Incentive Salience, not Psychomotor Sensitization or Tolerance, Drives Escalation of Cocaine Self-Administration in Heterogeneous Stock Rats

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Abstract:

Sensitization and tolerance are two phenomena often studied independently despite overlapping neurobiological substrates. Each has extensive research showing their influence on the development and maintenance of addiction, but the degree to which they drive escalation in cocaine self-administration is poorly understood. Using self-administration, intravenous noncontingent infusions, and pose-estimation machine vision, we find that incentive salience, not psychomotor sensitization or tolerance, drives the escalation of cocaine self-administration in heterogenous stock rats. Individual differences in psychomotor sensitization or tolerance were found to have no effect on cocaine intake. Incentive salience as measured by locomotion and active lever entrances per meter traveled occurring before the self-administration session began (pre-lever activity measures) during Short Access (2-hours) was found to predict intake during Long Access (6-hours). Both pre-lever locomotion and active lever entrances per meter were found to increase during Long Access and after two-to-three days of abstinence. Critically, rats with low pre-lever activity during Short Access escalated both their intake and pre-lever measures by the end of Long Access to levels comparable with high pre-lever activity rats who maintained their elevated responding. These findings support the notion that incentive salience during Short Access is a catalyst to escalated use and an early marker of addiction vulnerability. Moreover, they suggest that individuals initially resistant to incentive salience can, with sufficient exposure, become sensitized and escalate cocaine use to the same level as more susceptible individuals. Analysis of pre-lever activity offers a novel longitudinal behavioral marker to

predict vulnerability and provides a framework for understanding individual trajectories of addiction.

1. Introduction

Addiction is a chronic, relapsing disorder characterized by cycles of binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation, leading to long-lasting neuroadaptations in stress, executive function, and reward processing [1,2]. Within this framework, two of the most studied adaptations to repeated drug exposure are tolerance, a reduced response to the drug, and sensitization, an enhanced response. Both processes have been demonstrated in preclinical models of addiction, but the expression of either phenomenon varies widely depending on experimental parameters such as drug of choice, method of exposure (e.g., noncontingent injections versus self-administration), schedule of access (e.g. Short Access, Long Access, Intermittent, etc.), and outcome variables (e.g. locomotion, physiological measures, and operant behavior) [3-13]. Despite evidence that tolerance and sensitization share overlapping neurobiological substrates, they are often investigated in isolation [14-17], leading to two competing models in the transition to addiction.

According to the hedonic homeostatic dysregulation theory, 1) repeated drug use leads to tolerance to the positive reinforcing effects of drugs, and 2) the allostatic recruitment of stress circuitry, thereby shifting the hedonic set point and motivating continued use to restore homeostasis [1,18]. In contrast, the incentive-sensitization theory proposes that 1) repeated use hypersensitizes neural circuits leading to *incentive salience*—drug-related cues become increasingly "wanting" even as the “liking" diminishes—and 2) *behavioral sensitization*—a heightened psychomotor response observed primarily in psychostimulants like amphetamines and cocaine [19]. Many agree that both phenomena contribute to the addiction cycle; however, the degree to

which sensitization and tolerance drive escalation of cocaine self-administration is poorly understood. While a number of studies have demonstrated the expression of tolerance or sensitization to cocaine with noncontingent methods of continuous, repeated, or single infusions [7,9,20-23], or the use of different lengths of self-administration access [3-6,24-26], none have in a longitudinal paradigm employing noncontingent challenges, Short Access, and Long Access.

To address this gap, using an operant cocaine self-administration paradigm in heterogeneous stock rats in conjunction with machine vision pose-estimation, we assessed multiple behavioral indices to differentiate individual development of tolerance or sensitization across time and conditions and the subsequent influence on cocaine self-administration. These included: (1) locomotion following a single noncontingent intravenous infusion of cocaine at three time points: before Short Access, after Short Access 09, and after Long Access 14 (see **Fig. 1A** for timeline); (2) locomotion for 15 minutes before the self-administration session begins (pre-lever); (3) drug-seeking during pre-lever and Noncontingent sessions, measured as nose entrances into the active lever area per meter traveled; (4) cocaine intake within the first 15 minutes and (5) across the full session; and (6) breakpoint on a progressive ratio schedule. Tolerance and sensitization will be assessed by the measures of psychomotor locomotion during Noncontingent sessions. Incentive salience will be assessed by both locomotion and nose entrances into the active lever area per meter during the pre-lever phase of self-administration.

2. Materials and Methods

A more detailed account of procedures in sections 2.1-2.3 below has been published elsewhere [27-28]. ChatGPT4 was utilized for coding assistance in RStudio and python, authors take full responsibility for output.

2.1. Subjects

Heterogeneous stock rats (N=37; n=21 males; n=16 females) were used to mimic the genetic diversity seen in the human population. Rats were obtained from the from the University of California, San Diego colony [29] and housed in pairs on a 12-hour light/dark cycle. Rats were anesthetized with 1–5% isoflurane for jugular vein catheterization using Micro-Renathane tubing (Braintree Scientific) connected to a guide cannula that exits through the back and secured with mesh and dental acrylic. Post-surgery, rats received flunixin subcutaneously (2.5 mg/kg) and cefazolin intramuscularly (330 mg/kg). Catheters were flushed daily with heparinized saline (10 U/ml, American Pharmaceutical Partners) mixed with 0.9% bacteriostatic sodium chloride (Hospira) and cefazolin (52.4 mg/0.2 ml). Catheter patency was tested intravenously with brevital sodium (1.5 mg, American Pharmaceutical Partners) at experiment start and end, with failing rats removed from the experiment. Rats analyzed demonstrated catheter patency. All procedures were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees of UC San Diego.

2.2. Drugs

111 Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in
112 0.9% sterile saline. During self-administration sessions, a press of the active lever
113 resulted in an intravenous infusion of 0.1ml at 0.5 mg/kg over six seconds by pump,
114 followed by a 20-second timeout where the active lever has no programmed effect.
115 During Noncontingent sessions, a single intravenous infusion of 0.3ml at 1.5 mg/kg (i.e.
116 three times the dose received in self-administration) was delivered in under five
117 seconds in the same operant chamber as self-administration. Intravenous injections
118 were chosen to address the differences in absorption, clearance, and blood
119 concentrations between intravenous and intraperitoneal methods [30-32]. Furthermore,
120 administration within the same chamber and under five seconds were implemented to
121 address influences of environment and intravenous infusion rate on the development of
122 sensitization [33-36].

123

124 **2.3. Intravenous Self-Administration**

125 Over 8 weeks (Monday-Friday), rats participated in 30 self-administration
126 sessions with various lengths and ratios. Under fixed ratio one, rats participated in 10
127 two-hour Short Access sessions,15 six-hour Long Access sessions, and one Preshock
128 and one Shock session, each for one-hour. During Shock, each infusion had a 30%
129 chance to be paired with a footshock (0.3 mA for 0.5 sec) through the grid floor. Under
130 progressive ratio, rats participated in three six-hour sessions in which the response
131 requirements for an infusion followed the equation [5e(injection number × 0.2)] – 5. The
132 breakpoint was defined as the last ratio before a 60-minute period in which a ratio was

not completed, in which case the session ended. A complete experimental timeline is shown in **Fig. 1A**.

2.4. Noncontingent Sessions

Seven days after surgeries, rats underwent the first noncontingent session (Noncontingent 01), which occurred before Short Access 01. Rats were placed in the operant chamber and video recorded for 30 minutes to establish baseline measurements (Baseline 01). Rats were then given an intravenous saline injection, placed back in the chamber, and recorded for 30 minutes (Saline 01), followed by a noncontingent intravenous injection of cocaine (1.5 mg/kg) and recorded for 30 minutes (Drug 01). The same procedures were replicated for the subsequent noncontingent sessions following Short Access 09 (Noncontingent 02) and Long Access 14 (Noncontingent 03).

2.5. Video Recording and Processing

2.5.1. *Raspberry Pi Video Recording*

Video recordings of sessions were conducted as described previously [37] with a few alterations. A static FFmpeg build (ffmpeg-6.0-armhf-static; https://johnvansickle.com/ffmpeg/) was installed onto each Raspberry Pi for video processing to reduce video size threefold without sacrifice to video quality. All sessions were recorded for 30 minutes. During self-administration sessions, levers were extended in the chamber after 15 minutes of recording. Each comma-separated value

file (csv) file was processed in RStudio (R version 4.3.1 (2023-06-16 ucrt)) to ensure consistent length and alignment to the lever extension, resulting in ~25 minutes per csv, or ~12.5 minutes for both pre- and post-lever data. Out of a possible 1,110 csv files (37 rats over 30 sessions), 1,084 are present in the study due to exclusions for various issues such as catheter or infrared light disconnections. For eight rats during Long Access 04 the Med Associates program was initiated too early, resulting in no pre-lever data. For Noncontingent sessions, R processing resulted in ~25 minutes per csv. Out of a possible 333 csv files, 327 are present in the data, as six failed to track during Social LEAP Estimates Animal Poses (SLEAP) processing.

2.5.2. Social LEAP Estimates Animal Poses (SLEAP) Modeling

A pose-estimation model was trained using SLEAP on ~5200 labeled frames over 89 videos from past cohorts utilizing the top-down configuration and a unet backbone [38]. Thirteen nodes were tracked: nose, both ears, neck, four paws, base (where tail met the body), active and inactive levers, active lever cue light, and the catheter node, which was chosen as the model anchor point. A second model was trained to track the first Noncontingent session, where the rats were mistakenly not plugged into their catheter lines. This model focused on only six nodes to accelerate training: nose, both ears, neck, base, and the catheter as the anchor node, utilizing ~2200 labeled frames from 40 videos.

2.5.3. *High Throughput Computing on Open Science Grid (OSG)*

Video recordings were processed on the OSG Consortium supercomputer system [39-42]. A python script with SLEAP command line interface functions processed each video to identify one instance (track) using an “instance” identification method and Hungarian approach between frames. Results of each node for each frame per second inference registered as (*x*, *y*) coordinates were followed by an interpolation method to fill missing data points (https://sleap.ai/notebooks/Analysis_examples.html).

2.6. Dependent variable calculation

Calculations were conducted in R version 4.3.1 using RStudio [43-44]. Euclidian distances were used to calculate movement of the catheter (locomotion) and the nose (nose motion) nodes frame by frame and converted from pixels to centimeters using Image2 [45] and the known measurements of the ENV-005 grid. Given the barrel distortion of a fisheye lens, the conversion factor was computed by measuring the grid floor at five separate segments and averaging the two length measurements (left and right), averaging three width measurements (top, middle, and bottom), and taking the average of the two, resulting in ~0.111cm/px. Locomotion variables are the sum of the distances traveled and then smoothed by a window of 10 (10 FPS, i.e., 0.33 seconds), then converted to meters. Locomotion (m) pre-lever corresponds to the locomotion measured for the 15 minutes before the levers are extended into the chamber. Active and inactive lever entrances were calculated by producing a bounding box from the position of the (*x*,*y*) nodes, 30 +/- *x* and 18 +/- *y*, and counting each entrance of the nose node into the lever areas. Active and inactive lever entrances per meter are the total lever entrances normalized by locomotion. Cocaine infusions are the total infusions

delivered during the session, and cocaine infusions first 15 minutes are those that occurred during the video recordings. Session number corresponds to an individual session (e.g. Short Access 01). Session type refers to experimental phase (e.g. Long Access). Abstinence refers to the time between the end of one session and the beginning of the next, where 18 Hours translates to consecutive days, 66 Hours to a two-day weekend, and 90 Hours to a three-day weekend. Pre-lever variables were examined by abstinence to test for effects that could facilitate sensitization [6,14-17,20,33-35,46-47]. Analyses with sex as a factor were performed, but most effects were not sex dependent and therefore are reported in the Supplementary Material.

2.7. Statistical Analyses

Statistical comparisons were made by fitting a linear mixed effects model utilizing the lmerTest package [48-49] or a generalized linear mixed effects model utilizing the glmmTMB package [50] in R. Given that linear and generalized linear mixed-effects models are inherently robust to moderate deviations from normality and extreme values, outliers for all variables were winsorized at the 95th percentile to minimize their influence while retaining potentially meaningful behavioral variability. Inferential tests of fixed effects for linear mixed models were conducted using Type III ANOVA with Satterthwaite degrees of freedom approximations (Type III ANOVA test) which have been shown to control for Type I errors in hierarchical models [51], while for generalized linear models a Type III Wald chi-square analysis of deviance was conducted (Type III Wald test). Tests for model assumptions and fit were analyzed with the simulateResdiduals() function from the DHARMa package [52]. The model fitting

process and chosen models of each analysis can be found in the Supplementary Material with best adherence to suggestions previously published [53] but prioritizing the results of DHARMa tests. Transformations of response variables where necessary (see Supplemental Material) were achieved using Box-Cox or Yeo-Johnson after identifying the most effective lambda using powerTransform() from the car package and reported at two decimal places [54]. Post hoc analyses of all significance results were conducted using contrasts(), while direct comparisons to baseline used test(), both within the emmeans package and calculate the pairwise comparisons of the estimated marginal means of mixed effects models and are therefore model-derived ratios or mean differences [55]. All *p*-values were adjusted by the Benjamini-Yekutieli (BY) method due to its consideration of dependence and correlations in nested data such as mixed models [56]. From the rstatix package, Student's t-tests were conducted with t_test() with Bonferroni adjustments and correlations with cor_test() by either Pearson or Spearman Rank as determined by a normality test with shapiro_test() [57]. Plots represent the raw average values or percent difference from baseline.

3. Results

3.1. Cocaine Infusions

3.1.1. Total cocaine infusions escalate during both Short and Long Access

To determine whether cocaine intake changed during Short Access, a Type III Wald test was performed. A significant main effect of session number was observed ($\chi^2(9)=100.94$, $p<.001$). Post hoc analyses revealed all sessions to have significantly

more cocaine infusions in comparison to Short Access 01. These results confirm that cocaine intake increased as early as Short Access 02 (**Fig. 1B**).

To determine whether cocaine intake changed during Long Access, a Type III Wald test was performed. A significant main effect of session number was identified ($\chi^2(13)=173.01$, $p<.001$). Post hoc comparisons indicated that Long Access 09 through Long Access 14 had significantly more infusions relative to Long Access 01. These results show that cocaine intake continued to escalate into the third week of Long Access (**Fig. 1B**).

3.1.2. Cocaine infusions during the first 15 minutes escalate across sessions

To evaluate whether cocaine infusions during the initial 15 minutes of each session escalated, a Type III Wald test was conducted. A significant main effect of session number was identified ($\chi^2(23)=304.87$, $p<.001$). Post hoc analyses revealed that cocaine infusions were significantly greater from Short Access 07 through Long Access 14 compared to Short Access 01. Additionally, when compared to Long Access 01, only sessions Long Access 09, Long Access 13, and Long Access 14 showed significantly elevated infusions (**Fig. 1C**). These results indicate that escalation of cocaine intake during the first 15 minutes emerged during the later stages of the Short Access period and continued throughout Long Access, particularly after a period of abstinence.

