Unmasking Convergent Oxycodone Seeking and Consumption Driving Augmented Intake during Extended Access to Oral Operant Self-Administration

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Abstract

Individual vulnerability to opioid intake escalation is a critical but poorly understood aspect of addiction. Using genetically diverse inbred rat strains, we investigated operant oral oxycodone self-administration, identifying 'Augmenter' phenotypes that dramatically increased consumption during extended (16h vs. 4h) access—a vulnerability not predicted by standard motivation tests. A key innovation was applying lick microstructure analysis (LMA) to operationally distinguish 'consumption' from 'seeking' lick clusters within the inter-reward interval. During extended access, Augmenters of both sexes exhibited a striking surge in the *frequency* of both consumption and seeking clusters ($p<0.0001$), driving their escalated intake. Notably, female Augmenters also showed larger seeking cluster sizes ($p=0.006$), suggesting enhanced reward value specifically linked to seeking behavior. In contrast, interlick interval (a palatability measure) did not differentiate phenotypes. This LMA-based approach reveals that an increased drive to seek out additional oxycodone, rather than altered hedonic impact alone, underlies the augmentation of opioid intake, offering a nuanced rodent model of heightened vulnerability and a powerful tool to dissect reward dynamics.

Introduction

Rodent models of drug self-administration have revealed important distinctions between motivation, reward value, and pleasure in drug-seeking behavior. Both *internal state and reward value differentially influence motivation and impulsivity*, with internal state having a stronger effect on high-effort tasks[1]. Internal state primarily affects impulsive action, while reward value has minimal impact on impulsive choice (Albert-Lyons et al., 2024). The decision to engage cognitive control is driven by expected reward value, with individuals more likely to exert control when anticipating desired outcomes[2].

Research on brain structures controlling drug motivation and hedonic value reveals complex interactions between reward circuits. The nucleus accumbens, ventral pallidum, and ventral tegmental area form a key subcircuit necessary for drug-seeking behavior[3]. Dopamine release in these regions mediates both drug reinforcement and natural rewards, with chronic drug use leading to decreased dopamine function and altered prefrontal cortex activity [4]. Opioid stimulation in the nucleus accumbens enhances neuronal responses to hedonic "liking" for sucrose reward in the ventral pallidum, while dopamine stimulation selectively increases "wanting" without affecting hedonic impact[5]; and different neuronal subpopulations in the ventral pallidum mediate the signals for hedonic impact vs. incentive salience. Similar to the mechanisms of drug addiction, the homeostatic pathway regulates energy balance, while the hedonic pathway can override it for highly palatable foods[6]. These findings highlight the specific interconnected neural mediators of wanting, liking, and prediction within mesocorticolimbic circuitry for both drug and natural rewards.

Recent findings on the role of nucleus accumbens medium spiny neurons (MSNs) in the control of sugar intake suggest that D1 MSN activation, which is involved in the emission of licking bursts, might play a crucial role in response to novel rewards [7]. D2 MSN activation, which results in reduction of burst size and suppression of licking, might mediate the response to reward devaluation.

Lick microstructure analysis (LMA) is a valuable method for measuring reward value in rodents, providing insights into ingestive behavior and drug effects[7,8]. This analytical method involves recording individual licks and analyzing their frequency and clustering, which provides insights into palatability, motivation, and post-ingestive behaviors [8]. The number of licks per cluster (cluster size) is correlated with the palatability and hedonic reactions to a liquid [9]. Lick structure

analyses have helped clarify conditioned flavor preferences, contrast effects, and effort justification [9]. This approach has also been applied in studies of animal behavioral models of psychiatric disorders[9]. In general, reward is a complex concept, and behavioral measures may be influenced by multiple factors beyond reward sensitivity[10]. Nonetheless, lick microstructure analysis is a useful tool for studying the mechanisms controlling ingestion and drug effects on consumption[7].

Herein, we have applied LMA to elucidate potential differences in the reward value of oxycodone and to distinguish drug consumption from seeking during operant oral self-administration (SA) paradigm by multiple inbred rat strains. During 4 hour vs 16 hours SA sessions, strains that augmented their oxycodone intake by more than 3-fold were identified. LMA of Augmentor vs Non-Augmentor strains was conducted to determine if augmented oxycodone intake in 16 hour sessions was associated with increased reward value of oxycodone.

Results

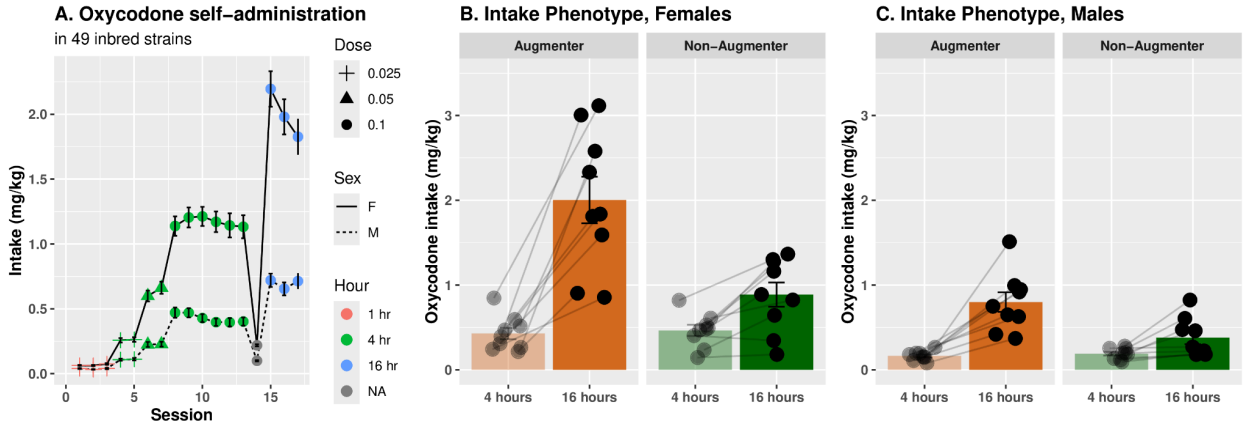


Figure 1. Augmenter and Non-Augmenter rat strains that greatly increased their oxycodone intake during 16h self-administration (SA) sessions were identified from a large panel of inbred strains. Panel A shows the progressive increase in mean oxycodone intake across stages (i.e., increasing concentration and duration of access to drug) SA by all female vs male rat strains in the full panel. Across the panel, oxycodone intake (mg/kg) increased as the dose and session length increased ($p < 2.0e-16$). Females showed higher overall intake than males ($p < 2.0e-16$). Panels B and C compare oxycodone intake by the two intake phenotypes, Augmenter strains ($n = 9$) and Non-Augmenter strains ($n = 9$), in 4h vs 16h SA sessions. In both sexes, intake was higher in Augmenters: females [panel B; main effects of intake phenotype ($p = 0.011$) and Schedule (4h vs 16h; $p < 2.0e-06$)]; males [panel C; main effects of intake phenotype ($p = 0.026$)

and Schedule ($p < 3.4e-06$)]. In both sexes, there was no difference in 4h oxycodone intake between Augmenters and Non-Augmenters (females, $p = 0.873$; males, $p = 0.795$).

