# The priming effect of rewarding brain stimulation persists following blockade of D2-like dopamine receptors

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# Abstract

Receipt of an intense reward boosts motivation to work for more of that reward. This phenomenon is called the *priming effect of rewards*. A previous study demonstrated that the priming effect of rewarding medial forebrain bundle stimulation was remarkably resilient following administration of medium-high doses of pimozide. Here, we used the operant-chamber paradigm described in the preceding paper to assess the dependence of the priming effect on D2-like dopamine receptors. We found that the priming effect also proved resilient to the effect of eticlopride, a selective D2-like receptor antagonist. The results are discussed within the framework of a new model of brain reward circuitry in which non-dopaminergic medial forebrain bundle fibers and dopamine axons provide parallel inputs to the final common paths for reward and incentive motivation.

# 1. Introduction

Performance for rewarding electrical brain stimulation depends on the integrity of dopaminergic neurotransmission (Crow, 1970; Franklin, 1978; Franklin & McCoy, 1979; C. R. Gallistel & Karras, 1984; Hernandez et al., 2010, 2012; Miliaressis et al., 1986; Trujillo-Pisanty et al., 2014; Wise & Rompré, 1989). Dopaminergic neurotransmission is also implicated in motivation (Berridge & Robinson, 1998; Ikemoto & Panksepp, 1999; Robinson & Berridge, 1993). The incentive-salience theory (Berridge & Robinson, 1998; Robinson & Berridge, 1993) has been particularly influential in this regard. That theory posits separate neural systems mediate *wanting* (incentive salience) and *liking* (hedonic value) of rewards; wanting is attributed to activation of dopamine neurons.

The priming effect of rewarding electrical brain stimulation shows both critical features of motivation: It both energizes and directs behavior. Following delivery of strong rewarding stimulation, rats seek out additional stimulation more vigorously, which demonstrates the energizing effect of priming (Edmonds & Gallistel, 1974; C. R. Gallistel et al., 1974; Sax & Gallistel, 1991). In the absence of priming, thirsty rats will choose to seek water in preference to rewarding brain stimulation (Wasserman et al., 1982). That preference is reversed by priming the rats with electrical brain stimulation, thus demonstrating that priming can shift the goal to which reward-seeking behavior is directed.

Given the motivational character of the priming effect and the implication of dopamine signaling in motivation, one might expect that blockade of dopamine receptors would weaken the priming effect. That commonsensical prediction was not borne out when the priming effect was challenged in the runway paradigm by the administration of the D2R antagonist, pimozide (Wasserman et al., 1982). Even

after having been treated with a very high dose, the rats showed a clear priming effect during the initial trials after the drug had taken effect. Pimozide also failed to block the priming-induced preference reversal: Following delivery of priming stimulation, thirsty rats continued to choose brain stimulation in lieu of water.

Here, we used the operant-chamber paradigm validated in the previous paper (Evangelista et al., 2022) to revisit the question of whether the priming effect of rewarding electrical brain stimulation depends on dopaminergic neurotransmission. Given that pimozide has affinity for $5HT_7$ receptors, we used a more specific D2-like receptor blocker instead, eticlopride (Hall et al., 1985; Martelle & Nader, 2008).

# 2. Method

## 2.1 Subjects, Electrode Implantation, Apparatus

Six male Long-Evans rats from the experiments described in the previous paper (Evangelista et al., 2022) served as subjects for this study. The housing conditions, procedures for electrode implantations, and apparatus for operant conditioning were identical to those described previously.

## 2.2 Procedures

**Testing.** The rats in this experiment had already undergone training and testing in the priming paradigm introduced in the previous paper (Evangelista et al., 2022), which was also used in the present study. The rats thus advanced to the present study without further training.

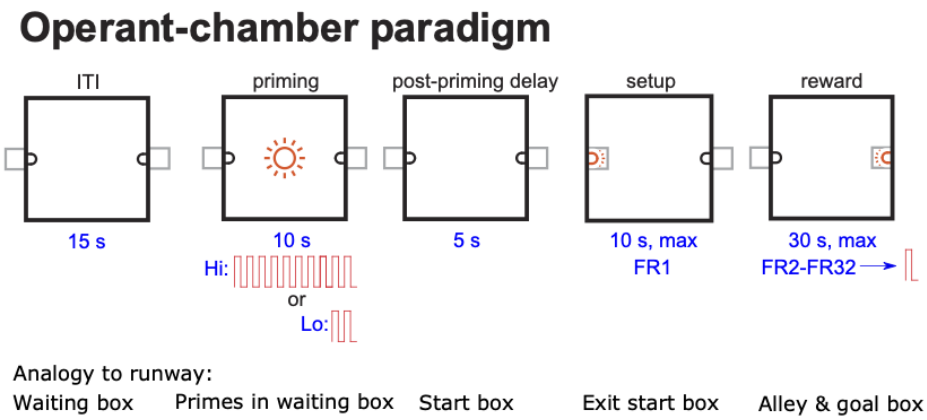


Figure 1: Paradigm for measuring the priming effects of rewards in operant-conditioning chambers (Evangelista et al., 2022).

**Testing: trial structure.** The trial structure is depicted in Figure 1.

- Both the setup and stimulation levers remain retracted during the 15 s ITI.
- On high-priming trials, the stimulation lever extends at the start of the priming phase, the light over the lever illuminates, the house light flashes (10 cycles of 0.5s on, 0.5s off), and a 20 s countdown begins. A single lever press triggers a train of priming stimulation and initiates retraction of the setup lever for 1 s, during which time the countdown pauses. The rat can earn up to 9 additional trains of priming before the countdown ends. Initiation of the tenth train or a countdown to zero terminates the priming phase.
    - The priming phase is identical on no priming trials except that the stimulation lever does not extend, the lever light does not illuminate, and no stimulation is available.

- There is a 5-s delay at the end of the priming phase, at which time the setup lever extends, the setup-lever light illuminates, a 10-s countdown is initiated, and the setup phase begins.
- The setup lever is armed on an FR8 schedule. If the rat completes the response requirement before the end of the 10-s countdown, the setup lever retracts, the setup lever light is extinguished, and the setup phase ends, thus triggering the start of the reward phase.
    - The stimulation lever then extends, the stimulation-lever light illuminates, and a 30-s countdown begins. A single depression of the stimulation lever triggers delivery of the rewarding stimulation and terminates the trial. If the rat fails to press the stimulation lever, the trial terminates after 30 s.
- If the rat fails to complete the response requirement during the setup phase before the end of the 10-s countdown, the setup lever retracts, the setup lever light is extinguished, and the setup phase terminates.
    - The stimulation lever does not extend at that time, nor is the stimulation-lever light illuminated. Instead, both levers remain retracted, and the trial ends 30 s later.

**Testing: Session structure.** Each test session is preceded by a warm-up session during which the rats pressed the stimulation lever for reward on an FR1 schedule for 2-3 minutes. Test sessions consist of 42 trials. The first two trials of a session are primed trials that served as learning trials and are excluded from analyses. The remaining 40 trials consist of four cycles of five primed and five non-primed trials.

Eticlopride (0.1 or 0.05 mg/kg, Sigma, St. Louis, MO) was dissolved in physiological saline (0.9%). These doses were based on data from a previous pilot study (data not shown) and Lazenka *et al.* (2016). Vehicle (0.9% physiological saline) or one of the two doses of eticlopride was administered intraperitoneally (i.p) 30 minutes prior to testing. Tests were conducted in three-day cycles consisting of a vehicle day, drug day, and washout day. We aimed to conduct ten of these 3-day cycles for each drug dose. However, there were instances where rats did not perform at all at the highest dose (0.1 mg/kg) of eticlopride and thus testing at that dose was discontinued. Tests were first conducted at the highest dose of eticlopride (0.1 mg/kg). Six rats underwent testing with the highest dose of eticlopride. However, only three rats (AM47, AM55, AM58) successfully completed testing at that dose, which induced catatonia in the other rats (AM46, AM48, AM50) following repeated administration. Those latter three rats underwent testing at the lowest dose of eticlopride. The rats that were tested at the highest dose of eticlopride were not tested with the lowest dose of eticlopride due to attrition (e.g., health, electrode stability, etc.).

