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Using pentobarbital to assess the sensitivity and independence of response-bout parameters in two mouse strains

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ABSTRACT

A recently developed model posits that a bout of operant responding comprises three different components: bout initiation rate, within-bout response rate and bout length. Each parameter is thought to be affected by different classes of variables. Pentobarbital was used to assess the independence and sensitivity of these parameters in two mouse strains, BALB/c and C57BL/6, selected because of their different behavioral characteristics. With or without a running wheel present, BALB/c and C57BL/6 mice nose-poked under a Percentile 10:0.5 schedule designed to select high response rates while holding reinforcement rate constant. Baseline rates of nose-poking were higher for BALB/c mice than for C57BL/6 mice, but no strain difference occurred in baseline distance run. Nose-poking occurred at a higher rate when the wheel was absent from the chamber for both strains, and this was due to longer bout lengths and higher bout initiation rates. Nose-poke rates were increased by the 5.6–17 mg/kg doses of pentobarbital, especially in C57BL/6 mice. Pentobarbital only decreased running. No strain differences in pentobarbital sensitivity were observed for running. Whether reinforcement was extrinsic or intrinsic to the response was hypothesized to influence pentobarbital's effects. The different bout parameters helped dissect pentobarbital's effects and were selectively affected by pentobarbital.

1. Introduction

Operant behavior under certain schedules of reinforcement can sometimes be viewed as occurring in bouts of responses that are separated by short interresponse times (IRTs). The bouts themselves are separated by long IRTs. A quantitative model based on the cumulative distribution of interresponse times (IRTs), has proven to be useful in quantifying the microstructure of these response bouts (Shull et al., 2001). In this analysis, IRTs are sorted from shortest to longest and expressed as a log survivor function. The horizontal axis is the IRT duration (from short to long) and the vertical axis is the log of the probability that an IRT is of a certain duration or longer. If responding can be described by two distinct distributions of IRTs, one representing within-bout responding and a second representing the rate at which bouts are initiated, then the log survivor plot has a "broken-stick" appearance, i.e., a steeply declining limb on the left end representing short IRTs and a more gradually declining limb on the right, representing long IRTs. The log-survival analysis of IRTs that have this broken-stick appearance can resolve three parameters: bout initiation rate, within-bout response rate and bout length.

Each parameter is thought to tap a separate influence over responding. Bout initiation rate reflects motivational aspects of responding, because of its sensitivity to reinforcement magnitude (Shull et al., 2001), food deprivation or satiation (Shull, 2004) or the availability of alternative reinforcers (Johnson et al., 2009). Bout length has been increased by using a *tandem* VI VR schedule, in which several responses, rather than one response, is required under the VI (Shull et al., 2004, 2001) and by increasing the overall reinforcement rate (Shull et al., 2004; Shull and Grimes, 2003). The determinants of within-bout response rate are not well understood, but are thought to reflect the physical characteristics of the response device or reinforcement contingencies that select short interresponse times (Shull and Grimes, 2003). In short, it is reasonable to think variables that affect motor behavior would affect within-bout response rate.

Drugs that disrupt the rate of operant behavior present an opportunity to test the independence of the model parameters, and pentobarbital offers some particularly interesting possibilities. Classified as a sedative–hypnotic drug, pentobarbital has been shown to increase the rate of operant behavior under fixed-ratio schedules of reinforcement at low doses (Dews, 1955; Herrnstein and Morse, 1957). At higher doses, pentobarbital causes sedation and motor deficits. Thus, a biphasic effect can be expected under certain schedule arrangements, especially those that tend to produce the short interresponse times, which the fixed-ratio schedule produces (Zeiler, 1977). Partitioning the response stream so as to examine the microstructure of responding could help to identify the determinants of pentobarbital's biphasic dose–effect relationship. For example, rate decreases at high doses might reflect motor slowing while rate

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increases at moderate doses could reflect the action of motivational variables.

The primary hypothesis of the present study was that the bout parameters would be influenced independently of each other and that their analysis would illuminate the source of rate changes seen with pentobarbital. Specifically, it was hypothesized that within-bout response rates would be decreased at high, motor-disrupting, doses of pentobarbital. It was not clear what form the rate increases would take at moderate doses, but it was hypothesized that it would be accompanied by increases in bout length or bout initiation rate, in the absence of increases in within-bout rate. The effects of the drug may then reveal other factors that alter the component measures of response rate.

Further examination of the model's generality was undertaken by examining two additional potential influences over bout structure. One is the presence of an alternative reinforcer, a running wheel, during an experimental session. Wheel running contrasts with nose-poking or other operant responses in that, it occurs at a high rate spontaneously, without training or support from experimenter-scheduled reinforcers. Wheel running provides an alternative measure of motor function and is often characterized as being intrinsically motivating, i.e., a reinforcer that is naturally related to the response that produced it (Catania, 1991{in Iversen and Lattal}; Sherwin, 1998). If the biphasic dose–response curve seen with pentobarbital is related to the degree to which the response is arbitrarily related to reinforcement, as in nose-poking or intrinsically linked to reinforcement, as in wheel running, then pentobarbital will affect the structure of wheel running bouts differently than it affects nose-poking bouts. Because bout initiation rate is associated with motivational influences over behavior, this measure might be especially sensitive to the nature of the reinforcer.

