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 repeated use hypersensitizes neural circuits leading to 1) *incentive salience*—drug-related cues become increasingly "wanting" even as “liking" diminishes—and 2) *behavioral sensitization*—a heightened psychomotor response observed primarily in psychostimulants [19]. Both phenomena can contribute to the addiction cycle; however, the degree to which sensitization and tolerance drive

escalation of cocaine self-administration is poorly understood. While several 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 conjunction with machine vision pose-estimation, we assessed multiple behavioral indices to differentiate individual development of tolerance or sensitization and the subsequent influence on cocaine self-administration. These included: 1) locomotion following a single noncontingent intravenous infusion of cocaine at three time points (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) full session; and 6) breakpoint on a progressive ratio schedule. Psychomotor tolerance and sensitization will be assessed by 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

Procedures in sections 2.1-2.3 below have been published elsewhere in more detail [27-28]. ChatGPT4 was utilized for coding assistance and authors take full responsibility for output.

2.1. Subjects

Heterogeneous stock rats (N=37; n=21 males; n=16 females) obtained from the UC San Diego colony [29] were used to mimic human genetic diversity 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. 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

Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in 0.9% sterile saline. During self-administration sessions, a press of the active lever

110 resulted in an intravenous infusion of 0.1ml at 0.5 mg/kg over six seconds by pump,
111 followed by a 20-second timeout where the active lever had no programmed effect.
112 During Noncontingent sessions, a single intravenous infusion of 0.3ml at 1.5 mg/kg (i.e.
113 three times the dose received in self-administration) was delivered in under five
114 seconds in the same operant chamber as self-administration to address influences of
115 environment and intravenous infusion rate on the development of sensitization [30-33].

116

117 **2.3. Self-Administration**

118 Over 8 weeks (Monday-Friday), rats participated in 30 self-administration
119 sessions of various lengths and ratios. Under fixed ratio one, rats participated in 10 two-
120 hour Short Access sessions,15 six-hour Long Access sessions, one one-hour Preshock,
121 and one one-hour Shock session. During Shock, each infusion had a 30% chance to be
122 paired with a footshock (0.3 mA for 0.5 sec) through the grid floor. Under progressive
123 ratio, rats participated in three six-hour sessions in which the response requirements for
124 an infusion followed the equation [5e(injection number × 0.2)] – 5. The breakpoint was
125 defined as the last ratio before a 60-minute period in which a ratio was not completed, in
126 which case the session ended. A complete experimental timeline is shown in **Fig. 1A**.

127

128 **2.4. Noncontingent Sessions**

129 Seven days after surgeries, rats underwent the first noncontingent session
130 (Noncontingent 01) which occurred before Short Access 01. Rats were first video
131 recorded for 30 minutes to establish baseline measurements (Baseline 01), then given

an intravenous saline injection 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.

2.5. Video Recording and Processing

2.5.1. Video Recording

Video recordings were conducted as described previously [34] 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 into the chamber after 15 minutes of recording. Each comma-separated value (csv) file was processed in R version 4.3.1 using RStudio [35-36] 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 1,110 csv files (37 rats over 30 sessions), 26 were excluded 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, post-processing resulted in ~25 minutes per csv. Out of 333 csv files, six files failed to track during Social LEAP Estimates Animal Poses (SLEAP) processing.

2.5.2. SLEAP Modeling

A pose-estimation model was trained using SLEAP on ~5200 labeled frames over 89 videos utilizing the top-down configuration and a unet backbone [37]. Thirteen nodes were tracked: nose, 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 Noncontingent 01 as rats were mistakenly not plugged into their catheter lines. This model focused on six nodes to accelerate training: nose, ears, neck, base, and catheter as the anchor node, utilizing ~2200 labeled frames from 40 videos.

2.5.3. High Throughput Computing on Open Science Grid

Video recordings were processed on the OSG Consortium supercomputer system [38-41]. A python script with SLEAP command line 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 inference registered as (x, y) coordinates were interpolated to fill missing data points (https://sleap.ai/notebooks/Analysis_examples.html).

2.6. Dependent and Independent Variables

Statistical calculations were conducted using RStudio. Euclidian distances calculated movement of the catheter (locomotion) and the nose (nose motion) nodes

175 frame-by-frame and converted from pixels to centimeters using Image2 [42] and the
176 known measurements of the ENV-005 grid. Given the barrel distortion of a fisheye lens,
177 the conversion factor was computed by averaging two length measurements (left and
178 right) and three width measurements (top, middle, and bottom), resulting in
179 ~0.111cm/px. Locomotion variables are the total distances traveled smoothed by a
180 window of 10 (10 FPS, i.e., 0.33 seconds) and converted to meters. Locomotion (m)
181 pre-lever is the distance traveled during the 15 minutes before the levers are extended
182 into the chamber. Active and inactive lever entrances were calculated by producing a
183 bounding box around the lever nodes (x,y) at 30 +/- x and 18 +/- y, and counting each
184 entrance of the nose node into the lever areas. Active and inactive lever entrances per
185 meter are the total entrances normalized by locomotion. Cocaine infusions are the total
186 infusions delivered during the session, and cocaine infusions first 15 minutes are those
187 that occurred during the video recordings. Session corresponds to an individual session
188 (e.g. Short Access 01). Session type refers to experimental phase (e.g. Long Access).
189 Abstinence refers to the time between the end of one session and the beginning of the
190 next, where 18 Hours translates to consecutive days, 66 Hours to a two-day weekend,
191 and 90 Hours to a three-day weekend. Pre-lever variables were examined by
192 abstinence to test for effects that could facilitate sensitization [6,14-17,20,30-34,43-44].
193 Analyses with sex as a factor were performed and reported in the Supplementary
194 Material as most effects were not sex dependent.