Oxycodone intake by a large panel of inbred rat strains and the Augmenters vs. Non-Augmenters from this panel

Augmenter and Non-Augmenter rat strains were identified from a large panel (Fig 1A) comprised of 47 inbred female strains (mean = 7.23 rats/strain, SE = 0.46) and 44 inbred male strains (mean = 6.30 rats/strain, SE = 0.34) that acquired oral operant oxycodone self-administration (SA). Across the entire panel, oxycodone intake (mg/kg) increased as the dose and session length increased (stage: $F(7, 12127) = 547.30$, $p < 2.0e-16$). Females showed higher overall intake than males (sex: $F(1, 12171) = 359.22$, $p < 2.0e-16$; female mean = 0.67 mg/kg, male mean = 0.25 mg/kg averaged over all stages). A significant interaction between sex and stage ($F(7, 12124) = 126.73$, $p < 2.0e-16$) indicated that the pattern of intake across sessions differed between sexes. Females reached peak intake of 1.17 mg/kg (SE = 0.033) during the 4h, 0.1 mg/ml stage and showed a large increase to 2.00 mg/kg (SE = 0.079) during the 16h stage, while males reached peak 4h intake at 0.43 mg/kg (SE = 0.014) and increased to 0.70 mg/kg (SE = 0.031) at the 16h stage, demonstrating that both dose/duration and sex influenced intake levels.

Rat strains were divided into two intake phenotypes, Augmenters (n = 9) and Non-Augmenters (n = 9), based on their increase in intake from the 4h, 0.1 mg/ml to the 16h, 0.1 mg/ml stages. In females (panel B), we found main effects of intake phenotype ($F(1, 16) = 8.22$, $p = 0.011$) and schedule (4h vs 16h; $F(1, 16) = 58.03$, $p < 2.0e-06$), along with an interaction of intake phenotype x schedule ($F(1, 16) = 19.32$, $p = 0.0005$). In females, during the 4h stage, there was no difference ($p = 0.8732$) in mean intake between Non-Augmenters (mg/kg, 0.46±0.066 SE) and Augmenters (mg/kg, 0.43±0.068 SE); however, at 16h, Augmenters (mg/kg, 2.00±0.275 SE) demonstrated higher intake ($p < 0.0001$) than Non-Augmenters (0.89±0.144 SE) . Similarly, in males (panel C), main effects were intake phenotype ($F(1, 16) = 6.00$, $p = 0.026$) and schedule ($F(1, 16) = 48.06$, $p < 3.4e-06$), as well as an intake phenotype x schedule interaction ($F(1, 16) = 14.05$, $p = 0.0018$). Similar to females, during the 4h stage, male Non-Augmenter oxycodone intake (0.19±0.022 SE) did not differ ($p = 0.7950$) from Augmenters (0.16±0.018 SE), but during the 16h stage, Augmenter males (0.80±0.116 SE) showed higher intake ($p = 0.0002$) compared to Non-Augmenters (0.38±0.076 SE). Therefore, in both sexes, augmented oxycodone intake was detected only in the Augmenters during 16h sessions.

Progressive ratio (PR) testing was conducted between the 4h and 16h SA stages. PR breakpoint was unaffected by intake phenotype ($F(1, 32) = 0.26$, $p = 0.6103$), sex ($F(1, 32) = 1.72$, $p = 0.1995$), or their interaction ($F(1, 32) = 0.29$, $p = 0.5920$). For PR break-point, female Non-Augmenters vs Augmenters were 0.88±0.08 SE and 0.88±0.10, respectively. Non-Augmenter males were 0.71±0.08 SE and Augmenters were 0.81±0.09 SE. Analysis using a median split within the dataset confirmed significant variation in PR break-point ($F(1, 32) = 62.16$, $p < 5.4e-09$), indicating that the similarity between intake phenotypes was not due to a lack of variability in the population. These findings suggest that the differentiation in oxycodone intake observed during maintenance self-administration (4h vs 16h) may not be due to variation in motivated responding.

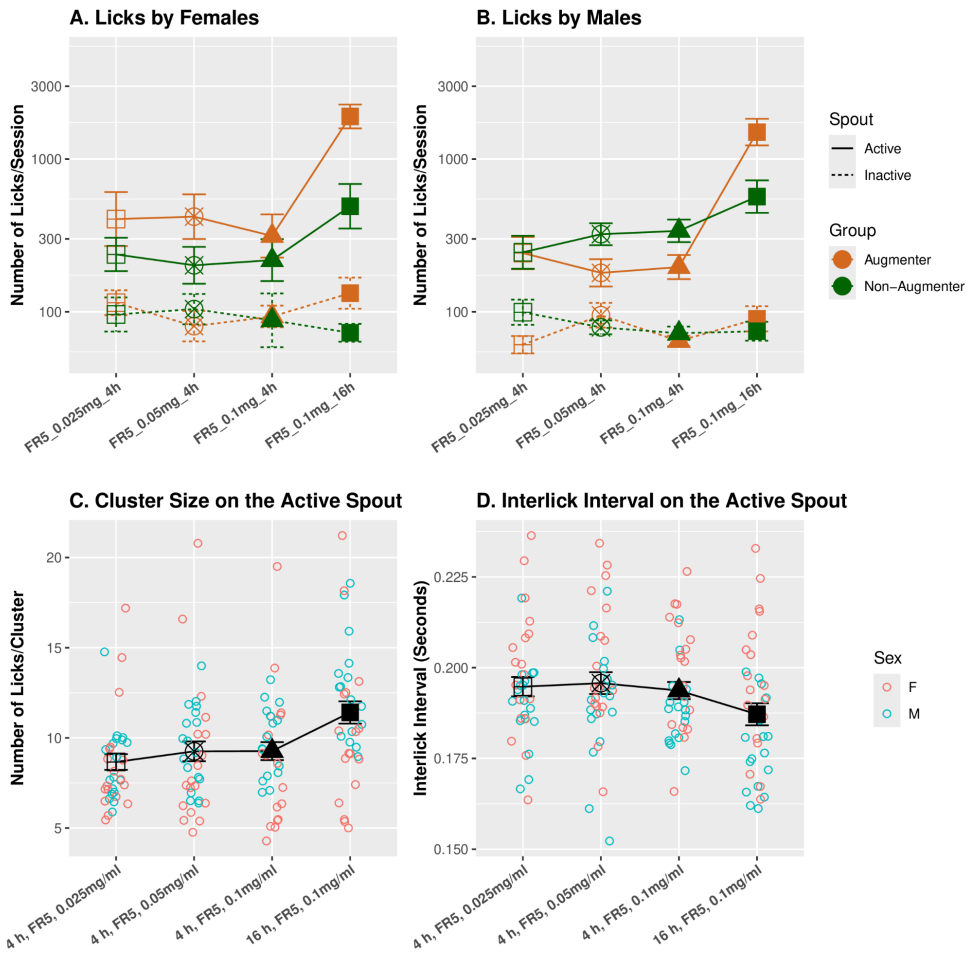


Figure 2. Total Licks on the active and inactive spouts by Augmenters vs Non-Augmenters in each sex by stage of oxycodone SA (panels A and B) and the dynamic ranges of lick cluster size (CS) and interlick interval (ILI) by stage of SA (panels C and D, respectively). In both sexes, a main effect of stage and an interaction of stage x intake phenotype was identified (panel A female, $p<0.001$ and $p = 0.001$, respectively; panel B, male, $p<0.001$ for both). In both sexes at 16h, the total number of licks was greater in Augmenters than Non-Augmenters (female and male, $p<0.001$). Licks on the inactive spout (panels A and B) did not differ between the two intake phenotypes in either sex. The dynamic range from 4h 0.025 mg/ml to 16h 0.1 mg/ml was significantly greater for CS (panel C) than ILI (panel D), $p < 0.001$.

