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, noncontingent methods, and pose-estimation, we demonstrate 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 occurring before the self-administration session began (pre-lever) during Short Access was found to predict intake during Long Access. 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]. Among 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, repeated drug use leads to tolerance to the positive reinforcing effects of drugs and 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 *incentive salience*—drug-related cues become increasingly "wanting" even as the “liking" diminishes—and to *behavioral sensitization*—a heightened psychomotor response observed primarily in psychostimulants like amphetamines and cocaine [19]. There is no doubt 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; (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; (4) cocaine intake within the first 15 minutes and (5) across the full session; and (6) breakpoint on a progressive ratio schedule.

2. Materials and Methods

A more detailed account of sections 2.1-2.3 below has been published elsewhere [27-28].

2.1. Subjects

88 Heterogeneous stock rats (N=37; n=21 males; n=16 females) were obtained from
89 the from the University of California, San Diego colony [29] and housed in pairs on a 12-
90 hour light/dark cycle. Rats were anesthetized with 1–5% isoflurane for jugular vein
91 catheterization using Micro-Renathane tubing (Braintree Scientific) connected to a guide
92 cannula that exits through the back and secured with mesh and dental acrylic. Post-
93 surgery, rats received flunixin subcutaneously (2.5 mg/kg) and cefazolin intramuscularly
94 (330 mg/kg). Catheters were flushed daily with heparinized saline (10 U/ml, American
95 Pharmaceutical Partners) mixed with 0.9% bacteriostatic sodium chloride (Hospira) and
96 cefazolin (52.4 mg/0.2 ml). Catheter patency was tested intravenously with brevital
97 sodium (1.5 mg, American Pharmaceutical Partners) at experiment start and end, with
98 failing rats removed from the experiment. Rats analyzed demonstrated catheter
99 patency. All procedures were conducted in strict adherence to the National Institutes of
100 Health Guide for the Care and Use of Laboratory Animals and were approved by the
101 Institutional Animal Care and Use Committees of UC San Diego.

102

103 **2.2. Drugs**

104 Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) is dissolved in
105 0.9% sterile saline. During self-administration sessions, a press of the active lever
106 resulted in an intravenous infusion of 0.1ml at 0.5 mg/kg over six seconds by pump,
107 followed by a 20-second timeout where the active lever is deactivated. During
108 Noncontingent sessions, a single intravenous infusion of 0.3ml at 1.5 mg/kg (i.e. three
109 times received in self-administration) was delivered in under five seconds. Intravenous
110 injections were chosen to address the differences in absorption, clearance, and blood

concentrations between intravenous and intraperitoneal methods and the influence of intravenous infusion rate on the development of sensitization [30-36].

2.3. Intravenous Self-Administration

Over 8 weeks (Monday-Friday), rats participated in 30 self-administration sessions with various lengths and ratios. Under fixed ratio one, rats participated in 10 two-hour Short Access sessions,15 six-hour Long Access sessions, and one-hour PRESHOCK and SHOCK sessions. During SHOCK, rats had a 30% chance to have an infusion paired with a footshock (0.3 mA for 0.5 sec) through the grid floor. Under progressive ratio, rats participated in three six-hour sessions in which infusions followed the equation [5e(injection number × 0.2)] – 5. The 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). 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 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 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 SLEAP processing.

2.5.2. Social LEAP Estimates Animal Poses (SLEAP) Modeling

A pose-estimation model was trained using SLEAP [38] on ~5200 labeled frames over 89 videos from past cohorts utilizing the top-down configuration and a unet backbone. 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 code 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 RStudio version 4.3.1. 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 [43] 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). Deprivation 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 as a two-day weekend, and 90 Hours as a three-day weekend. Pre-lever variables were examined by deprivation to account for abstinence effects that could facilitate sensitization [6,14-17,20,33-35].

2.7. Statistical Analyses

Statistical comparisons were made by fitting a linear mixed effects model utilizing the lmerTest package [44-45] or a generalized linear mixed effects model utilizing the

