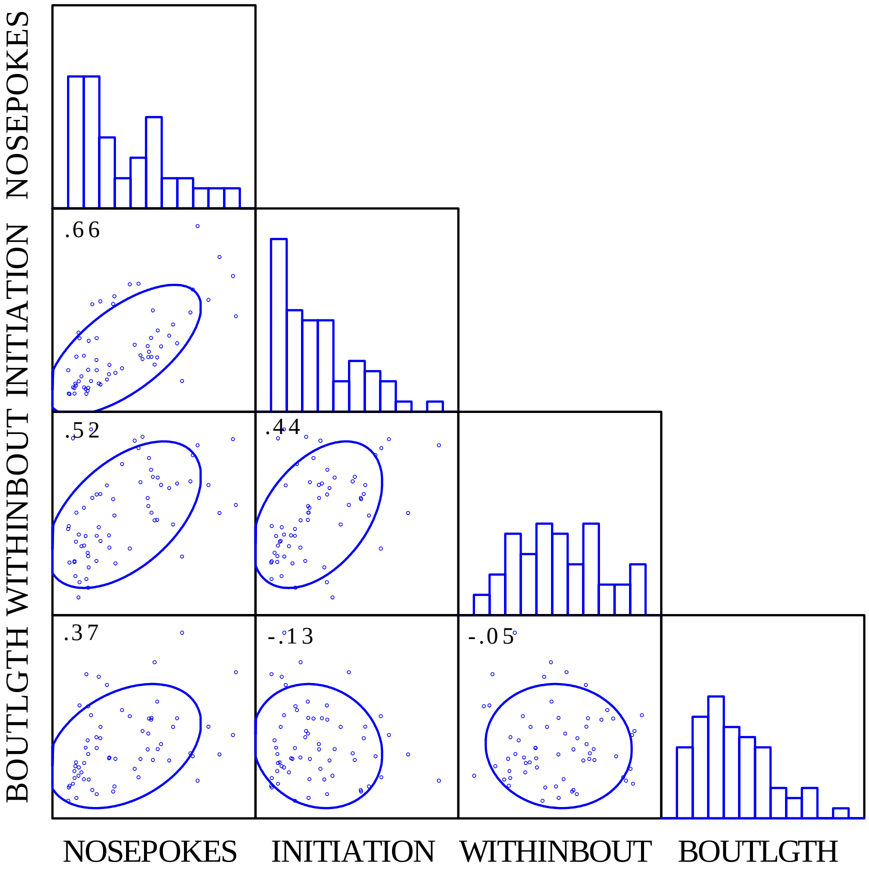

Figure 5. Correlation matrix that features both C57BL/6 and BALB/c mice combined across all behavioral dimensions. Total nose pokes, bout initiation rate, within-bout response rate and bout length are all featured as variables. Pearson R values are located in the upper left corner of each panel.

Figure 6: Correlation matrix for C57BL/6 mice.