Analysis of total Active and Inactive Licks at each stage of oxycodone SA

Analysis of total licks on the active spout in female rats (Fig 2A) showed main effects of intake phenotype ($F(1, 16) = 4.99$, $p = 0.040$) and stage ($F(3, 48) = 24.25$, $p < 0.001$), and an intake phenotype x stage interaction ($F(3, 48) = 6.40$, $p = 0.001$). There was no difference in active licks between female Augmenters and Non-Augmenters during the 4h stages (0.025mg, 4h: $t = 1.49$, $p = 0.148$; 0.05mg, 4h: $t = 1.32$, $p = 0.197$; 0.1mg, 4h: $t = 0.69$, $p = 0.495$). However, Augmenters licked significantly more than Non-Augmenters during the final 16-hour session (2120.6±300.0 SE vs 766.2±268.7 SE, respectively; $t = 4.13$, $p < 0.001$). For female inactive spout licking (Fig 2A), there was a main effect of stage ($F(3, 48) = 3.30$, $p = 0.028$), but no effects of intake phenotype ($F(1, 16) = 2.88$, $p = 0.109$) or intake phenotype x stage ($F(3, 48) = 0.38$, $p = 0.765$).

In male rats, active spout licking (Fig 2B) showed a main effect of stage ($F(3, 48) = 26.81$, $p < 0.001$), an intake phenotype x stage interaction ($F(3, 48) = 10.12$, $p < 0.001$), but no effect of intake phenotype ($F(1, 16) = 1.57$, $p = 0.229$). Similar to females, there was no difference in active spout licking between Augmenters and Non-Augmenters during the 4h stages (0.025mg, $t = -0.07$, $p = 0.942$; 0.05mg, $t = -0.75$, $p = 0.456$; 0.1mg, $t = -0.79$, $p = 0.431$). However, Augmenter males had more active spout licks than Non-Augmenters during the 16-hour session (1744.4±322.3 SE vs 722.5±180.4 SE; $t = 4.93$, $p < 0.001$). Inactive spout licking (Fig 2B) showed no main effects of intake phenotype ($F(1, 16) = 1.44$, $p = 0.248$) or stage ($F(3, 48) = 1.20$, $p = 0.321$), and no intake phenotype x stage interaction ($F(3, 48) = 2.28$, $p = 0.092$). Therefore, only in 16h SA sessions did male Augmenters emit a significantly greater number of active spout licks than Non-Augmenters, and inactive licks were unaffected

LIck microstructure analysis of oxycodone SA: the dynamic range of lick cluster size (CS) and interlick interval (ILI)

LMA of licks on active and inactive spouts focuses on the two key LMA parameters of cluster size (CS) and interlick interval (ILI). To determine and compare the overall dynamic range of these two parameters (Figure 2C and D) in the combined population of Augmenters and Non-Augmenters, the incremental change from the first 4h stage to the 16h stage in active spout CS and IRI was calculated for each parameter and then normalized to its first 4h stage. The mean change in the CS parameter (0.3 ±0.07 SE) was significantly greater ($t(37.82) = 5.57$, $p < 0.001$) than the mean change in ILI (-0.04± 0.01 SE). The effect size for the CS and ILI data (Fig 2E and F) was also analyzed by Cohen's d from the initial 4h to 16h for CS and ILI: the increase in CS (Cohen's d = 0.86, large effect) was greater than the decrease in ILI (Cohen's d = -0.44, small effect).

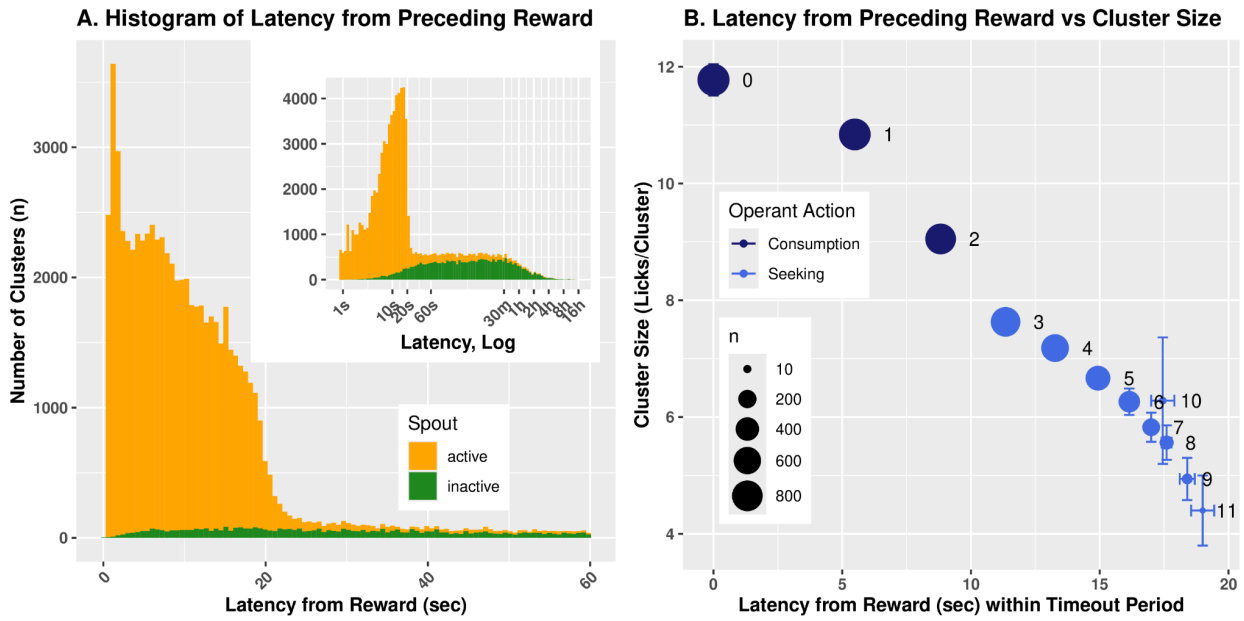


Figure 3. Classifying consummatory vs. seeking behavior based on the post-reward microstructure of active spout lick clusters. A. Latency is the time (sec) from a reward-associated cluster generated on the active spout to all subsequent non-reward clusters occurring before the very next reward cluster. Active spout non-reward clusters (orange) were mostly emitted during the 20 sec time-out interval, whereas a vastly smaller number occurred after the time-out. In order to display data for the entire 16h session (inset), the latencies of each cluster were log transformed. B. Analysis of lick cluster size (licks/cluster; CS) in clusters emitted consecutively after a reward-associated cluster (position 0) and the latency (sec) of each of these clusters emitted within the 20-sec timeout period revealed a distinct pattern. The reward-triggering cluster (i.e. 0) and the first two subsequent clusters (dark blue) were significantly larger than later clusters (light blue,

positions 3-11), with the most substantial decrease in CS occurring after cluster number 2. This temporal decay in CS (shown by circle size) informed the operational classification, designating the initial, larger clusters (0-2) as "Consumption" clusters and the later, smaller clusters (3+) as "Seeking." In each panel, the total number of clusters (n = 5,013) emitted on the active spout by Augmenter and Non-Augmenter strains in both sexes are included. Data points are mean ± SEM.