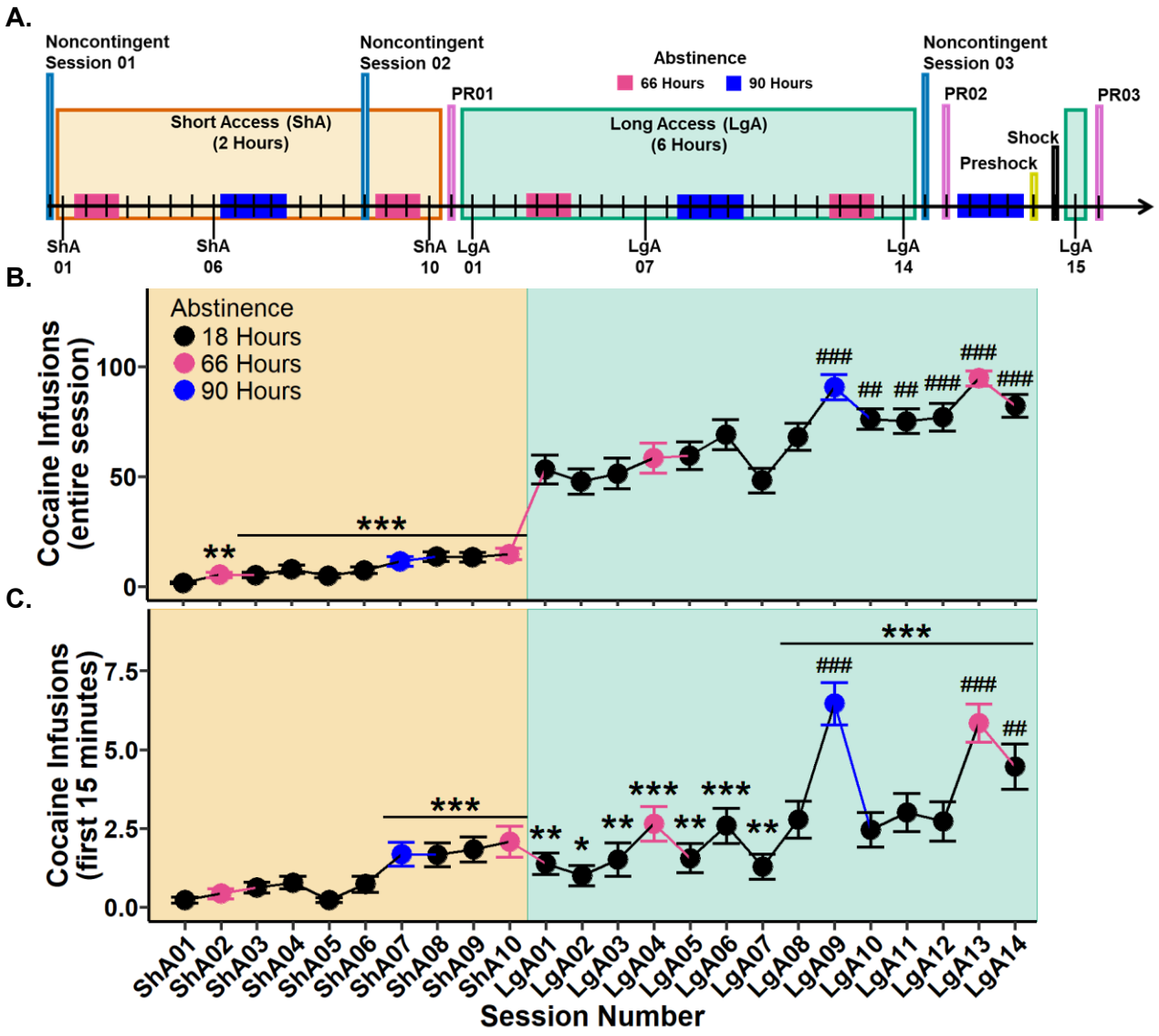


Figure 1. Experimental timeline and cocaine infusions during Short (ShA) and Long Access (LgA). (A.) Experimental timeline with 66 hours (pink) and 90 hours (blue) indicating instances of abstinence. **(B.)** Cocaine infusions during the entire session. **(C.)** Cocaine infusions during the first 15 minutes of the session. Plots represent means and SEM. *p<0.05, **p<0.01, ***p<.001 vs. Short Access 01; ##p<.01, ##p<.001 vs. Long Access 01.

3.2. Locomotion During Noncontingent Sessions

3.2.1. Locomotion increases after noncontingent cocaine administration with no evidence of psychomotor sensitization or tolerance after Short or Long Access to cocaine self-administration.

To determine if raw locomotion differs between Noncontingent sessions following a noncontingent intravenous infusion of cocaine, a Type III ANOVA test was performed. A significant main effect of session number was found ($F(8, 290.88)=17.38$, $p<.001$). Post hoc analyses only found significant differences within each respective Noncontingent session. Specifically, Drug sessions had significantly greater locomotion than their respective Baseline and Saline sessions (**Fig. 2A**).

To assess the change in the locomotor response to noncontingent cocaine administration, data was normalized to each rat's baseline and analyzed as percent difference from the respective Baseline sessions. A Type III ANOVA revealed a significant main effect of session number ($F(5, 176.97)=16.36$, $p<.001$). Post hoc analyses revealed similar significant differences, where Drug sessions were significantly greater than their respective Saline and Baseline sessions, but no significant differences between Drug sessions (**Fig. 2B**). However, Saline 02 was significantly greater than Baseline 02, potentially an anticipatory conditioned stimulus effect as the last time they were handled as such, they received a noncontingent infusion of cocaine (Drug 01). The fact that this effect is not seen following Long Access suggests that the conditioned stimulus effect of picking up the animals and administering saline has disappeared, perhaps because at this stage (Long Access 14+) the animals have been handled extensively (~4 weeks) and therefore the act of being picked up from the operant

chamber is not associated anymore with cocaine. These results show that locomotion did not increase in successive Noncontingent sessions following a noncontingent intravenous infusion of cocaine at the population level.

3.2.2. Rats differentially develop psychomotor sensitization or tolerance after a history of cocaine self-administration

To assess each individual rat's response to successive noncontingent infusions, rats were grouped by their locomotion difference from Drug 01 to Drug 03. Rats who *increased* were labeled "Sensitization" and rats who *decreased* were labeled as "Tolerance". The locomotion in each Drug session was then analyzed by a percentage change from Baseline (Drug 01). Following a 0.13 lambda Box-Cox power transformation, a Type III Wald test found no significant main effect of behavioral expression ($\chi^2(1)=4.8$, $p=.059$), but a significant effect of session number ($\chi^2(1)=6.47$, $p=.031$) and their interaction ($\chi^2(1)=15.2$, $p<.001$). Post hoc analyses revealed rats in the Tolerance group to have significantly decreased in their locomotion as early as Drug 02, with a further decrease in Drug 03. Similarly, although not significantly increased from baseline, rats in the Sensitization group showed greater locomotion in Drug 02 than the Tolerance group that progressed further in Drug 03. These results demonstrate markedly divergent profiles in the psychomotor response to cocaine that begin as early as the first nine Short Access sessions and continue through Long Access (**Fig. 2C**). The effect observed was not due to stereotypies in Tolerant rats (**Fig. S1**).

3.2.3. *Psychomotor sensitization and tolerance show no effect on cocaine self-administration*

To test whether the development of psychomotor sensitization or tolerance had an effect on cocaine self-administration over the entire session, a Type III Wald test was performed for both Short and Long Access. During Short Access, a main effect of session number was found ($\chi^2(9)=64.36$, $p<.001$), but not of behavioral expression ($\chi^2(1)=0.98$, $p=.967$), or their interaction ($\chi^2(9)=8.72$, $p=.967$. Similarly, in Long Access, a main effect of session number was found ($\chi^2(13)=107.44$, $p<.001$), but not of behavioral expression ($\chi^2(1)=0.47$, $p=1$), or their interaction ($\chi^2(13)=11.54$, $p=1$). These results suggest that the development of psychomotor sensitization or tolerance to the effect of cocaine has no effect on cocaine intake during Short or Long Access (**Fig. 2D**).

To determine if the development of psychomotor sensitization or tolerance has an effect on cocaine infusions in the first 15 minutes, a Type III Wald test was performed. A main effect of session number was found ($\chi^2(23)=154.13$, $p<.001$), but not of behavioral expression ($\chi^2(1)=0.003$, $p=1$), or their interaction ($\chi^2(23)=30.78$, $p=.356$). These results confirm that the development of psychomotor sensitization or tolerance to the effect of cocaine has no effect on the loading behavior during cocaine self-administration (**Fig. 2E**).

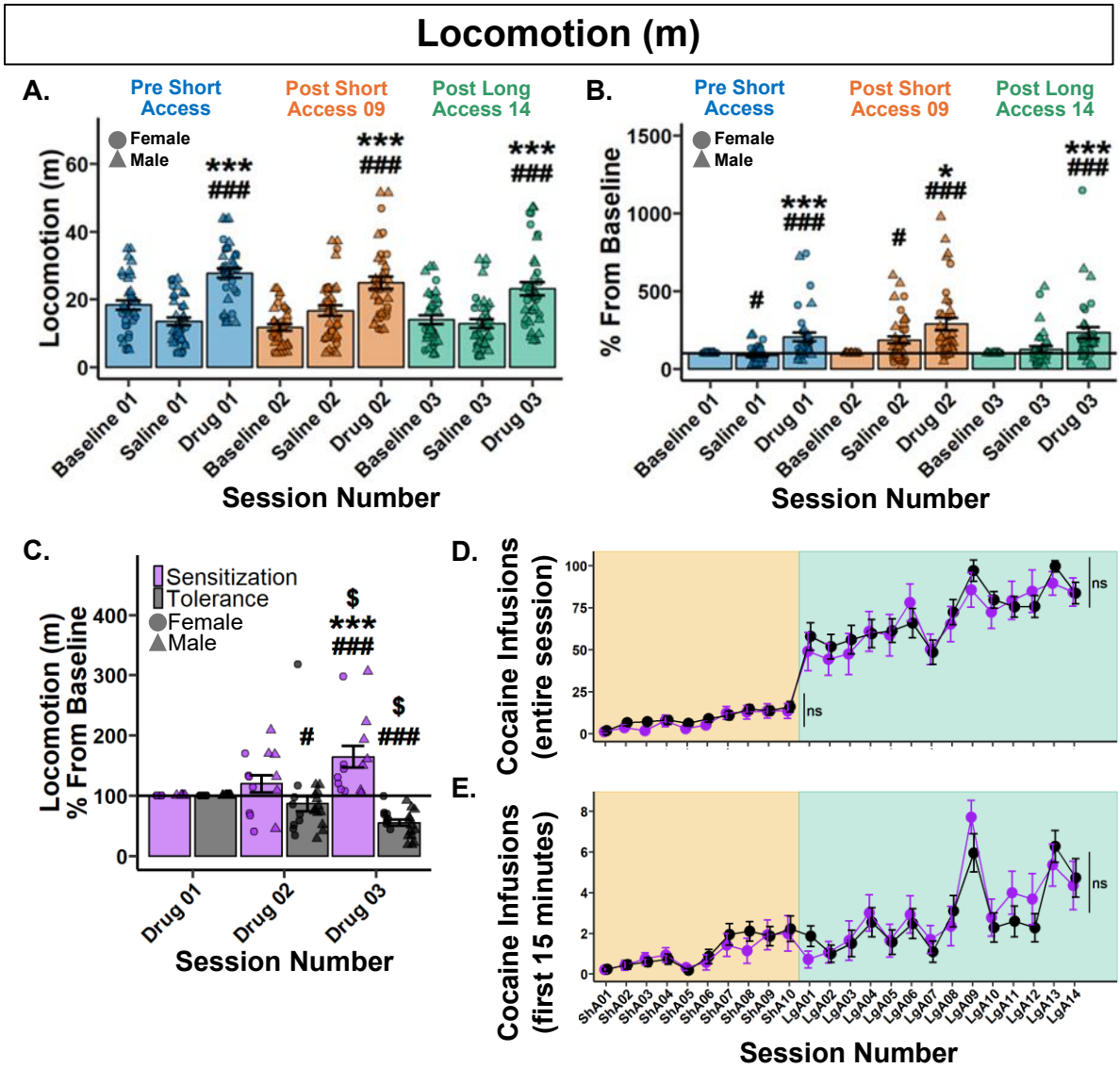


Figure 2. Psychomotor sensitization and tolerance show no effect on cocaine self-administration. (A.) Raw locomotion (m) across Noncontingent sessions. ###*p*<.001 vs Baseline; ***p<.001 vs Saline. **(B.)** Locomotion (m) percent difference from Baseline. #*p*<.05, ##*p*<.01, ###*p*<.001 vs Baseline; *p<.05, ***p<.001 vs Saline. **(C.)** Locomotion (m) percentage difference from Baseline (Drug 01) grouped by behavioral expression. Drug 01 < Drug 03, Sensitization (n = 14); Drug 01 ≥ Drug 03, Tolerance (n = 22). #*p*<.05, ###*p*<.001 vs Baseline; Within: $*p*<.05 vs Drug 02;

Between: ***p<.001 vs Tolerance Drug 03. **(D-E.)** Cocaine infusions over the entire session (D.) and within the first 15 minutes (E.) grouped by behavioral expression. Plots represent means and SEM.

3.3. **Pre-Lever Activity**

3.3.1. Locomotion pre-lever increases from Short to Long Access and predicts cocaine intake during the first 15 minutes of self-administration

To determine if locomotion pre-lever increased over the experiment, a Type III Wald test was performed. A significant main effect of session number was found following a 0.29 lambda Box-Cox power transformation ($\chi^2(23)=204.7$, $p<.001$). Post hoc analyses revealed significant increases at Short Access 07, Short Access 08, Long Access 09, and Long Access 13 in comparison with Short Access 01 (**Fig. 3A**). These results indicate that locomotion pre-lever increased from Short to Long Access.

To assess the influence of session type and abstinence on locomotion pre-lever, a separate analysis was conducted following a 0.28 lambda Box-Cox transformation. A Type III Wald test found significant main effects of session type ($\chi^2(1)=11.93$, $p=.002$), abstinence ($\chi^2(2)=14$, $p=.002$), and their interaction ($\chi^2(2)=27.57$, $p<.001$). Within Short Access, post hoc analyses revealed locomotion pre-lever at 90 hours was significantly greater than 18 hours. Within Long Access, 66 and 90 hours were found to be significantly greater than 18 hours. Interactively, at 18 hours there was a significant decrease in locomotion pre-lever from Short to Long Access. At 66 hours, there was a significant increase from Short to Long Access. Overall, locomotion pre-lever was

366 significantly greater in Long Access than in Short Access across abstinence (**Fig. 3B**).
367 These results show that locomotion pre-lever increases with drug history and hours of
368 abstinence.

369 To assess locomotion pre-lever predictability of cocaine intake in the first 15
370 minutes of the session and break point, correlation analyses were performed. Between
371 locomotion pre-lever and cocaine infusions during the first 15 minutes, a positive
372 correlation was found during Short Access ($r_s(4222)=.5$, $p=.002$) (**Fig. 3C**), and Long
373 Access ($r(4.29)=.59$, $p<.001$, 95% CI [0.33, 0.77]) (**Fig. 3E**), but not during Shock (r-
374 $_s(6952)=.18$, $p=.298$) (**Fig. 3G**). Between locomotion pre-lever and breaking point, no
375 correlations were found in both Progressive Ratio 01 ($r_s(7497)=.04$, $p=.84$) or
376 Progressive Ratio 02 ($r_s(6689)=.21$, $p=.219$) (**Fig. 3D & 3F**). These results indicate that
377 locomotion pre-lever is predictive of cocaine intake during the first 15 minutes in Short
378 and Long Access, but is not predictive of the motivation to seek cocaine as measured
379 by breaking point.

380

381 *3.3.2. Active lever entrances per meter pre-lever escalate from Short to Long Access*
382 *and predict cocaine intake during the first 15 minutes of self-administration*

383 Initial analyses indicated that nose entrances into the active lever area may be
384 predictive of intake, however, there were large individual variability in locomotion. To
385 account for animals with higher general activity having a greater probability of entering
386 the active lever area by chance, we normalized the number of active lever area
387 entrances to the overall locomotion in the chamber (active lever entrances per meter).

To determine if active lever entrances per meter increased over the experiment, a Type III Wald test was performed. A significant main effect of session number was found ($\chi^2(23)=234.44$, $p<.001$). Post hoc comparisons with Short Access 01 revealed significant increases in all sessions from Short Access 07 to Long Access 14, excluding Long Access 01, 03, and 07 (**Fig. 3H**). These results confirm that active lever entrances per meter increased over the experiment.

To assess the effects of session type and abstinence on active lever entrances per meter, a separate Type III Wald test was performed. No effect of abstinence was found ($\chi^2(2)=1.7$, $p=.89$), but a significant main effect of session type ($\chi^2(1)=13.66$, $p<.001$), and their interaction ($\chi^2(2)=17.47$, $p<.001$). Post hoc analyses revealed no significant differences between abstinence levels within Short Access. Within Long Access, significant increases were found at 66 and 90 hours in comparison to 18 hours. Interactively, at every level of abstinence there was found to be a significant increase from Short to Long Access (**Fig. 3I**). These results confirm that active lever entrances per meter increase with drug experience, with the strongest increases occurring after longer periods of abstinence.