198 glmmTMB package [46] in R. Given that linear and generalized linear mixed-effects
199 models are inherently robust to moderate deviations from normality and extreme values,
200 outliers for all variables were winsorized at the 95th percentile to minimize their
201 influence while retaining potentially meaningful behavioral variability. Inferential tests of
202 fixed effects for linear mixed models were conducted using Type III ANOVA with
203 Satterthwaite degrees of freedom approximations (Type III ANOVA test) which have
204 been shown to control for Type I errors in hierarchical models [47], while for generalized
205 linear models a Type III Wald chi-square analysis of deviance was conducted (Type III
206 Wald test). Tests for model assumptions and fit were analyzed with the
207 simulateResdiduals() function from the DHARMa package [48]. The model fitting
208 process and chosen models of each analysis can be found in the Supplementary
209 Material with best adherence to suggestions previously published [49] but prioritizing the
210 results of DHARMa tests. Transformations of response variables where necessary were
211 achieved using Box-Cox or Yeo-Johnson after identifying the most effective lambda
212 using powerTransform() from the car package and reported at two decimal places [50].
213 Post-hoc analyses of all significance results were conducted using contrasts(), while
214 direct comparisons to baseline used test(), both within the emmeans package and
215 calculate the pairwise comparisons of the estimated marginal means of mixed effects
216 models and are therefore model-derived ratios or mean differences [51]. All *p*-values
217 were adjusted by the Benjamini-Yekutieli (BY) method due to its consideration of
218 dependence and correlations in nested data such as mixed models [52]. From the
219 rstatix package, Student's t-tests were conducted with t_test() with Bonferroni
220 adjustments and correlations with cor_test() by either Pearson or Spearman Rank as

221 determined by a normality test with shapiro_test() [53]. Plots represent the raw or
222 percent difference from baseline.

223

224 **3. Results**

225 **3.1. Cocaine Infusions**

226 *3.1.1. Total cocaine infusions escalate during both Short and Long Access*

227 To determine whether cocaine intake changed during Short Access, a Type III
228 Wald test was performed. A significant main effect of session number was observed
229 ($\chi^2(9)=100.94$, $p<.001$). Post-hoc analyses revealed all sessions to have significantly
230 more cocaine infusions in comparison to Short Access 01. These results confirm that
231 cocaine intake increased as early as Short Access 02 (**Fig. 1B**).

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

238

239 *3.1.2. Cocaine infusions during the first 15 minutes escalate across sessions*

240 To evaluate whether cocaine infusions during the initial 15 minutes of each
241 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 deprivation.