Classification of post-reward lick clusters into consummatory vs seeking clusters.

To differentiate between consummatory and seeking behaviors within the timeout period following oxycodone reward, we analyzed the microstructure of licking based on the sequential position of active spout lick clusters relative to the reward-triggering cluster (Figure 3B; cluster 0). Cluster size significantly decreased as a function of the sequential position of each active spout cluster generated within the timeout period ($F(12, 4805.1) = 81.755$, $p < 2.2e-16$). Specifically, the reward-triggering cluster (11.77 ± 0.27 licks/cluster) was significantly larger ($p = 0.0014$) than the subsequent active spout cluster (i.e., cluster 1; 10.84 ± 0.21 licks/cluster), which was larger ($p < 0.0001$) than the next cluster (i.e. cluster 2; 9.05 ± 0.18 licks/cluster). Cluster size further decreased ($p < 0.0001$) between the 2nd and 3rd subsequent clusters (cluster 3; 7.63 ± 0.16 licks/cluster), but the size of the 3rd cluster was not different ($p = 0.8269$) from the 4th active spout cluster (cluster 4; 7.18 ± 0.15 licks/cluster).

We also examined the inter-cluster interval (ICI), defined as the time from the end of the preceding cluster to the start of the next cluster. ICI also varied with sequential position ($F(11, 4739.1) = 195.33$, $p < 2.2e-16$), primarily driven by very long latencies preceding the reward-triggering (1642.89 ± 61.18 s) and 1st subsequent cluster (575.90 ± 44.58 s), likely reflecting the inter-reward interval and initial post-reward pause, respectively. Notably, the ICI preceding the 2nd subsequent cluster dropped dramatically (2.31 ± 0.05 s) and remained relatively short at ± 0.05 s. Based on the parallel decline of both cluster size and ICI after the 2nd subsequent cluster, we classified the reward-triggering cluster 0 and the first two subsequent clusters (i.e. clusters 1 and 2) as "Consumption clusters," and the 3rd and all following active spout clusters (i.e, 3-11) as "Seeking clusters." While the interlick interval (ILI) within clusters also showed a small but significant overall change across sequential clusters ($F(11, 4785.6) = 3.6443$, $p = 3.638e-05$), the pattern was less pronounced than for cluster size or ICI. In summary, this classification provides a functional distinction between the initial large

and widely separated licking clusters that mediate consumption, while later, smaller, more rapidly initiated (i.e. shorter ICI) licking clusters reflect seeking behavior.

Figure 3A shows the frequency (mean) of all clusters (n = 5,013) emitted by Augmenters and Non-Augmenters at increasing latencies from the previous active spout reward-associated cluster. Following the high frequency of consumption clusters, shown in the orange bars out to a latency of approximately 8.5 sec, the seeking phase of clusters was characterized by a rapid, accelerating decline in cluster frequencies. Hence, active spout seeking clusters were mostly emitted during the 20 sec time-out interval following the reward-associated cluster, whereas a vastly smaller number occurred after the time-out. As shown in green, licking on the inactive spout was minimal during this seeking phase of the 20 sec time-out. This provides further evidence supporting the functional specificity of the seeking clusters generated during the time-out. In order to display cluster data for the entire 16h session (inset), the latencies of each cluster were also log transformed.

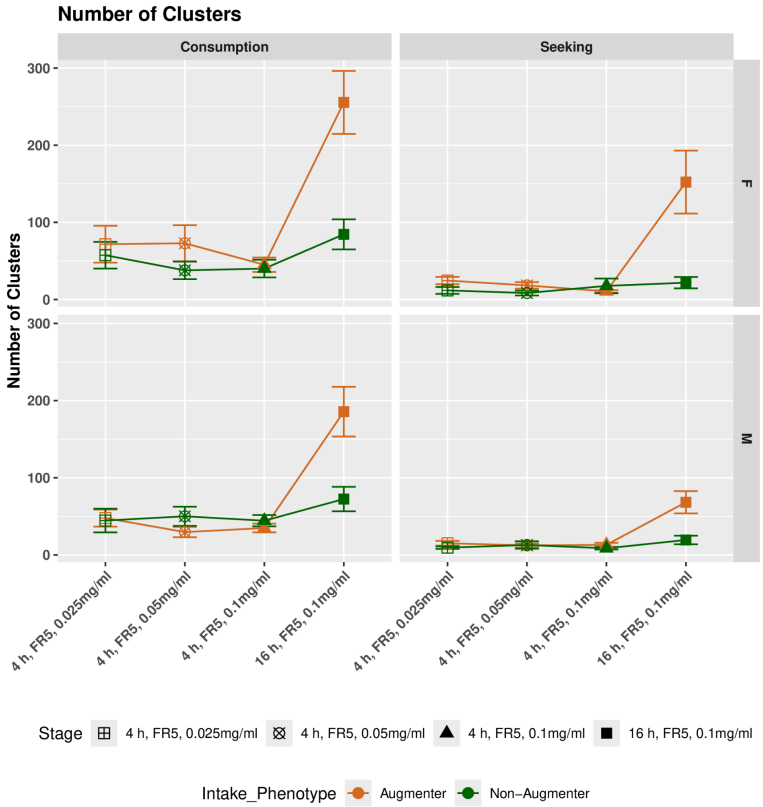
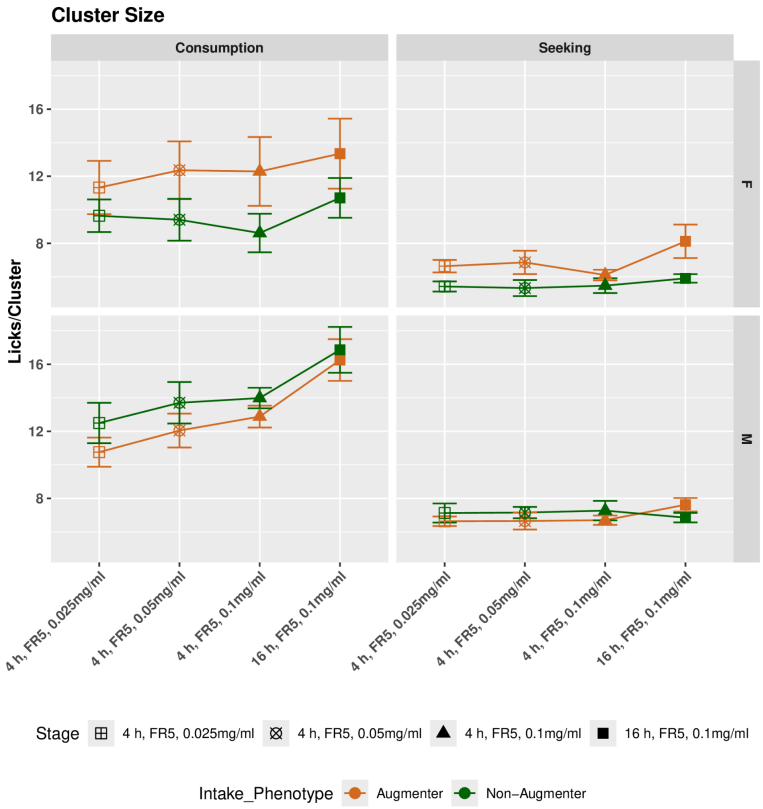