Another variable considered is mouse strain. Two commonly used inbred strains of mice, C57BL/6 and BALB/c, provide excellent templates for assessing the hypothesized drug-behavior interactions. C57BL/6 mice show high levels of open field locomotion, while BALB/c mice are commonly among the lowest responders in such tasks (Crawley et al., 1997; Crabbe, 1986; Mathis et al., 1994). BALB/c mice lever or disk-press and nose-poke at higher rates and with greater force than C57BL/6 mice (McKerchar and Fowler, 2005; Fowler et al., 2001; Johnson et al., 2009) and BALB/c mice show substantially better performance on complex learning tasks than C57BL/6 mice (Johnson et al., 2010). Based on their disparate behavioral profiles, BALB/c and C57BL/6 mice may show differential sensitivity to pentobarbital on the percentile task that is employed in the present study.

2. Material and methods

2.1. Subjects

Ten C57BL/6 and seven BALB/c male mice approximately one year old at the start of the experiment were obtained from Harlan laboratories (Indianapolis, IN) and used in the current experiment. All mice were housed individually in duplex Plexiglas (clear plastic) cages (10.5″L × 5.5″W × 5″H per side) with wood chip bedding. BALB/c and C57BL/6 mice were maintained at 24–26 g and 27–29 g, respectively. These weights are approximately 85% of the estimated adult free-feeding weight for these strains, with mild adjustments as required to establish sucrose as an effective reinforcer. Animals were housed in a room with 12-h light/dark cycles (lights on at 6:00 am lights off at 6:00 pm) in an AAALAC-accredited facility. All experiments were approved by the Auburn University Animal Care and Use Committee.

2.2. Apparatus

Four MedPC (St. Albans, VT) rat operant chambers fitted to accommodate mice were situated inside sound attenuating ventilated shells. Each chamber contained a photo-beam-based nose-poke device (model no. ENV-313M), a lever that was not used in the present study, and a 17.78 cm diameter running wheel containing magnets that generated four response-pulses per revolution that could be monitored and stored by the software controlling sessions. 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. Signal lights were located above both the nose-poke device and the lever. A houselight was provided near the top of the chamber directly above the food tray. A pellet dispenser delivered 20 mg sucrose pellets. MedPC (St Albans, VT) was used to program the experiments and collect data with 0.01 s resolution.

2.3. Procedure

Experimental sessions were 30 min long and occurred five days per week (Monday–Friday). Animals were used in a previous study in which they nose-poked under a second order random interval 60 s (Percentile 10:0.5) schedule of reinforcement, as in the current study (Johnson et al., 2009). The random interval (RI) 60 s schedule reinforced criterion responses (or, more precisely, criterion inter-response times (IRTs)) randomly but on an average of once/min. To qualify for reinforcement under the Percentile 10:0.5 schedule, an IRT had to be shorter than 50% of the previous 10 IRTs. This arrangement maintains high response rates (short IRTs) while continuously adjusting the response criterion according to recent performance. The RI schedule maintained a constant rate of reinforcement across a broad range of response rates (or IRT distributions). Additional details can be found in Johnson et al., 2009.

Pentobarbital sodium (Sigma-Aldrich Co., St Louis, Missouri, USA) was dissolved in a 0.9% saline solution, which also served as the vehicle, and administered 15 min prior to behavioral sessions. Doses of 3, 5.6, 10, 17, and 30 mg/kg (as the salt) were administered *ip*, in an ascending fashion on Tuesdays and Fridays. For the first dose–effect determination animals nose-poked for sucrose reinforcement without a running wheel available in the experimental chamber. For the second determination, a running wheel was added to the rear portion of the experimental chamber; but otherwise the schedule for obtaining sucrose pellets by nose-poking remained the same.

2.4. Log survivor analysis

A log survivor analysis was applied to the IRTs from each mouse, from each session in order to differentiate between bout initiations and within-bout responses on an individual basis (Shull et al., 2001, 2004). IRTs following reinforcer delivery (i.e. post-reinforcer pause times) were removed from the IRT distribution of nose-pokes before analysis. Sometimes the mice produced a few IRTs in the 0.25 s to 0.5 s second range after the sucrose pellet dispenser was activated (and coded as such in the session record). These IRTs were on the order of 60 to about 500 ms and, in this, resembled the high within-bout response rate. To ensure that these very short IRTs were not counted as a post-reinforcer pause, the IRTs following reinforcer delivery were screened so that a reasonable candidate for a post-reinforcer pause could be identified. Specifically, the five IRTs following the activation of the sucrose pellet dispenser were reviewed and the pause time was considered to be either any value greater than two seconds or the longest of those five IRTs. This process was arrived at after inspection of many sequences of IRTs that followed activation of the pellet dispenser. All the IRTs (exclusive of post-reinforcer pauses) from a session were recorded in deciseconds, collated and sorted in ascending fashion, from shortest to longest. The duration of the shortest IRT was subtracted from the entire distribution so that the function would cross the Y axis at the value (0,1). This permitted a more precise estimate of the within-bout response rate. The longest 0.5% and 1% of the IRTs were removed for wheel running and nose-

poking respectively because preliminary analyses indicated that they exerted excessive influence over the parameter estimates and resulted in visually poorer fits. A two-exponential function (Eq. (1)) was fitted to this survival function of IRTs

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

using nonlinear least squares regression. The Y(t) term represents the proportion of IRTs>t seconds; p is the proportion of responses that initiate a bout, and (1 − p) is the proportion of responses that are within a bout; *b* represents the bout initiation rate in bouts/s ; *w* represents the within-bout response rate in responses/s. Bout length is 1/*p* responses/bout. 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 nonlinear regressions required to estimate the bout parameters automatically each day.