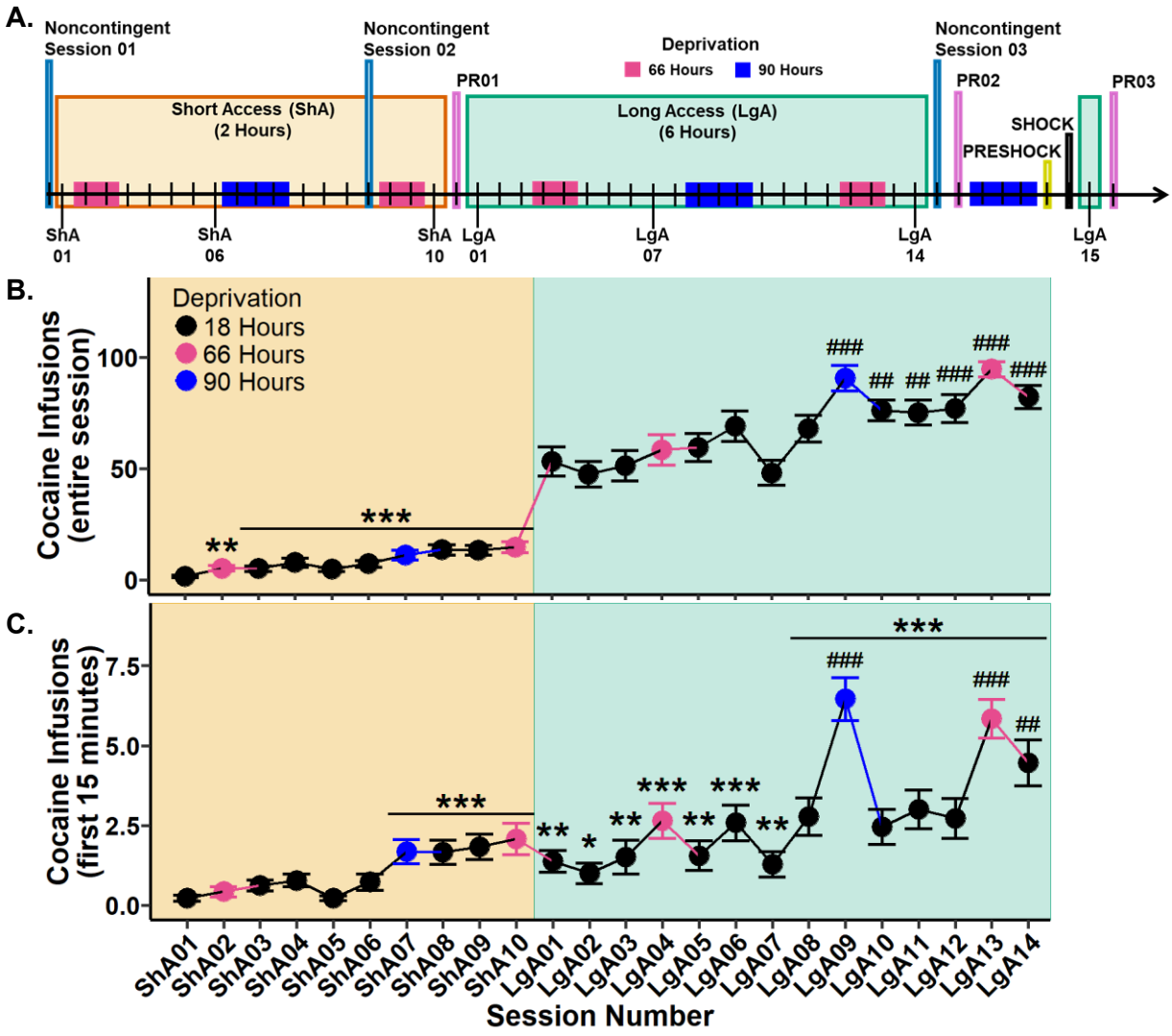


Figure 1. Experimental timeline and cocaine infusions during Short (ShA) and Long Access (LgA). (A.) Experimental timeline with 66 hours (pink) and 90 hours (blue) indicating instances of deprivation. **(B.)** Cocaine infusions during the entire session. **(C.)** Cocaine infusions during the first 15 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 revealed significant differences only within Noncontingent sessions, as each Drug session 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**). 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 Noncontingent 02, with a further decrease in Noncontingent 03. Similarly, although not significantly increased from baseline, rats in the Sensitization group showed greater locomotion in Noncontingent 02 than the Tolerance group that progressed further in Noncontingent 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**).

295

296 3.2.3. *Psychomotor sensitization and tolerance show no effect on cocaine self-*
297 *administration*

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

307 To determine if the development of psychomotor sensitization or tolerance has an
308 effect on cocaine infusions in the first 15 minutes, a Type III Wald test was performed. A
309 main effect of session number was found ($\chi^2(23)=154.13$, $p<.001$), but not of behavioral
310 expression ($\chi^2(1)=0.003$, $p=1$), or their interaction ($\chi^2(23)=30.78$, $p=.356$). These results
311 confirm that the development of psychomotor sensitization or tolerance to the effect of
312 cocaine has no effect on the loading behavior during cocaine self-administration (**Fig.**
313 **2E**).