## 2.3 Measures of response vigor

**Initial Speed**. Initial speed is the inverse of the latency (s) to press the setup lever following its extension. A larger initial speed number indicates that a rat was quicker to initiate the first press on the setup lever. This is akin to how fast a rat exited the start box in the runway paradigm.

**Reward Rate.** Reward rate is the inverse of the total time (s) elapsed between the start of the trial and the delivery of the reward. Higher reward rates reflect more vigorous reward pursuit. This transformation is analogous to the running speed measure reported in Gallistel's investigations on the priming effect of electrical brain stimulation (Gallistel et al., 1974).

**Tempo**. Tempo is the response rate during the setup phase. It is calculated by dividing the number of setup responses by the interval between the first and last responses. Higher tempos indicate faster reward pursuit. This is analogous to running speed in the alley alone.

## 2.3 Statistical Analyses

The statistical analyses were identical to those conducted in the previous study (Evangelista et al., 2022). For each rat, data were analyzed for each priming condition (high, none). Means, medians, robust means (the location parameter obtained from application of the Tukey bisquare estimator (Hoaglin et al., 2000) and medians were calculated for each measure. Data were analyzed, and graphs were plotted using MATLAB (The Mathworks, Natick, MA). Cliff's Delta (Cliff, 1993) served as the measure of effect size. Priming effects are considered statistically reliable when the 95% confidence interval surrounding Cliff's Delta, computed by means of resampling (Efron & Tibshirani, 1993), excludes zero. Priming effects are considered to be meaningful when the Difference-Ratio measure described in the previous paper (Evangelista et al., 2022) exceeds 0.05. That measure scales the difference between the robust means for the priming and no-priming conditions by the grand robust mean (the robust mean across priming conditions).

In addition to analyzing data for each priming condition (high or no priming) for each rat, the results obtained at each drug dose (saline, 0.1, or 0.5 mg/kg of eticlopride) were analyzed separately.

# 3. Results

The priming effect was seen in both the vehicle and drug conditions, even at the highest dose of eticlopride (0.1 mg/kg). This is illustrated in Figures 1-3 in the case of rat AM47. Only four testing

sessions were completed by this rat under the effects of the 0.1 mg/kg dose, but the variance is low, and a clear effect of priming stimulation is evident. In the remaining drug sessions, rat AM47 initiated responding on the setup lever on a total of only 35 of the 240 trials (6 sessions × 40 trials/session) and only harvested a reward on 17 of these trials. In contrast, all 10 test sessions were completed successfully following vehicle administration. Although priming continued to boost performance substantially under the influence of 0.1 mg/kg of eticlopride in rat AM47, the initiation of responding in the setup phase (Figure 1) and rate of responding on the setup and reward phases (Figure 2) were slowed. However, once responding had been initiated, response tempo was equal or greater than that observed in the vehicle condition (Figure 3).

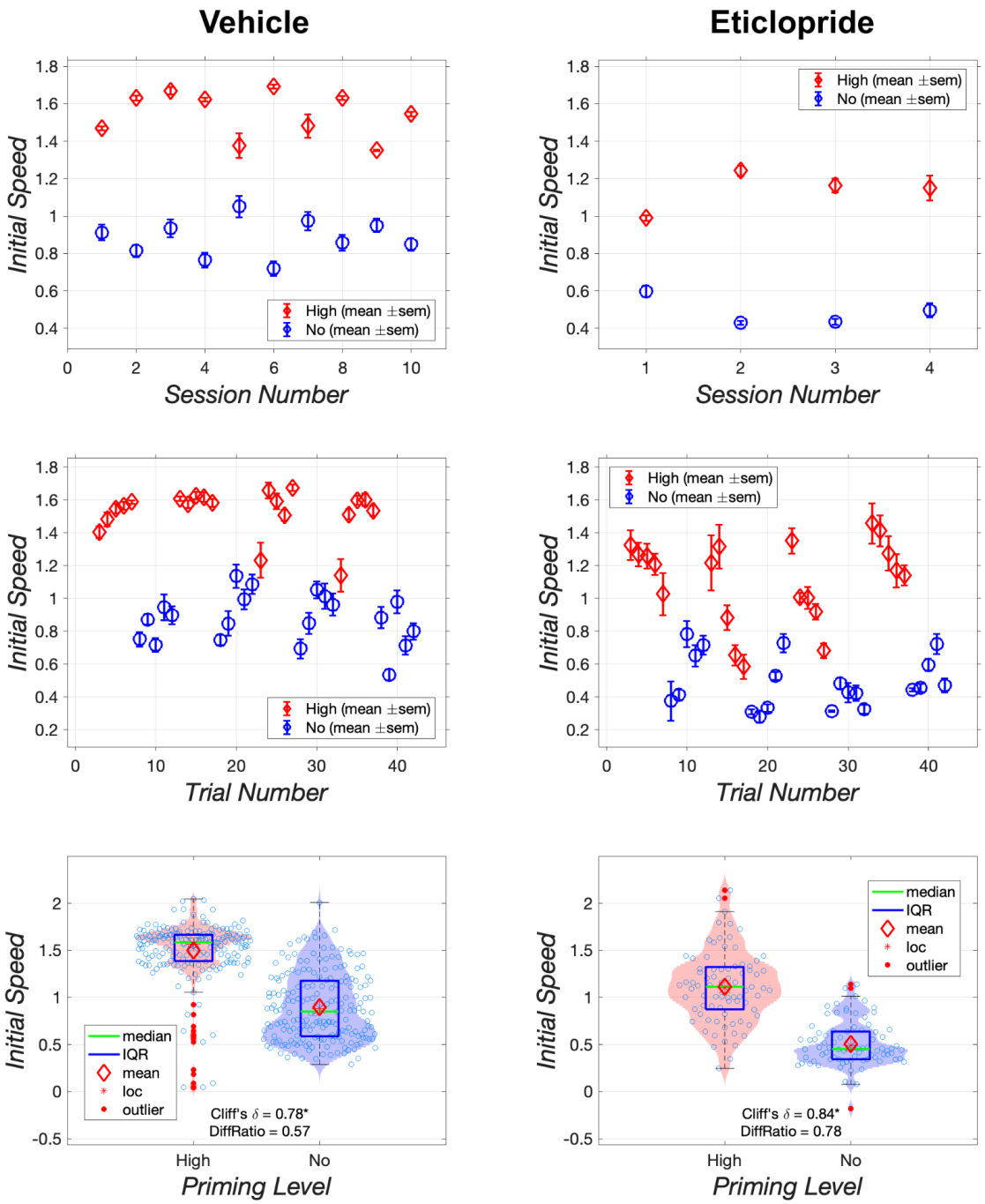


***Figure 1.*** Effect of vehicle and 0.1 mg/kg of eticlopride on the initial speed to initiate responding by rat AM47. The upper row shows results collapsed over trials, whereas the middle row shows results collapsed over sessions. The violin plots in the bottom row show the kernel density of the data.

Individual observations are denoted by open circles. The red diamond denotes the robust mean, the green line denotes the median, the vertical extent of the blue rectangle shows the interquartile range, and filled red circles denote outliers.