To assess active lever entrances per meter predictability of cocaine intake in the first 15 minutes of the session and break point, correlation analyses were performed. Between active lever entrances per meter and cocaine infusions during the first 15 minutes, a significant positive correlation was found during Short Access ($r_s(3002)=.64$, $p<.001$) (**Fig. 3J**), Long Access ($r(4.65)=.62$, $p<.001$, 95% CI [0.37, 0.79]) (**Fig. 3L**), and Shock ($r_s(5199)=.38$, $p=.019$) (**Fig. 3N**). Between active lever entrances per meter and break point, a significant positive correlation was found during Progressive Ratio 01 (*r*-

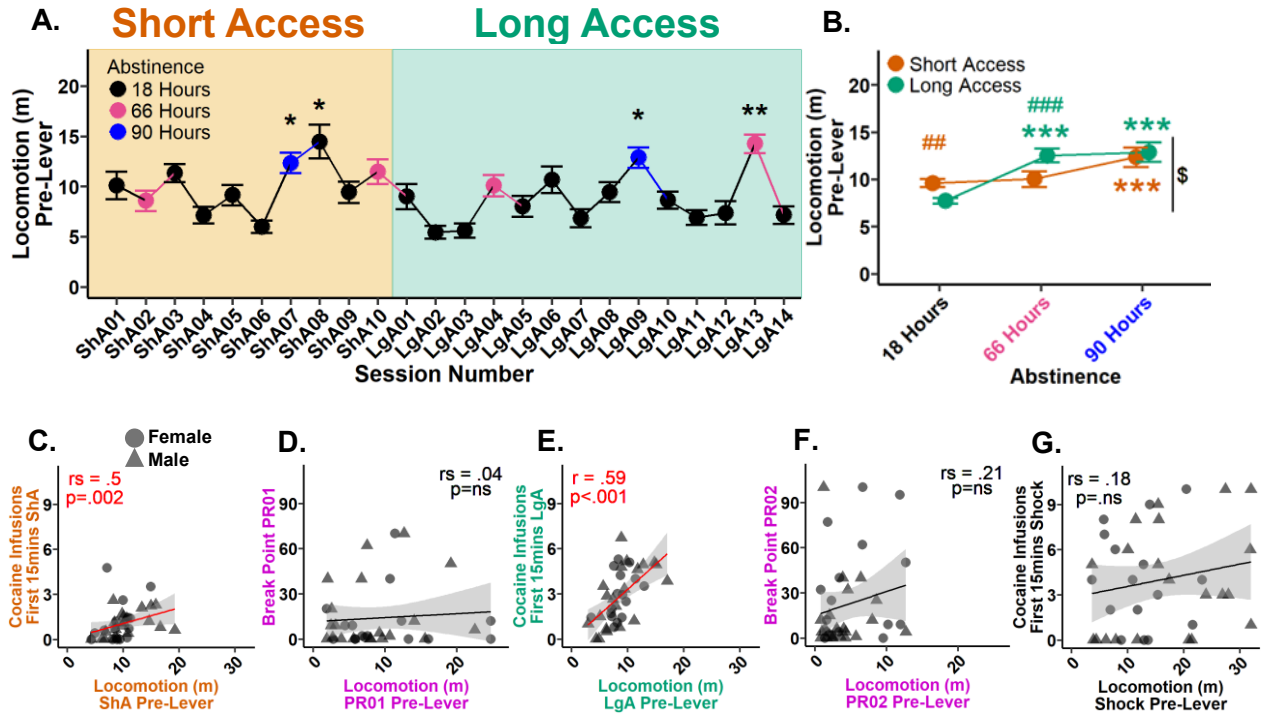
411 $_s$(5173)=.33, p=.046) (**Fig. 3K**), but not Progressive Ratio 02 (r_s(7985)=.05, p=.753)

412 (**Fig. 3M**). These results show that active lever entrances per meter pre-lever are

413 predictive of both cocaine intake within the first 15 minutes and motivation measured as

414 break point following Short Access, but not after Long Access.

Locomotion (m) Pre-Lever



Active Lever Entrances per Meter Pre-Lever

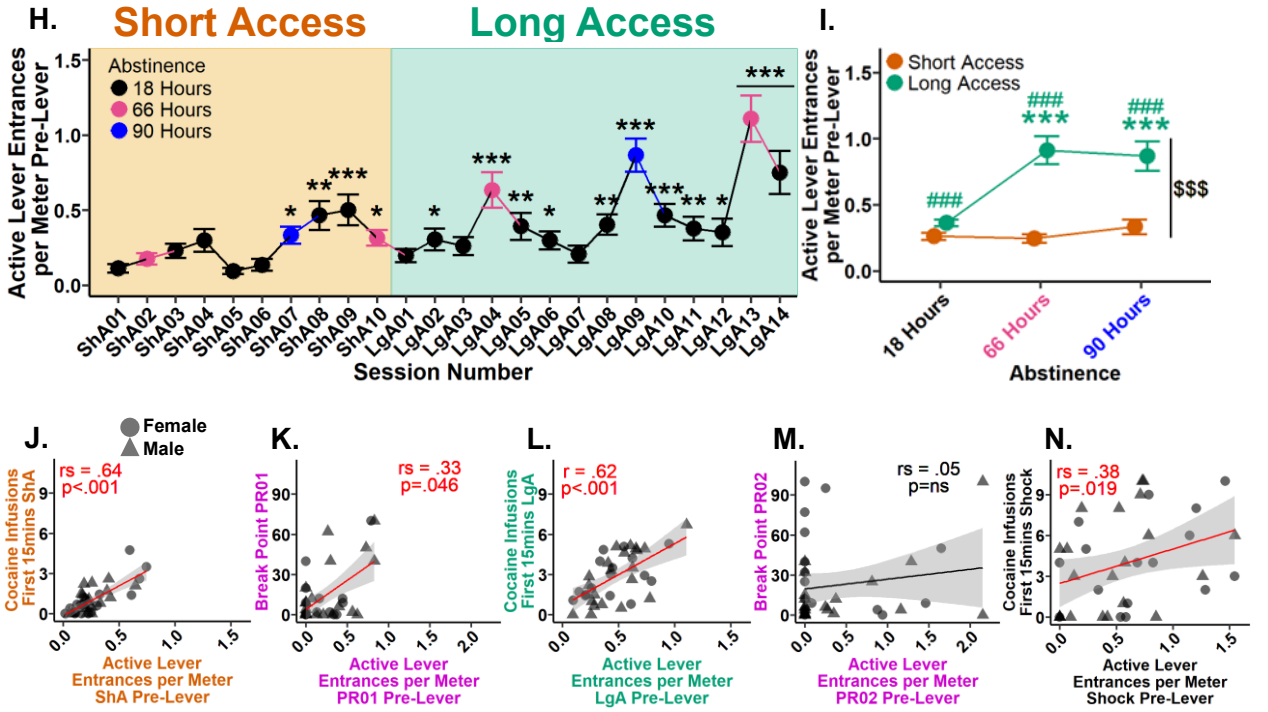


Figure 3. Pre-lever activity increases with Long Access, hours of abstinence, and is predictive of cocaine infusions within the first 15 minutes of self-administration. (A.) Locomotion pre-lever across experiment. *p<.05, **p<.01 vs Short Access 01. **(B.)** Comparison of abstinence levels within and between session types. Within session type: ***p<.001 vs 18 hours; between session type and abstinence: ##p<.01 vs 18 hours Long Access, ###p<.001 vs 66 hours Short Access; between session type: $$$$p$<.001 vs Short Access. **(C.)** Mean locomotion pre-lever during Short Access correlates with mean cocaine infusions during first 15 minutes of Short Access. **(D.)** Locomotion pre-lever during Progressive Ratio 01 does not correlate with break point. **(E.)** Mean locomotion pre-lever during Long Access correlates with mean cocaine infusions during first 15 minutes of Long Access. **(F.)** Locomotion pre-lever during Progressive Ratio 02 does not correlate with break point. **(G.)** Locomotion pre-lever during Shock does not correlate with cocaine infusions during first 15 minutes of Shock. **(H.)** Active lever entrances per meter pre-lever across experiment. *p<.05, **p<.01, ***p<.001 vs ShA01. **(I.)** Comparison of abstinence levels within and between session types. Within session type: ***p<.001 vs 18 hours; between session type and abstinence: ##p<.01 vs 18, 66, and 90 hours Short Access; between session type: $$$$p$<.001 vs Short Access. **(J.)** Mean active lever entrances per meter pre-lever during Short Access correlates with mean cocaine infusions during first 15 minutes of Short Access. **(K.)** Active lever entrances per meter pre-lever during Progressive Ratio 01 correlates with break point. **(L.)** Mean active lever entrances per meter pre-lever during Long Access correlates with mean cocaine infusions during first 15 minutes of Long Access. **(M.)** Active lever entrances per meter pre-lever during Progressive Ratio 02

does not correlate with break point. **(N.)** Active lever entrances per meter pre-lever during Shock correlates with cocaine infusions during first 15 minutes of Shock. Plots A, B, H, and I represent mean and SEM. Correlation plots E and L are by Pearson, while remaining correlations are by Spearman Rank.

3.4. Lever Entrances During Noncontingent Sessions

3.4.1. Lever entrances per meter as percent difference from Noncontingent 01 show a preference for the active lever after a history of self-administration

To determine if seeking behavior was expressed during Noncontingent sessions, entrances per meter into the active and inactive lever areas were analyzed. Considering the levers never extend during Noncontingent sessions they were treated as pre-lever sessions. Examination of raw data showed low variation within Noncontingent sessions and therefore all three sessions (e.g. Baseline, Saline, and Drug) were grouped to analyze nose entrances into the active and inactive lever areas per meter. Noncontingent 01 was then treated as the Baseline, and Noncontingent 02 and 03 active and inactive lever entrances per meter for each rat were normalized by the group mean of Noncontingent 01 to account for high individual variation. Following a 0.23 lambda Yeo-Johnson power transformation, a Type III Wald test indicated a significant main effect of session number ($\chi^2(1)=12.46$, $p=.002$), but not a significant main effect of levers ($\chi^2(1)=3.12$, $p=.162$) or their interaction ($\chi^2(1)=3.49$, $p=.162$). Post hoc analyses revealed Noncontingent 03 to be significantly greater than Noncontingent 02, prompting a paired Student's t-test between the active and inactive levers in Noncontingent 03

where active was found to be greater than inactive. Direct comparisons to Noncontingent 01 found that the inactive lever entrances per meter in Noncontingent 02 were significantly less than Noncontingent 01, while the active lever entrances per meter in Noncontingent 03 were significantly greater than baseline. (**Fig. 4A**). These results indicate that by Noncontingent 03 rats demonstrate preferential seeking behavior toward the drug-associated active lever area.

To visualize the development of interest in the active lever, density heat maps of each rat's nose node coordinates (x, y) for all sessions within each Noncontingent session were overlain. Bounding boxes corresponding to the active and inactive lever areas and the rounded mean lever entrances into each lever area were also plotted (Noncontingent 01 active: M=22.2, SD=13.9; inactive: M=23.4, SD=12.3; Noncontingent 02 active: M=21.8, SD=16.1; inactive: M=18.4, SD=16.5; Noncontingent 03 active: M=43.5, SD=42.1; inactive: M=24.7, SD=22.1). Student's t-tests between active and inactive lever entrances within Noncontingent sessions revealed only Noncontingent 03 following a 0.23 lambda Yeo-Johnson power transformation achieved significance, with active greater than inactive (**Fig. 4B**). These results highlight the development of interest in the active lever after a history or self-administration as the high activity regions migrate from the inactive side of the chamber in Noncontingent 01 to the active side in Noncontingent 03.

3.4.2. Pre-lever activity during Short Access sessions 07-09 predicts active lever entrances per meter during Noncontingent sessions.

To determine if the interest in the active lever was developed during self-administration, rats were grouped as “High” (n=19) or “Low” (n=18) by a median-split z-score of their pre-lever activity measures (locomotion and active lever entrances per meter) during Short Access 07-09. These sessions were chosen as they were the first to show significance compared to Short Access 01 and are the last Short Access sessions before Noncontingent 02. First, the average of each variable was computed across the three sessions before being standardized within the respective variables. The two z-scores were then summed and divided by 2, where higher scores equate to greater pre-session activity. The median of the group was then taken, and those greater than or equal to the median were deemed “High”, and those less than were deemed “Low”.

Following a 0.2 lambda Yeo-Johnson power transformation, a Type III Wald test indicated no main effect of session number ($\chi^2(1)=4.81$, $p=.059$), but a significant effect of pre-lever activity score ($\chi^2(1)=29.53$, $p<.001$) and their interaction ($\chi^2(1)=9.39$, $p=.006$). Post hoc analyses revealed that both Noncontingent 02 and 03 in the High pre-lever activity group had significantly greater active lever entrances per meter than baseline, and versus the Low pre-lever activity group in Noncontingent 02. For the Low pre-lever activity group, Noncontingent 02 was significantly less than both baseline and Noncontingent 03. There was no difference between groups in Noncontingent 03 (**Fig. 4C**). These results confirm that rats with high pre-lever activity during Short Access 07-09 led to significant seeking in Noncontingent 02 that persisted in Noncontingent 03. Furthermore, the Low pre-lever activity group developed an interest during Long Access before Noncontingent 03.

506 *3.4.3. Pre-lever activity during Short Access sessions 07-09 predicts cocaine self-*
507 *administration in Short and Long Access*

508 To determine if pre-lever activity during Short Access sessions 07-09 predicts
509 cocaine intake over the entire session in Short Access, a Type III Wald test was
510 performed. A significant main effect was found in session number ($\chi^2(9)=125.52$,
511 $p<.001$), but not a significant effect of pre-lever activity score ($\chi^2(1)=3.51$, $p=.169$) or
512 their interaction ($\chi^2(9)=16.9$, $p=.169$) (**Fig. 4D**). These results show that cocaine intake
513 did not differ between groups in Short Access.

514 To determine if pre-lever activity during Short Access sessions 07-09 predicts
515 cocaine intake over the entire session in Long Access, a Type III Wald test was
516 performed. Significant main effects were found in session number ($\chi^2(13)=62.03$,
517 $p<.001$), pre-lever activity score ($\chi^2(1)=13.49$, $p<.001$) and their interaction
518 ($\chi^2(13)=28.79$, $p=.015$). Post hoc analyses revealed the High pre-lever activity group to
519 have significantly greater cocaine infusions over the entire session in all Long Access
520 sessions except Long Access 11, 13, and 14 (**Fig. 4D**). These results confirm that pre-
521 lever activity during Short Access 07-09 was initially predictive of cocaine intake during
522 Long Access, but that groups eventually converged. An analysis including sex as a
523 factor showed that among rats with low pre-lever activity, females escalated their
524 cocaine intake more rapidly than males during Long Access, indicating that the female
525 bias in vulnerability emerges specifically in individuals with low initial pre-lever activity
526 measures (**Fig. S2**, see Supplementary Material for sex-specific effects).

527 To determine if pre-lever activity during Short Access sessions 07-09 predicts
528 cocaine intake within the first 15 minutes of the session, a Type III Wald test was

performed. A significant main effect was found in session number ($\chi^2(23)=208.9$, $p<.001$), but not in pre-lever activity score ($\chi^2(1)=0.85$, $p=.742$) or their interaction ($\chi^2(23)=35.32$, $p=.134$) (**Fig 4E**). These results suggest that pre-lever activity during Short Access 07-09 is not predictive of loading behavior between groups.

Considering the incongruency of the significant association between pre-lever variables and cocaine intake variables (**Fig. 3**), and the previous nonsignificant results between pre-lever activity groups and cocaine infusions in the first 15 minutes (**Fig. 4D**), correlations were performed between cocaine intake variables and pre-lever activity z-scores in Short Access and three separate phases of Long Access. For cocaine infusions during the entire session and pre-lever activity, significant positive correlations were found in Short Access ($r_s(2960)=.65$, $p<.001$; **Fig. 4F**), Long Access 01-05 ($r_s(3284)=.61$, $p<.001$; **Fig. 4G**), Long Access 06-10 ($r_s(3422)=.59$, $p<.001$; **Fig. 4H**), but not during Long Access 11-14 ($r_s(7013)=.17$, $p=.318$; **Fig. 4I**). For cocaine infusions during the first 15 minutes and pre-lever activity, significant positive correlations were similarly found in Short Access ($r_s(3094)=.63$, $p<.001$; **Fig. 4J**), Long Access 01-05 ($r_s(3301)=.61$, $p<.001$; **Fig. 4K**), Long Access 06-10 ($r_s(4023)=.52$, $p<.001$; **Fig. 4L**), but not during Long Access 11-14 ($r_s(6735)=.2$, $p=.231$; **Fig. 4M**). These results confirm that rats who showed markedly increased pre-lever activity during Short Access sessions 07-09 were more likely to consume more cocaine during the first 15 minutes and over the entire session in both Short and Long Access, but converged by the end of Long Access. Furthermore, these results support those shown in **Fig. 4C,** where no significant difference was found in active lever entrances per meter in Noncontingent 03, which occurs following Long Access 14.