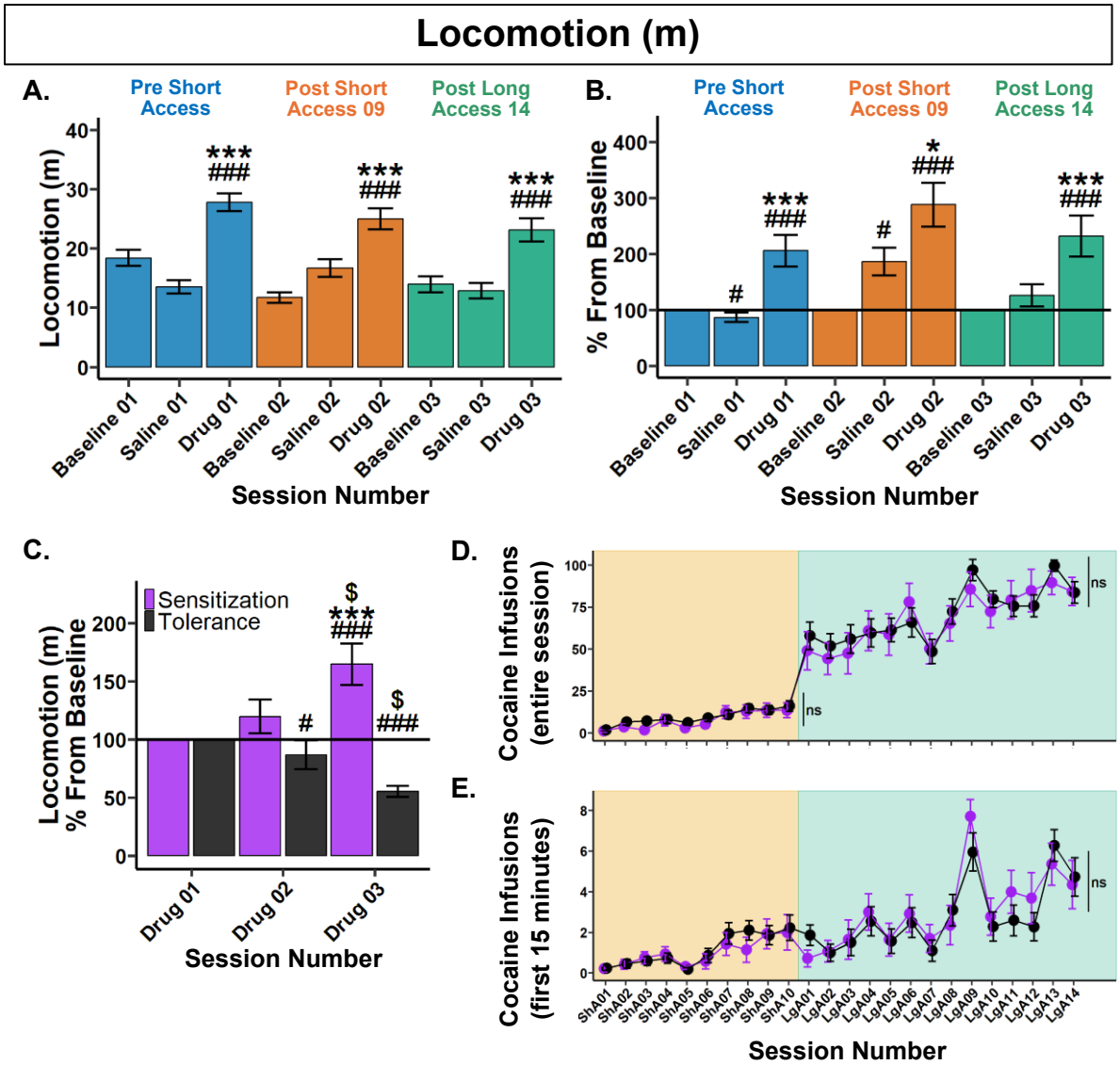


Figure 2. Psychomotor sensitization and tolerance show no effect on cocaine self-administration. (A.) Raw locomotion (m) across Noncontingent sessions. ###*p*<.001 vs Baseline; ***p<.001 vs Saline. **(B.)** Locomotion (m) percent difference from Baseline. #*p*<.05, ##*p*<.01, ###*p*<.001 vs Baseline; *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 deprivation 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 ($\chi^2(1)=11.93$, $p=.002$), deprivation ($\chi^2(2)=14$, $p=.002$), 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

344 significantly greater in Long Access than in Short Access across deprivation (**Fig. 3B**).
345 These results show that locomotion pre-lever increases with drug history and hours of
346 deprivation.

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

358

359 *3.3.2. Active lever entrances per meter pre-lever escalate from Short to Long Access*
360 *and predict cocaine intake during the first 15 minutes of self-administration*

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

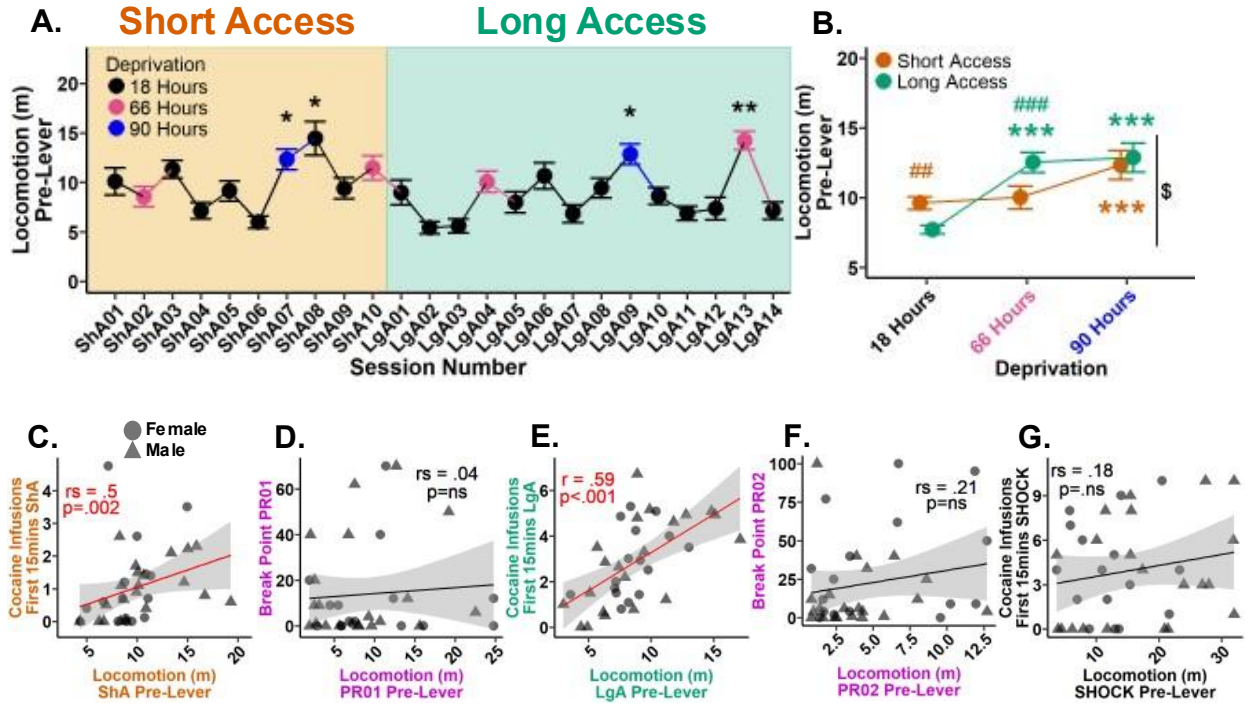
To determine if active lever entrances per meter increased over the experiment, a Type III Wald test was performed. A significant main effect of session number was found ($\chi^2(23)=234.44$, $p<.001$). Post-hoc comparisons with Short Access 01 revealed significant increases in all sessions from Short 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 deprivation on active lever entrances per meter, a separate Type III Wald test was performed. No effect of deprivation 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 deprivation 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 deprivation 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_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 predictive of both cocaine intake within the first 15 minutes and motivation measured as break point following Short Access, but not after Long Access.