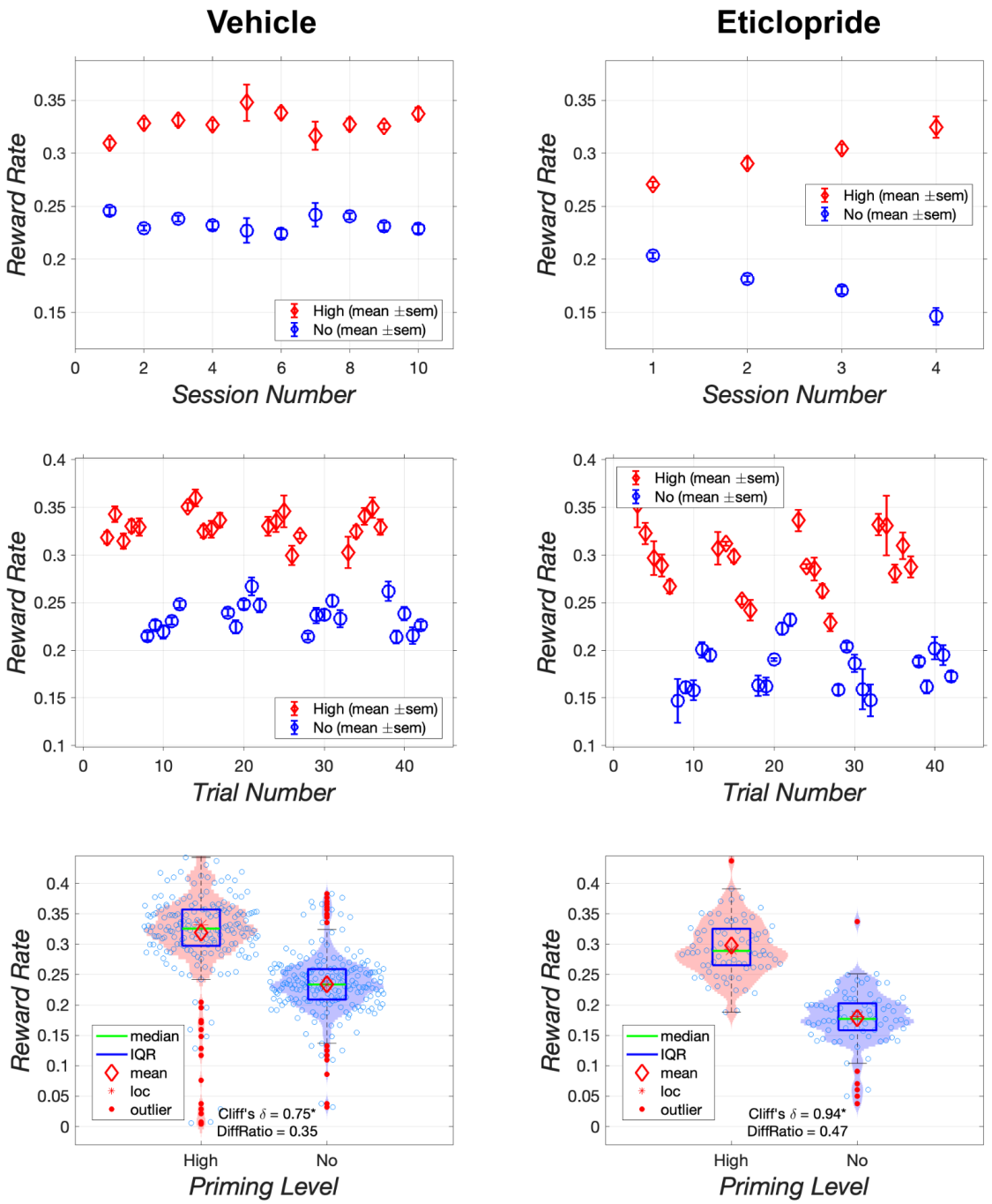


***Figure 2.*** Effect of vehicle and 0.1 mg/kg of eticlopride on reward rate in rat AM47. The upper row shows results collapsed over trials, the middle row shows results collapsed over sessions, and the bottom row shows violin plots of the kernel density of the data.

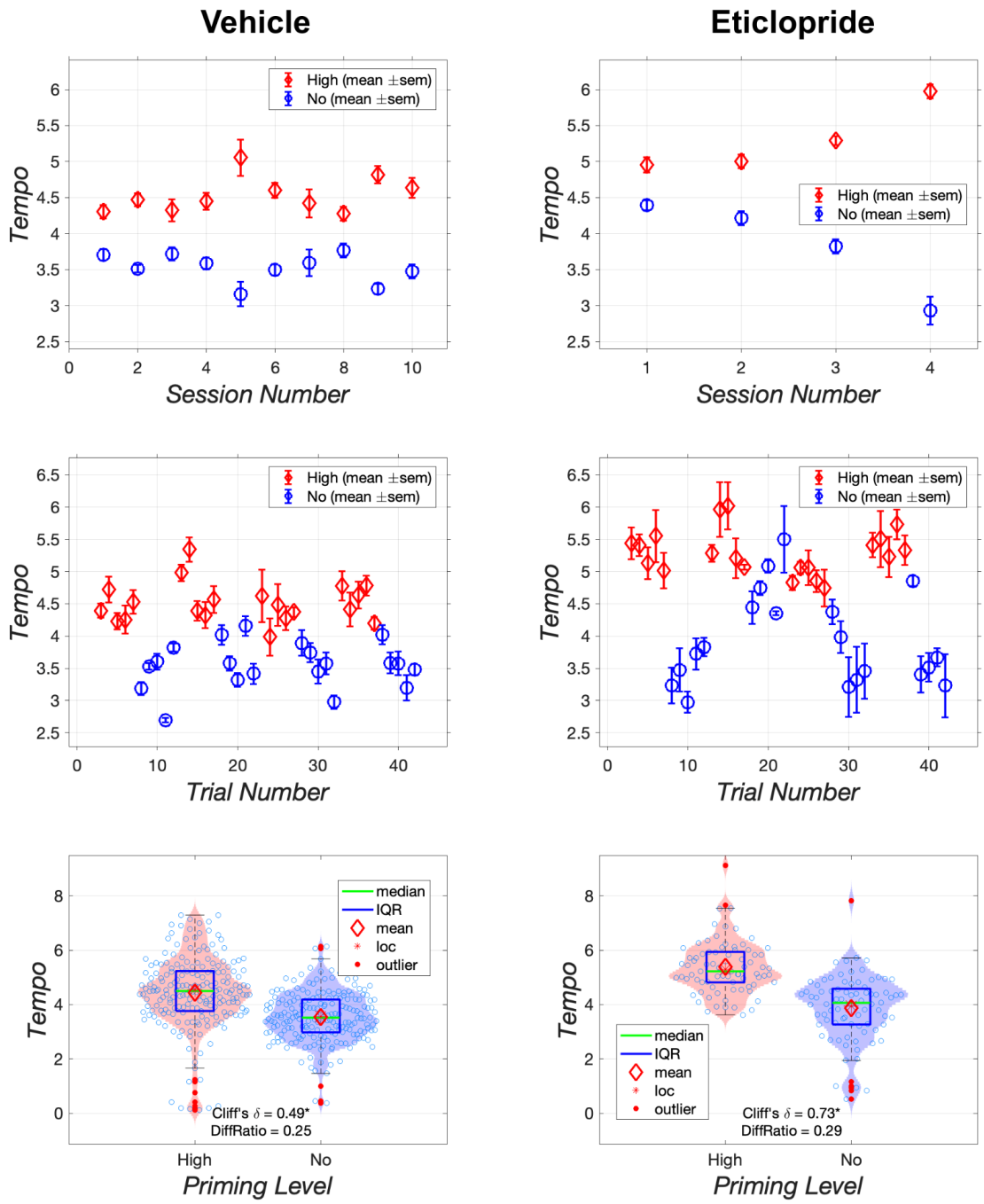


***Figure 3.*** Effect of vehicle and 0.1 mg/kg of eticlopride on the tempo of responding during the setup phase in rat AM47. The upper row shows results collapsed over trials, whereas the middle row shows results collapsed over sessions, and the bottom row shows violin plots of the kernel density of the data.