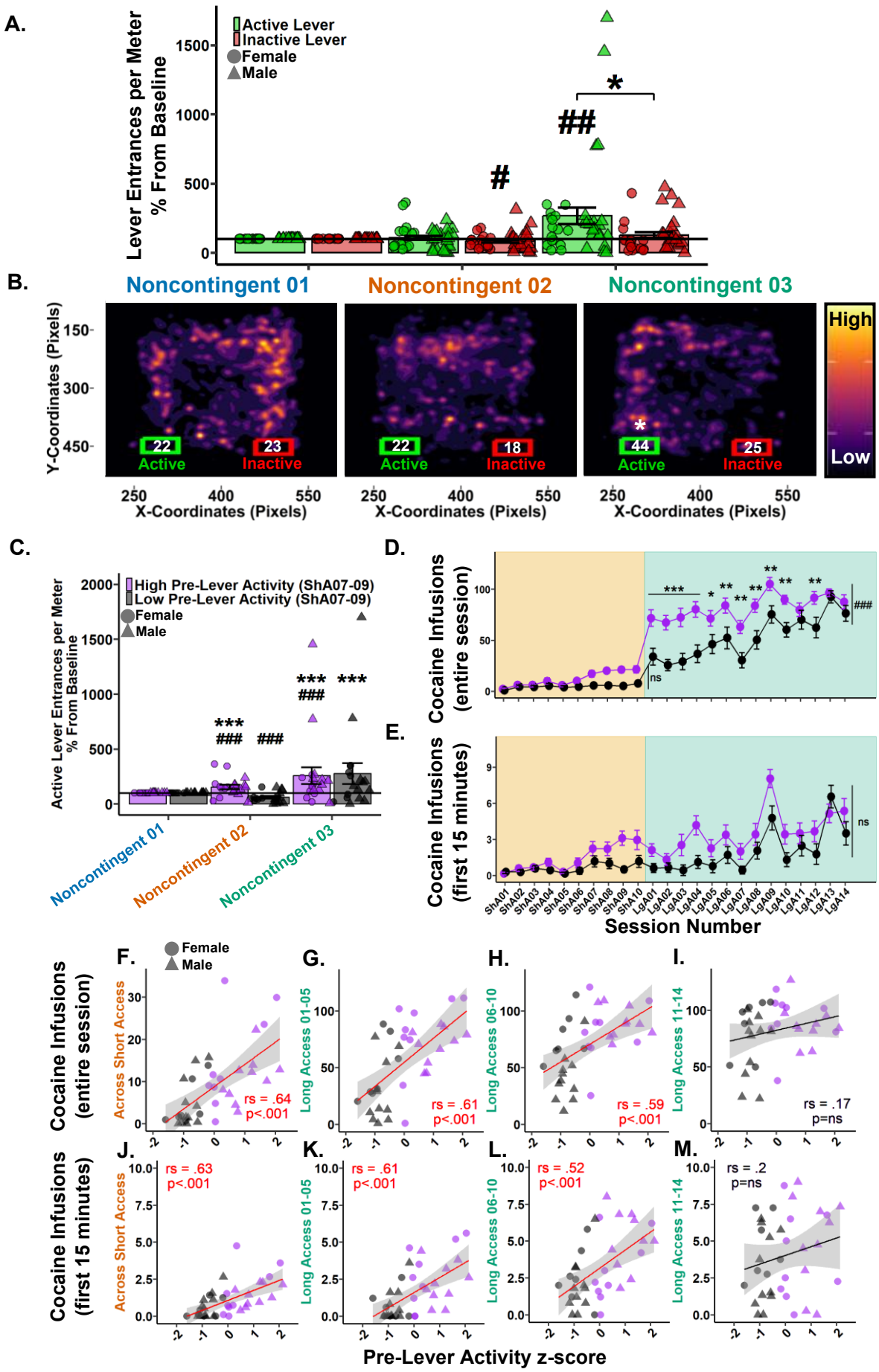

A.
Active Lever
Inactive Lever
Female
Male
Lever Entrances per Meter
% From Baseline
B.
Noncontingent 01
Noncontingent 02
Noncontingent 03
Y-Coordinates (Pixels)
X-Coordinates (Pixels)
Active
Inactive
High
Low
C.
High Pre-Lever Activity (ShA07-09)
Low Pre-Lever Activity (ShA07-09)
Active Lever Entrances per Meter
% From Baseline
D.
Cocaine Infusions
(entire session)
E.
Cocaine Infusions
(first 15 minutes)
Session Number
F.
G.
H.
I.
J.
K.
L.
M.
Across Short Access
Long Access 01-05
Long Access 06-10
Long Access 11-14
rs = .64
p<.001
rs = .61
p<.001
rs = .59
p<.001
rs = .17
p=ns
rs = .63
p<.001
rs = .61
p<.001
rs = .52
p<.001
rs = .2
p=ns
Pre-Lever Activity z-score

Figure 4. Pre-lever activity during Short Access 07-09 predicts cocaine intake in Short and Long Access and seeking behavior during Noncontingent sessions. **(A.)** Active (green) and inactive (red) lever entrances per meter as percentage difference from Baseline (Noncontingent 01). ##*p*<.01, ###*p*<.001 vs Baseline*;* **p*<.05 vs inactive lever. **(B.)** Nose (*x, y)* pixel coordinate heatmaps of all rats within each Noncontingent session in relation to active (green) and inactive (red) Levers. Each plot represents ~109 sessions overlain revealing high and low activity zones. Numbers within lever bounding boxes are the mean sum of active and inactive lever entrances for all rats within each Noncontingent session. **p*<.05 vs inactive lever. **(C.)** Active lever entrances per meter percent difference from Baseline (Noncontingent 01) grouped by pre-lever activity z-score during Short Access 07-09. ###*p*<.001 vs Baseline*;* ****p*<.001 vs Noncontingent 02 Low group. **(D-E.)** Cocaine infusions over the entire session (D.) and within the first 15 minutes (E.) grouped by Short Access 07-09 pre-lever activity z-score. **p*<.05, ***p*<.01, ****p*<.001, ###*p*<.001 vs Low. **(F-I.)** Cocaine infusions over the entire session correlated using Spearman Rank with Short Access 07-09 pre-lever activity z-score in Short Access (F.), Long Access 01-05 (G.), Long Access 06-10 (H.), but not during Long Access 11-14 (I.). **(J-M.)** Cocaine infusions within the first 15 minutes correlated using Spearman Rank with Short Access 07-09 pre-lever activity z-score in Short Access (J.), Long Access 01-05 (K.), Long Access 06-10 (L.), but not during Long Access 11-14 (M.). Plots represent means and SEM.

4. Discussion

Here we demonstrate that cocaine infusions over the full session and within the first 15 minutes escalated during Short and Long Access in heterogeneous stock rats. Locomotion increased after a noncontingent cocaine infusion, and group-level analysis showed no signs of psychomotor sensitization or tolerance following Short Access (Noncontingent 02) or Long Access (Noncontingent 03). Individual analyses revealed strong divergence with some rats developing psychomotor sensitization and others showing tolerance, though neither influenced the level of cocaine self-administration. Pre-lever locomotion and active lever entrances per meter escalated from Short to Long Access, increased after instances of abstinence, and predicted early cocaine intake. Rats with high pre-lever activity during Short Access sessions 07-09 escalated lever entries across Noncontingent sessions and earned more infusions during Short Access and first two weeks of Long Access than low pre-lever activity rats, but these differences disappeared by Long Access 13 as low-activity rats escalated to similar levels. Together, the data dissociate psychomotor sensitization and tolerance from cocaine intake and identify pre-lever activity as a robust predictor of cocaine intake and escalation.

4.1. Cocaine Intake

Rats increased total cocaine intake as early as the second Short Access session and within the first 15 minutes as early as Short Access 07, while exhibiting significant escalation of intake after two weeks of Long Access. These findings align with our previous reports that heterogenous stock rats show increased cocaine intake during Short and Long Access [28], and the larger literature that Long Access induces escalation of intake [3,4,24,58].

597 ### 4.2. Psychomotor Expression

598 Rats showed individual differences in the propensity to develop psychomotor
599 sensitization or tolerance to noncontingent cocaine administration. One alternative
600 interpretation is that analyzing only ambulatory activity may misclassify some rats
601 displaying stereotypic behavior, including head bobbing and weaving, sniffing or
602 gnawing at high cocaine doses [8,59]. However, this is unlikely for several reasons.
603 First, the dose used (1.5 mg/kg) is known to produce minimal stereotypic behavior and
604 robust sensitization [6,33-35]. Second, the nose motion (a proxy for head bobbing,
605 weaving, sniffing or gnawing) was not found to increase in Tolerant rats (**Fig. S1**).Third,
606 visual inspection of the videos show that the most tolerant rat (M2677) did not exhibit
607 stereotypic behavior while the most sensitized rat (M2668) showed pronounced
608 psychomotor sensitization. A possible explanation for the difference between sensitized
609 vs. tolerant rats is that rats classified as sensitized consumed less cocaine, while
610 tolerant rats consumed more. However, intake analyses do not support this hypothesis:
611 the two groups showed no differences in cocaine intake across the Short or Long
612 Access session or within the first 15 minutes. These findings challenge the idea that
613 Short Access produces only sensitization and Long Access only tolerance to the effects
614 of cocaine [1,3-6,18,24], and they show that neither tolerance nor sensitization reliably
615 predict future cocaine intake.

616 ### 4.3. Pre-Lever Activity Measures

617 Pre-lever variables locomotion and active lever entrances per meter increased
618 from Short to Long Access and intensified following two-to-three days of abstinence.

619 Similarly, rats showed a significant increase in the active lever area entrances per meter
620 in Noncontingent 03 in comparison to Noncontingent 01, suggesting sensitization of
621 drug-seeking behavior. In contrast to measures of psychomotor sensitization or
622 tolerance expression, both locomotion and active lever entrances per meter were highly
623 predictive of intake. These findings align with the incentive salience literature, showing
624 that repeated drug use leads to salience attribution to cues and environments that
625 particularly manifests following a period of abstinence [5,6,15-16,19-20,33-35,46-47,61-
626 68].

627 **4.4. Individual Differences in Pre-Lever Activity Measures**

628 To investigate the individual propensity to attribute salience to drug-associated
629 cues, a median z-score of pre-lever activity was calculated. Short Access sessions 07–
630 09 were selected as they occurred within the same week (i.e., no abstinence periods)
631 and were the first sessions in which both pre-lever variables (**Fig. 3A & 3H**) and
632 infusions within the first 15 minutes (**Fig. 1C**) reached significance. Comparisons
633 between High and Low pre-lever activity groups revealed that High pre-lever activity
634 promoted an early escalation of cocaine intake during Long Access, but ultimately both
635 High and Low pre-lever activity groups reached the same level of escalation after two
636 weeks. Although no sex differences were observed in rates of pre-lever activity, females
637 who exhibited low pre-lever activity escalated in cocaine infusions before low pre-lever
638 activity males in Long Access (**Fig. S2**, see Supplementary for sex-specific effects). One
639 possible explanation is that rats in the Low pre-lever activity group in Short Access was
640 slower in developing incentive salience, and once expressed, their intake escalated to
641 levels comparable to the High pre-lever activity group. Indeed, the significant difference

642 in active lever entrances per meter between activity groups in Noncontingent 02 and its
643 absence in Noncontingent 03 supports this interpretation. These findings align with
644 previous reports that individuals differ in their susceptibility to incentive salience
645 attribution [59,62,64-65], and that incentive salience may act as a catalyst of escalation
646 and an early predictor of vulnerability [26]. Another interpretation is that negative affect
647 or allostatic load may be driving the incentive salience and escalation in Long Access
648 [1,3,58,60]. While the current report does not test this specific hypothesis, there is
649 compelling evidence in both animal models and humans that acute withdrawal,
650 withdrawal-conditioned cues, negative affect, and psychosocial stress increase drug-
651 induced craving, drug-induced seeking, and amplifies drug cue-evoked brain activity.
652 While it is unlikely that these phenomena contribute to the effects observed in this report
653 during Short Access (although not impossible), it is likely that they contribute during
654 Long Access as animals experience acute withdrawal and negative emotional state and
655 that abstinence produces a large increase in pre-lever activity measures before the
656 session only in Long Access rats [69-73].

657 The phenotype we observed (rats with high pre-lever activity measured as
658 elevated active lever entrances per meter and locomotion) shares conceptual parallels
659 with the sign-tracker/goal-tracker framework [62]. Sign-trackers preferentially interact
660 with a discrete cue (the lever CS), while goal-trackers orient to the site of reward
661 delivery. Sign-trackers have been shown to initially self-administer more cocaine [66]
662 and choose cocaine over food [74]. However, important differences exist: sign-tracking
663 is defined in a purely Pavlovian context, with lever pressing having no instrumental
664 consequence, whereas our measure reflects operant-conditioned lever-directed

exploration. Notably, recent work has shown that sign-/goal-tracker status does not reliably predict overall cocaine self-administration or escalation, although sign-trackers may show greater resistance to punishment [75]. Thus, our results extend the sign-/goal-tracker framework into an operant cocaine paradigm, highlighting that cue-bias and responsivity can shape early intake and drive escalation of use even in resistant populations with prolonged access.

4.5. Limitations

The present study has a few limitations. One is the lack of a control group without periods of abstinence (no weekends). A separate group participating in all sessions in succession without instances of abstinence could further confirm the influence of abstinence on incentive salience incubation. However, noncontingent sessions 02 and 03 did not follow a period of abstinence, and expressions of incentive salience and both psychomotor sensitization and tolerance were still observed. Another limitation is the use of pose-estimation over manual scoring of behavior could result in inaccuracies. However, pose-estimation with SLEAP is remarkably accurate [38,76], and the instance rendered sample videos display precise tracking of the catheter and nose nodes (see supplementary videos).

4.6. Summary

To summarize, this report demonstrates that individual differences in psychomotor sensitization or tolerance had no influence on cocaine intake. In contrast, sensitization of incentive salience during Short Access acts as a catalyst or early predictor of escalation in cocaine self-administration during the first two weeks of Long

687 Access, but does not predict established escalation of cocaine intake after three weeks
688 of Long Access. Moreover, individuals initially resistant to incentive salience can, with
689 sufficient exposure, become sensitized and escalate cocaine use to the same level as
690 more susceptible individuals. Expression of incentive salience sensitization as reflected
691 in pre-lever activity measures of active lever entrances per meter and locomotion and
692 their subsequent influence on cocaine intake supports the idea that incentive salience
693 and psychomotor sensitization are mediated by distinct mechanisms [6-7,21,34]. The
694 development of the incentive salience profiles is accelerated by periods of abstinence
695 (weekend), which allows for incubation of craving, a progressive increase of craving
696 during early days of abstinence that can remain elevated following prolonged
697 abstinence [47]. The measure of approach toward the active lever area using video
698 recording before the start of the self-administration session may represent a novel
699 longitudinal behavioral marker of addiction vulnerability that can precisely track the
700 motivation for the drug with high accuracy without using any operant measures, the
701 drug itself, or any other training or conditioning that could interfere with the drug self-
702 administration. Such a measure may also be useful for medication development to allow
703 dissociation between drug-dependent and drug-independent effect of experimental
704 medication on drug seeking and taking.

705

706 **Data Availability Statement**

707 The datasets generated during and/or analyzed during the current study are
708 available from the corresponding author on reasonable request.

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Lieselot L. G. Carrette, PhD: Experimental design, manuscript drafting and revising

Olivier George, PhD: Manuscript drafting and revising, experimental design, data analysis and interpretation, funding and resources, final approval

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Competing Interests

Authors declare no competing interests.

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Supplementary Material

Incentive Salience, not Psychomotor Sensitization or Tolerance, Drives Escalation of Cocaine Self-Administration in Heterogeneous Stock Rats

Introduction

The following document provides a detailed account of the model fitting and choice process for the linear and generalized mixed models utilized in the repeated measure ANOVA statistical analyses, a test of stereotypies to accompany **Fig. 2C**, and analyses with sex as a factor. First is the step-by-step process of testing and comparing models with different model fits. Next are the assumption test visualizations provided by the simulateResiduals() function from the RStudio DHARMa package (Hartig, 2024) for each final mixed model, including the analyses of sex and stereotypies. The function had the parameters “refit = FALSE” and “n = 500” for residual simulations. The default number of simulations is 250, and 500 was chosen for computation time and greater precision (Hartig, 2024). Each plot is identified alphabetically for how they appear in the manuscript, and each model’s statistics can be found in the excel spreadsheet table. Next are the results and figure for the stereotypies analysis. And lastly are the analyses with sex included as a factor.

1. Model Testing and Comparison of Fit Process

To best explain the process of testing and comparing different linear and generalized linear mixed model combinations, an example is used to fit the steps in context. The chosen example is the first statistical analysis in the manuscript and

supplementary excel spreadsheet, i.e., answering the question: “Do cocaine infusions in the entire session differ between sessions in Short Access”? Therefore, the response variable is Cocaine Infusions, the fixed effect is Session Number, and given this is a repeated measure analysis, the random effect is each individual Rat. The formula, as it would appear in RStudio, would then be:

response variable ~ fixed effects + random effects

or in this example,

Cocaine Infusions ~ Session Number + (1|Rat)

This formula is then used to fit a model of the data using the linear mixed effects model function lmer() (Bates et al., 2015; Kuznetsova, Brockhoff, & Christensen, 2017) or a generalized linear mixed effects model with glmmTMB() (Brooks et al., 2017). All models were fit with maximum likelihood to allow valid statistical comparisons of fixed effects structures and to support post hoc testing as recommended (Bates et al., 2015; Luke, 2017; Meteyard & Davies, 2020). The process is as follows:

1. The first step was to determine the optimal lambda for Box-Cox of Yeo-Johnson power transformations to achieve normality for a linear regression model. Box-Cox are for response variables that do not contain zero or negatives, and Yeo-Johnson for those that do. For this example, a Yeo-Johnson was performed.
2. Two linear mixed models using lmer() were then produced: one without power transformation, and one with power transformation.
3. The two linear mixed models were then compared with a likelihood ratio test.

4. The two linear mixed models were then visualized with simulateResiduals() in the order of the likelihood ratio test results, i.e., worst to best, and their results documented. These steps elucidate the modeling done by linear methods.
5. Next, a number of generalized linear mixed models are fit using glmmTMB. Unlike linear mixed models, generalized mixed models allow for specification of both the distribution family (e.g., gaussian, logarithmic, tweedie, etc.) and the dispersion structure, enabling more flexible modeling of data with non-normal distributions or heterogeneous variance. For example, some distribution families require nonnegative and whole numbers (negative binomial distributions), while others do not have such constraints (gaussian). For this example, six separate base models were fit to the data: without transformation, with a Yeo-Johnson transformation, with a tweedie distribution, and all three available negative binomial distributions.
6. Similarly to the linear mixed models, the generalized mixed models were then compared using a likelihood ratio test.
7. The generalized mixed models were then visualized with simulateResiduals() in the order of the likelihood ratio test results from worst to best. Steps 5-7 reveals the best family distribution to be used.
8. The best-fitting model from the previous step is then used as a baseline to evaluate alternative dispersion structures within the generalized linear mixed model. Whereas linear mixed models estimate only the conditional mean of the outcome variable (e.g., $Y \sim X$), generalized linear mixed models permit simultaneous modeling of both the mean and the variance. By default, variance

is assumed to follow the form dictated by the specified distributional family. However, the inclusion of dispersion parameters enables the modeling of heteroskedasticity by allowing fixed effects (e.g., session number, session type, etc.) to account for variation in the residual variance. This step evaluates whether explicitly modeling dispersion improves overall model fit and residual behavior. In the case of this example, a few of the dispersion parameters fit were:

Cocaine Infusions ~ Session Number + (1|Rat)

Dispersion = Session Number

Dispersion = Session Number + (1|Rat)

Dispersion = Abstinence

Dispersion = Abstinence + (1|Rat)

These specifications allow the variance to vary as a function of Session, as a function of both Session and individual Rats, by each level of Abstinence, or Abstinence and each individual Rat.