This technique was also used to determine the microstructure of wheel running. Each 1/4 wheel revolution generated a pulse that was treated as an individual response. Inter-pulse intervals, representing the time required to turn the wheel 1/4 revolution, were sorted and subjected to a log-survival analysis as described for nose-poke IRTs. The results were then converted to distance.

2.5. Inferential statistics

All error bars represent standard error of the mean, and all cases are included in each analysis unless specified otherwise. The baseline values were calculated using the average of the last 5 control sessions. The dependent measures analyzed include: nose-poke rate, bout initiation rate, within-bout response rate, bout length and distance run. If zero nose-pokes were emitted during a session, bout parameter values were not calculated and coded as zeroes. An α of 0.05 was used.

Strain differences in baseline levels of wheel running were evaluated using an independent samples *t*-test. A two-way ANOVA (strain × wheel) was used to analyze main effects on nose-poking of strain and the presence of the wheel and their interactions during baseline. Drug effects were evaluated using two-way repeated measures ANOVAs with pentobarbital dose as the within-subjects factor and strain as the between-subjects factor. This was conducted separately for the wheel and no-wheel conditions. Drug and saline effects were expressed as percent of baseline values. This way, the sensitivity to pentobarbital for each animal could be evaluated as a function of that animal's baseline performance.

3. Results

3.1. Sample survivor plots

The left panel of Fig. 1 contains representative log survivor plots. The plots represent an individual animal's IRT distribution for one session. Plots are shown both for a BALB/c mouse (mouse 126) and a C57BL/6 mouse (mouse 221) for a non-injection control session and a session where 10 mg/kg of pentobarbital was administered prior to the start of the session. The equation used to fit the data is included inside the log survivor plot. Pentobarbital administration resulted in faster bout initiation rates for both strains which generate a steeper slope of the right leg and a larger value for the second exponential term.

The right panel of Fig. 1 contains the cumulative record data corresponding to the same sessions used to generate the log survivor plots. The BALB/c mice (top two rows) nose-poked more than C57BL/6 mice (bottom two rows), and administration of 10 mg/kg of pentobarbital reduced the interresponse time. By reducing interresponse time, more nose-pokes were emitted, which can be seen in the steeper and more frequent peaks in the cumulative records.

3.2. Baseline

BALB/c mice nose-poked more than C57BL/6 mice ($F(1,15) = 30.31$, $p<0.001$) and both strains nose-poked more when the wheel was absent from the experimental chamber than when present (Fig. 2, $F(1,15) = 26.32$, $p<0.001$). BALB/c mice initiated nose-poke bouts at a higher rate than C57BL/6 mice ($F(1,15) = 19.39$, $p = 0.001$). The bout initiation rate was higher when the wheel was absent in the experimental chamber than when it was present ($F(1,15) = 38.06$, $p<0.001$). A significant interaction ($F(1,15) = 14.56$, $p = 0.002$) indicated that the wheel caused a larger reduction in initiation rate for BALB/c mice than for C57BL/6 mice. BALB/c mice also had a higher within-bout response rate than C57BL/6 mice ($F(1,15) = 14.91$, $p = 0.002$) and there was a significant wheel × strain interaction ($F(1,15) = 8.75$, $p = 0.01$). Nose-poking bouts were longer for BALB/c than C57BL/6 mice ($F(1,15) = 10.18$, $p = 0.006$).

Post-reinforcer pauses were monitored and analyzed, but not graphed. During baseline, the BALB/c and C57BL/6 mice had pauses of about 1 s and 2.5 s, respectively ($F(1,15) = 10.59$, $p = 0.005$), regardless of the presence of the running wheel.

The right panel of Fig. 2 shows the bout structure of running. There was no significant effect of strain on distance run ($t(15) = -1.8$, $p = 0.091$). There was, however, a strain difference in bout initiation rate and within-bout rate. C57BL/6 mice initiated more bouts ($t(15) = -2.7$, $p = 0.016$) and had higher within-bout speeds than BALB/c mice ($t(15) = -2.18$, $p = 0.045$) but no distinguishable strain difference occurred in bout length.