Results from the other 2 rats that completed at least four sessions under the effects of the 0.1 mg/kg dose (AM55, AM58) are shown in Figures S1-S6. Rat AM55 completed all 10 vehicle sessions and five drug sessions, whereas rat AM58 completed nine of 10 vehicle sessions and five drug sessions. Figures 1-3 and S1-S6 show only the results obtained on the completed sessions.

Tables 1, 3, and 5 compile the values of the Difference Ratio and Cliff's Delta on the three measures of vigor in the vehicle condition for all three rats that received the 0.1 mg/kg dose of eticlopride. The results from the drug condition are shown in Tables 2, 4, and 6. The reward-rate and tempo results meet our criteria for a meaningful (Difference Ratio $\geqq$ 0.05) and reliable (confidence interval around Cliff's Delta excludes 0) effect in both the vehicle and drug conditions in all three rats. All three rats showed a meaningful and reliable priming effect in the vehicle condition on the initial-speed measure, whereas in the eticlopride condition, rats AM47 and AM55 met the criteria for a meaningful and reliable priming effect but AM58 did not. Both the Difference Ratio and Cliff's Delta scores for all three vigor measures were higher in the eticlopride data from rat AM47 than in the vehicle data, and the confidence intervals around Cliff's Delta for the drug condition do not overlap those for the vehicle condition in the cases of the reward-rate and tempo measures. In other words, the priming effect in this rat was generally stronger under the influence of eticlopride than in the vehicle condition.

## Initial Speed: Vehicle

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM47 | 1.565 | 0.881 | 0.684 | 1.203 | 0.569 | 0.779 | 0.849 | 0.708 | TRUE |
| AM55 | 1.233 | 0.711 | 0.522 | 0.969 | 0.539 | 0.665 | 0.744 | 0.578 | TRUE |
| AM58 | 1.228 | 0.981 | 0.248 | 1.108 | 0.224 | 0.313 | 0.432 | 0.197 | TRUE |
| Median | 1.233 | 0.881 | 0.522 | 1.108 | 0.539 | 0.665 | 0.744 | 0.578 | |
| Max | 1.565 | 0.981 | 0.684 | 1.203 | 0.569 | 0.779 | 0.849 | 0.708 | |
| Min | 1.228 | 0.711 | 0.248 | 0.969 | 0.224 | 0.313 | 0.432 | 0.197 | |
| met criterion | | | | | 3 | | | 3 | 3 |

## Initial Speed: 0.1 mg/kg Eticlopride

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM47 | 1.101 | 0.488 | 0.613 | 0.788 | 0.778 | 0.842 | 0.920 | 0.738 | TRUE |
| AM55 | 0.648 | 0.555 | 0.092 | 0.603 | 0.153 | 0.140 | 0.263 | 0.014 | TRUE |
| AM58 | 0.692 | 0.641 | 0.051 | 0.653 | 0.078 | 0.169 | 0.330 | -0.009 | FALSE |
| Median | 0.692 | 0.555 | 0.092 | 0.653 | 0.153 | 0.169 | 0.330 | 0.014 | |
| Max | 1.101 | 0.641 | 0.613 | 0.788 | 0.778 | 0.842 | 0.920 | 0.738 | |
| Min | 0.648 | 0.488 | 0.051 | 0.603 | 0.078 | 0.140 | 0.263 | -0.009 | |
| met criterion | | | | | 3 | | | 2 | 2 |

***Tables 1 (top) and 2 (bottom):*** Initial speed to initiate responding in the setup phase in rats AM47, AM55, and AM58 following administration of the vehicle (top) or 0.1 mg/kg eticlopride (bottom).

## Reward Rate: Vehicle

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM47 | 0.331 | 0.233 | 0.098 | 0.278 | 0.351 | 0.758 | 0.836 | 0.671 | TRUE |
| AM55 | 0.250 | 0.189 | 0.060 | 0.218 | 0.277 | 0.704 | 0.779 | 0.618 | TRUE |
| AM58 | 0.222 | 0.195 | 0.027 | 0.210 | 0.130 | 0.387 | 0.500 | 0.272 | TRUE |
| Median | 0.250 | 0.195 | 0.060 | 0.218 | 0.277 | 0.704 | 0.779 | 0.618 | |
| Max | 0.331 | 0.233 | 0.098 | 0.278 | 0.351 | 0.758 | 0.836 | 0.671 | |
| Min | 0.222 | 0.189 | 0.027 | 0.210 | 0.130 | 0.387 | 0.500 | 0.272 | |
| met criterion | | | | | 3 | | | 3 | 3 |

## Reward Rate: 0.1 mg/kg Eticlopride

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM47 | 0.291 | 0.181 | 0.110 | 0.236 | 0.467 | 0.940 | 0.986 | 0.871 | TRUE |
| AM55 | 0.206 | 0.183 | 0.024 | 0.193 | 0.123 | 0.284 | 0.396 | 0.162 | TRUE |
| AM58 | 0.184 | 0.148 | 0.036 | 0.170 | 0.213 | 0.371 | 0.528 | 0.190 | TRUE |
| Median | 0.206 | 0.181 | 0.036 | 0.193 | 0.213 | 0.371 | 0.528 | 0.190 | |
| Max | 0.291 | 0.183 | 0.110 | 0.236 | 0.467 | 0.940 | 0.986 | 0.871 | |
| Min | 0.184 | 0.148 | 0.024 | 0.170 | 0.123 | 0.284 | 0.396 | 0.162 | |
| met criterion | | | | | 3 | | | 3 | 3 |

***Tables 3 (top) and 4 (bottom):*** Reward rate in rats AM47, AM55, and AM58 following administration of the vehicle (top) or 0.1 mg/kg eticlopride (bottom).

**Tempo: Vehicle**

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaCIhi | DeltaCIlo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM47 | 4.552 | 3.551 | 1.000 | 4.006 | 0.250 | 0.495 | 0.593 | 0.394 | TRUE |
| AM55 | 3.446 | 2.807 | 0.639 | 3.116 | 0.205 | 0.411 | 0.524 | 0.296 | TRUE |
| AM58 | 2.622 | 2.371 | 0.251 | 2.507 | 0.100 | 0.308 | 0.421 | 0.178 | TRUE |
| Median | 3.446 | 2.807 | 0.639 | 3.116 | 0.205 | 0.411 | 0.524 | 0.296 | |
| Max | 4.552 | 3.551 | 1.000 | 4.006 | 0.250 | 0.495 | 0.593 | 0.394 | |
| Min | 2.622 | 2.371 | 0.251 | 2.507 | 0.100 | 0.308 | 0.421 | 0.178 | |
| met criterion | | | | | 3 | | | 3 | 3 |

**Tempo: 0.1 mg/kg Eticlopride**

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaCIhi | DeltaCIlo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM47 | 5.304 | 3.938 | 1.366 | 4.659 | 0.293 | 0.722 | 0.827 | 0.609 | TRUE |
| AM55 | 3.507 | 3.023 | 0.484 | 3.251 | 0.149 | 0.345 | 0.452 | 0.239 | TRUE |
| AM58 | 2.501 | 1.832 | 0.669 | 2.181 | 0.307 | 0.463 | 0.597 | 0.314 | TRUE |
| Median | 3.507 | 3.023 | 0.669 | 3.251 | 0.293 | 0.463 | 0.597 | 0.314 | |
| Max | 5.304 | 3.938 | 1.366 | 4.659 | 0.307 | 0.722 | 0.827 | 0.609 | |
| Min | 2.501 | 1.832 | 0.484 | 2.181 | 0.149 | 0.345 | 0.452 | 0.239 | |
| met criterion | | | | | 3 | | | 3 | 3 |

***Tables 5 (top) and 6 (bottom):*** Response tempo during the setup phase in rats AM47, AM55, and AM58 following administration of the vehicle (top) or 0.1 mg/kg eticlopride (bottom).