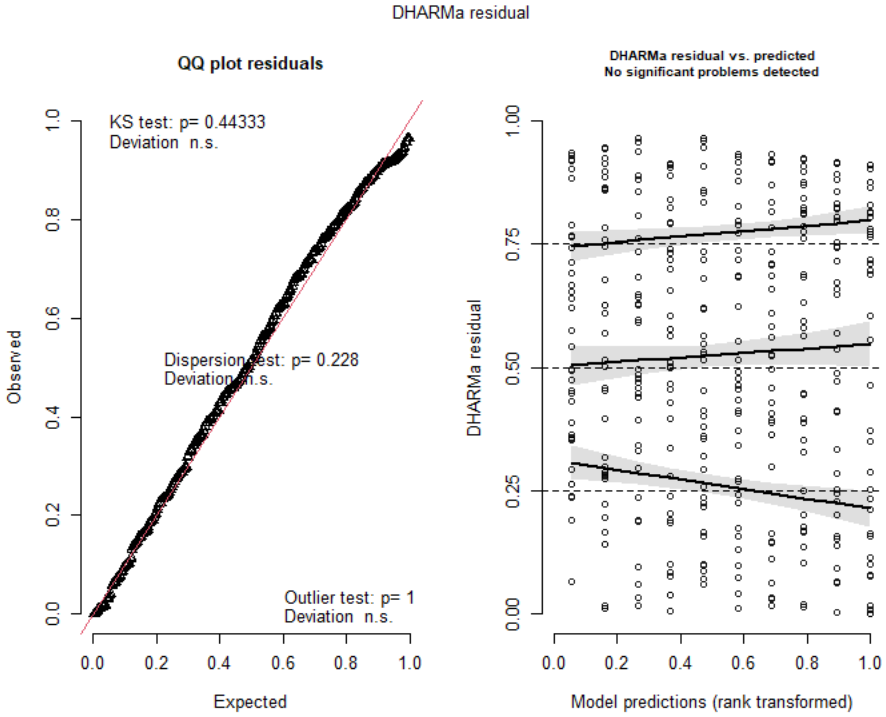
9. Each generalized mixed model from step 8 was then compared using a likelihood ratio test.
10. Each model from step 8 was then visualized with simulateResiduals() in the order of the likelihood ratio test results. It is in this step that the best linear mixed model simulateResiduals() results from above in step 4 is compared with the generalized mixed model combinations.
11. The model in Step 10 that yielded the most favorable simulateResiduals() diagnostics was selected as the final model. Primary emphasis was placed on

the Kolmogorov–Smirnov test to assess residual uniformity and overall model fit to the data. However, in instances where warranted, a balanced interpretation of all diagnostic outputs—which include dispersion, outlier detection, and observed versus predicted relationships—was preferred, as the Kolmogorov–Smirnov test is known to be sensitive to large sample sizes, which may yield statistically significant deviations despite an acceptable practical fit (Hartig, 2024; Razali & Wah, 2011).

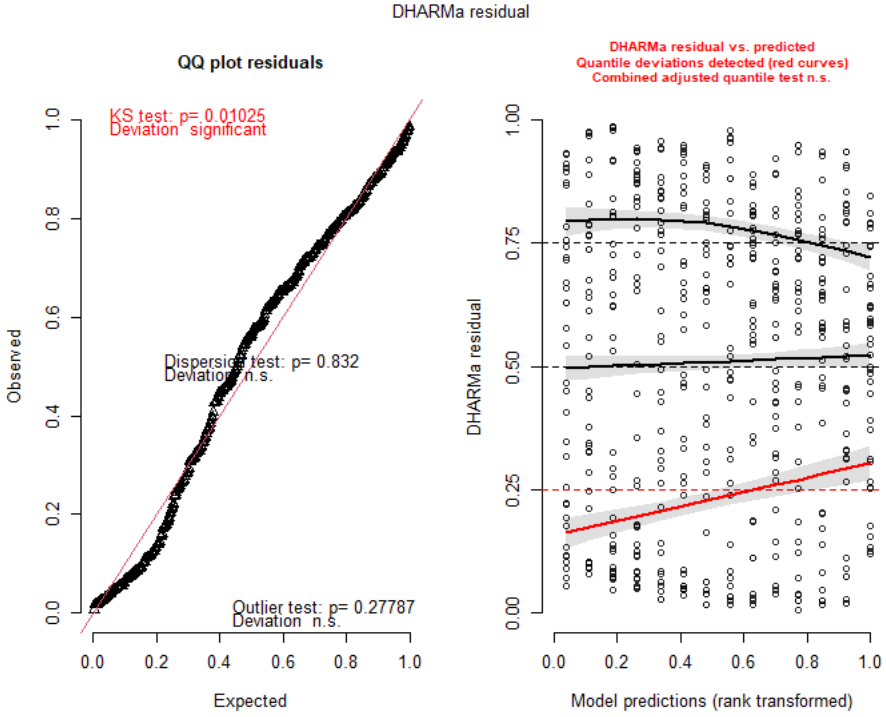
2. DHARMa simulateResiduals() Assumption Check Results

Below are the plotted outputs generated by simulateResiduals() for the final model used in each of the repeated measure statistical analyses found in the manuscript. Red text indicates a failure of an assumption test. The plots are alphabetical in the order in which they appear in the manuscript and in the accompanying supplementary excel spreadsheet. The excel spreadsheet contains a table with the final formula of the models as well as the statistical results generated by summary(). Results of models not selected are available from the corresponding author on reasonable request.

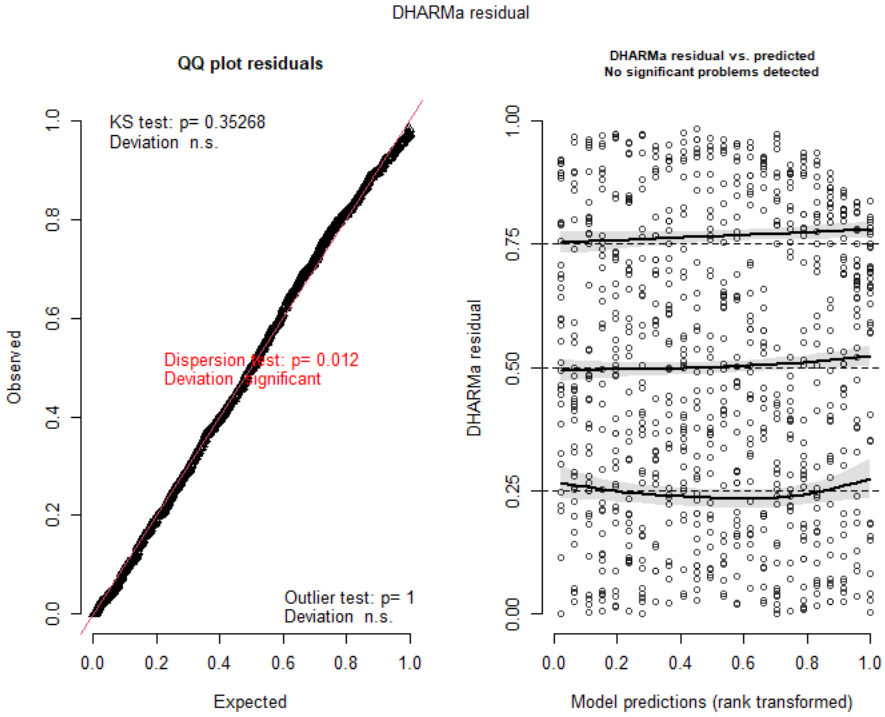
A. Cocaine Infusions in Short Access



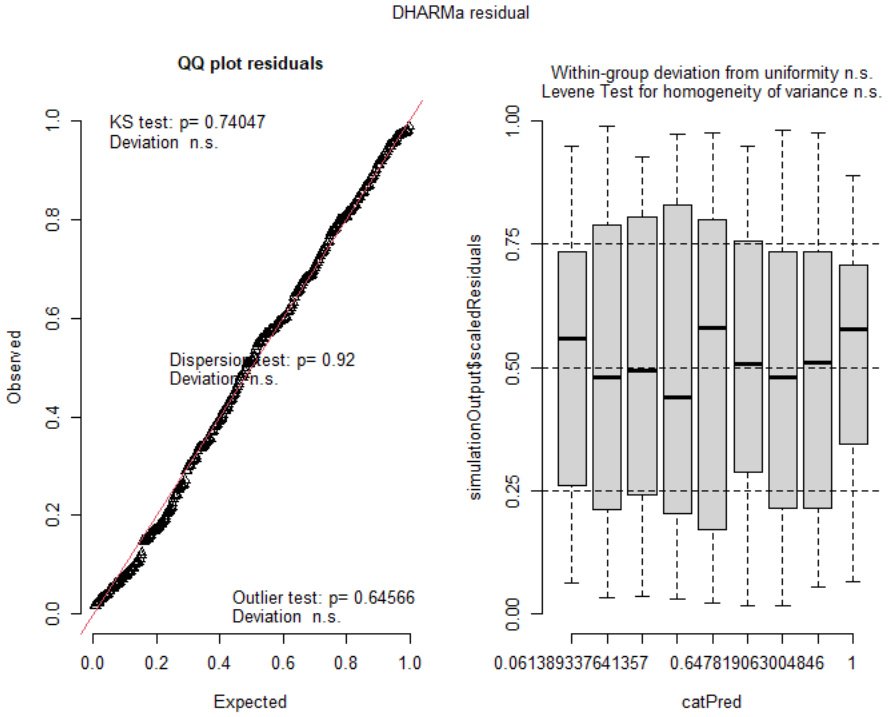
B. Cocaine Infusions in Long Access



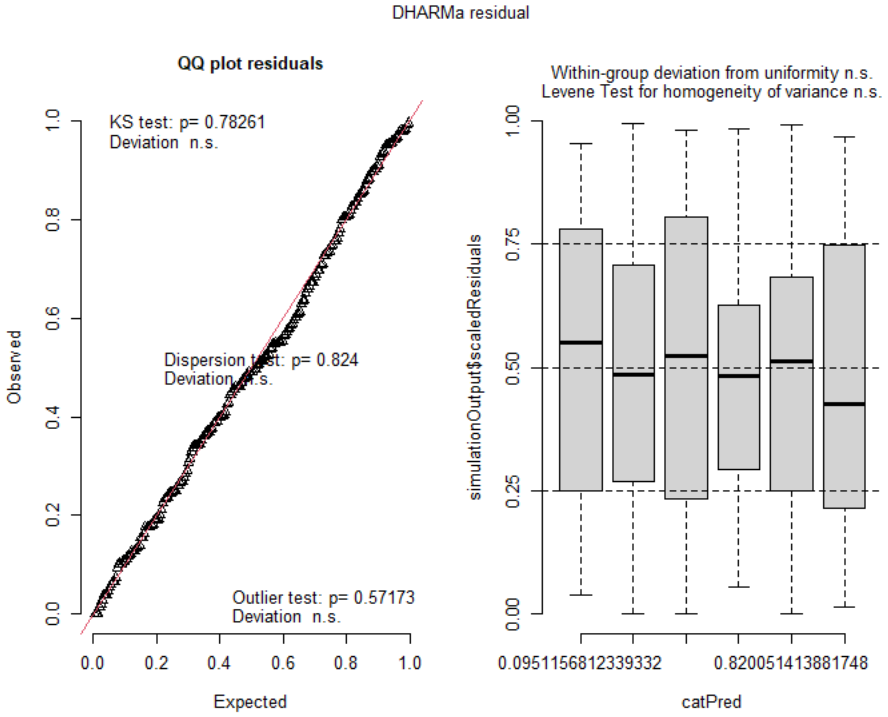
C. Cocaine Infusions First Fifteen Minutes



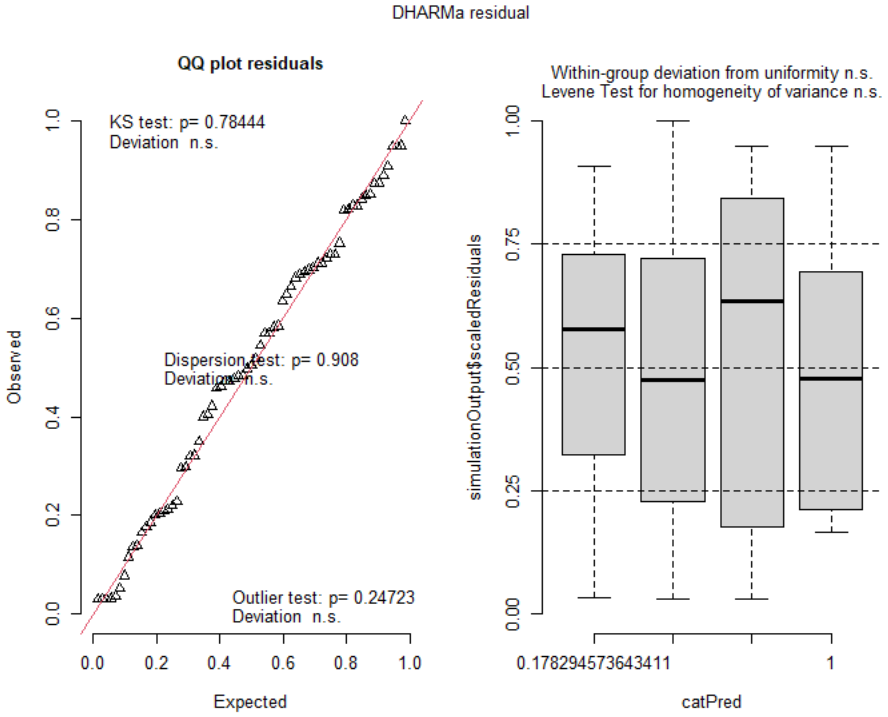
D. Locomotion Raw in Noncontingent Sessions



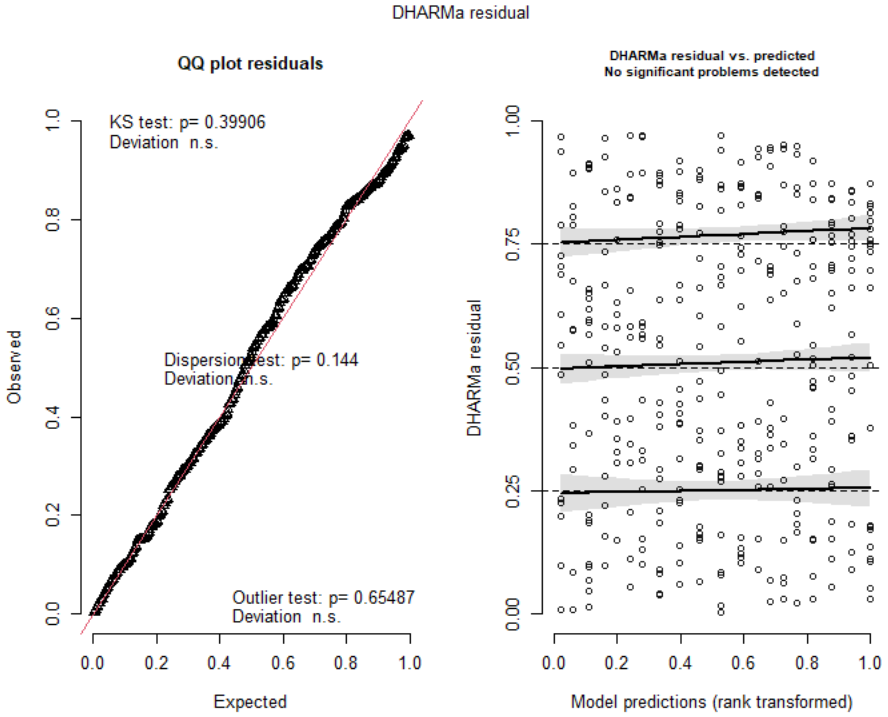
E. Locomotion Percent Difference From Baseline in Noncontingent Sessions