Figure 4. **During 16h sessions of oxycodone SA, the frequency of lick clusters during both consumption and seeking on the active spout differentiates Augmenters from Non-Augmenters.** Inclusive analysis of the four panels showed the number of lick clusters during Consumption and Seeking varied across stages ($p < 2.2e-16$) and differed between sexes (female > male; $p = 0.031$) and intake phenotypes (Augmenter: orange circle; Non-Augmenter: green circle; $p = 6.4e-05$). While cluster counts were comparable between phenotypes during standard 4h access at 0.1mg/ml, Augmenters in both sexes exhibited a pronounced escalation in the frequency of both consumption and seeking clusters relative to Non-Augmenters, specifically during the extended 16h access condition (consumption and seeking, $p < 0.0001$) . Data are mean ± SEM.

The frequency of lick clusters during consumption vs seeking in Augmenters and Non-Augmenters

We then examined the number of consumption and seeking clusters on the active spout across stages, sex and intake phenotype (Figure 4). Inclusive analysis of the four panels showed more consumption clusters (73.34 ± 6.65 clusters/session) than seeking clusters (26.52 ± 4.03 clusters/session; $F(1, 229.363) = 83.58$, $p < 2.2e-16$). We identified main effects of stage ($F(3, 229.363) = 56.58$, $p < 2.2e-16$), sex (Females > Males; $F(1, 214.394) = 4.72$, $p = 0.031$), and intake phenotype (Augmenters > Non-Augmenters; $F(1, 87.109) = 17.64$, $p = 6.4e-05$). Critically, an interaction between stage x intake phenotype emerged ($F(3, 229.363) = 29.86$, $p < 2.2e-16$). Within panels, a main effect of intake phenotype was observed in females during consumption ($F(1, 16) = 7.981$, $p = 0.012$) and in both sexes during seeking (female, $F(1, 16) = 9.170$, $p = 0.0080$; male, $F(1, 16) = 8.098$, $p = 0.012$). Although no significant difference in the number of clusters between intake phenotypes was detected for males during consumption ($F(1, 16) = 1.973$, $p = 0.179$), there was a significant interaction between intake phenotype and SA stage ($F(3,48) = 11.7$, $p = 7.12e-6$).

Post-hoc tests revealed no significant difference in the number of consumption or seeking clusters between Augmenters and Non-Augmenters during the 4h, 0.1mg/ml stage in either sex (all $p > 0.3$). However, during the subsequent 16h stage, Augmenters displayed more consumption clusters than Non-Augmenters in both females (Aug, 255.50 ± 40.81 vs Non-Aug, 84.51 ± 19.48; $p < 0.0001$) and males (Aug, 185.67 ± 32.21 vs Non-Aug, 72.43 ± 15.88; $p < 0.0001$). Similarly, Augmenters emitted more seeking clusters than Non-Augmenters during the 16h stage in both females (Aug, 152.22 ± 40.78 vs Non-Aug, 21.87 ± 7.38; $p < 0.0001$) and males (Aug, 68.27 ± 14.47 vs Non-Aug, 19.37 ± 5.57; $p < 0.0001$). Hence, the enhanced intake



observed in Augmenters during extended 16h SA sessions is driven by an increased frequency of both consumption- and seeking-related licking bouts.

Figure 5. Lick Cluster Size (CS) shows a limited association with intake phenotype. Overall, consumption clusters (defined in Fig. 3) were consistently much larger (i.e.,licks/cluster) than seeking clusters ($p < 2.2e-16$). Combined analysis of all 4 panels found main effects of stage ($p = 2.8e-05$), with CS generally increasing at the 16h stage; sex ($p = 0.003$), with male CS greater than female; and intake phenotype ($p = 0.0003$), with Augmenters having slightly larger CS than Non-Augmenters. CS generally increased during extended (16h) access compared to prior sessions and differed slightly between sexes and phenotypes. At 16h, an increase in seeking CS was observed in female Augmenters vs Non-Augmenters ($p = 0.0062$). Data are mean ± SEM.

Cluster Size during Consumption and Seeking in Augmenters and Non-Augmenters throughout the time-out time interval following the delivery of an oxycodone reward.

Based on the four panels, we analyzed the average size of the consumption and seeking clusters previously defined in Figure 3. As expected, combined analysis of Augmenters and

Non-Augmenters showed that consumption clusters (12.31 ± 0.37 licks/cluster) were larger than seeking clusters (6.62 ± 0.13 licks/cluster; F(1, 225.77) = 388.74, p < 2.2e-16). Main effects were found for stage (F(3, 225.76) = 8.33, p = 2.8e-05), with cluster sizes generally increasing at the 16h stage; sex (F(1, 250.96) = 8.90, p = 0.003), with males (10.27 ± 0.36 licks/cluster) having larger clusters than females (8.60 ± 0.36 licks/cluster); and intake phenotype (F(1, 186.37) = 13.35, p = 0.0003), Examining lick cluster size (CS) by intake phenotype only within the right upper panel, we found a main effect of intake phenotype on seeking in females [F(1, 16) = 5.310, p = 0.035)]. Augmenters had larger seeking clusters than Non-Augmenters at the 4h 0.05 mg/ml and 16h stages (right upper panel, Aug: 6.87±0.70 and 8.12 ± 1.00 vs Non-Aug: 5.33±0.48 and 5.91 ± 0.25 licks/cluster; p = 0.049 and p = 0.0062, respectively). This suggests that while cluster number (Fig. 4) is the primary driver of phenotype differences, female Augmenters also exhibit more reward-enhanced seeking behavior during extended access.