Locomotion (m) Pre-Lever



Active Lever Entrances per Meter Pre-Lever

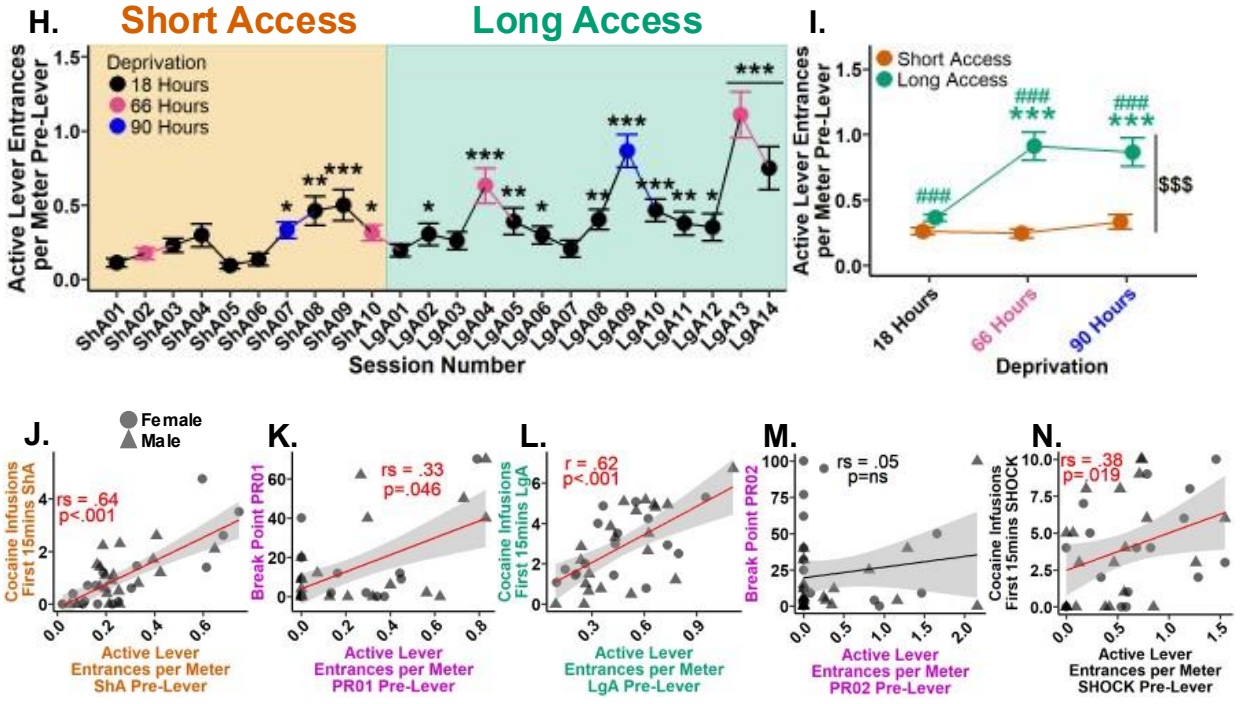


Figure 3. Pre-lever activity increases with Long Access, hours of deprivation, and is predictive of cocaine infusions within the first 15 minutes of self-administration. (A.) Locomotion pre-lever across experiment. *p<.05, **p<.01 vs Short Access 01. **(B.)** Comparison of deprivation levels within and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