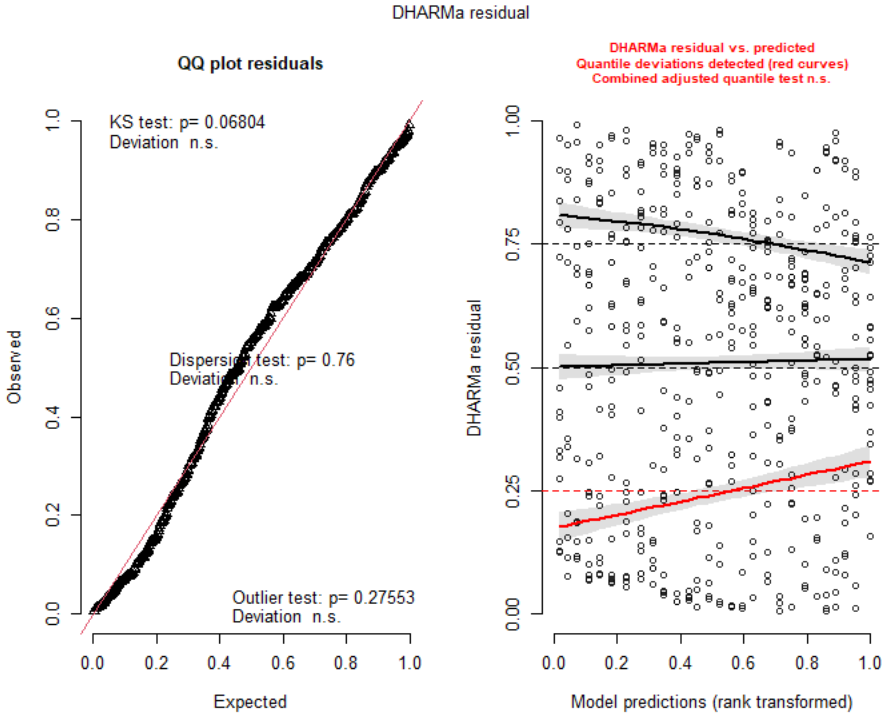
F. Locomotion Percent Difference From Drug 01 by Behavioral Expression



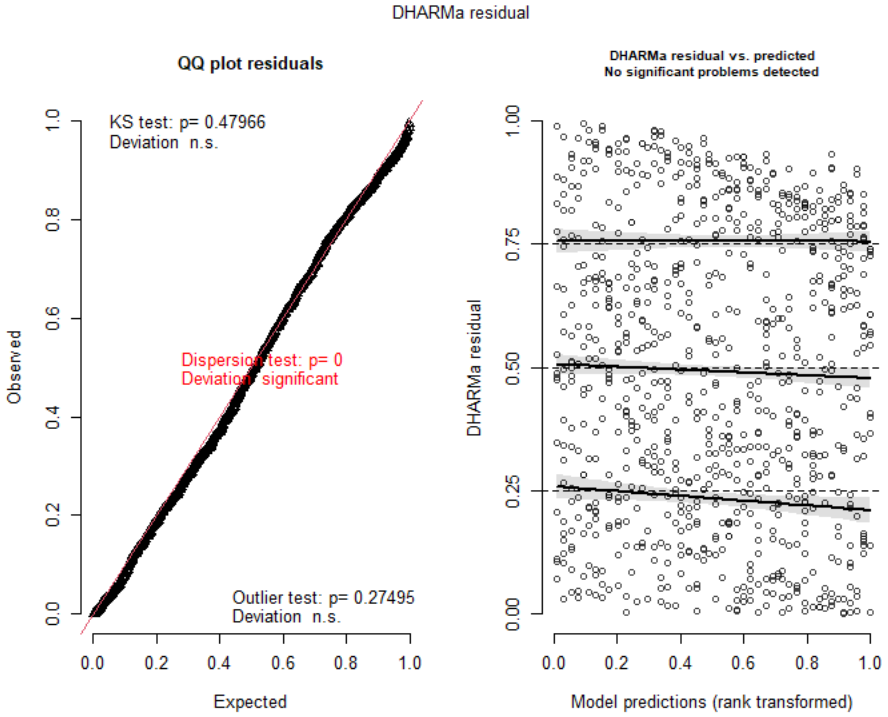
G. Cocaine Infusions in Short Access by Behavioral Expression



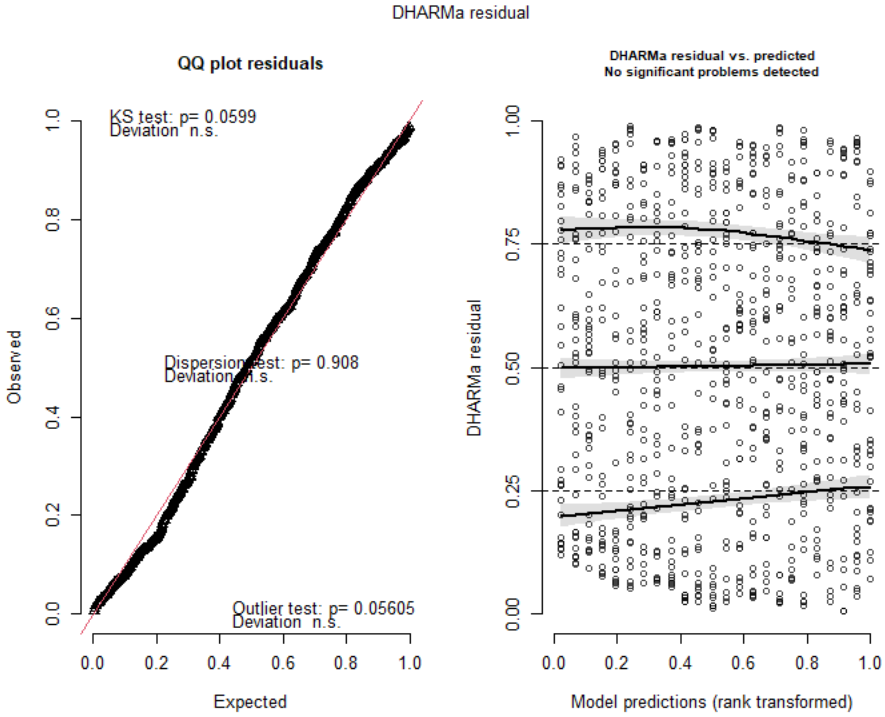
H. Cocaine Infusions in Long Access by Behavioral Expression



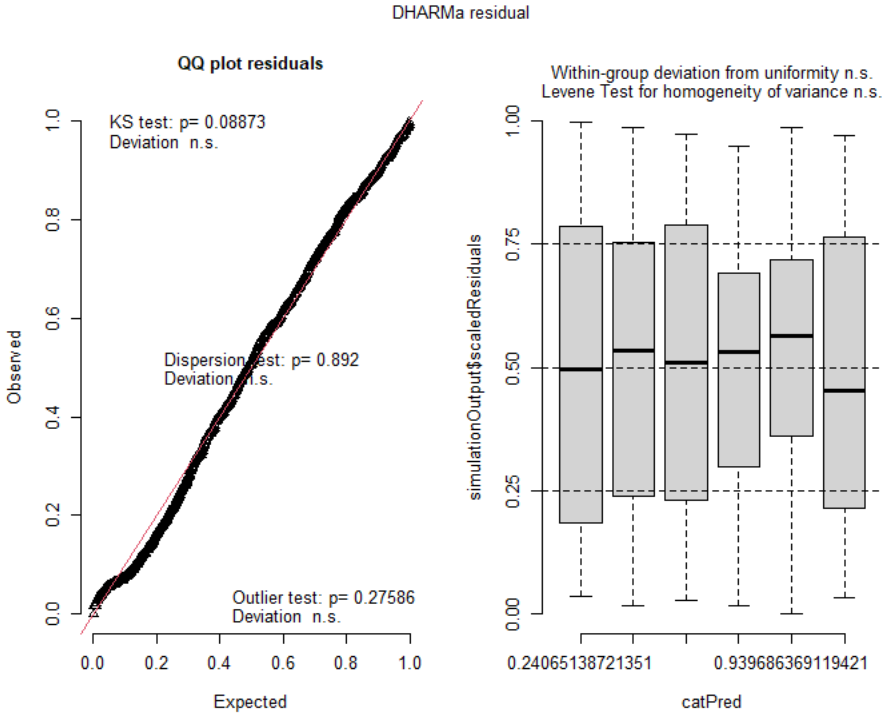
I. Cocaine Infusions First 15 Minutes by Behavioral Expression



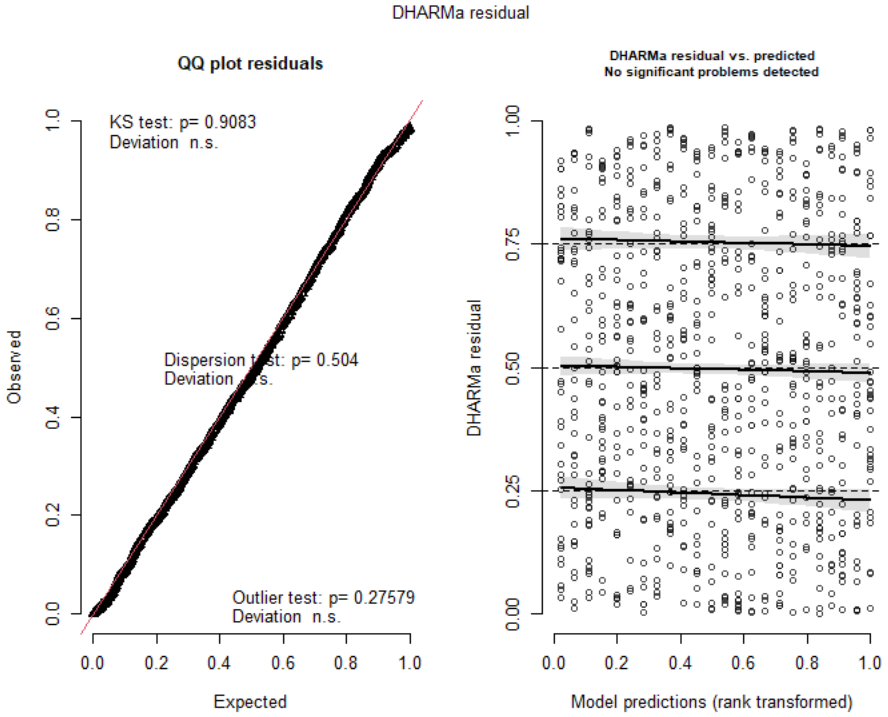
J. Locomotion Pre-Lever



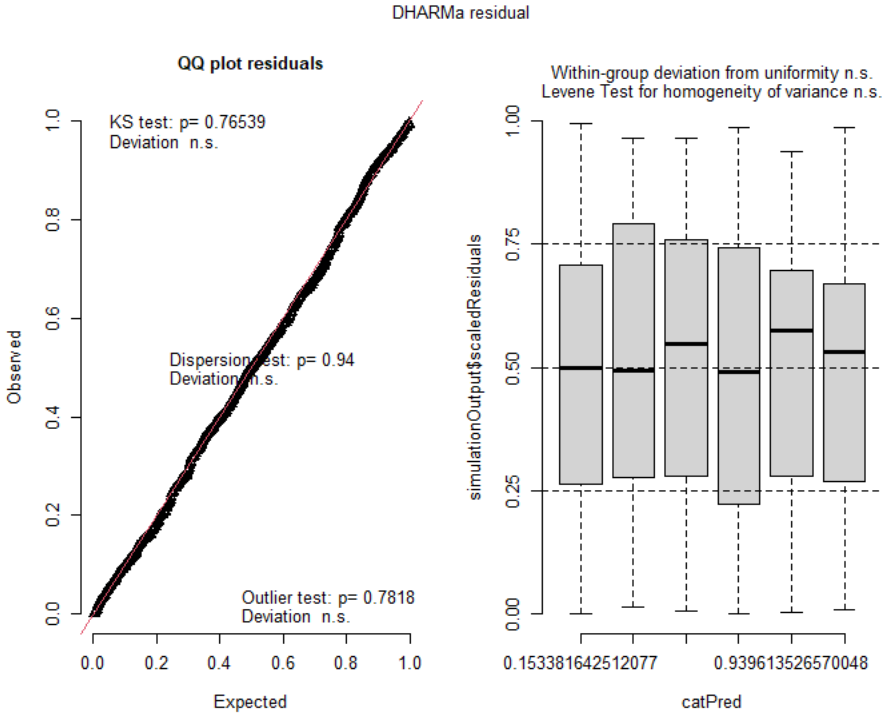
K. Locomotion Pre-Lever by Session Type and Abstinence



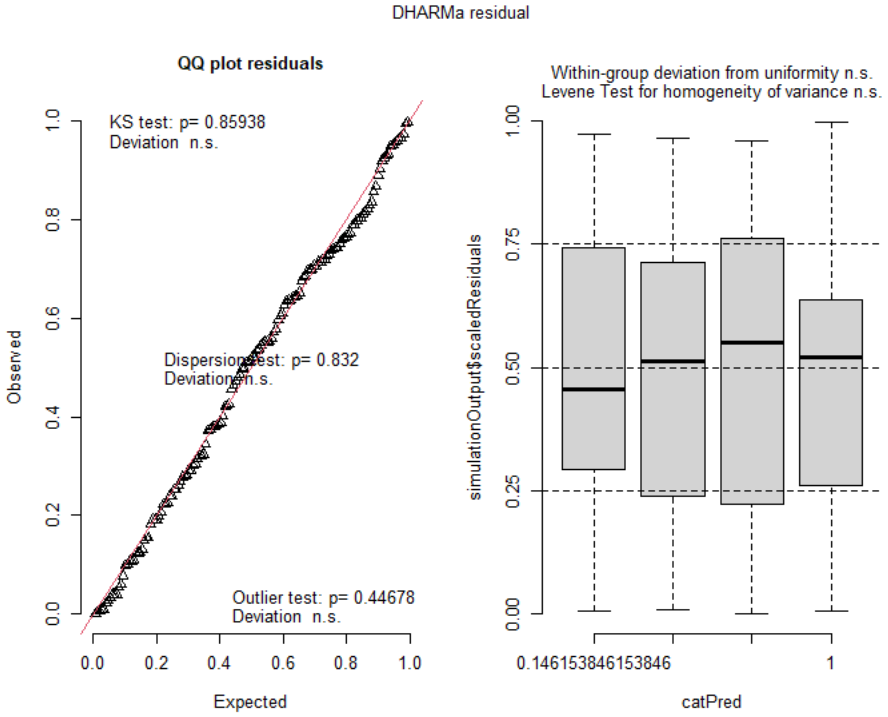
L. Active Lever Entrances per Meter Pre-Lever



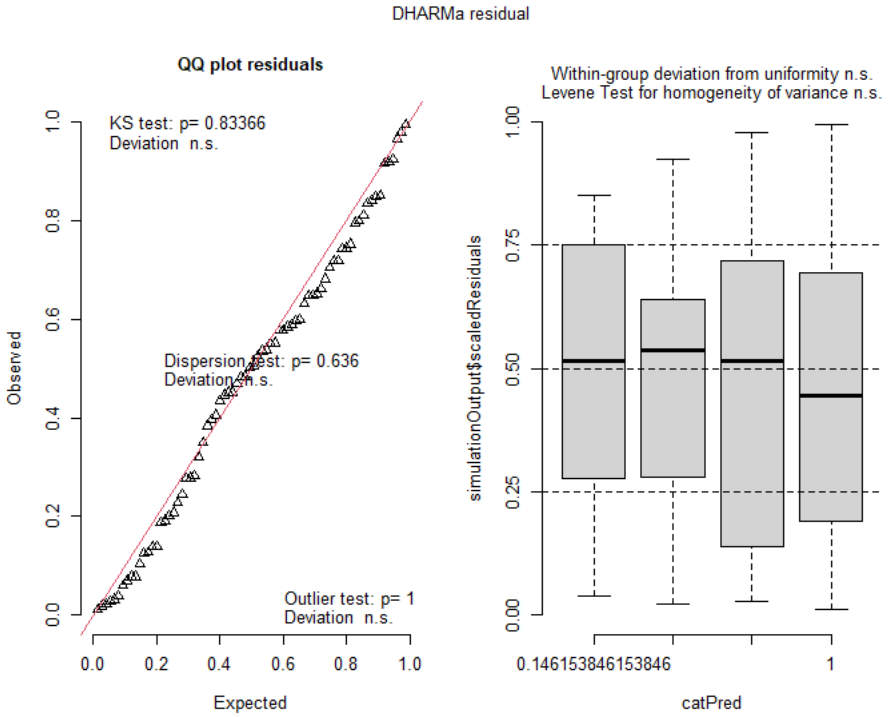
M. Active Lever Entrances per Meter by Session Type and Abstinence