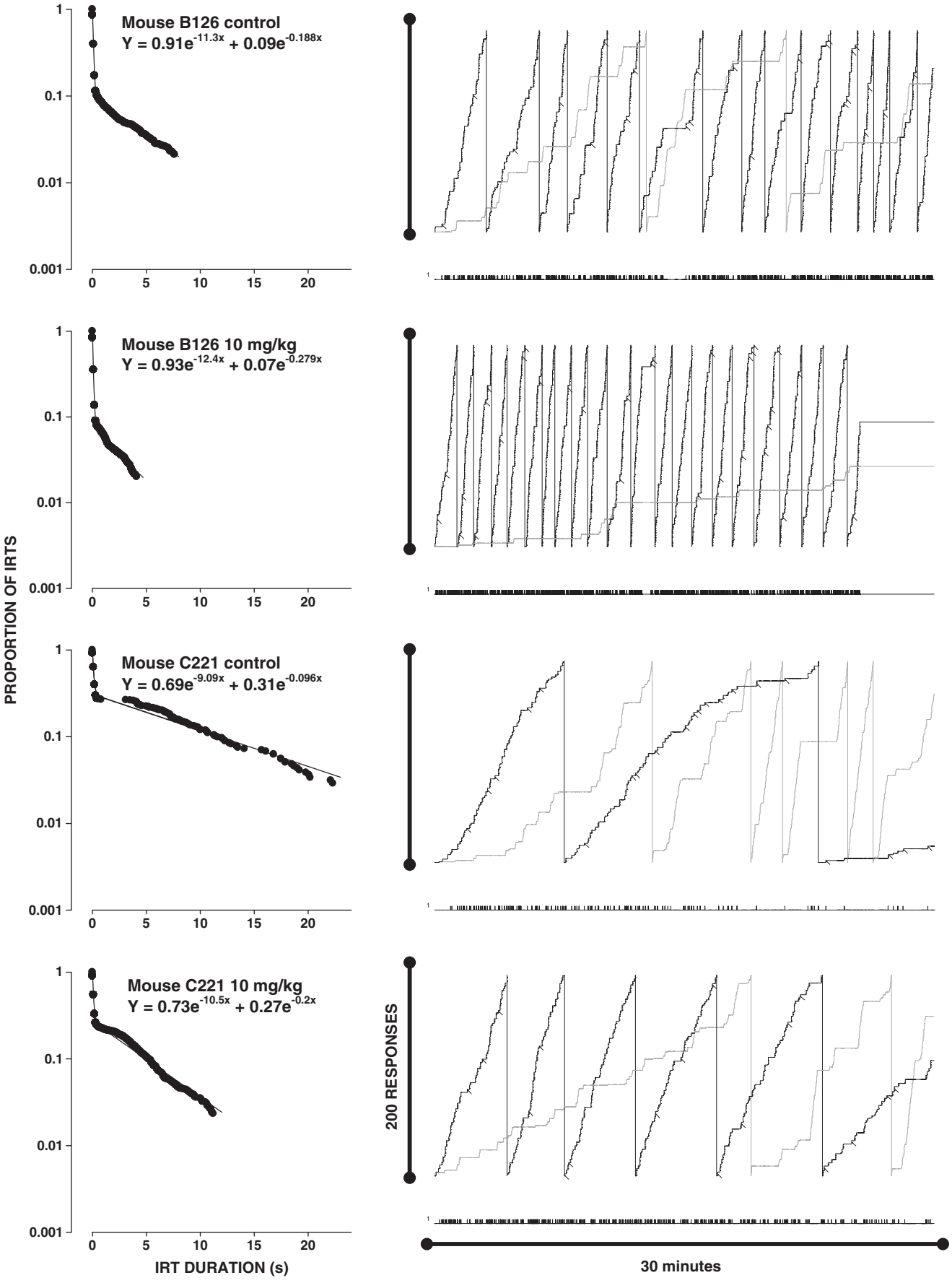
3.3. Strain differences in sensitivity to pentobarbital

As seen in the left portion of Fig. 3, a biphasic dose–effect curve appeared in overall nose-poking for both strains when the wheel was present; nose-poking increased at low to moderate doses and decreased at high doses of pentobarbital for both strains ($F(4,60) = 7.41$, $p<0.01$). Pentobarbital increased nose-poking for the C57BL/6 mice more (up to 100%) than for BALB/c mice (up to 50%) relative to their baseline rates ($F(1,15) = 9.33$, $p = 0.008$). bout initiation rate was increased more, relative to baseline, for C57BL/6 mice ($F(1,15) = 11.17$, $p = 0.04$) and increased at doses of 5.6–17 mg/kg. This parameter was unaffected at moderate doses and decreased at the highest dose for BALB/c mice. Within-bout response rate decreased to about half of baseline rates at the highest dose for both strains ($F(4,60) = 11.91$, $p<0.01$). There was a significant main effect of dose ($F(4,60) = 4.64$, $p<0.01$), driven by an increase in pause time, at the highest dose of 30 mg/kg (to about 3.0 s for the C57BL/6 and 2.6 s for the BALB/c mice) and a slight decrease at the 10 mg/kg dose (to about 1.5 s for the C57BL/6 and 0.8 s for the BALB/c mice).

When the wheel was absent, there was a main effect of dose on nose-poking ($F(4,68) = 24.237$, $p<.001$) and there was a significant dose × strain interaction ($F(4,68) = 3.805$, $p = .008$) such that pentobarbital produced a 2-fold rate increase in nose-poking for the C57BL/6 mice but no detectable change for the BALB/c mice. There was also a main effect of strain ($F(1,17) = 24.122$, $p<.001$).

With respect to the bout parameters, when the wheel was absent there was a main effect of pentobarbital ($F(4,68) = 3.192$, $p = .018$)

Fig. 1. Log survivor plots (left column) are displayed for both a representative BALB/c and C57BL/6 mouse nose-poking under drug and non-drug sessions when a wheel was available in the experimental chamber. A point on the plot represents IRTs that are longer than the corresponding value on the X-axis. The equation used to generate the fit line is also included in the figure. Cumulative records (right column) show both nose-poking (black line) and wheel running (grey line) through the course of an experimental session. Event records are provided under the cumulative records and each vertical line represents a nose-poke.