Figures 4-6 illustrate the effect of the lower dose of eticlopride (0.05 mg/kg) on the three measures of vigor in rat AM46, one of the three rats that were unable to complete at least four sessions under the influence of the higher dose (0.1 mg/kg). This rat completed 9 of ten test sessions in both the vehicle and drug conditions, and a clear priming effect is seen in both conditions.

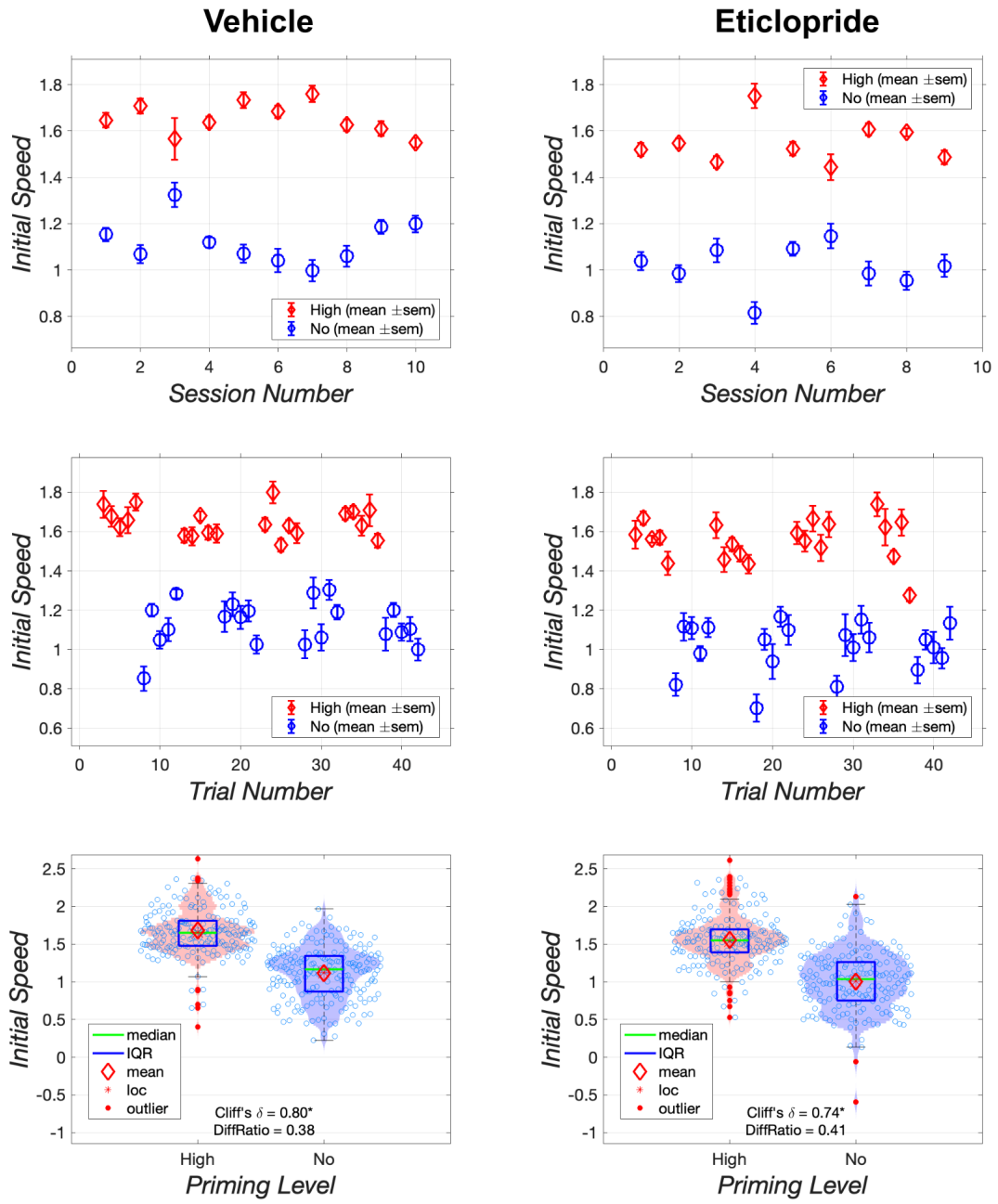


***Figure 4.*** Effect of vehicle and 0.05 mg/kg of eticlopride on the initial speed to initiate responding by rat AM46. The upper row shows results collapsed over trials, the middle row shows results collapsed over sessions, and the bottom row shows violin plots of the kernel density of the data.

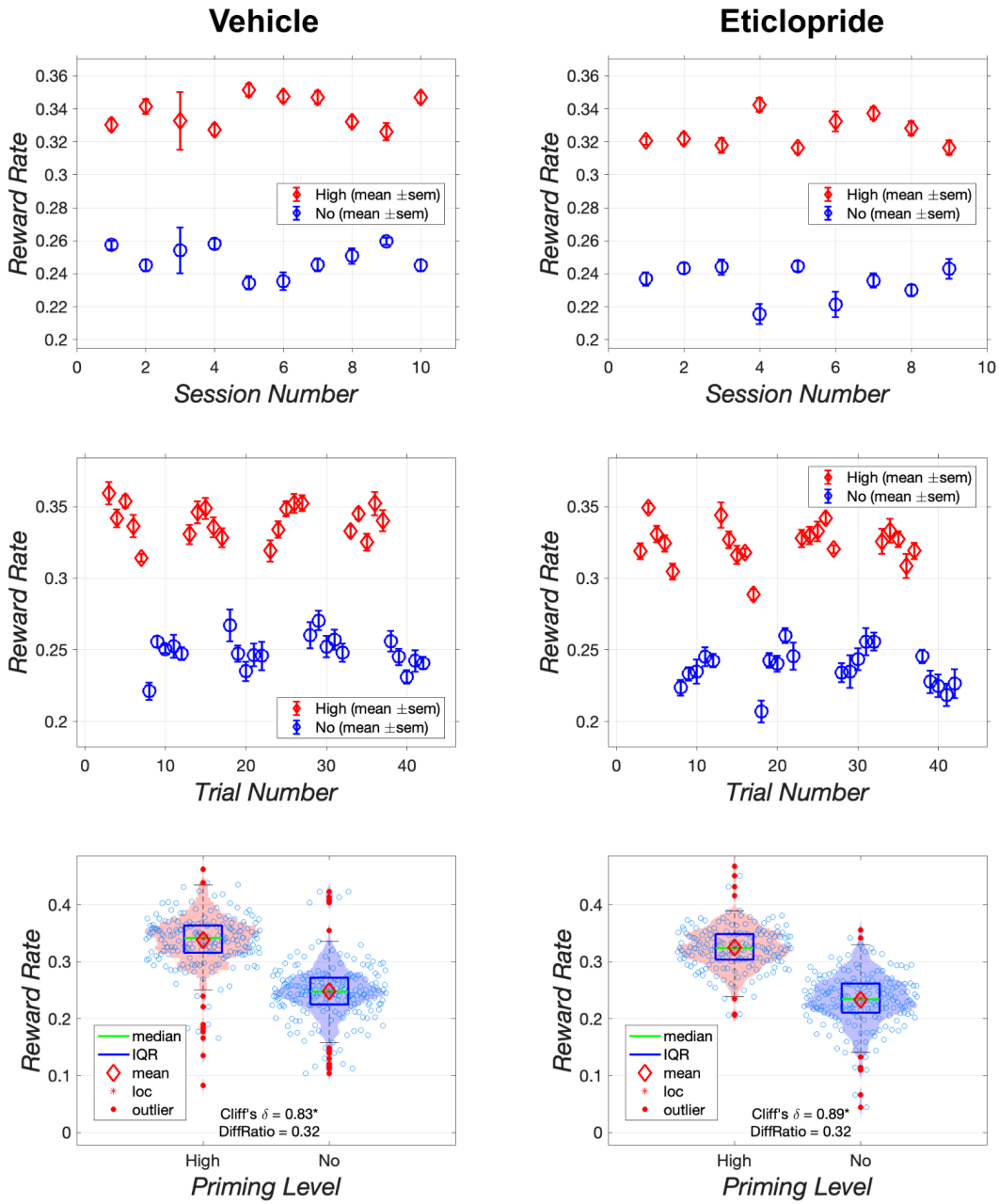


***Figure 5:*** Effect of vehicle and 0.05 mg/kg of eticlopride on reward rate in rat AM4g. The upper row shows results collapsed over trials, the middle row shows results collapsed over sessions, and the bottom row shows violin plots of the kernel density of the data.