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

445 To visualize the development of interest in the active lever, density heat maps of
446 each rat's nose node coordinates (*x*, *y*) for all sessions within each Noncontingent
447 session were overlain. Bounding boxes corresponding to the active and inactive lever
448 areas and the rounded mean lever entrances into each lever area were also plotted
449 (Noncontingent 01 active: *M*=22.2, *SD*=13.9; inactive: *M*=23.4, *SD*=12.3; Noncontingent
450 02 active: *M*=21.8, *SD*=16.1; inactive: *M*=18.4, *SD*=16.5; Noncontingent 03 active:
451 *M*=43.5, *SD*=42.1; inactive: *M*=24.7, *SD*=22.1). Student's t-tests between active and
452 inactive lever entrances within Noncontingent sessions revealed only Noncontingent 03
453 following a 0.23 lambda Yeo-Johnson power transformation achieved significance, with
454 active greater than inactive (**Fig. 4B**). These results highlight the development of
455 interest in the active lever after a history or self-administration as the high activity
456 regions migrate from the inactive side of the chamber in Noncontingent 01 to the active
457 side in Noncontingent 03.

458

459 *3.4.2. Pre-lever activity during Short Access sessions 07-09 predicts active lever*
460 *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. 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.

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 number ($\chi^2(9)=125.52$,

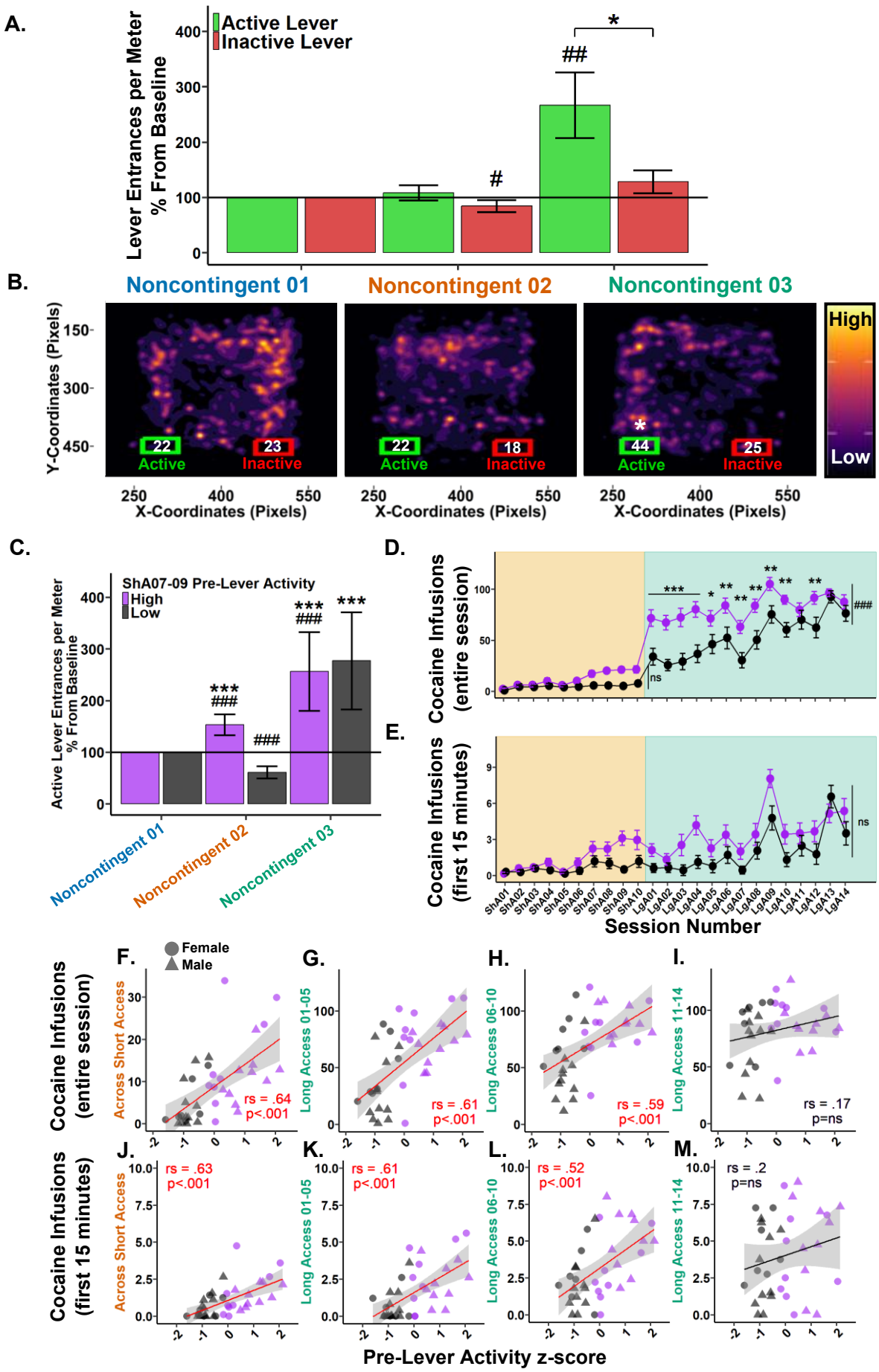
484 $p<.001$), but not a significant effect of pre-lever activity score ($\chi^2(1)=3.51$, $p=.169$) or
485 their interaction ($\chi^2(9)=16.9$, $p=.169$) (**Fig. 4D**). These results show that cocaine intake
486 did not differ between groups in Short Access.