195

196 **2.7. Statistical Analyses**

Statistical comparisons were made by fitting a linear mixed-effects model utilizing the lmerTest package [45-46] or a generalized linear mixed-effects model utilizing the glmmTMB package [47] in R. Given that linear and generalized linear models are inherently robust to moderate deviations from normality and extreme values, outliers for all dependent variables were winsorized at the 95th percentile to minimize their influence while retaining potentially meaningful behavioral variability. Inferential tests of fixed effects for linear 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 [48], while generalized linear models a Type III Wald chi-square analysis of deviance was conducted (Type III Wald test). Model assumption tests and fit were analyzed with the simulateResdiduals() function from the DHARMa package [49]. The model fitting process and chosen models of each analysis can be found in the Supplementary Material with best adherence to suggestions previously published [50] 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 [51]. Post hoc analyses were conducted using contrasts(), while direct baseline comparisons used test(), both within the emmeans package and calculate the pairwise comparisons of the estimated marginal means and are therefore model-derived ratios or mean differences [52]. 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 [53]. 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() [54]. Plots represent the raw average values or percent difference from baseline.

3. Results

3.1. Cocaine Infusions

3.1.1. 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 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 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 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, 13, and 14 showed significantly elevated infusions (**Fig. 1C**). These results indicate that escalation of cocaine intake during the first 15 minutes emerged at the end of Short Access and continued throughout Long Access.

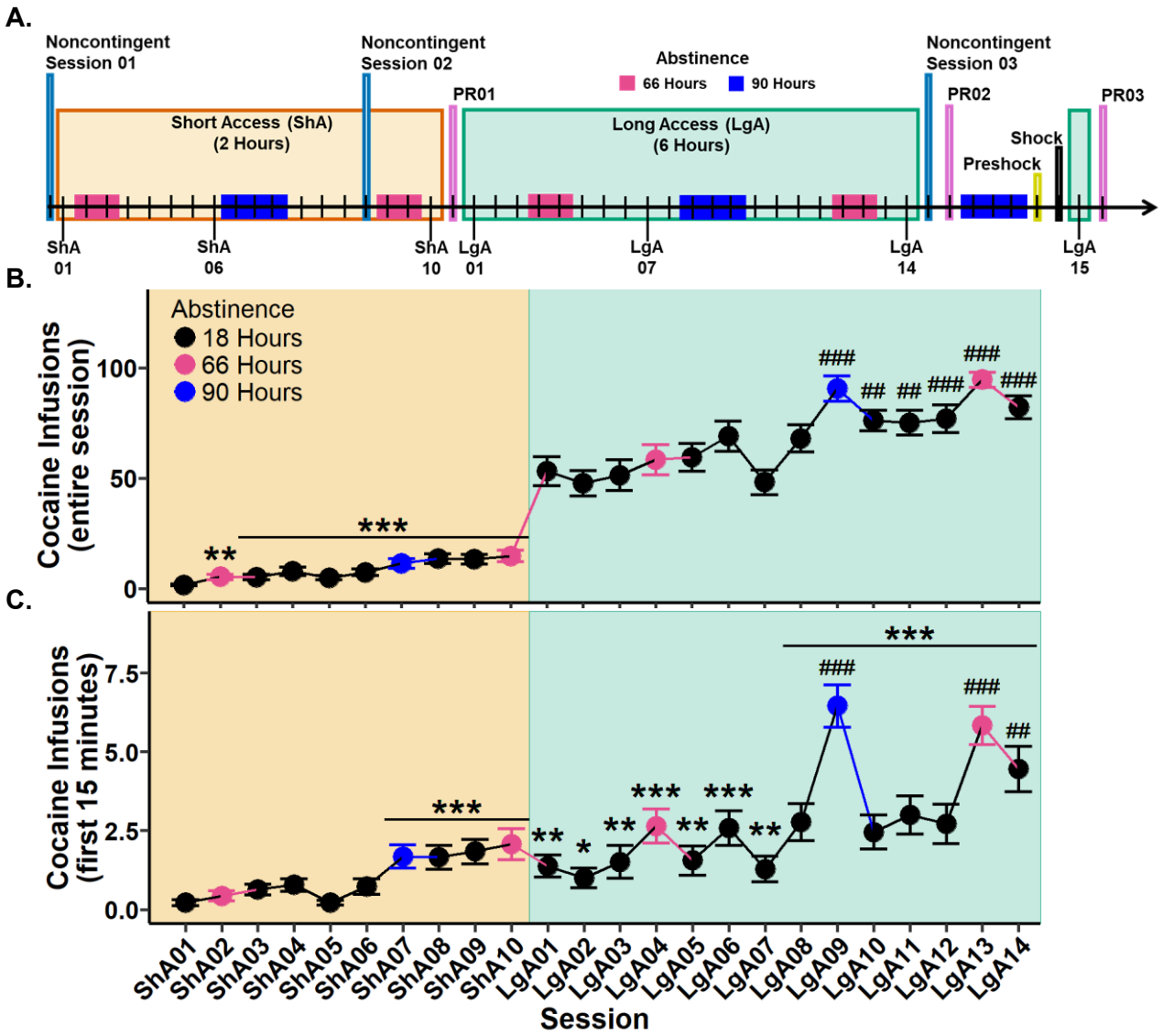


Figure 1. Experimental timeline and cocaine infusions during Short (ShA) and Long Access (LgA). (A.) Experimental timeline with 66 hours (pink) and 90 hours (blue) indicating instances of abstinence. **(B.)** Cocaine infusions during the entire session. **(C.)** Cocaine infusions during the first 15 minutes of the session. Plots represent means and SEM. **p*<.05, ***p*<.01, ****p*<.001 vs. Short Access 01; ##*p*<.01, ##*p*<.001 vs. Long Access 01.