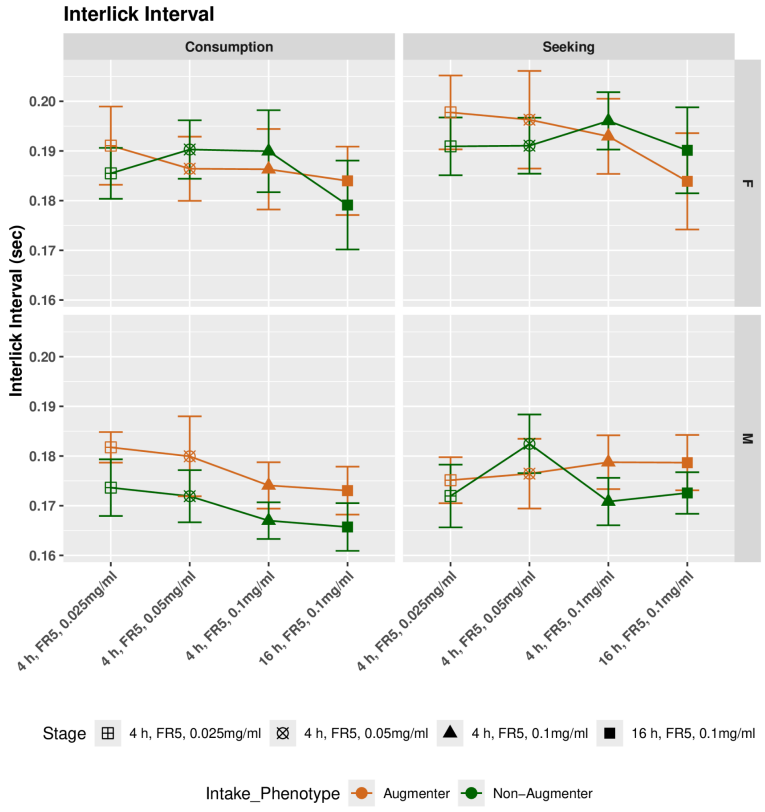


Figure 6. interlick interval remains stable between Augmenters vs Non-Augmenters despite divergent intake levels. Based on a four panel analysis of the average interlick interval (ILI, sec) within consumption and seeking clusters, a potential indicator of reward valuation, we found main effects of sex (p = 5.3e-06), with longer ILIs (i.e. slower licking) in females (0.190 ± 0.002 s) than males (0.175 ± 0.001 s), and a small effect of operant action (p = 0.033), with seeking cluster

ILIs (0.184 ± 0.002 s) slightly longer than consumption clusters (0.180 ± 0.002 s). However, there were no main effects of stage ($p = 0.13$) or intake phenotype ($p = 0.08$). Although males generally displayed faster licking (shorter ILI) than females, no significant differences in ILI were found between Augmenter and Non-Augmenter phenotypes for either cluster type. Data are mean ± SEM.

Analysis of interlick intervals

Lastly, we examined the ILI, a measure of subjective reward, within consumption and seeking clusters (Figure 6). Overall, there was a main effect of sex ($F(1, 230.41) = 21.73$, $p = 5.3e-06$), with females having longer ILIs (slower licking; 0.190 ± 0.002 s) than males (0.175 ± 0.001 s), and a small but significant effect of operant action ($F(1, 202.97) = 4.58$, $p = 0.033$), with seeking clusters (0.184 ± 0.002 s) having slightly longer ILIs than consumption clusters (0.180 ± 0.002 s). However, there were no significant main effects of stage ($F(3, 202.9) = 1.92$; $p = 0.13$) or intake phenotype ($F(1, 159.59) = 3.03$; $p = 0.08$). Additionally, no significant interactions were found between stage x intake phenotype (all $p > 0.4$). Post-hoc comparisons confirmed no significant differences in ILI between Augmenters and Non-Augmenters for either consumption or seeking clusters in either sex (all $p > 0.1$).

Discussion

The present study utilized a large panel of genetically diverse inbred rat strains to investigate individual differences in oral oxycodone self-administration, particularly focusing on the augmentation of intake during extended access. Our findings reveal the emergence of two distinct intake phenotypes, termed 'Augmenters' and 'Non-Augmenters', originating from nine strains each, which exhibited similar oxycodone consumption under standard 4-hour access but diverged dramatically during 16-hour sessions. Augmenters, comprising strains from diverse genetic backgrounds, significantly increased their intake, suggesting a convergent phenotype emerging under conditions of extended access.

A key innovation of this work was the application of lick microstructure analysis (LMA) to operationally define and quantify 'consumption' versus 'seeking' related lick clusters within the post-reward time-out interval, offering a novel approach to assess the behavioral dynamics underlying intake patterns. We observed a striking increase in the frequency of both consumption and seeking clusters specifically in Augmenter strains during 16-hour sessions,

driving their enhanced intake. This effect was particularly pronounced for seeking clusters, which showed a sharp increase in both sexes at 16 hours, with females exhibiting a greater increase than males. Furthermore, only female Augmenters displayed a significant increase in the size of their seeking clusters during these extended sessions, suggesting enhanced value of the anticipated oxycodone reward, specifically during seeking behavior. Notably, while baseline sex differences in LMA parameters were observed (males generally showing larger cluster sizes and faster intra-cluster licking/shorter ILIs), these overall LMA metrics did not consistently differentiate the intake phenotypes as clearly as cluster frequency, especially the sharp increase in seeking clusters. Crucially, these nuanced differences in enhanced oxycodone intake and lick microstructure were not predicted by standard progressive ratio (PR) breakpoint measures obtained prior to the extended access phase. This underscores the limitations of PR in capturing the propensity for intake escalation under conditions of prolonged drug availability and the unique insights provided by the LMA-enabled classification of lick clusters into consumption- and seeking-related.

The emergence of the Augmenter phenotype only during extended 16-hour access in specific inbred strains, following a period of chronic 4-hour daily exposure, strongly suggests that neuroadaptations occurring in response to repeated opioid administration interact with genetic predispositions to drive augmented intake when access limitations are relaxed (i.e. 16h). This aligns with models proposing that chronic drug exposure induces persistent changes, potentially including genetic or epigenetic modifications within reward and stress circuits, that sensitize individuals to subsequent drug seeking and taking, particularly when environmental constraints change [11,12]. The fact that nine genetically distinct strains converge in this Augmenter phenotype, despite likely differences in their specific underlying genetic architecture, supports a 'convergence model' where various genetic vulnerabilities can lead to common behavioral outcome (augmented intake) pathways following sufficient drug history and under permissive environmental conditions (e.g., extended access). This extended access to opioid likely unmasks latent vulnerabilities or potentiates drug-induced adaptations that are not apparent under more restricted access schedules or captured by effort-based measures like PR testing.

A major methodological innovation presented here is the structural and functional classification of active spout lick clusters into 'consumption' versus 'seeking' based on their size and temporal position relative to reward delivery within the post-reward timeout. This distinction is grounded in the observation that lick cluster size, a well-established correlate of palatability and hedonic

impact [9], rapidly decays in the clusters immediately following reward delivery (Figure 3). We operationally defined the initial, larger clusters with a greater reward value as reflecting consummatory behavior – the direct engagement with the obtained reward – while the subsequent, smaller, and more rapidly initiated clusters represent seeking behavior aimed at obtaining another oxycodone reward[13]. The validity of this classification rests on the distinct temporal dynamics and cluster size characteristics observed, mirroring patterns seen in natural ingestive behaviors where initial engagement differs from subsequent motivated actions. This novel approach moves beyond simply quantifying total licks or rewards earned by deconstructing undifferentiated licking behavior into distinct functional phases. Importantly, by analyzing parameters like CS and ILI within these functionally defined clusters, this study represents, to our knowledge, one of the first attempts to probe the subjective reward or 'liking' aspects of opioid self-administration within a rodent operant licking paradigm, complementing traditional measures more closely associated with 'wanting' or motivation[5,14].