Mouse B126 control
Y = 0.91e-11.3x + 0.09e-0.188x
Mouse B126 10 mg/kg
Y = 0.93e-12.4x + 0.07e-0.279x
Mouse C221 control
Y = 0.69e-9.09x + 0.31e-0.096x
Mouse C221 10 mg/kg
Y = 0.73e-10.5x + 0.27e-0.2x
PROPORTION OF IRTS
1
0.1
0.01
0.001
0
5
10
15
20
IRT DURATION (s)
200 RESPONSES
30 minutes

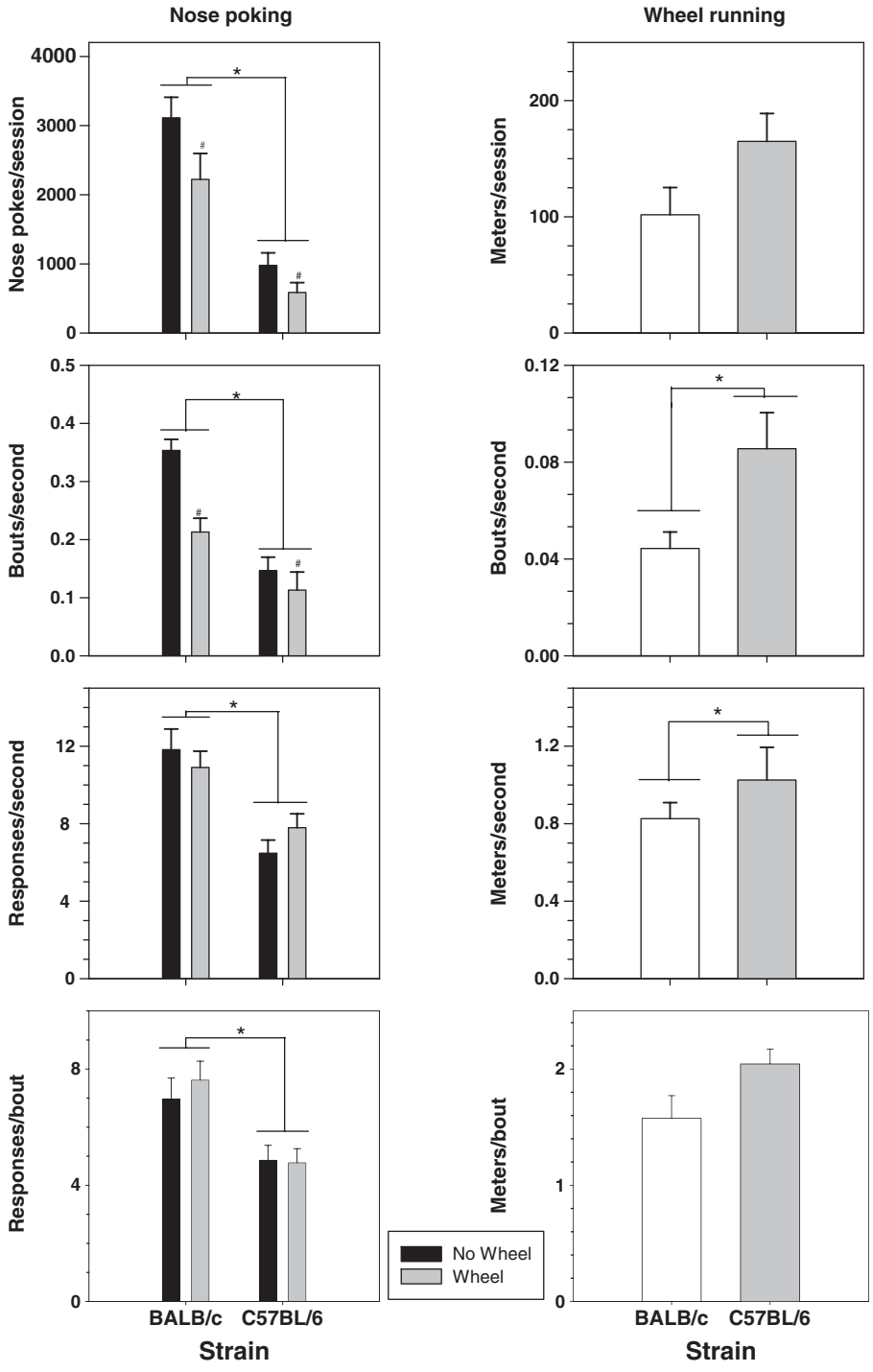
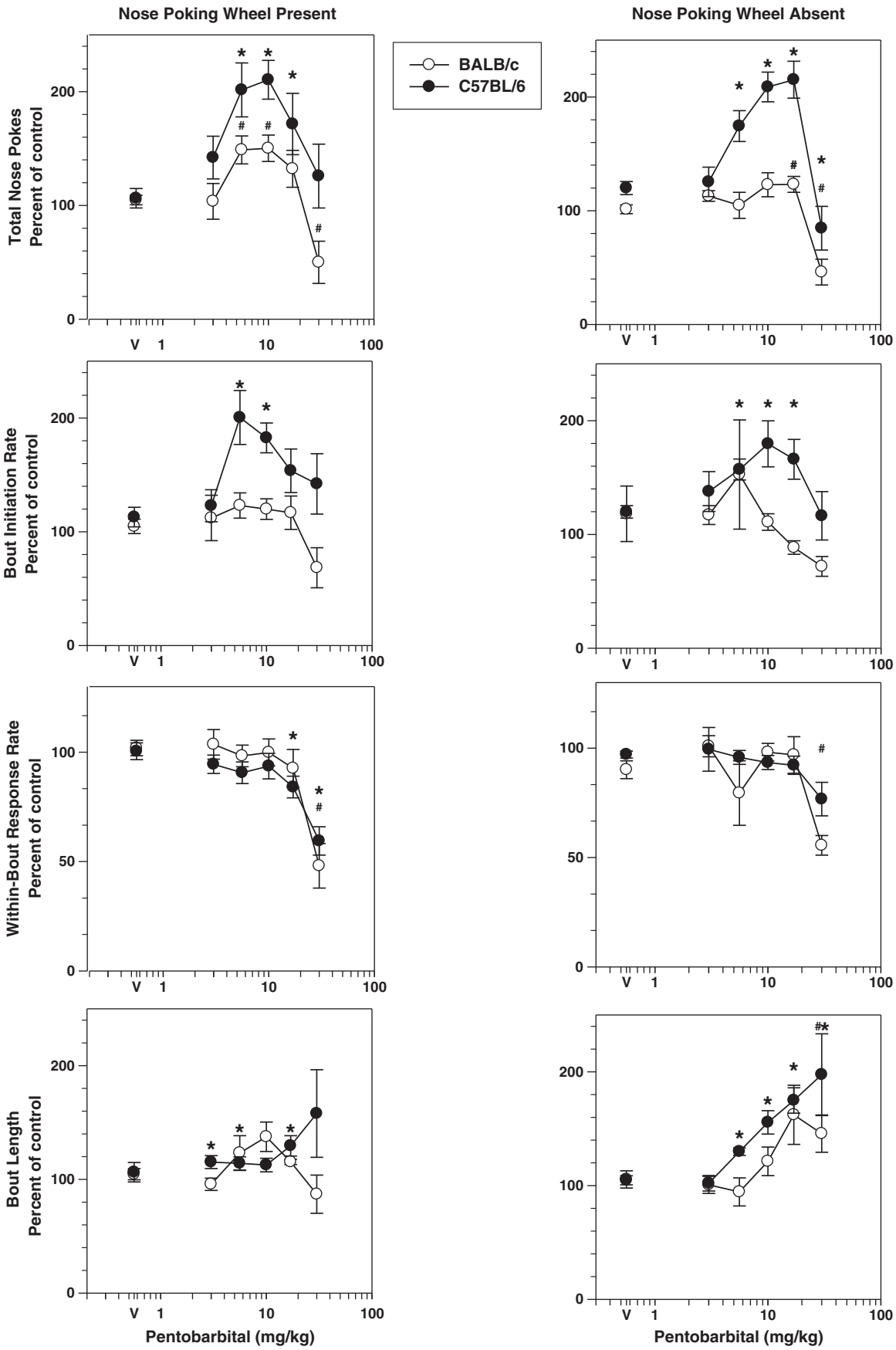