256 **3.2. Locomotion During Noncontingent Sessions**

257 *3.2.1. Noncontingent cocaine administration increases locomotion with no evidence of*
258 *psychomotor sensitization or tolerance following Short or Long Access cocaine*
259 *self-administration.*

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

266 To assess the change in the locomotor response to noncontingent cocaine
267 administration, data was analyzed as percent difference from each rat's respective
268 Baseline session. A Type III ANOVA revealed a significant main effect of session
269 ($F(5,176.97)=16.36$, $p<.001$). Post hoc analyses revealed that Drug sessions were
270 significantly greater than their respective Saline and Baseline sessions, but no
271 significant differences between Drug sessions (**Fig. 2B**). These results show that
272 locomotion did not differ between Noncontingent sessions following noncontingent
273 administration of cocaine at the population level.

274

275 *3.2.2. Rats differentially develop psychomotor sensitization or tolerance after a history*
276 *of cocaine self-administration*

To assess each individual rat's response to 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". Locomotion between Drug sessions 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 ($\chi^2(1)=6.47$, $p=.031$) and their interaction ($\chi^2(1)=15.2$, $p<.001$). Post hoc analyses revealed that Tolerant rats significantly decreased in locomotion in Drug 02, with a further decrease in Drug 03. Conversely, Sensitized rats increased in Drug 03 and were greater than Tolerant rats in both Drug 02 and 03. These results demonstrate divergent profiles in the psychomotor response to cocaine that begins after just nine Short Access sessions and continues 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 influenced cocaine self-administration, a Type III Wald test was performed for both Short and Long Access. During Short Access, a main effect of session was found ($\chi^2(9)=64.36$, $p<.001$), but not 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 was found ($\chi^2(13)=107.44$, $p<.001$), but not 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

300 psychomotor sensitization or tolerance to the effect of cocaine has no effect on cocaine
301 intake during Short or Long Access (**Fig. 2D**).

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

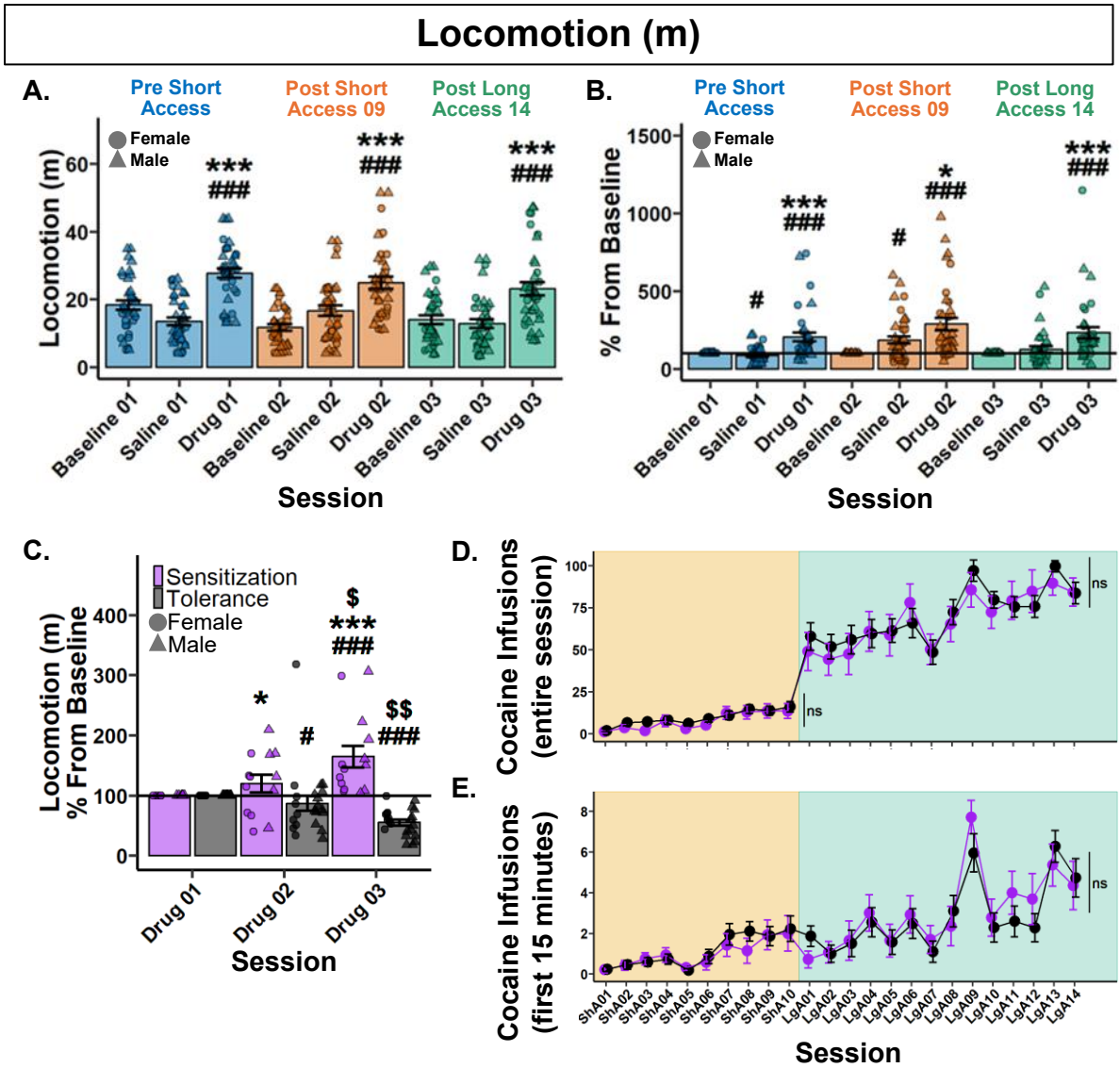


Figure 2. Psychomotor sensitization and tolerance show no effect on cocaine self-administration. (A.) Raw locomotion (m) across Noncontingent sessions. ###*p*<.001 vs Baseline; ***p<.001 vs Saline. **(B.)** Locomotion (m) percent difference from Baseline. #*p*<.05, ###*p*<.001 vs Baseline; *p<.05, ***p<.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 behavioral expression groups: $*p*<.05,