The LMA revealed critical differences between the Augmenter and Non-Augmenter phenotypes, primarily driven by the frequency of initiating licking bouts. The dramatic increase in both consumption and seeking cluster frequency in Augmenters during 16h sessions (Figure 4) indicates an enhanced drive to both initiate consumption and to continue working for the drug during the time-out inter-reward interval. This pattern contrasts with the much more stable frequency observed in Non-Augmenters. While CS and ILI showed less robust phenotype differentiation overall, the specific increase in seeking cluster size among female Augmenters at 16h (Figure 5) is particularly insightful. It suggests that for these females, the motivation to seek additional oxycodone during extended access is coupled with an increase in the perceived hedonic value, which may further amplify that seeking behavior itself. This finding provides a potential microstructural signature for the enhanced vulnerability to augmentation of oxycodone intake observed in this female subgroup. These findings add a new dimension to the extensive literature on intravenous opioid self-administration (IVSA) in rats. While IVSA models have clearly demonstrated intake escalation with extended access [15], they typically lack the fine-grained analysis of consummatory behavior afforded by LMA. Our results suggest that the augmentation phenomenon involves not just increased work output but also a profound increase in the initiation of both consummatory and seeking bouts, with potential changes in the perceived reward value driving seeking, particularly in females. The Augmenter phenotype identified here may represent a rodent parallel to human opioid users who rapidly increase

consumption when constraints are removed, driven by potentiation of both consummatory drive and drug-seeking motivation.

Sex differences were evident throughout the study. Females exhibited higher overall oxycodone intake across SA stages (Figure 1A), consistent with numerous reports in both preclinical models and human addiction patterns [16,17]. While both sexes displayed the Augmenter/Non-Augmenter phenotypes, the specific LMA signatures differed. The more pronounced increase in seeking cluster frequency in females (Figure 4) and the unique increase in seeking cluster size in female Augmenters (Figure 5) point towards potentially distinct mechanisms or sensitivities driving escalation between the sexes. Females may be more susceptible to the motivational and reward-enhancing effects of prolonged oxycodone access manifest during seeking periods. Conversely, males consistently showed somewhat larger cluster sizes and shorter ILIs overall (Figures 5 & 6), potentially indicating a higher baseline hedonic impact or greater efficiency in consumption, though this did not translate into greater escalation propensity compared to females within the Augmenter group. These complex sex-specific patterns in LMA parameters warrant further investigation into the underlying neurobiological substrates.

Several limitations should be acknowledged. Firstly, the classification of lick clusters into consumption and seeking is an operational definition based on empirically observed patterns; while justifiable based on objective lick microstructure principles and measurements, the attribution of seeking remains an inference about the animal's behavioral intent. Secondly, oral self-administration involves pharmacokinetic factors (absorption, metabolism) and potential taste confounds that differ from IVSA paradigms, although the consistent licking patterns suggest reinforcing efficacy overcame any potential aversion. Finally, while the use of multiple inbred strains provides genetic diversity, the specific genetic factors driving the Augmenter phenotype remain unidentified and generalizability to outbred populations requires confirmation.

In conclusion, this study demonstrates the power of combining an extended access oral oxycodone self-administration model across genetically diverse rat strains with detailed lick microstructure analysis. We identified distinct Augmenter and Non-Augmenter intake phenotypes characterized primarily by large differences in the frequency of both consumption and seeking lick clusters during prolonged access. This novel classification of lick clusters provides a valuable tool for dissecting the motivational components of drug intake, revealing a

dramatic increase in seeking behavior in Augmenters, especially females, which was associated with larger seeking cluster sizes indicative of enhanced reward value. These findings underscore the importance of considering access conditions and employing fine-grained behavioral analyses like LMA coupled with novel analytical distinctions to understand the mechanisms driving augmented opioid intake and the subjective rewarding effects of drugs. This innovative analytical method moves beyond traditional measures of motivation (i.e. progressive ratio test), which did not detect the motivation involved in persistent operant seeking for additional oxycodone by the Augmenter strains in these studies.

Methods

Animals

Breeders from the HRDP were obtained from Dr. Melinda R. Dwinell at the Medical College of Wisconsin. All rats were bred on campus and housed in groups in a room with a 12:12 h reversed light cycle (lights off: 9AM-9PM) at the University of Tennessee Health Science Center. Experiments were conducted during the dark phase of this cycle. Each rat, including breeders and offspring, had a radio frequency identification (RFID) tag implanted under its skin for identification purposes. Adult rats (PND 65-90) of both sexes were used in oxycodone self-administration. The Animal Care and Use Committee of The University of Tennessee Health Science Center approved all procedures, which complied with NIH Guidelines for the Care and Use of Laboratory Animals. Animals were sacrificed by thoracotomy under deep isoflurane anesthesia.

Drugs

Oxycodone HCl, a kind gift by Noramco (Wilmington, DE), was dissolved in distilled water.

Oral operant oxycodone self-administration

We employed the same methodology as previously reported[18,19]. The operant chamber (Med Associates) featured two lickometers: licks on the active spout following a fixed ratio 5 (FR5) schedule triggered the immediate release of a 60 µl oxycodone solution (0.025–0.10 mg/ml) onto the spout's tip, along with the activation of an LED visual cue. A 20-second timeout followed the drug delivery, during which licks on the active spout and any licks on the inactive spout were recorded but had no programmed consequences. Rats had free access to food and water and were kept under a reversed light cycle, being tested in their dark phase.

Training commenced with three daily 1-h FR5 sessions at 0.025 mg/ml oxycodone concentration. Subsequent sessions extended to 4-h and occurred every other day. From session four onwards, we doubled the dose every two sessions up to the maximum dose of 0.1 mg/ml. Rats underwent six sessions at this highest dose, followed by a progressive ratio test on session fourteen. During the progressive ratio (PR) test, the number of licks to obtain a subsequent reward was determined using the exponential formula 5e0.2 × injections − 5, such that the required responses per injection were as follows: 1, 2, 4, 9, 12, 15, 20, 25, 32, 40, 50, etc[20]. The PR sessions ended after 20 min of inactivity. The final ratio completed is the breakpoint. Session lengths then increased to 16-h (4 PM - 8 AM) for three sessions. . Extinction sessions were conducted for 1-h daily, without programmed consequences, until licks on the active spout decreased to less than fifty for two consecutive sessions, when, in general, the number of licks on the active vs inactive sprouts were no longer significantly different. A final reinstatement session was carried out where active spout licks triggered only visual cue delivery without oxycodone. Final reinstatement data were not collected on a few strains due to campus closures from unexpected weather conditions. We recorded both the number and timing of oxycodone deliveries as well as licks on active and inactive spouts. Full strain names and sexes of rats involved in oxycodone self-administration, along with the number of rats per strain (minimum is 3) and sex are listed in Table 1.