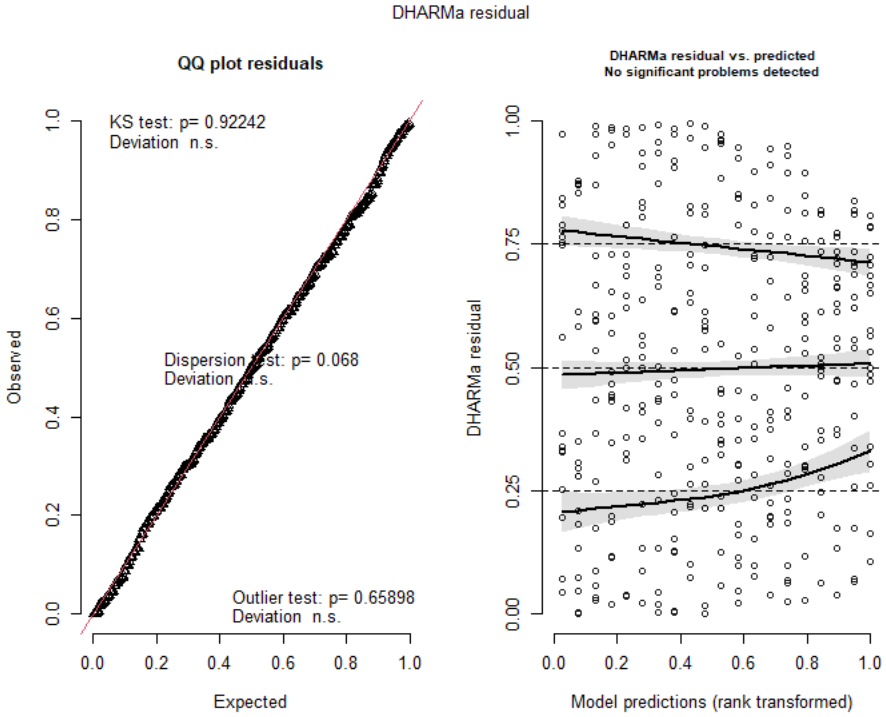
N. Entrances per Meter Percent Difference From Noncontingent 01



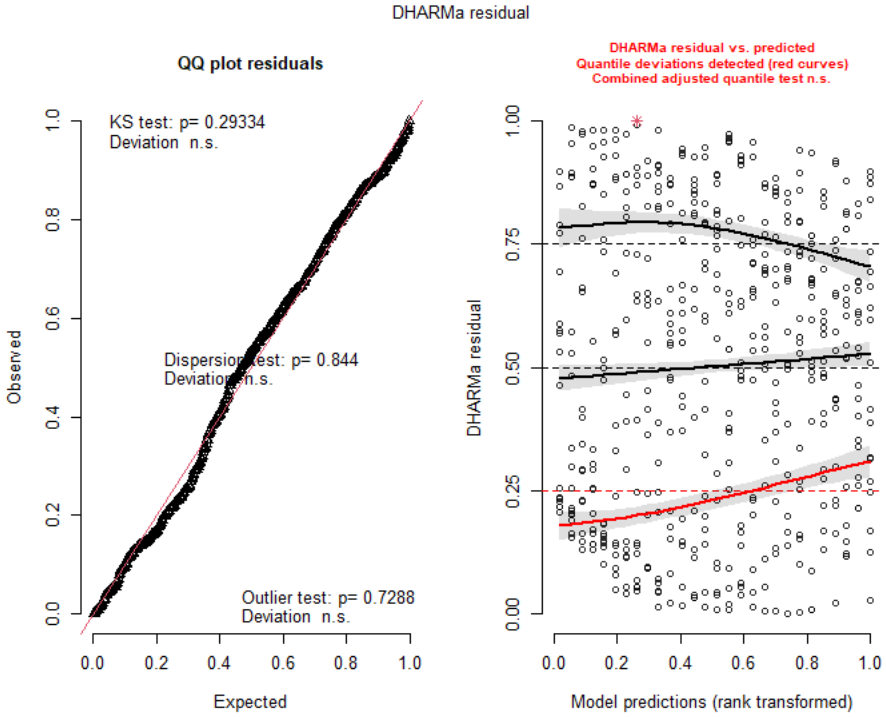
O. Active Lever Entrances per Meter Percent Difference From Noncontingent 01 by Pre-Lever Activity



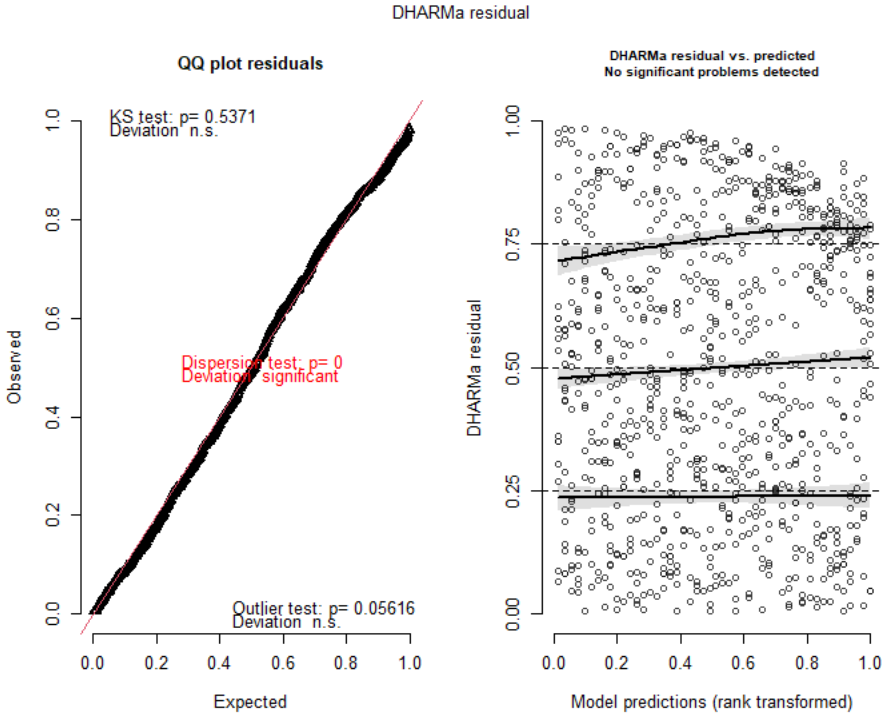
P. Cocaine Infusions in Short Access by Pre-Lever Activity



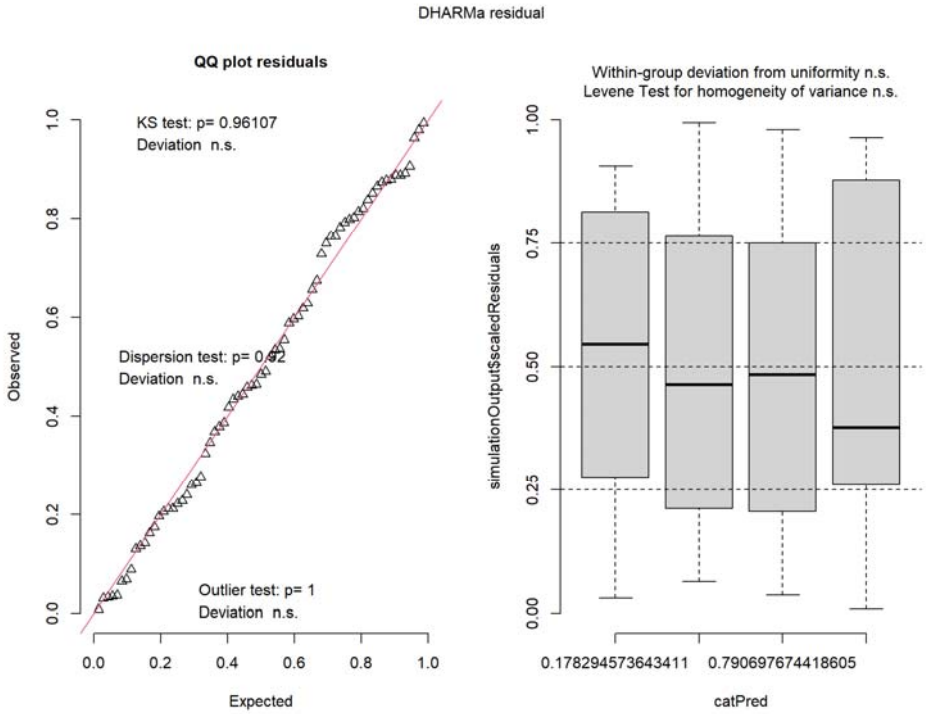
Q. Cocaine Infusions in Long Access by Pre-Lever Activity



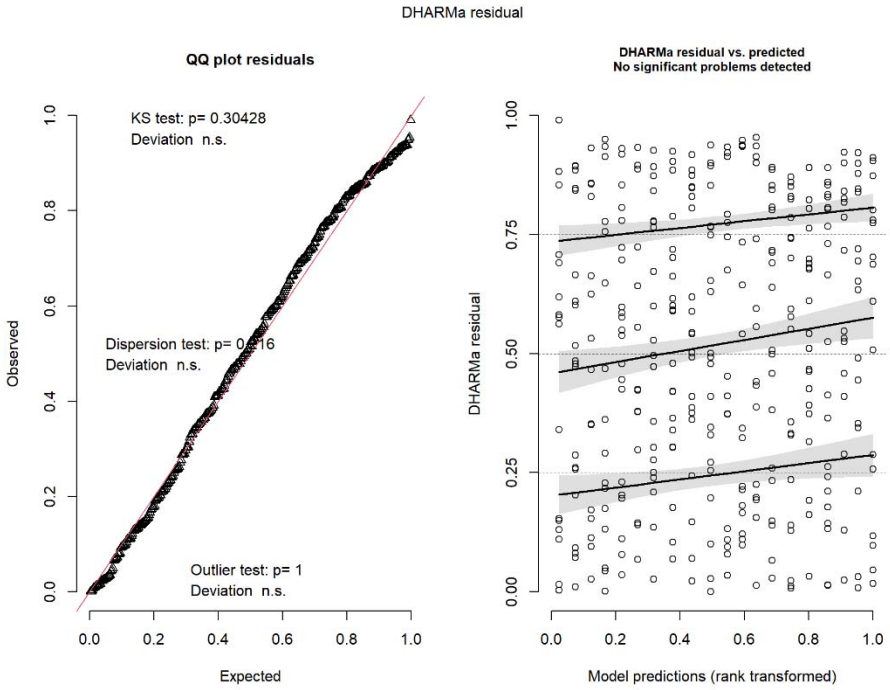
R. Cocaine Infusions First 15 Minutes by Pre-Lever Activity



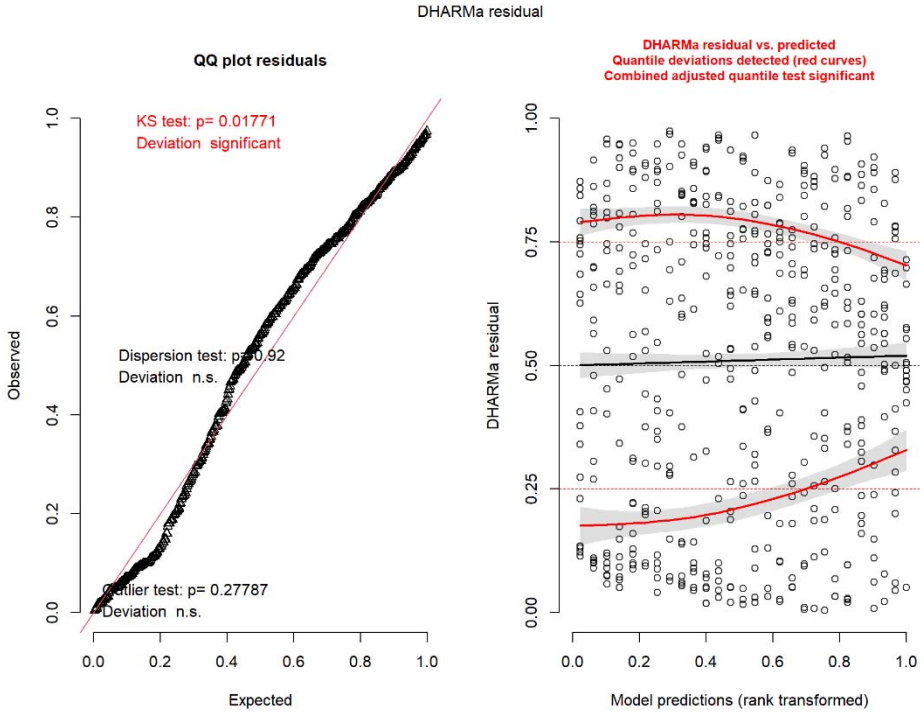
S. Nose Motion Percent Difference From Drug 01 by Behavioral Expression



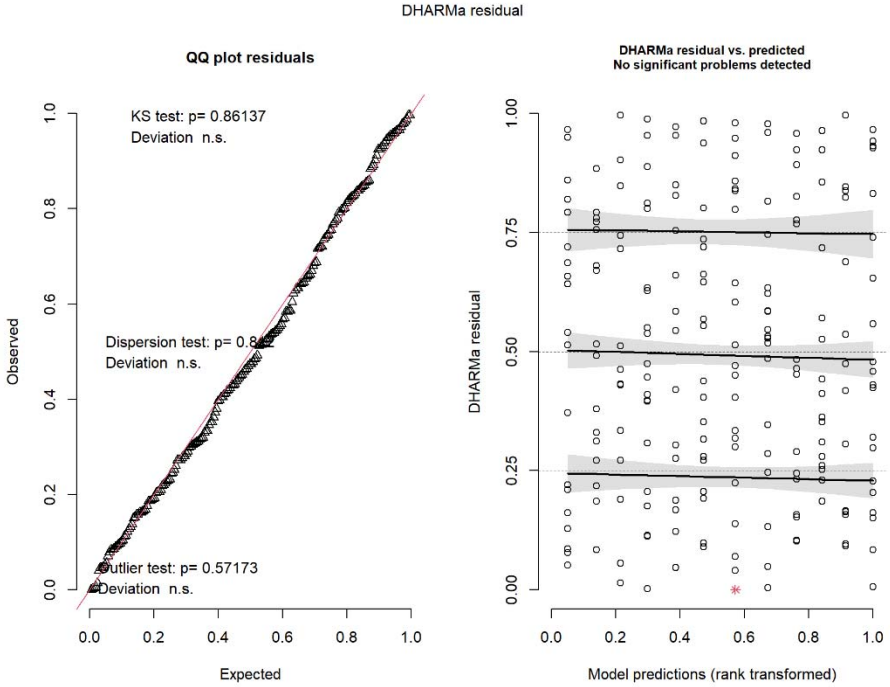
T. Cocaine Infusions in Short Access by Sex



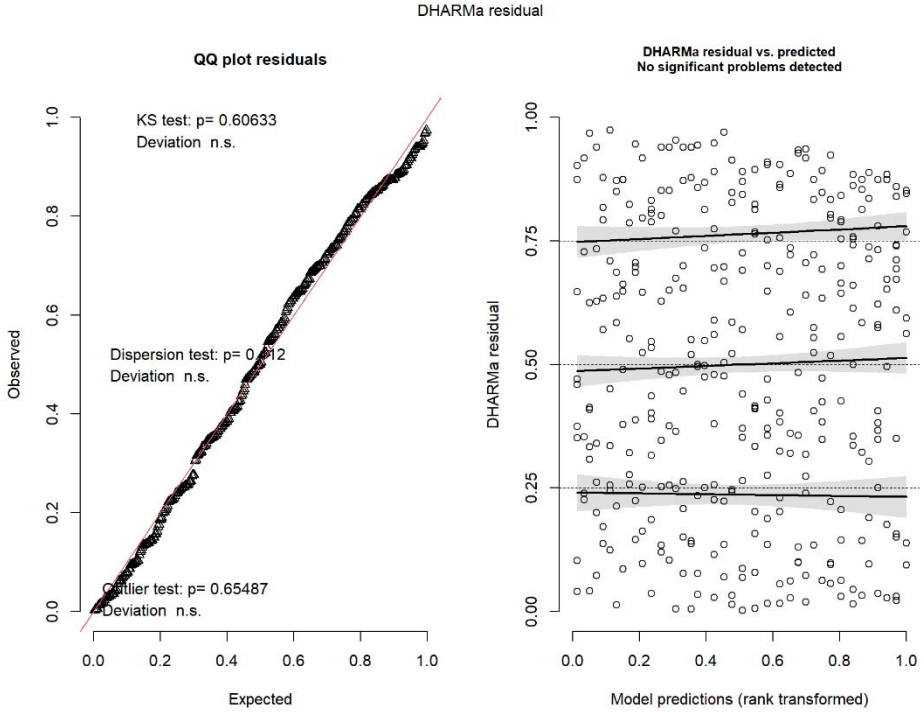
U. Cocaine Infusions in Long Access by Sex