316 $$$p$<.01 vs Drug 02; Between behavioral expression groups: *p<.05 vs Tolerance Drug
317 02, ***p<.001 vs Tolerance Drug 03. **(D-E.)** Cocaine infusions over the entire session
318 (D.) and within the first 15 minutes (E.) grouped by behavioral expression. Plots
319 represent means and SEM.

320

321 **3.3. Pre-Lever Activity**

322 *3.3.1. Locomotion pre-lever increases from Short to Long Access and predicts cocaine*
323 *intake during the first 15 minutes of self-administration*

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

330 To assess the influence of session type and abstinence on locomotion pre-lever,
331 a Type III Wald test was conducted following a 0.28 lambda Box-Cox transformation.
332 Significant main effects of session type ($\chi^2(1)=11.93$, $p=.002$), abstinence ($\chi^2(2)=14$,
333 $p=.002$), and their interaction ($\chi^2(2)=27.57$, $p<.001$) were found. Within Short Access,
334 post hoc analyses revealed locomotion pre-lever at 90 hours was significantly greater
335 than 18 hours. Within Long Access, 66 and 90 hours was significantly greater than 18
336 hours. Interactively, locomotion pre-lever significantly decreased at 18 hours from Short
337 to Long Access. At 66 hours, there was a significant increase from Short to Long

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

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

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

Initial analyses indicated that nose entrances into the active lever area may be predictive of intake. However, there was large individual variability in locomotion. To account for animals with higher general activity having a greater probability of entering the active lever area by chance, active lever area entrances were normalized by locomotion. 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 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.

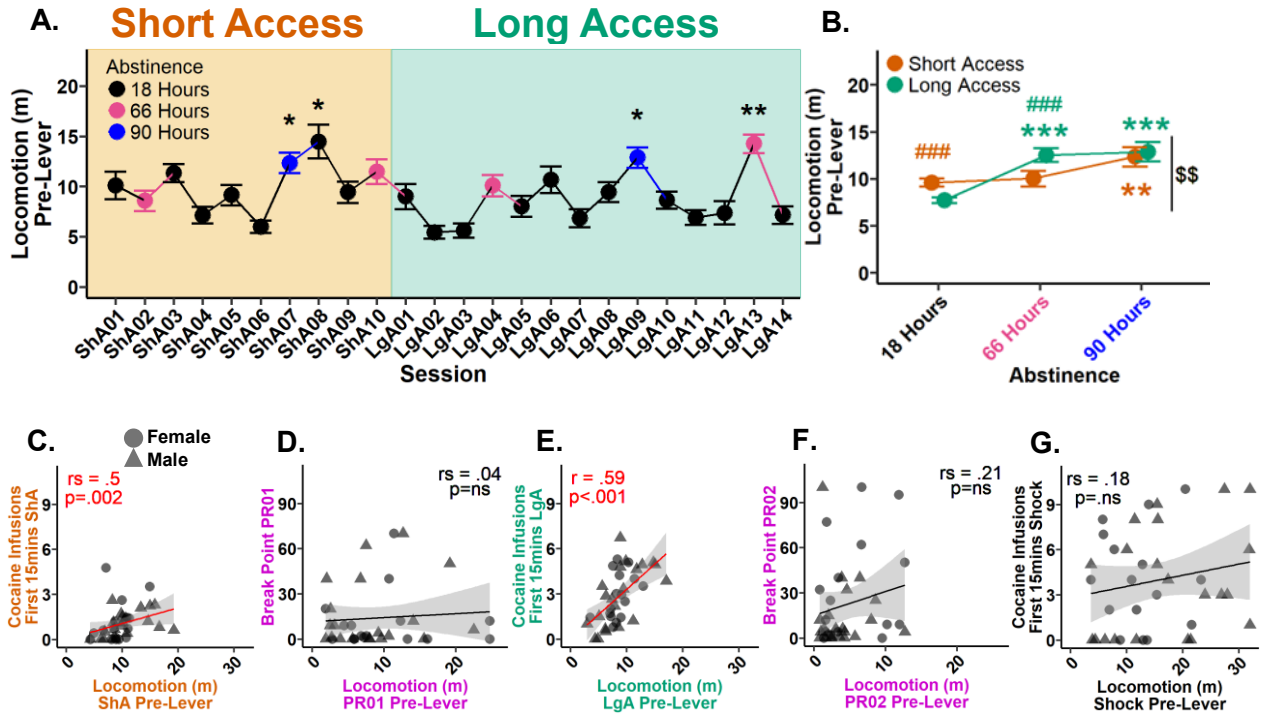
To assess the effects of session type and abstinence on active lever entrances per meter, a Type III Wald test was performed. No effect of abstinence was found ($\chi^2(2)=1.7$, $p=.89$), but a significant main effect of session type ($\chi^2(1)=13.66$, $p<.001$), and their interaction ($\chi^2(2)=17.47$, $p<.001$). 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, particularly 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 ($r_s(5173)=.33$, $p=.046$) (**Fig. 3K**), but not Progressive Ratio 02 ($r_s(7985)=.05$, $p=.753$) (**Fig. 3M**). These results show that active lever entrances per meter pre-lever are