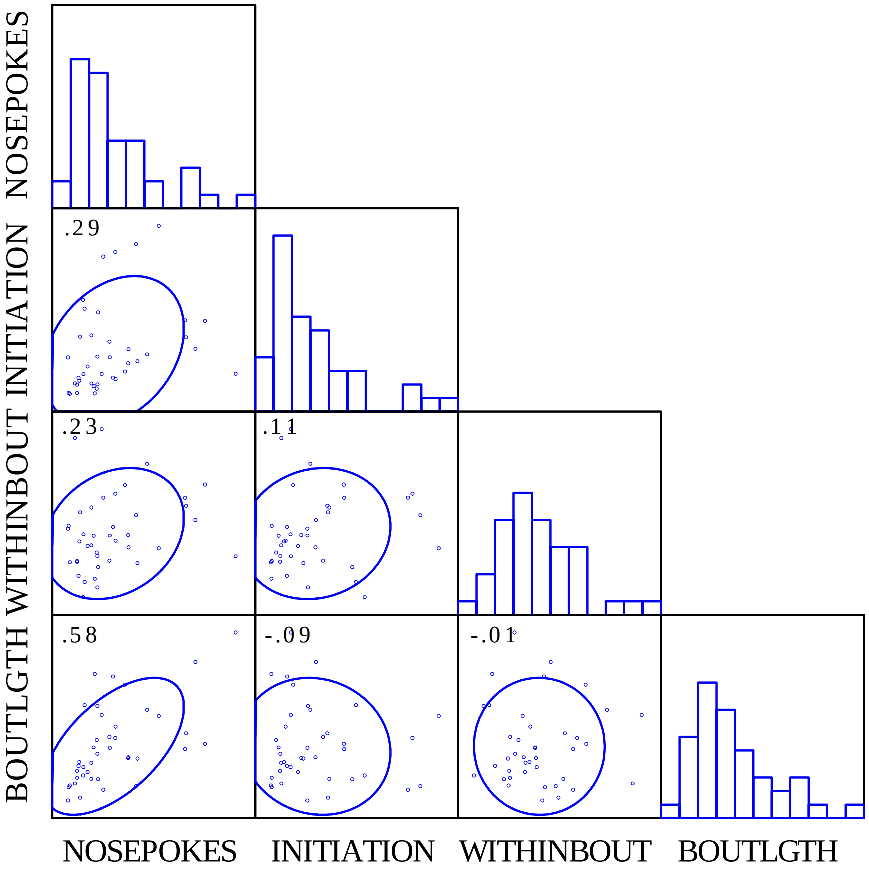

Figure 6. Correlation matrix that features C57BL/6 mice exclusively. Total nose pokes, bout initiation rate, within-bout response rate and bout length were combined across all behavioral interventions. Pearson R values are located in the upper left corner of each panel.

Figure 7: Correlation matrix for BALB/c mice.

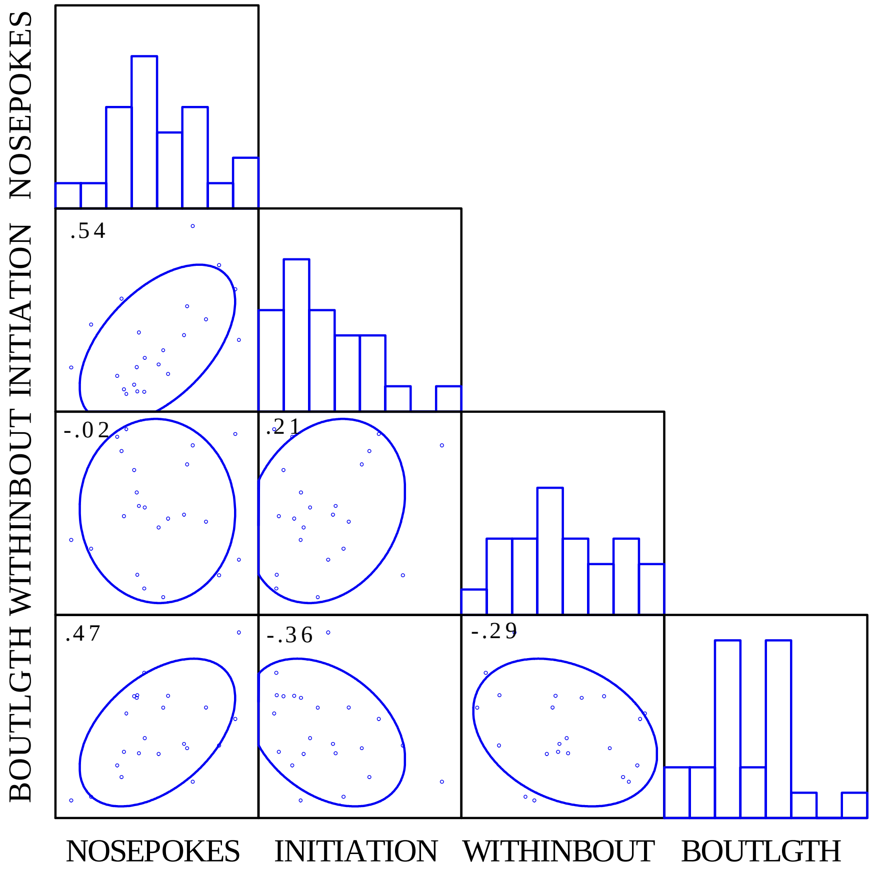

Figure 7. Correlation matrix that features BALB/c mice exclusively. Total nose pokes, bout initiation rate, within-bout response rate and bout length were combined across all behavioral interventions. Pearson R values are located in the upper left corner of each panel.

Table 1: Reinforcement rate across conditions.

BALB/c			C57BL/6		
Condition	Total nose pokes*	Reinforcement rate*	Condition	Total nose pokes*	Reinforcement rate*
Baseline	2186.41	27.5	Baseline	1077.61	23
Wheel	1721.25	28.41	Wheel	595.33	22.8
Food restriction	3248.83	29.66	Food restriction	1035.16	24.71
Free feeding	2064.75	24.83	Free feeding	341	13.04
Normal light cycle	2322.33	24.66	Normal light cycle	871.8	22.56
Reverse light cycle	2091.33	28.55	Reverse light cycle	685.33	21.71

* Variables were calculated as the average of the last three sessions for all animals