487 To determine if pre-lever activity during Short Access sessions 07-09 predicts
488 cocaine intake over the entire session in Long Access, a Type III Wald test was
489 performed. Significant main effects were found in session number ($\chi^2(13)=62.03$,
490 $p<.001$), pre-lever activity score ($\chi^2(1)=13.49$, $p<.001$) and their interaction
491 ($\chi^2(13)=28.79$, $p=.015$). Post-hoc analyses revealed the High pre-lever activity group to
492 have significantly greater cocaine infusions over the entire session in all Long Access
493 sessions except Long Access 11, 13, and 14 (**Fig. 4D**). These results confirm that pre-
494 lever activity during Short Access 07-09 was initially predictive of cocaine intake during
495 Long Access, but that groups eventually converged.

496 To determine if pre-lever activity during Short Access sessions 07-09 predicts
497 cocaine intake within the first 15 minutes of the session, a Type III Wald test was
498 performed. A significant main effect was found in session number ($\chi^2(23)=208.9$,
499 $p<.001$), but not in pre-lever activity score ($\chi^2(1)=0.85$, $p=.742$) or their interaction
500 ($\chi^2(23)=35.32$, $p=.134$) (**Fig 4E**). These results suggest that pre-lever activity during
501 Short Access 07-09 is not predictive of loading behavior between groups.

502 Considering the incongruency of the significant association between pre-lever
503 variables and cocaine intake variables (**Fig. 3**), and the previous nonsignificant results
504 between pre-lever activity groups and cocaine infusions in the first 15 minutes (**Fig. 4D**),
505 correlations were performed between cocaine intake variables and pre-lever activity z-
506 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
Lever Entrances per Meter
% From Baseline
B.
Noncontingent 01
Noncontingent 02
Noncontingent 03
Y-Coordinates (Pixels)
X-Coordinates (Pixels)
Active
Inactive
High
Low
C.
ShA07-09 Pre-Lever Activity
High
Low
Active Lever Entrances per Meter
% From Baseline
D.
Cocaine Infusions
(entire session)
E.
Cocaine Infusions
(first 15 minutes)
Session Number
F.
G.
H.
I.
Female
Male
Cocaine Infusions
(entire session)
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
J.
K.
L.
M.
Cocaine Infusions
(first 15 minutes)
rs = .63
p<.001
rs = .61
p<.001
rs = .52
p<.001
rs = .2
p=ns
Pre-Lever Activity z-score

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

542

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 deprivation, 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.

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 [3,4,24,54].

565 Rats showed individual differences in the propensity to develop psychomotor
566 sensitization or tolerance to noncontingent cocaine administration. One alternative
567 interpretation is that analyzing only ambulatory activity may misclassify some rats
568 displaying stereotypic behavior, including head bobbing and weaving, sniffing or
569 gnawing at high cocaine doses [8,55]. However, this is unlikely for several reasons.
570 First, the dose used (1.5 mg/kg) is known to produce minimal stereotypic behavior and
571 robust sensitization [6,33-35]. Second, the nose motion (a proxy for head bobbing,
572 weaving, sniffing or gnawing) was highly correlated with locomotion ($r_s(606)=.93$,
573 $p<.001$), suggesting very few stereotypic behaviors. Third, visual inspection of the
574 videos show that the most tolerant rat (M2677) did not exhibit stereotypic behavior while
575 the most sensitized rat (M2668) shows pronounced psychomotor sensitization. A
576 possible explanation for the difference between sensitized vs. tolerant rats is that rats
577 classified as sensitized consumed less cocaine, while tolerant rats consumed more.
578 However, intake analyses do not support this hypothesis: the two groups showed no
579 differences in cocaine intake across the Short or Long Access session or within the first
580 15 minutes. These findings challenge the idea that Short Access produces only
581 sensitization and Long Access only tolerance to the effects of cocaine [1,3-6,18,24], and
582 they show that neither tolerance nor sensitization reliably predict future cocaine intake.