Table 1. Sample size of oxycodone oral self-administration data by strain

Strain	F	M
ACI/EurMcwi	9	6
BDIX/NemOda	5	5
BN-Lx/Cub	7	7
BN/NHsdMcwi	16	8
BUF/Mna	3	2
BXH12/Cub	5	8
BXH13/Cub	6	3
BXH2	9	7
BXH6/Cub	10	5
F344/DuCrl	6	4
F344/NHsd	6	NA
F344/Stm	8	7

FHH/EurMcwi	7	6
FXLE12/Stm	11	13
FXLE13/Stm	2	4
FXLE14/Stm	3	6
FXLE15/Stm	7	8
FXLE17/Stm	15	9
FXLE19/Stm	5	7
HXB1	8	3
HXB10	10	10
HXB10XWMI	7	7
HXB13	3	5
HXB17	5	4
HXB18	5	5
HXB2	5	7
HXB23	5	6
HXB31	11	9
HXB4	1	8
HXB7	4	3
LE/Stm	5	4
Lew/NHsd	8	8
LEXF2B/Stm	5	5
LEXF5/Stm	4	5
M520/NMcwi	9	5
MNS/N	13	6
MR/N	5	4
MWF	5	6
SHR/NCrl	6	NA
SHR/OlaIpcv	8	6
SHR/OlaIpcvxBn-Lx/Cub	6	6
SHR/OlaIpcvxBN/NHsdMcwi	4	2
SR/JrHsd	6	2
SS/JrHsdMcwi	8	12
SS/JrHsdMcwi	9	4

Crl		
SS/JrHsdMcwix SHR/Olalpcv	9	7
WAG/RijCrl	6	7
WLI/Eer	6	3
WMI/Eer	6	5

Identification of Augmenter vs Non-Augmenter strains

As described before, in each sex, HRDP strains with more than a threefold increase in oxycodone (0.1 mg/ml) intake during 16-h vs 4-h sessions were classified as augmenters: 9 females,BDIX/NemOda, BN/NHsdMcwi, FXLE14/Stm, FXLE15/Stm, FXLE19/Stm, LEXF2B/Stm, LEXF5/Stm, (SHR/Olalpcv x BN/NHsdMcwi) F1, WAG/RijCrl and 9 males: BDIX/NemOda, BN-Lx/Cub, BN/NHsdMcwi, BXH2, F344/Stm, FXLE15/Stm, LE/Stm, LEXF5/Stm, M520/NMcwi. In each sex, all HRDP strains were then ranked by intak e in 4-h sessions. For each augmenter strain, one strain that ranked immediately above it (i.e., higher 4-h intake) and not already identified as an augmenter or non-augmenter was specified as the control non-augmenter strain. These are: females, BXH2, F344/Stm, FXLE17/Stm, HXB17, HXB18, HXB2, HXB23, M520/NMcwi, (SHR/Olalpcv x Bn-Lx/Cub) F1, males, BXH6/Cub, F344/DuCrl, FXLE12/Stm, FXLE13/Stm, FXLE14/Stm, FXLE17/Stm, HXB23, LEXF2B/Stm, MNS/N.

Lick microstructure analysis

Lick microstructure analysis was performed on lick timing data collected from active and inactive spouts, along with corresponding reward delivery times extracted from MedPC data files. Custom R scripts (R Version 4.4.3)[21] utilizing the dplyr[22] and lubridate[23] packages were employed for this analysis. Initial versions of the lick microstructure function were generated with the assistance of Google Gemini 2.5 Pro Experimental (released on 2025-03-25). The output of all parameters from the microstructure function were manually validated using a small synthetic dataset and a few real datasets before the function was used to analyze the entire dataset. Lick clusters were identified based on temporal proximity. A cluster was initiated by a lick, and subsequent licks if the ILI did not exceed a pre-defined threshold of 0.5 seconds. A cluster concluded when this ILI threshold was surpassed or when no further licks occurred. Only clusters comprising at least three licks were included in subsequent analyses. For each valid cluster, several characteristics were quantified: the total number of licks within the cluster (cluster size), the average ILI between consecutive licks within that cluster, and its classification

based on reward proximity. Clusters were designated as 'Overlap' if a reward delivery occurred within the cluster's temporal boundaries, or 'No_Overlap' otherwise. The time elapsed between the end of one cluster and the start of the next (inter-cluster interval, ICI) was also calculated, specifically the interval preceding the current cluster and the interval following it. To examine licking patterns specifically following a reward, 'No_Overlap' clusters were assigned a sequential run index. This index started at 1 for the first 'No_Overlap' cluster after a reward delivery (or after the session start if no prior reward had occurred) and incremented for each subsequent consecutive 'No_Overlap' cluster. This count was reset to zero if an 'Overlap' cluster occurred or if a reward was delivered between clusters. For analytical consistency, 'Overlap' clusters were assigned a run index of 0. A 20-second post-reward timeout period was also considered, where clusters initiated within this window following a reward were specifically analyzed for certain aspects, such as determining operant action thresholds.

Classification of lick clusters

Licking behavior was subsequently categorized into distinct operant actions based on the cluster type and its sequential run index following a reward. 'Consumption' was defined as licking behavior primarily associated with reinforcer intake, encompassing all 'Overlap' clusters (which had a run index of 0) and 'No_Overlap' clusters that were the first or second in sequence after a reward. 'Seeking' was defined as licking behavior not immediately tied to reward consumption, consisting of 'No_Overlap' clusters that were third or later in the sequence following a reward. This classification scheme was informed by preliminary mixed-effects model analyses of cluster characteristics (e.g., size, ILI, ICIs) against the sequential run index, which revealed distinct properties for these initial post-reward clusters compared to later ones.

Statistical analysis

Outliers were addressed by first applying a logarithmic transformation to each variable to normalize distributions. Outliers were then identified on this transformed scale as values falling below the first quartile minus twice the interquartile range (IQR) or above the third quartile plus twice the IQR. These identified outliers were treated as missing data, and the remaining data were subsequently back-transformed to their original scale. To prepare data for statistical comparisons between Augmenter and Non-Augmenter intake phenotypes, the detailed cluster data, following outlier removal and operant action categorization, underwent a two-stage aggregation process. First, daily mean values for the number of clusters, total licks, average cluster size, and average intra-cluster ILI were computed for each individual animal, within each

experimental stage, sex, and assigned intake phenotype. These calculations were performed separately for clusters categorized as 'Consumption' and 'Seeking'. Second, these daily animal means were further averaged across all experimental days to yield a single mean value per strain for each combination of sex, intake phenotype, experimental stage, and operant action. These strain-level means served as the primary data points for subsequent statistical modeling.

All statistical analyses were performed using R (Version 4.4.3). Linear mixed-effects models (LMMs) were implemented using the lme4[24] and lmerTest[25] packages, with post-hoc comparisons conducted via the emmeans[26] package. The dependent variables, derived from the strain-level aggregated data, included the number of clusters, total licks, mean cluster size, and mean intra-cluster ILI. The LMMs incorporated experimental stage, sex, intake phenotype, and operant action as fixed effects, along with all their potential interactions. To account for inherent variability due to genetic background, 'strain' was included as a random intercept in these models. The significance of fixed effects and their interactions was assessed using Type III Analysis of Variance (ANOVA) tables, with Satterthwaite's method for approximating degrees of freedom. Following significant main effects or interactions, post-hoc pairwise comparisons of estimated marginal means were performed, applying Tukey's HSD method for multiple comparison adjustment. A significance level of $p < 0.05$ was adopted for all statistical inferences.

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