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

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

Locomotion (m) Pre-Lever



Active Lever Entrances per Meter Pre-Lever

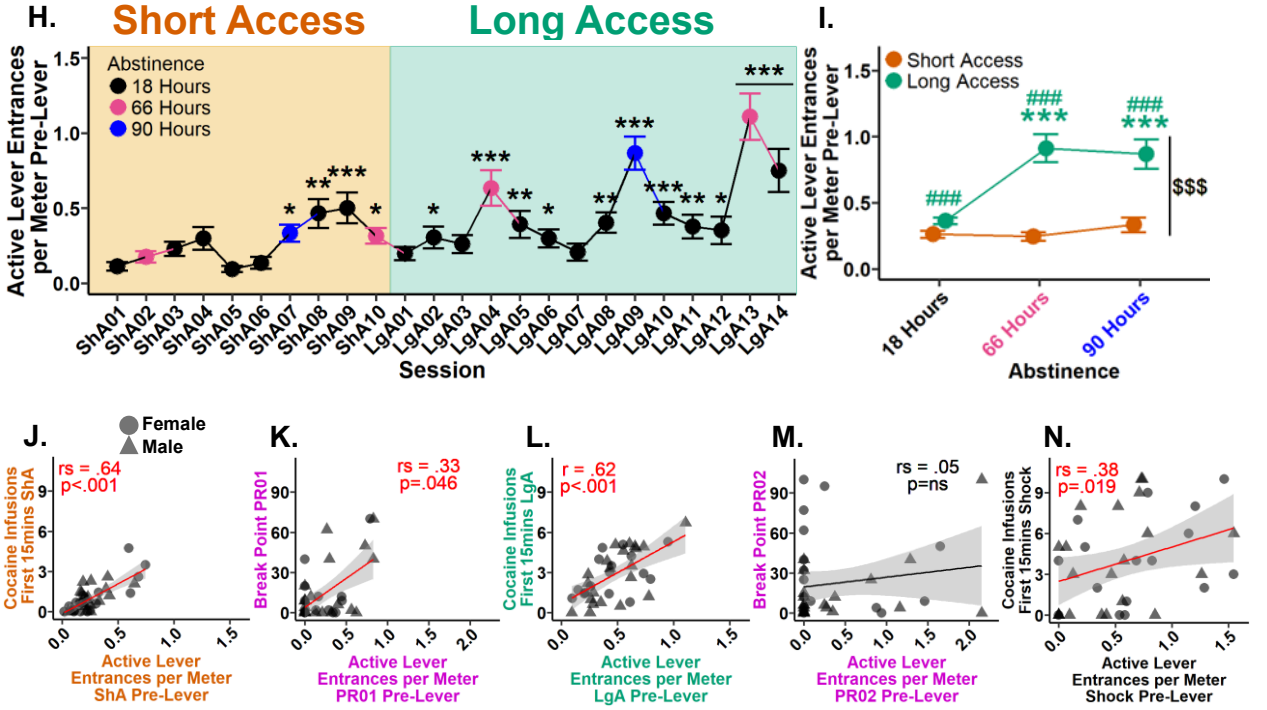


Figure 3. Pre-lever activity increases with Long Access, hours of abstinence, and is predictive of cocaine infusions within the first 15 minutes of self-administration. (A.) Locomotion pre-lever across experiment. *p<.05, **p<.01 vs Short Access 01. **(B.)** Comparison of abstinence levels within and between session types. Within session type: **p<.01, ***p<.001 vs 18 hours; between session type and abstinence: ###p<.001 vs 18 hours Long Access, ###p<.001 vs 66 hours Short Access; between session type: $$$p$<.01 vs Short Access. **(C.)** Mean locomotion pre-lever during Short Access correlates with mean cocaine infusions during first 15 minutes of Short Access. **(D.)** Locomotion pre-lever during Progressive Ratio 01 does not correlate with break point. **(E.)** Mean locomotion pre-lever during Long Access correlates with mean cocaine infusions during first 15 minutes of Long Access. **(F.)** Locomotion pre-lever during Progressive Ratio 02 does not correlate with break point. **(G.)** Locomotion pre-lever during Shock does not correlate with cocaine infusions during first 15 minutes of Shock. **(H.)** Active lever entrances per meter pre-lever across experiment. *p<.05, **p<.01, ***p<.001 vs ShA01. **(I.)** Comparison of abstinence levels within and between session types. Within session type: ***p<.001 vs 18 hours; between session type and abstinence: ###p<.001 vs 18, 66, and 90 hours Short Access; between session type: $$$$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 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 for analyses. Noncontingent 01 was then treated as the Baseline, and Noncontingent 02 and 03 active and inactive lever entrances per meter 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 ($\chi^2(1)=12.46$, $p=.002$), 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 baseline, 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 were plotted with the rounded mean lever entrances. 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 to the active lever side by 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 averaged, where higher scores equate to greater pre-session activity. Following a median z-score split, 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 ($\chi^2(1)=4.81$, $p=.059$), but a significant effect of pre-lever activity ($\chi^2(1)=29.53$, $p<.001$) and their interaction ($\chi^2(1)=9.39$, $p=.006$). Post hoc analyses revealed the High pre-lever activity group had significantly greater active lever entrances per meter in both Noncontingent 02 and 03 than baseline and versus the Low pre-lever activity group within 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.