Fig. 2. Baseline measures of nose-poking with and without the wheel present (left panels) and wheel running (right panels) for both strains. Total responding and individual bout parameters are in separate panels. Session duration is 30 min. Asterisks (*) signify significant main effects of strain, while a pound sign (#) signifies a significant difference between conditions (wheel or no wheel). The legend corresponds to the left most panels only.

and strain ($F(1,17) = 7.297$, $p = .015$) on bout initiation rate, but no strain × drug interaction was detected. Moderate doses increased bout initiation rate. A monotonic dose-related decrease in within-bout rate occurred for both strains ($F(4,68) = 7.598$, $p < .001$) but no main effect and no strain × drug interaction was detected. Pentobarbital increased bout length ($F(4,64) = 6.690$, $p < .001$), more for the C57BL/6 mice than BALB/c mice ($F(1,17) = 5.199$, $p = .036$). Post-reinforcer pauses (not graphed) increased at the high doses ($F(4,60) = 4.64$, $p = 0.003$) and there was no difference between the two strains ($p = 0.166$). A post-hoc analysis of post-reinforcer pauses revealed significant

Fig. 3. Pentobarbital's effect on nose-poking rates and bout parameters with (left) and without (right) a running wheel. The data are expressed as a proportion of control to highlight strain differences in pentobarbital's effects independent of control rate differences. The letter V represents experimental sessions where the vehicle (saline) was administered prior to the start of the session. Individual doses that significantly differ from vehicle for C57BL/6 mice are denoted by the symbol (*). Individual doses that significantly differ from vehicle for BALB/c mice are denoted by the symbol (#).


Nose Poking Wheel Present
Nose Poking Wheel Absent
BALB/c
C57BL/6
Total Nose Pokes
Percent of control
Bout Initiation Rate
Percent of control
Within-Bout Response Rate
Percent of control
Bout Length
Percent of control
V 1 10 100
Pentobarbital (mg/kg)