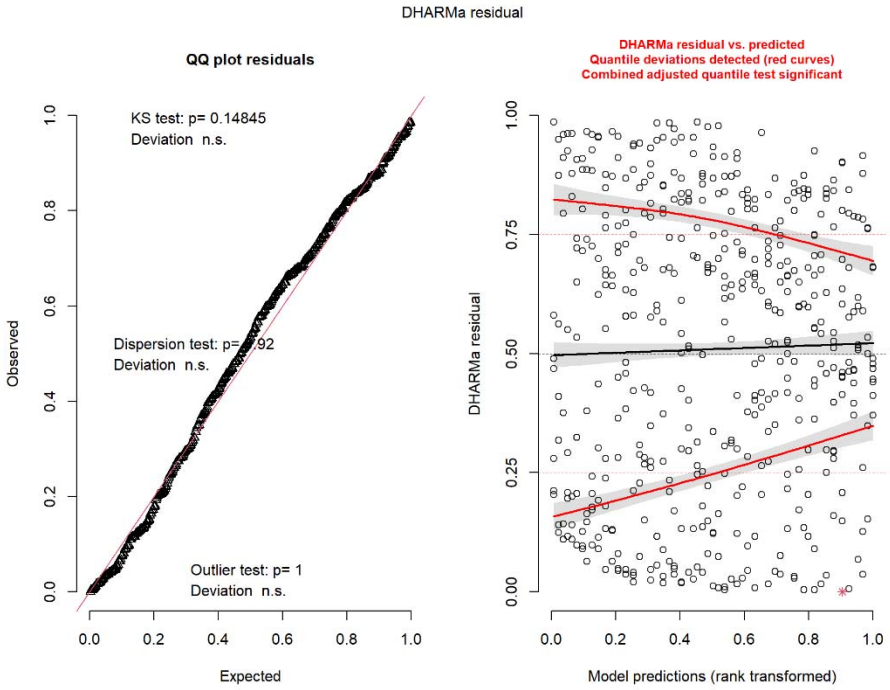
V. Locomotion Percent Difference From Baseline in Noncontingent Sessions by Sex



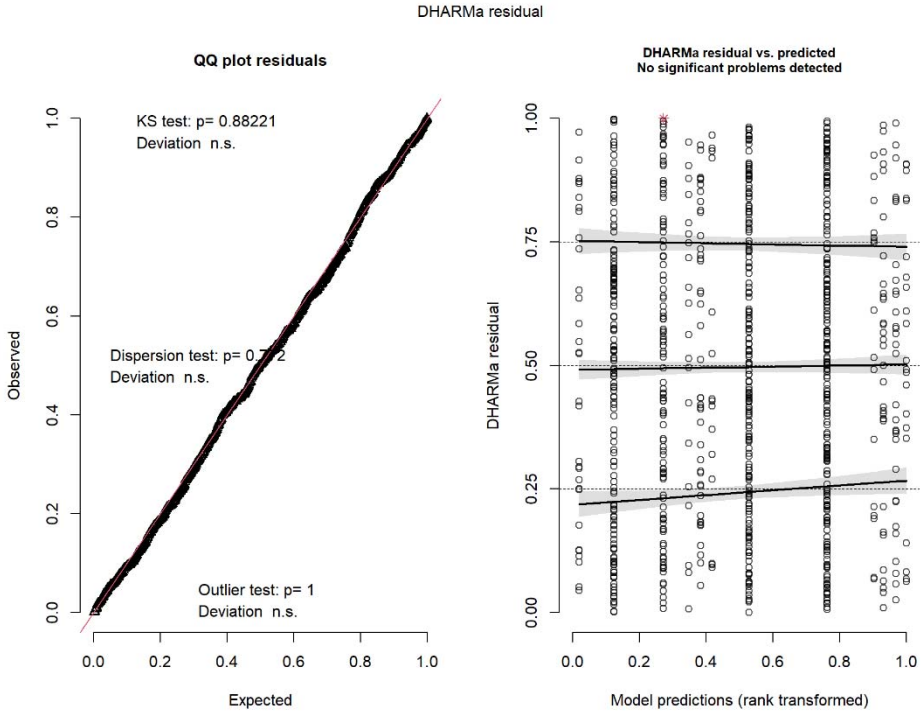
W. Cocaine Infusions in Short Access by Behavioral Expression and Sex



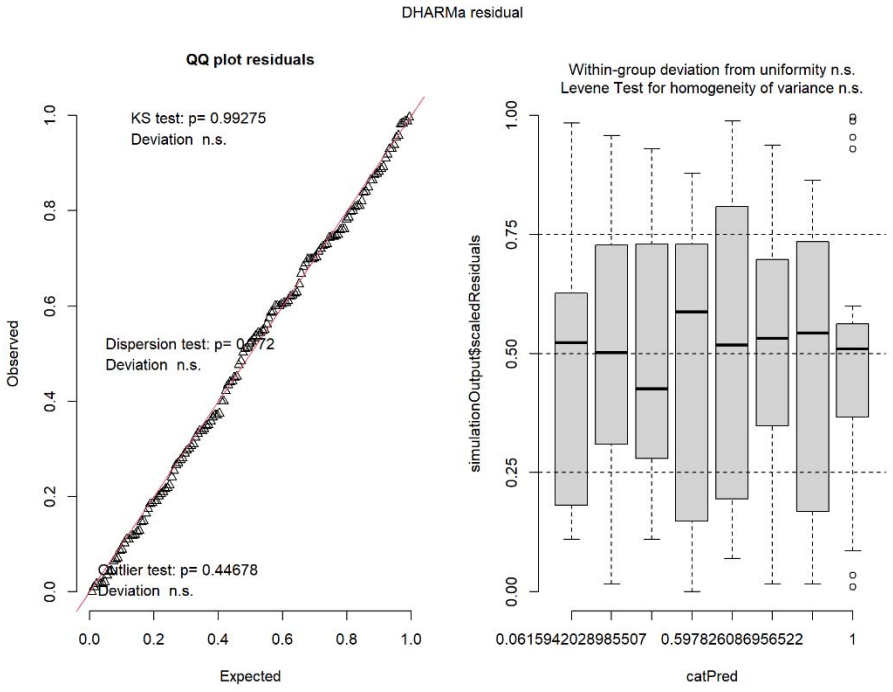
X. Cocaine Infusions in Long Access by Behavioral Expression by Sex



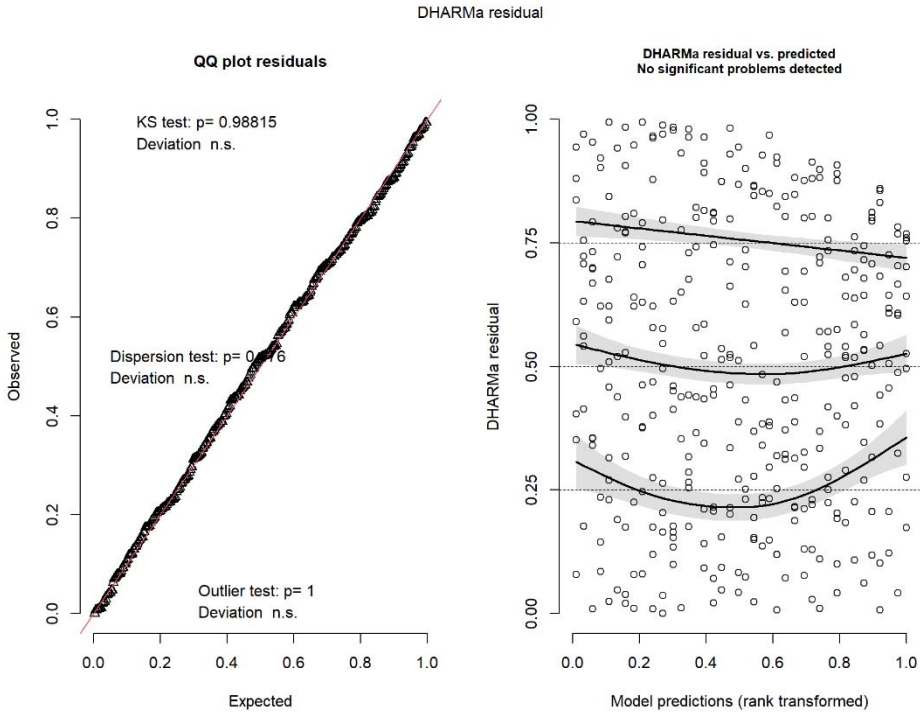
Y. Active Lever Entrances per Meter by Session Type, Abstinence, and Sex



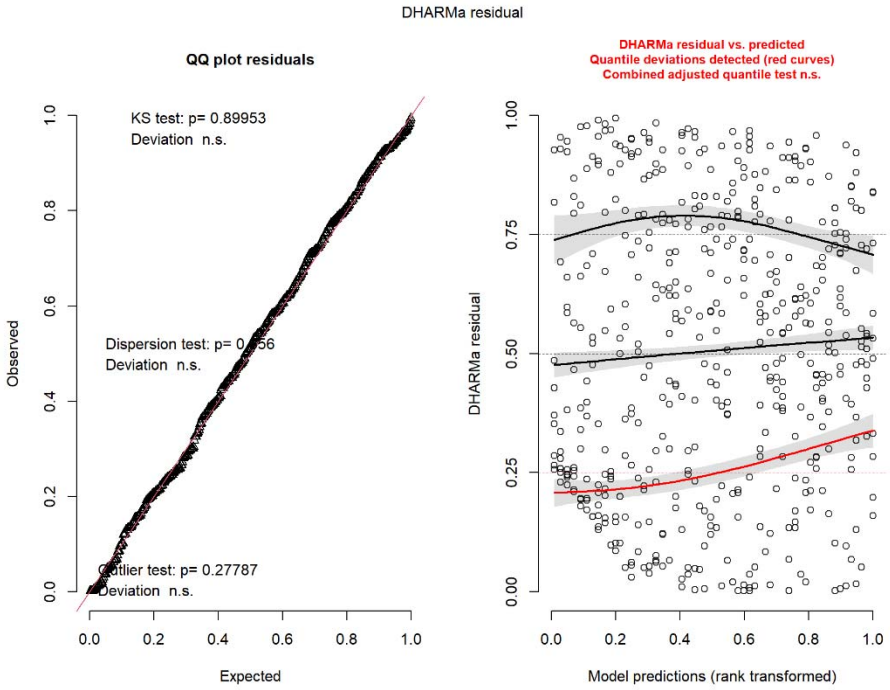
Z. Entrances per Meter Percent Difference From Noncontingent 01 by Sex



AA. Cocaine Infusions in Short Access by Pre-Lever Activity and Sex



BB. Cocaine Infusions in Long Access by Pre-Lever Activity and Sex

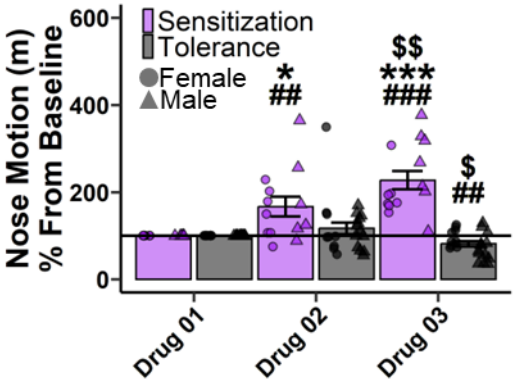


3. Nose Motion Analysis

3.1. Tolerant rats show no evidence of stereotypies during Drug sessions

To assess if rats in the Tolerance group were miscategorized due to stereotypies, nose motion percentage change from Baseline (Drug 01) between behavioral expression groups was analyzed. Following a 0.0002 lambda Box-Cox power transformation, a Type III Wald test found a significant main effect of behavioral expression ($\chi^2(1)=5.77$, $p=.034$), of session number ($\chi^2(1)=9.87$, $p=.005$) and their interaction ($\chi^2(1)=18.95$, $p<.001$). Post hoc analyses revealed rats in the Tolerance group to have significantly decreased their nose motion in Drug 03 compared to both baseline and Drug 02. Conversely, the Sensitization group showed significantly increased nose motion in both Drug 02 and Drug 03 compared to baseline, and Drug 03

greater than Drug 02. Comparatively, the Sensitization group had greater nose motion within Drug 02 and Drug 03 than the Tolerance group (**Fig. S1**). These results demonstrate that the observed effect seen in **Fig. 2C** was not due to stereotypies, and that behavioral expression groups were sufficiently categorized based on their locomotor difference from Drug 01 to Drug 03.



Supplementary Figure 1. Tolerant rats show no evidence of stereotypies during Drug sessions. Nose motion (m) percentage difference from Baseline (Drug 01) grouped by psychomotor behavioral expression. ##*p*<.01, ###*p*<.001 vs Baseline*;* Within group: $*p*<.05, $$*p*<.01 vs Drug 02; Between groups: **p*<.05 vs Tolerance Drug 02; ***p*<.001 vs Tolerance Drug 03.

4. Sex as a Factor Analyses

The following analyses were conducted with sex included as a factor. These specific tests were chosen as they are critical results to the manuscript interpretation. The main focus was the full interaction between factors and reported below.

4.1. No effect of sex on cocaine infusions in Short Access

To determine if sexes differed in cocaine infusions during Short Access, a Type III Wald test was performed. Results showed no significant interaction between sex and session number ($\chi^2(9)=14.3$, $p=.233$). These results show that cocaine intake did not differ between sexes in Short Access.

4.2. No effect of sex on cocaine infusions in Long Access

To determine if sexes differed in cocaine infusions during Long Access, a Type III Wald test was performed. Results showed no significant interaction between sex and session number ($\chi^2(13)=16.77$, $p=.583$). These results show that cocaine intake did not differ between sexes in Long Access.

4.3. No effect of sex on locomotion percent difference from baseline during Noncontingent sessions

To determine if sexes differed in their locomotion perfect difference from baseline during Noncontingent sessions, a Type III Wald test was performed. Results showed no significant interaction between sex and session number ($\chi^2(5)=10.72$, $p=.159$). These results show that locomotion percent difference from baseline did not differ between sexes in Noncontingent sessions.

4.4. No effect of sex x behavioral expression on cocaine infusions across Short Access sessions

To determine if a sex x behavioral expression x session number interaction had an effect on cocaine infusions during Short Access, a Type III Wald test was performed. Results showed the interaction was not significant ($\chi^2(9)=14.31$, $p=.607$), indicating that

cocaine intake did not differ between sexes as a function of behavioral expression across Short Access sessions.

4.5. No effect of sex x behavioral expression on cocaine infusions across Long Access sessions

To determine if a sex x behavioral expression x session number interaction had an effect on cocaine infusions during Long Access, a Type III Wald test was performed. Results showed no significant interaction effect ($\chi^2(13)=9.48$, $p=.736$), indicating that cocaine intake did not differ between sexes as a function of behavioral expression across Long Access sessions.

4.6. No effect of sex x session type x abstinence on active lever entrances per meter pre-lever

To determine if a sex x session type x abstinence interaction had an effect on active lever entrances per meter pre-lever, a Type III Wald test was performed. Results showed no significant interaction effect ($\chi^2(2)=3.96$, $p=.138$), indicating that active lever entrances per meter did not differ between sexes as a function of session type and abstinence.

4.7. No effect of sex x levers on entrances per meter percent difference from Noncontingent 01

To determine if a sex x levers interaction had an effect on entrances per meter perfect difference from Noncontingent 01, a Type III Wald test was performed. Results showed no significant interaction effect ($\chi^2(1)=0.05$, $p=1$), indicating that entrances per

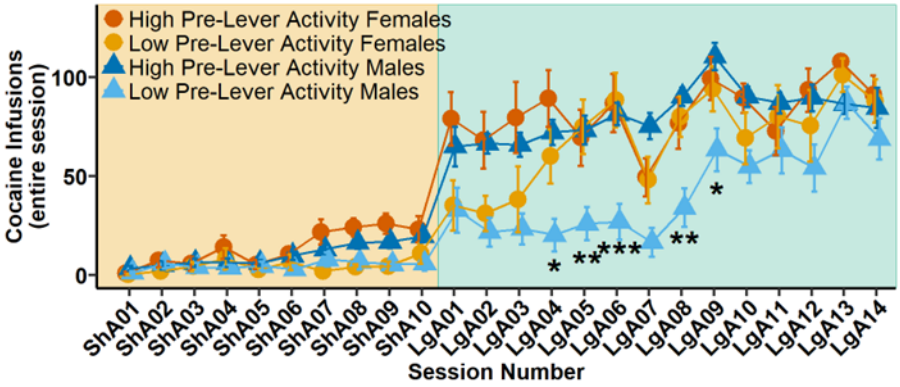
meter percent difference from Noncontingent 01 did not differ between sexes towards either the active or inactive lever during Noncontingent sessions.

4.8. No effect of sex x pre-lever activity group on cocaine infusions across Short Access

To determine if a sex x pre-lever activity group interaction had an effect on cocaine infusions during Short Access, a Type III Wald test was performed. Results showed no significant interaction between sex and pre-lever activity groups ($\chi^2(9)=15.61$, $p=.438$). These results show that cocaine intake did not differ between sexes as a function of pre-lever activity group across Short Access sessions.

4.9. An effect of sex x pre-lever activity group observed on cocaine infusions across Long Access

To determine if a sex x pre-lever activity group interaction had an effect on cocaine infusions during Long Access, a Type III Wald test was performed. Results showed a significant interaction between sex x pre-lever activity group x session number ($F(13,467.25)=2.34$, $p=.023$). Post hoc analyses revealed Low Pre-Lever Activity females to have greater cocaine infusions than Low Pre-Lever Activity males from Long Access 04 to Long Access 09, excluding Long Access 07, suggesting that females are more sensitive to escalation of cocaine intake (**Fig. S2**). These results align with previous reports that females escalate in use before males (de Guglielmo et al., 2024).



Supplementary Figure 2. Low Pre-Lever Activity Female rats receive greater cocaine infusions than Low Pre-Lever Activity Male rats during Long Access. Cocaine infusions across Short and Long Access by sex and pre-lever activity group. Within Low Pre-Lever Activity group: *p<.05, **p<.01, ***p<.001 vs Females.

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