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

To determine if pre-lever activity during Short Access sessions 07-09 predicts cocaine intake over the entire session in Short Access, a Type III Wald test was performed. A significant main effect was found in session ($\chi^2(9)=125.52$, $p<.001$), but

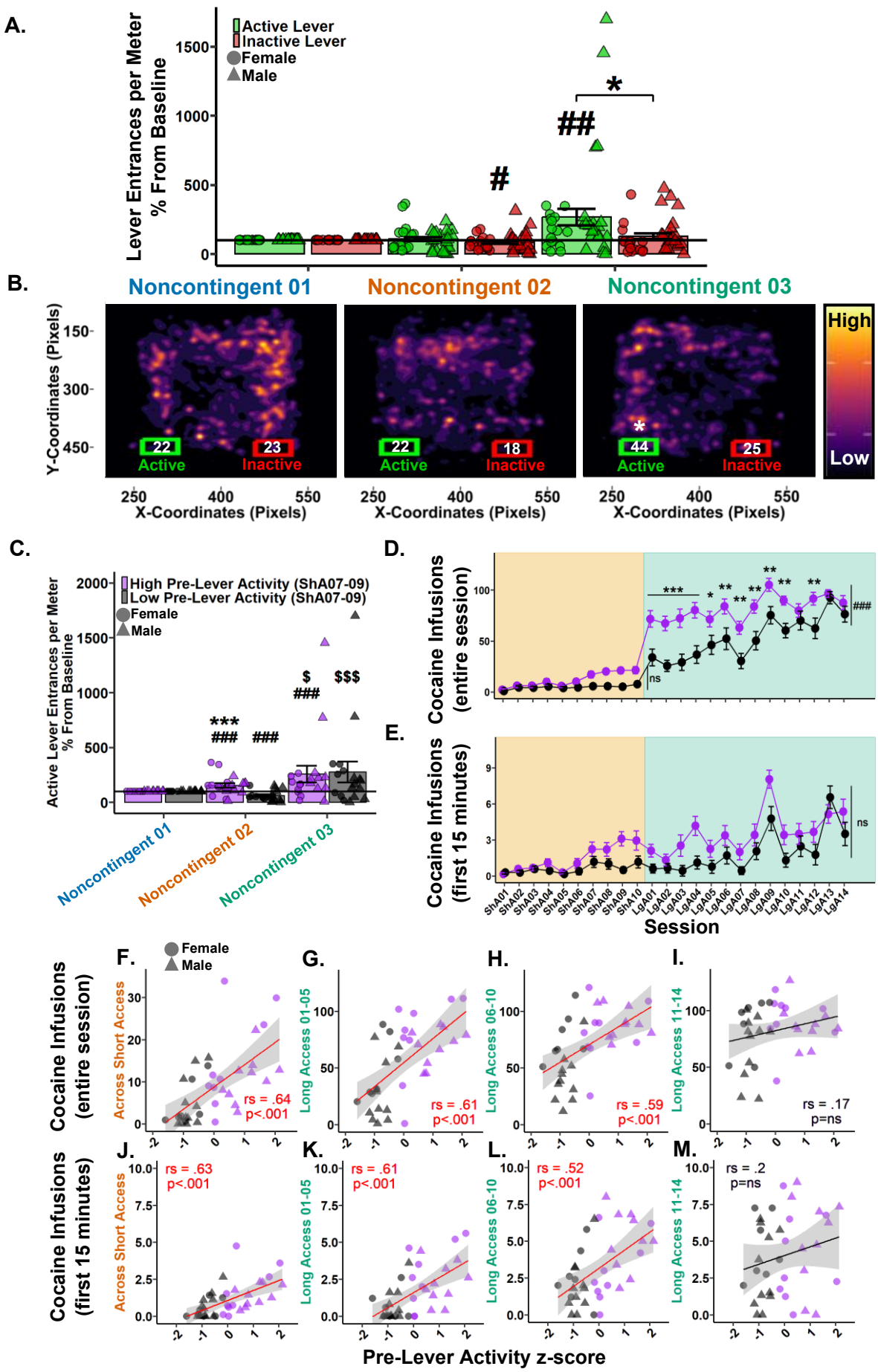
475 not a significant effect of pre-lever activity ($\chi^2(1)=3.51$, $p=.169$) or their interaction
476 ($\chi^2(9)=16.9$, $p=.169$) (**Fig. 4D**). These results show that cocaine intake did not differ
477 between groups in Short Access.

478 To determine if pre-lever activity during Short Access sessions 07-09 predicts
479 cocaine intake over the entire session in Long Access, a Type III Wald test was
480 performed. Significant main effects were found in session ($\chi^2(13)=62.03$, $p<.001$), pre-
481 lever activity ($\chi^2(1)=13.49$, $p<.001$) and their interaction ($\chi^2(13)=28.79$, $p=.015$). Post
482 hoc analyses revealed the High pre-lever activity group to have significantly greater
483 cocaine infusions over the entire session in all Long Access sessions except Long
484 Access 11, 13, and 14 (**Fig. 4D**). These results confirm that pre-lever activity during
485 Short Access 07-09 was initially predictive of cocaine intake during Long Access, but
486 that groups eventually converged.

487 To determine if pre-lever activity during Short Access sessions 07-09 predicts
488 cocaine intake within the first 15 minutes of the session, a Type III Wald test was
489 performed. A significant main effect was found in session ($\chi^2(23)=208.9$, $p<.001$), but
490 not in pre-lever activity ($\chi^2(1)=0.85$, $p=.742$) or their interaction ($\chi^2(23)=35.32$, $p=.134$)
491 (**Fig 4E**). These results suggest that pre-lever activity during Short Access 07-09 is not
492 predictive of loading behavior between groups.