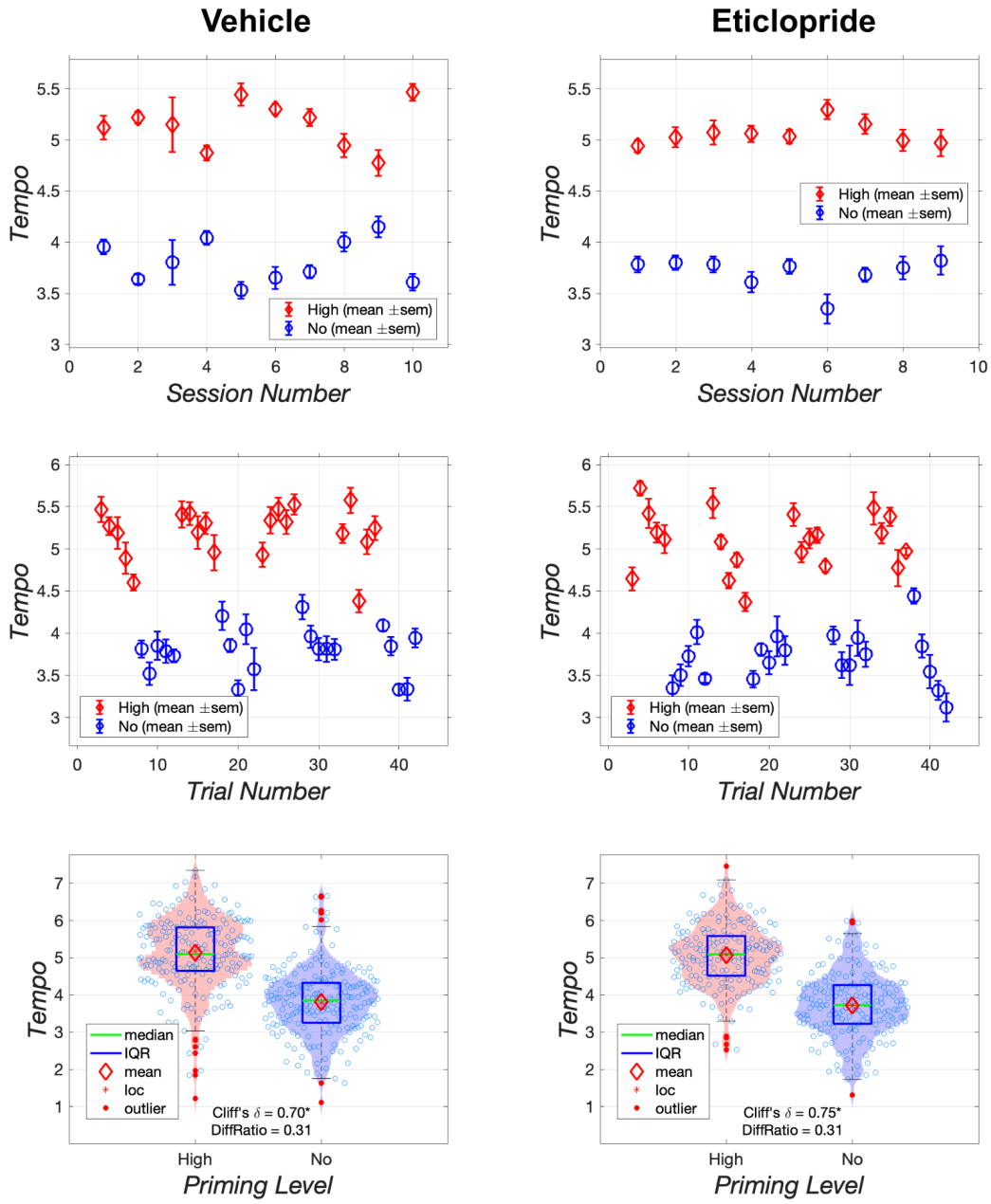


***Figure 6:*** Effect of vehicle and 0.05 mg/kg of eticlopride on the tempo of responding during the setup phase in rat AM46. The upper row shows results collapsed over trials, the middle row shows results collapsed over sessions, and the bottom row shows violin plots of the kernel density of the data.

The results from the other two rats tested at this dose (AM48, AM50) are shown in Figures S7-S12. Rat AM48 completed 9 of ten test sessions in the vehicle condition and four of 10 sessions in the drug condition, whereas rat AM50 completed all ten test sessions in the vehicle condition and nine of 10 sessions in the drug condition.

Tables 7, 9, and 11 compile the values of the Difference Ratio and Cliff's Delta on the three measures of vigor in the vehicle condition for all three rats that received the 0.0.05 mg/kg dose of eticlopride. The results from the drug condition are shown in Tables 8, 10, and 12. The initial-speed and reward-rate results meet our criteria for a meaningful (Difference Ratio $\geqq$ 0.05) and reliable (confidence interval around Cliff's Delta excludes 0) effect in both the vehicle and drug conditions in all three rats. This was also the case for the tempo measure in rats AM46 and AM50. Rat AM48 did not show a priming effect on the tempo measure in either the vehicle or the drug condition.

**Initial Speed: Vehicle**

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaCIhi | DeltaCIlo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM46 | 1.656 | 1.124 | 0.531 | 1.398 | 0.380 | 0.801 | 0.860 | 0.729 | TRUE |
| AM48 | 0.938 | 0.807 | 0.130 | 0.878 | 0.148 | 0.186 | 0.314 | 0.061 | TRUE |
| AM50 | 3.124 | 1.436 | 1.688 | 1.921 | 0.879 | 0.771 | 0.838 | 0.693 | TRUE |
| Median | 1.656 | 1.124 | 0.531 | 1.398 | 0.380 | 0.771 | 0.838 | 0.693 | |
| Max | 3.124 | 1.436 | 1.688 | 1.921 | 0.879 | 0.801 | 0.860 | 0.729 | |
| Min | 0.938 | 0.807 | 0.130 | 0.878 | 0.148 | 0.186 | 0.314 | 0.061 | |
| met criterion | | | | | 3 | | | 3 | 3 |

**Initial Speed: 0.05 mg/kg Eticlopride**

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaCIhi | DeltaCIlo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM46 | 1.540 | 1.010 | 0.531 | 1.291 | 0.411 | 0.740 | 0.818 | 0.655 | TRUE |
| AM48 | 0.975 | 0.804 | 0.171 | 0.890 | 0.192 | 0.283 | 0.445 | 0.115 | TRUE |
| AM50 | 2.288 | 1.298 | 0.991 | 1.709 | 0.580 | 0.710 | 0.779 | 0.621 | TRUE |
| Median | 1.540 | 1.010 | 0.531 | 1.291 | 0.411 | 0.710 | 0.779 | 0.621 | |
| Max | 2.288 | 1.298 | 0.991 | 1.709 | 0.580 | 0.740 | 0.818 | 0.655 | |
| Min | 0.975 | 0.804 | 0.171 | 0.890 | 0.192 | 0.283 | 0.445 | 0.115 | |
| met criterion | | | | | 3 | | | 3 | 3 |

Tables 7 (top) and 8 (bottom): Initial speed to initiate responding in the setup phase in rats AM46, AM48, and AM50 following administration of the vehicle (top) or 0.05 mg/kg eticlopride (bottom).