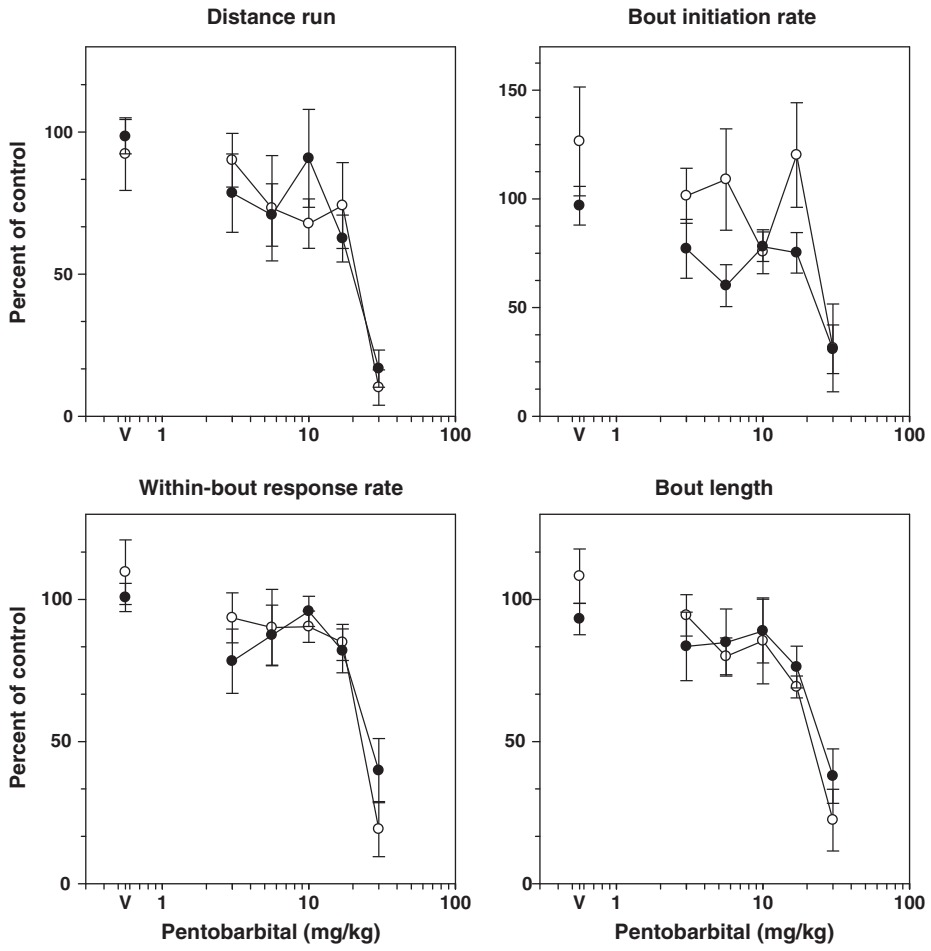


Fig. 4. Pentobarbital's effects on distance run and bout parameters of wheel running. The letter V represents experimental sessions where the vehicle (saline) was administered prior to the start of the session. Individual doses that significantly differ from vehicle for C57BL/6 mice are denoted by the symbol (*). Individual doses that significantly differ from vehicle for BALB/c mice are denoted by the symbol (#).

decreases from saline occurred for the BALB/c mice at the 5.6 mg/kg dose (an 18% decrease, $p<.05$) and for the C57BL/6 mice at doses of 5.6 and 10 mg/kg ($p<.05$).

Fig. 4 shows pentobarbital's effects on wheel running. High doses of pentobarbital decreased wheel running, usually at the highest dose (30 mg/kg) ($F(4,60)=10.37$, $p<0.01$). Similarly bout initiation rate ($F(4,60)=7$, $p<0.01$), within-bout response rate ($F(4,60)=14.35$, $p<0.01$) and bout length ($F(4,60)=10.88$, $p<0.01$) all decreased at the highest dose. Visual inspection confirmed this effect was driven by the 30 mg/kg dose of pentobarbital. The only strain differences in wheel running was a larger decrease in initiation rate for C57BL/6 mice at the moderate doses ($F(1,15)=6.18$, $p=0.025$) than for BALB/c mice.

3.4. Intercorrelations among parameters

The intercorrelations among the parameters: PRP, bout initiation rate, within-bout rate, and bout length were examined by combining these parameters across both strains and all drug conditions. The conditions were combined so as to provide a wide range of values for this analysis. Correlations were conducted with 110 degrees of freedom. The three parameters that were derived from the log survivor analysis were weakly correlated with one another, with values of 0.20 (bout length and bout initiation rate), 0.24 (within-bout rate and bout initiation rate) and 0.35 (bout length and within-bout rate). The PRP was correlated with bout initiation rate, within-bout rate, and bout length with values of −0.66, −0.41, and −0.45, respectively. All correlations were significant with p's ≤ 0.05.

4. Discussion

The present study provided behavioral and pharmacological support for a quantitative model that conceptualizes responding as occurring in bouts (Shull et al., 2001). Specifically, these data confirm and extend earlier reports that the parameters interpreted as within-bout response rate, bout initiation rate, and bout length contribute separate descriptors of the structure of response bouts for both nose-poking and wheel running. The analysis of the biphasic dose–effect relationship describing pentobarbital's behavioral effects offers a novel test case for this model. While pentobarbital is a sedative at high doses, a rate increase in the already high rates maintained by fixed-ratio schedules has been reported after acute administration of low to moderate doses (Dews, 1955; Herrnstein and Morse, 1957), even when the response is effortful (Newland and Weiss, 1990). Here, high rate responding was maintained not by a fixed-ratio schedule but rather by a percentile schedule that selected short IRTs while dynamically adjusting the definition of the criterion by which an IRT is eligible for reinforcement. For comparison, high rates of wheel running were also produced simply by making a running wheel available.

Low to moderate doses of pentobarbital increased nose-poking maintained under the Percentile 10:0.5 schedule, although the increase from control for BALB/c mice in the absence of the wheel was small. These increases were due to longer bout lengths or higher bout initiation rates, depending on strain and the availability of a wheel. Within-bout response rates for nose-poking, a measure that is thought to reflect motor influences (Shull et al., 2001), was unaffected

by the lower doses of pentobarbital. At the highest dose, however, within-bout rates were reduced by as much as 50% of control values. Overall, the descending leg of the dose–effect curve was most consistently associated with a decreased within-bout rate. Therefore pentobarbital, at low to moderate doses, increased bout initiation rate and bout length but did not affect within-bout response rate for nose-poking.

PRP was negatively correlated with all three bout parameters with correlations ranging from 0.4 to 0.66, indicating that measures that corresponded to increased responding (longer, faster, and more frequent bouts) were also correlated with shorter PRPs. The three bout parameters were weakly related to one another, with correlations ranging from 0.20 to 0.35, or variances accounted for ranging from 4% to 12%. In a previous study, for which the number of comparisons was smaller ($df = 62$) it was reported that the correlations among these parameters were indistinguishable from zero because they were less than 0.25 (Johnson et al., 2009). The analysis here has greater power because there are more degrees of freedom (110) and a wider range of values due to the inclusion of a broad range of pentobarbital doses, two strains and conditions with and without a running wheel available. The weakness of the correlation and the presence of different dose–effect relationships for within-bout rate, bout initiation rate and bout length suggest that these parameters provide separable measures of bout structure.