493 Considering the incongruency of the significant association between pre-lever
494 variables and cocaine intake variables (**Fig. 3**), and the previous nonsignificant results
495 between pre-lever activity groups and cocaine infusions in the first 15 minutes (**Fig. 4D**),
496 correlations were performed between cocaine intake variables and pre-lever activity z-
497 scores in Short Access and three separate phases of Long Access. For cocaine

498 infusions during the entire session and pre-lever activity, significant positive correlations
499 were found in Short Access (r_s(2960)=.65, p<.001; **Fig. 4F**), Long Access 01-05 (r-
500 $_s$(3284)=.61, p<.001; **Fig. 4G**), Long Access 06-10 (r_s(3422)=.59, p<.001; **Fig. 4H**), but
501 not during Long Access 11-14 (r_s(7013)=.17, p=.318; **Fig. 4I**). For cocaine infusions
502 during the first 15 minutes and pre-lever activity, significant positive correlations were
503 similarly found in Short Access (r_s(3094)=.63, p<.001; **Fig. 4J**), Long Access 01-05 (r_s
504 (3301)=.61, p<.001; **Fig. 4K**), Long Access 06-10 (r_s(4023)=.52, p<.001; **Fig. 4L**), but
505 not during Long Access 11-14 (r_s(6735)=.2, p=.231; **Fig. 4M**). These results confirm that
506 rats who showed markedly increased pre-lever activity during Short Access sessions
507 07-09 were more likely to consume more cocaine during the first 15 minutes and over
508 the entire session in both Short and Long Access, but converged by the end of Long
509 Access. Furthermore, these results support those shown in **Fig. 4C,** where no
510 significant difference was found in active lever entrances per meter in Noncontingent
511 03, which occurs after 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
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

513 **Figure 4. Pre-lever activity during Short Access 07-09 predicts cocaine intake in**
514 **Short and Long Access and seeking behavior during Noncontingent sessions.**
515 **(A.)** Active (green) and inactive (red) lever entrances per meter as percentage
516 difference from Baseline (Noncontingent 01). #p<.05, ##p<.01 vs Baseline; *p<.05 vs
517 inactive lever. **(B.)** Nose (*x, y)* pixel coordinate heatmaps of all rats within each
518 Noncontingent session in relation to active (green) and inactive (red) Levers. Each plot
519 represents ~109 sessions overlain revealing high and low activity zones. Numbers
520 within lever bounding boxes are the mean sum of active and inactive lever entrances for
521 all rats within each Noncontingent session (Noncontingent 01 active: M=22.2, SD=13.9;
522 inactive: M=23.4, SD=12.3; Noncontingent 02 active: M=21.8, SD=16.1; inactive:
523 M=18.4, SD=16.5; Noncontingent 03 active: M=43.5, SD=42.1; inactive: M=24.7,
524 SD=22.1). *p<.05 vs inactive lever. **(C.)** Active lever entrances per meter percent
525 difference from Baseline (Noncontingent 01) grouped by pre-lever activity z-score
526 during Short Access 07-09. ###p<.001 vs Baseline; Within pre-lever activity group:
527 $$p$<.05, $$$$p$<.001 vs Noncontingent 02; Between pre-lever activity groups: ***p<.001
528 vs Noncontingent 02 Low group. **(D-E.)** Cocaine infusions over the entire session (D.)
529 and within the first 15 minutes (E.) grouped by Short Access 07-09 pre-lever activity z-
530 score. *p<.05, **p<.01, ***p<.001, ###p<.001 vs Low. **(F-I.)** Cocaine infusions over the
531 entire session correlated using Spearman Rank with Short Access 07-09 pre-lever
532 activity z-score in Short Access (F.), Long Access 01-05 (G.), Long Access 06-10 (H.),
533 but not during Long Access 11-14 (I.). **(J-M.)** Cocaine infusions within the first 15
534 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 population-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 in psychomotor sensitization or tolerance expression, though neither influenced 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 session intake. Rats with high pre-lever activity during Short Access sessions 07-09 escalated active lever entrances 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 Short Access 02 and within the first 15 minutes as early as Short Access 07, while exhibiting significant escalation of intake

557 after two weeks of Long Access. These findings align with our previous reports that
558 heterogenous stock rats show increased cocaine intake during Short and Long Access
559 [28], and the larger literature that Long Access induces escalation of intake [3,4,24,55].

560 **4.2. Psychomotor Expression**

561 Population-level analyses revealed locomotion increased following a
562 noncontingent infusion of cocaine, but no differences between Drug sessions. Saline 02
563 was significantly greater than Baseline 02, suggesting an anticipatory conditioned
564 stimulus effect as the last time they were handled as such they received a
565 noncontingent infusion of cocaine (Drug 01). However, the effect is not seen following
566 Long Access, suggesting that this conditioned responding is short-lasting and unlikely to
567 drive escalation of cocaine self-administration. Individually, rats did show differences in
568 the propensity to develop psychomotor sensitization or tolerance to noncontingent
569 cocaine administration. One interpretation is that analyzing only ambulatory activity may
570 misclassify some rats displaying stereotypic behavior, such as head bobbing, weaving,
571 sniffing, or gnawing at high cocaine doses [8,56]. However, this is unlikely for several
572 reasons. First, the dose used (1.5 mg/kg) is known to produce minimal stereotypic
573 behavior and robust sensitization [6,30-32]. Second, the nose motion (a proxy for
574 stereotypic behavior) was not found to increase in Tolerant rats (**Fig. S1**). Third, visual
575 inspection of the videos show that the most tolerant rat (M2677) did not exhibit
576 stereotypic behavior while the most sensitized rat (M2668) showed pronounced
577 psychomotor sensitization. A possible explanation for the difference between sensitized
578 vs. tolerant rats is that sensitized rats consumed less cocaine, while tolerant rats
579 consumed more. However, intake analyses do not support this hypothesis: groups

showed no differences in cocaine intake in Short or Long Access, or within the first 15 minutes. These findings challenge the idea that Short Access produces only sensitization and Long Access only tolerance to the effects of cocaine [1,3-6,18,24,57], and show that neither reliably predicts cocaine intake.