583 Pre-lever variables locomotion and active lever entrances per meter increased
584 from Short to Long Access and intensified following two-to-three days of abstinence.
585 Similarly, rats showed a significant increase in the active lever area entrances per meter
586 in Noncontingent 03 in comparison to Noncontingent 01, suggesting sensitization of
587 drug-seeking behavior. In contrast to measures of psychomotor sensitization or

588 tolerance expression, both locomotion and active lever entrances per meter were highly
589 predictive of intake. These findings align with the incentive salience literature, showing
590 that repeated drug use leads to salience attribution to cues and environments that
591 particularly manifests following a period of abstinence [5,6,15-16,19-20,33-35,57-64].

592 To investigate the individual propensity to attribute salience to drug-associated
593 cues, a median z-score of pre-lever activity was calculated. Short Access sessions 07–
594 09 were selected as they occurred within the same week (i.e., no deprivation periods)
595 and were the first sessions in which both pre-lever variables (**Fig. 3A & 3H**) and
596 infusions within the first 15 minutes (**Fig. 1C**) reached significance. Comparisons
597 between High and Low pre-lever activity groups revealed that High pre-lever activity
598 promoted an early escalation of cocaine intake during Long Access, but ultimately both
599 High and Low pre-lever activity groups reached the same level of escalation after two
600 weeks. One possible explanation is that the Low pre-lever activity group in Short Access
601 was slower in developing incentive salience, and once expressed, their intake escalated
602 to levels comparable to the High pre-lever activity group. Indeed, the significant
603 difference in active lever entrances per meter between activity groups in Noncontingent
604 02 and its absence in Noncontingent 03 supports this interpretation. These findings
605 align with previous reports that individuals differ in their susceptibility to incentive
606 salience attribution [55,58,60-61], and that incentive salience may act as a catalyst of
607 escalation and an early predictor of vulnerability [26]. Another interpretation is that
608 negative affect or allostatic load may be driving the incentive salience and escalation in
609 Long Access [1,3,54,56].

The phenotype we observed (rats with high pre-lever activity measured as elevated active lever entrances per meter and locomotion) shares conceptual parallels with the sign-tracker/goal-tracker framework [58]. 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 [62] and choose cocaine over food [65]. 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 [66]. 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.

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 deprivation could further confirm the influence of deprivation on incentive salience incubation. However, noncontingent sessions 02 and 03 did not follow a period of deprivation, 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 [38,67], and the instance

632 rendered sample videos display precise tracking of the catheter and nose nodes (see
633 supplementary videos).

634 In summary, this report demonstrates that individual differences in psychomotor
635 sensitization or tolerance had no influence on cocaine intake. In contrast, sensitization
636 of incentive salience during Short Access acts as a catalyst or early predictor of
637 escalation in cocaine self-administration during the first two weeks of Long Access, but
638 does not predict established escalation of cocaine intake after three weeks of Long
639 Access. Moreover, individuals initially resistant to incentive salience can, with sufficient
640 exposure, become sensitized and escalate cocaine use to the same level as more
641 susceptible individuals. Expression of incentive salience sensitization as reflected in
642 pre-lever activity measures of active lever entrances per meter and locomotion and their
643 subsequent influence on cocaine intake supports the idea that incentive salience and
644 psychomotor sensitization are mediated by distinct mechanisms [6-7,21,34]. The
645 development of the incentive salience profiles is accelerated by periods of abstinence
646 (weekend), which allows for incubation of craving. The measure of approach toward the
647 active lever area using video recording before the start of the self-administration session
648 may represent a novel longitudinal behavioral marker of addiction vulnerability that can
649 precisely track the motivation for the drug with very high accuracy without using any
650 operant measures, the drug itself, or any other training or conditioning that could
651 interfere with the drug self-administration. Such a measure may also be useful for
652 medication development to allow dissociation between drug-dependent and drug-
653 independent effect of experimental medication on drug seeking and taking.

654

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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Competing Interests

Authors declare no competing interests.

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