## Reward Rate: Vehicle

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM46 | 0.341 | 0.247 | 0.093 | 0.293 | 0.319 | 0.834 | 0.898 | 0.762 | TRUE |
| AM48 | 0.188 | 0.179 | 0.009 | 0.183 | 0.051 | 0.159 | 0.281 | 0.035 | TRUE |
| AM50 | 0.378 | 0.306 | 0.071 | 0.339 | 0.211 | 0.708 | 0.784 | 0.628 | TRUE |
| Median | 0.341 | 0.247 | 0.071 | 0.293 | 0.211 | 0.708 | 0.784 | 0.628 | |
| Max | 0.378 | 0.306 | 0.093 | 0.339 | 0.319 | 0.834 | 0.898 | 0.762 | |
| Min | 0.188 | 0.179 | 0.009 | 0.183 | 0.051 | 0.159 | 0.281 | 0.035 | |
| met criterion | | | | | 3 | | | 3 | 3 |

## Reward Rate: 0.05 mg/kg Eticlopride

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM46 | 0.325 | 0.236 | 0.089 | 0.281 | 0.316 | 0.889 | 0.933 | 0.838 | TRUE |
| AM48 | 0.191 | 0.174 | 0.016 | 0.181 | 0.090 | 0.354 | 0.513 | 0.189 | TRUE |
| AM50 | 0.371 | 0.287 | 0.083 | 0.327 | 0.255 | 0.821 | 0.885 | 0.755 | TRUE |
| Median | 0.325 | 0.236 | 0.083 | 0.281 | 0.255 | 0.821 | 0.885 | 0.755 | |
| Max | 0.371 | 0.287 | 0.089 | 0.327 | 0.316 | 0.889 | 0.933 | 0.838 | |
| Min | 0.191 | 0.174 | 0.016 | 0.181 | 0.090 | 0.354 | 0.513 | 0.189 | |
| met criterion | | | | | 3 | | | 3 | 3 |

Tables 9 (top) and 10 (bottom): Reward rate in rats AM46, AM48, and AM50 following administration of the vehicle (top) or 0.05 mg/kg eticlopride (bottom).

## Tempo: Vehicle

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM46 | 5.173 | 3.785 | 1.387 | 4.455 | 0.311 | 0.707 | 0.778 | 0.625 | TRUE |
| AM48 | 2.165 | 2.171 | -0.006 | 2.169 | -0.003 | 0.008 | 0.143 | -0.112 | FALSE |
| AM50 | 4.695 | 4.205 | 0.490 | 4.432 | 0.111 | 0.295 | 0.402 | 0.180 | TRUE |
| Median | 4.695 | 3.785 | 0.490 | 4.432 | 0.111 | 0.295 | 0.402 | 0.180 | |
| Max | 5.173 | 4.205 | 1.387 | 4.455 | 0.311 | 0.707 | 0.778 | 0.625 | |
| Min | 2.165 | 2.171 | -0.006 | 2.169 | -0.003 | 0.008 | 0.143 | -0.112 | |
| met criterion | | | | | 2 | | | 2 | 2 |

## Tempo: 0.05 mg/kg Eticlopride

| Rat | HP | NP | Diff | Grand | DiffRatio | Delta | DeltaClhi | DeltaCllo | met both |
|---|---|---|---|---|---|---|---|---|---|
| AM46 | 5.072 | 3.714 | 1.359 | 4.393 | 0.309 | 0.753 | 0.819 | 0.673 | TRUE |
| AM48 | 2.197 | 2.099 | 0.098 | 2.146 | 0.045 | 0.135 | 0.317 | -0.045 | FALSE |
| AM50 | 5.099 | 4.020 | 1.079 | 4.569 | 0.236 | 0.589 | 0.682 | 0.490 | TRUE |
| Median | 5.072 | 3.714 | 1.079 | 4.393 | 0.236 | 0.589 | 0.682 | 0.490 | |
| Max | 5.099 | 4.020 | 1.359 | 4.569 | 0.309 | 0.753 | 0.819 | 0.673 | |
| Min | 2.197 | 2.099 | 0.098 | 2.146 | 0.045 | 0.135 | 0.317 | -0.045 | |
| met criterion | | | | | 2 | | | 2 | 2 |

***Tables 11 (top) and 12 (bottom):*** Response tempo during the setup phase in rats AM46, AM48, and AM50 following administration of the vehicle (top) or 0.05 mg/kg eticlopride (bottom).

# 4. Discussion

Wasserman, Gomita and Gallistel (1982) used the runway paradigm to determine whether the priming effect of rewarding electrical brain stimulation persists under the influence of moderate to very high doses of pimozide. They found that the drug failed to block the energizing effect of the priming stimulation: On the first few trials following the onset of drug action, the rats traversed the runway more quickly after having received priming stimulation prior to being placed in the start box. Wasserman et al. also showed that the directing effect of the priming stimulation persisted under the influence of the drug: Thirsty rats chose brain stimulation in preference to water following priming but preferred water in the absence of priming.

We assessed the role of dopamine D2-like receptors in the priming effect in a manner complementary to the methods used by Wasserman et al. Instead of administering pimozide, which blocks serotonin 5HT7 receptors in addition to blocking dopamine D2 receptors, we administered eticlopride, a more specific D2-like antagonist (Hall et al., 1985; Martelle & Nader, 2008). We used the operant-conditioning chamber paradigm introduced in Experiment 2 to assess the priming effect in lieu of the runway paradigm. The drug doses employed were sufficiently low to allow the rats to complete multiple test sessions. Within each session, blocks of high-priming and no-priming trials alternated, thus providing multiple measurements of the priming effect. Our results are entirely consistent with the findings of Wasserman et al.

All three rats tested at the 0.1 mg/kg dose of eticlopride and all three rats tested at the 0.05 mg/kg dose showed reliable and meaningful priming effects under the influence of the drug on two of the three measures of vigor; two rats in each dose group also showed reliable and meaningful priming effects

under the influence of the drug on the third measure of vigor. In five of six rats, the robust grand mean of the initial-speed and reward-rate measures was lower under the influence of the drug than in the vehicle condition (Tables 10-13, 16-19; see also Figures 10-11, 13-14, S16-S17, S19-20, S22-S23, S25-S26). This shows that both doses of the drug were behaviorally effective but nonetheless failed to block the priming effect.

Below, we discuss the implications of these findings for contemporary models of brain reward circuitry and the role of dopaminergic neurotransmission within them.

## 4.1 Neural circuitry underlying priming and reward

The account of intracranial self-stimulation that Deutsch derived from his theory of reward and reinforcement (Deutsch, 1960; Deutsch & Howarth, 1963) predicts that two different neural systems are directly activated by rewarding brain stimulation, one responsible for priming and the second for reward. Subsequent psychophysical measurements of strength-duration functions (Matthews, 1977) and recovery from refractoriness (Gallistel, Shizgal & Yeomans, 1981) fail to provide support for that hypothesis. The simplest explanation of this failure is that the very same directly activated neurons give rise to both the priming and reward effects.