4.3. Pre-Lever Activity Measures

Pre-lever variables locomotion and active lever entrances per meter increased from Short to Long Access and intensified following two-to-three days of abstinence. Similarly, rats showed a significant increase in the active lever area entrances per meter in Noncontingent 03 in comparison to Noncontingent 01, suggesting sensitization of drug-seeking behavior. In contrast to measures of psychomotor sensitization or tolerance, both locomotion and active lever entrances per meter were highly predictive of intake. These findings align with the incentive salience literature, showing that repeated drug use leads to salience attribution to cues and environments that particularly manifests following a period of abstinence [5-6,15-16,19-20,30-32,46-47,58-65].

4.4. Individual Differences in Pre-Lever Activity Measures

To investigate the individual propensity to attribute salience to drug-associated cues, a median z-score of pre-lever activity was calculated. Short Access sessions 07–09 were selected as they occurred within the same week (no abstinence periods) and were the first sessions in which both pre-lever variables and infusions within the first 15 minutes reached significance (**Fig. 1C, 3A, & 3H**). Comparisons between High and Low pre-lever activity groups revealed that high-activity rats exhibited an early escalation of

602 cocaine intake during Long Access, but ultimately both pre-lever activity groups reached
603 a ceiling level of intake after two weeks when groups no longer differed.

604 One possible explanation is that Low pre-lever activity rats in Short Access were
605 slower in developing incentive salience, and once expressed, escalated their intake to
606 levels comparable to High pre-lever activity rats. Indeed, the significant difference in
607 active lever entrances per meter between pre-lever activity groups in Noncontingent 02
608 and its absence in Noncontingent 03 supports this interpretation. These findings align
609 with previous reports that individuals differ in their susceptibility to attribute incentive
610 salience, which may serve as an early predictor of vulnerability [56,59,62-63].
611 Furthermore, they suggest that incentive salience is a catalyst of escalation, contrary to
612 previous findings that incentive salience and escalation are dissociable [26].

613 Another interpretation is that escalating pre-lever activity during Long Access
614 reflects the combined influence of allostatic load [1,3,55,57], incentive habit formation
615 [66-68], and the incubation of craving [69-70]. Allostasis theory predicts that repeated
616 use progressively recruits stress systems and negative affect, shifting the hedonic set
617 point and motivating drug seeking to alleviate withdrawal-related distress. Consistent
618 with this view, both animal and human studies show that acute withdrawal, withdrawal-
619 conditioned cues, negative affect, and psychosocial stress increase craving, invigorate
620 drug seeking, and amplify cue-evoked neural activity [1,3,55,57,71-75]. The incentive
621 habit framework suggests that repeated drug-cue pairing transforms early cue reactivity
622 into motivationally charged habitual responses, which may lead to automatic approach
623 toward drug-associated cues even before drug availability [66-68]. Finally, incubation of
624 craving provides an additional, complementary mechanism: periods of abstinence

without obvious negative emotional states enhance the motivational impact of drug cues over time, strengthening cue-triggered approach behavior [69-70]. Follow up studies will be needed to dissect the role of each of these processes.

The high pre-lever activity phenotype we observed shares conceptual parallels with the sign-tracker/goal-tracker framework [59]. Sign-trackers preferentially interact with a discrete cue (the lever CS), while goal-trackers orient to the site of reward delivery. Sign-trackers have been shown to initially self-administer more cocaine [63] and choose cocaine over food [76]. However, important differences exist: sign-tracking is defined in a purely Pavlovian context, with lever pressing having no instrumental 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 [77]. 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 intake with prolonged access even in initially resistant animals.

4.5. Limitations

The present study has a few limitations. One is the lack of a control group without periods of abstinence. A control group participating in successive sessions 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

648 of behavior could result in inaccuracies. However, pose-estimation with SLEAP is
649 remarkably accurate [37,78], and the instance rendered sample videos display precise
650 tracking of the catheter and nose nodes (see supplementary videos).

651 **4.6. Summary**

652 In summary, this report demonstrates that individual differences in psychomotor
653 sensitization or tolerance had no influence on cocaine intake. In contrast, incentive
654 salience during Short Access acts as a catalyst and early predictor of escalation in
655 cocaine self-administration during the first two weeks of Long Access, but does not
656 predict established escalation of cocaine intake after three weeks of Long Access.
657 Moreover, individuals initially resistant to incentive salience can, with sufficient
658 exposure, become sensitized and escalate cocaine use to the same level as more
659 susceptible individuals. Expression of incentive salience sensitization as reflected in
660 pre-lever activity measures and their subsequent influence on cocaine intake supports
661 the idea that incentive salience and psychomotor sensitization are mediated by distinct
662 mechanisms [6-7,21,31]. The development of the incentive salience profiles is
663 accelerated by periods of abstinence (weekend), which allows for incubation of craving,
664 a progressive increase of craving during early days of abstinence that can remain
665 elevated following prolonged abstinence [44,69-70]. The measure of approach toward
666 the active lever area using video recording before the start of the self-administration
667 session may represent a novel behavioral marker of addiction vulnerability that can
668 precisely track the motivation for the drug with high accuracy without using any operant
669 measures, the drug itself, or any other training or conditioning that could interfere with
670 the drug self-administration. Such a measure may also be useful for medication

development to allow dissociation between drug-dependent and drug-independent effects of experimental medication on drug seeking and taking.

Data Availability Statement

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

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

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Competing Interests

Authors declare no competing interests.

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