Pentobarbital produced longer bouts of nose-poking in both strains, especially when the wheel was absent. Interestingly, none of these effects of pentobarbital appeared in wheel running. Three possible reasons for this discrepancy might be noted. One is that wheel running was never examined in the absence of the opportunity to nose-poke, and the availability of the latter response could have dampened rate-increasing effects of pentobarbital.

A second possible reason for the discrepancy between nose-poking and wheel running drug effects is the baseline rate of responding. Many behaviorally active drugs have effects that are rate-dependent, such that low rates are increased while high rates are decreased (McKim, 1973; Dews, 1958; Kelleher et al., 1961). Normally, a rate-dependency hypothesis would be difficult to address here because of the qualitative difference in response types: nose-poking is a discrete response while wheel running is continuous. The partitioning of responding into bouts, however, provides a common metric that can be used for comparison: bout initiation rate. For nose-poking, pentobarbital increased the low bout initiation rates at doses that did not alter the high within-bout rates (or wheel running). The pentobarbital-induced rate increase in nose-poking occurred in both strains when the wheel was present and nose-poke rates were low. When the wheel was absent, an increase in nose-poking occurred only in the C57BL/6 mice, the strain that reliably nose-poked at a low rate. Unfortunately, a rate-dependency account cannot accommodate pentobarbital's effects on wheel running. During baseline, bout initiation rates of wheel running were an order of magnitude lower than within-bout rates and substantially lower than those of nose-poking. Thus, one might expect an increase in the low rate of initiating wheel running bouts, but none was seen at any dose for either strain.

A third possibility might be whether reinforcement was intrinsic or extrinsic. Intrinsic reinforcers have a natural relation to the response that produces it which is the case for wheel running (Catania, 1991; Sherwin, 1998). In contrast, an extrinsic reinforcer has an arbitrary relationship to the response, such as sucrose pellets reinforcing nose-poking (Catania, 1991). Nose-poking occurred only when explicitly reinforced while wheel running appeared "spontaneously," with no training and no extrinsic reinforcement. It can be hypothesized that pentobarbital, at low doses, increases response rates of behavior when maintained by extrinsic, but not intrinsic, reinforcers. This suggests that the drug shifted behavior toward this explicitly reinforced response at the expense of other activity.

A strain difference in baseline replicates a previous report (Johnson et al., 2009) that BALB/c mice nose-poked more than C57BL/6 mice regardless of the presence or absence of a running wheel in the experimental chamber. Specifically, BALB/c mice produce more bouts, and the response rates within those bouts are higher, than C57BL/6 mice. This strain difference has been documented (Johnson et al., 2009; Wang and Fowler, 1999; Zarcone et al., 2004) and likely reflects the disparate behavioral profiles of these two inbred mouse strains.

In the present study, the addition of a running wheel was reported to affect bout length and the bout initiation rate parameters differently than what was reported in a previous study (Johnson et al., 2009). In the present study, adding the running wheel reduced bout initiation rate and had little effect on bout length. In the previous paper (Johnson et al., 2009) adding the running wheel was reported to increase bout initiation rate and decreased bout length. In addition, bout lengths were shorter in the present paper than in the previous one. It is not clear why these discrepancies exist, but one difference can be noted. They might be due to differences in how individual IRT's and post-reinforcer pauses were distinguished. If a reinforcer was delivered in the middle of a bout, then a few nose-pokes may occur after the pellet dispenser was activated. In the analyses conducted for the present paper, but not for the 2009 paper, these nose-pokes were screened and considered as within-bout nose-pokes while a long IRT that occurred within a few responses of the delivery of a food pellet was recorded separately as a post-reinforcer pause, as described in the methods section. This removed post-reinforcer pauses, which would include the time required to consume a sucrose pellet, from the IRTs used to calculate bout length and initiation rates and resulted in more accurate estimates of these parameters. We feel that the removal of post-reinforcer pauses in this way provides a more robust, and accurate, depiction of the effects of adding a running wheel on the microstructure of nose-poking.

There was a clear strain difference in pentobarbital's effects on nose-poking, but not on wheel running. This provides further support that the behavioral task under examination could be an important determinant of strain differences in drug effects (Crabbe et al., 1992, 1994). It can be noted that there was no significant effect on total wheel running despite changes on bout initiation and within-bout rates. This may be because the variability in total wheel running was relatively high as compared with the variability in the specific bout parameter. For those aspects of the microstructure of responding that reflect motor deficits, namely running speed and within-bout nose-poke rates, there were no strain differences in sensitivity to pentobarbital.

In sum, pentobarbital altered the microstructure of nose-poking and wheel running in different ways. The quantitative model was successful in separating motor and motivational influences of pentobarbital. Moderate doses of pentobarbital selectively altered the microstructure of nose-poking, but not wheel running, as revealed by the bout parameters. The influence of baseline response rates and whether reinforcement was extrinsic or intrinsic to the response, as in wheel running, were noted as possible influences over this effect of pentobarbital. Also, behavioral task proved to be one important determinant of strain differences in drug effects. This quantitative model is appropriate to be used as a tool to separate motivational and motor effects of a drug.

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