Sax and Gallistel (1991) used psychophysical methods to infer temporal integration characteristics of the neural substrates for priming and reward. These characteristics (e.g., reciprocity between the current and pulse frequency required to produce an effect of a given strength) almost certainly arise in postsynaptic targets of the directly stimulated substrate. They found that the resulting trade-off functions differ only in scale and not in shape. The similarity in shape is consistent with a common neural substrate for priming and reward encompassing at least the first two stages of the

circuitry. Sax and Gallistel interpreted the scale difference in terms of the hypothesis that it is "the experience of several recent large rewards that excites the transient motivational state called the priming effect of the stimulation, the state that makes the animal avidly pursue more brain stimulation." Stated otherwise, the quantity of stimulation required to produce a behaviorally measurable priming effect exceeds the quantity required to produce a behaviorally measurable reward effect.

If common neural circuitry subserves both the priming and rewarding effects, how is one to explain the fact that the priming effect is transient and the reward effect is enduring? The neural mechanisms underlying the two effects must ultimately diverge. Sax and Gallistel's account places this divergence beyond the mono-synaptically activated stage. They propose that after the divergence, the branch subserving the reward effect translates the transient stimulation-induced activation into an engram, whereas the branch subserving the priming effect provides a potentiating input to the neural circuitry subserving the goal-seeking actions. The results of Experiment 3 and those of Wasserman et al. (1982) bear directly on the question of where, in the diverging pathway, the midbrain dopamine neurons intervene.

## 4.2 The role of dopaminergic neurotransmission

Extensive psychophysical data argue that the directly activated stage of the circuitry responsible for self-stimulation of the MFB consists largely of neurons with myelinated, non-dopaminergic axons that are continuous between the lateral hypothalamus and ventral tegmental area (Bielajew et al., 1982; C. Gallistel et al., 1981; Shizgal et al., 1980). The behaviorally consequential direction of conduction in at least some of these fibers is rostro-caudal (Bielajew & Shizgal, 1986). The reason that these non-dopaminergic, myelinated axons predominate in the directly stimulated stage is that they are much

more highly excitable to extracellular currents than the fine, unmyelinated, dopaminergic axons interspersed among them (Anderson et al., 1996; Yeomans et al., 1988).

To reconcile the extensive data implicating dopaminergic neurotransmission in MFB self-stimulation with the evidence that the directly activated neurons are at least largely non-dopaminergic, a “series-circuit” model was proposed (Bielajew & Shizgal, 1986; Shizgal et al., 1980; Wise, 1980). On that view, the midbrain dopamine neurons relay the activation of the directly stimulated fibers to the later stages of the neural circuitry, the stages that subserve the reward-seeking actions and engram formation. The combination of the series-circuit model and the evidence suggesting that both priming and reward arise from excitation of the same directly stimulated neurons thus predict that blockade of dopaminergic neurotransmission will block both the priming and reward effects. However, Wasserman et al. (1982) showed that this is not the case: The priming effect survives blockade of dopamine D2-like receptors by pimozide. We show in Experiment 3 that this is also the case with the more specific action of eticlopride on these receptors. An alternative to the series-circuit model is required to explain these findings. The convergence model proposed by Trujillo-Pisanty and Shizgal (2020) fits the bill.

The convergence model holds that signals arising in the directly activated myelinated fibers composing the MFB substrate reach the final common path for the rewarding effect in parallel to signals arising from activation of midbrain dopamine neurons. That view is readily combined with the hypothesis that the initial stages of the neural circuitry subserving the priming and rewarding effects are the same (Sax & Gallistel, 1991) and with the data demonstrating the resistance of the priming effect to perturbation of dopaminergic neurotransmission. The key concept that enables this combination is the bifurcation Sax and Gallistel propose in the causal pathways for the priming and rewarding effects. They

argue that this bifurcation occurs beyond both the directly stimulated stage and the subsequent stage(s) underlying spatio-temporal integration. Prior to the bifurcation point, the electrically induced activity has been integrated to yield a unidimensional signal. Beyond the bifurcation, one branch accumulates the unidimensional signals produced by multiple stimulation trains, provided that they are separated by 1-2 s and provided that their strength exceeds the strength required to maximize behavioral performance (Sax & Gallistel, 1991). Following the termination of the series of trains, the accumulated excitation decays over tens of seconds. That is the excitation that is expressed as the priming effect. The second branch generates the rewarding effect under dopaminergic modulation, translating the transient stimulation-induced activation into an enduring entry into multiple mnemonic representations, thus capturing, at the very least, the magnitude of the activation as well as the time and place at which it was triggered (C. R. Gallistel, 2020).

Work by Shizgal and colleagues (Hernandez et al., 2010, 2012; Trujillo-Pisanty et al., 2014) constrains the manner in which the dopaminergic modulation may occur. The option that meshes most cleanly with the Sax and Gallistel hypothesis is that dopaminergic drive scales the reward-intensity value encoded in memory. On that view, this is why the rats that had medium-high doses of pimozide ceased to run down the alley in the Wasserman et al. (1982) study and why three of the rats in the present study ceased to work in the operant-chamber priming paradigm on most sessions following administration of the higher dose of eticlopride. Under the influence of the lower dose of eticlopride in the present study or a low dose of pimozide (0.1 mg/kg) in the Trujillo-Pisanty et al. study (2014), performance could be sustained, but the latter study shows that the cost of the reward had to be reduced to compensate for the effect of the drug.

## 4.3 Priming and incentive motivation

The priming effect of rewarding electrical brain stimulation is a special case of incentive motivation (Bindra, 1968, 1969), one in which the motivation-enhancing effect of exposure to a recent reward arises from direct stimulation of central neurons. The results of this experiment, coupled with the prior findings of Wasserman et al. (1982) show that this effect is resistant to blockade of dopamine D2-like receptors. That finding generalizes beyond the effects of MFB stimulation. We have shown that the priming effect produced by consumption of highly palatable food also resists blockade of dopamine D2-like receptors as well as blockade of dopamine D1-like receptors (Evangelista et al., 2019). How are such findings to be reconciled with the abundant data implicating dopaminergic neurotransmission in incentive motivation (e.g., (Berridge & Robinson, 2003; Robinson & Berridge, 1993))? The key is to keep firmly in mind the convergent causation of so many behavioral phenomena and to resist the temptation to assume covertly that the neural substrate for any particular causal factor is the final common path for all the others.

The convergence model of brain stimulation reward in no way disputes the ability of dopaminergic activation to produce powerfully rewarding effects. Instead, it merely asserts that there are other means of generating these effects that operate in parallel to dopaminergic neurons. Similarly, we argue here that non-dopaminergic neurons can produce incentive-motivational effects in parallel to the circuitry underlying those generated by dopaminergic activation. On that view, the final common path for incentive motivational effects lies beyond the point of convergence between inputs that relay signals of dopaminergic and non-dopaminergic origin. We know a great deal about the dopaminergic inputs. The results reported here and in prior work on the priming effects of rewarding electrical brain

stimulation and palatable food argue that it would be worthwhile to learn much more about the significance, function, and neural instantiation of the non-dopaminergic inputs.

## 4.4 Future directions

One question that begs to be addressed using the operant-chamber paradigm is whether the priming effect of rewarding electrical brain stimulation resists blockade of dopamine D1-like receptors. We found that the priming effect of palatable food was intact following blockade of bothe D1-like and D2-like receptors. Is this also the case for the priming effect of rewarding brain stimulation? Another question of interest is whether a priming effect is generated by direct, specific, optical activation of midbrain dopamine neurons. That question addresses the hypothesis that dopaminergic and non-dopaminergic branches of brain reward circuitry posited by the convergence model (Trujillo-Pisanty et al., 2020) also provide converging input to the circuitry responsible for the transient incentive-motivational aftereffect.

It has been several decades since the priming effects of rewards have been studied in earnest. Given the explosive development of powerful neuroscientific methods for tracing the projections of neurochemically characterized neurons and for linking the activation of these neurons to behaviors and psychological processes of interest, we argue that it is high time to take up this work again.

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