Sex and heredity are determinants of oral oxycodone self-administration in 36 Inbred Rat Strains: correlations with behavioral tests of anxiety and novelty-seeking

Burt M Sharp[1], Shuangying Leng[1], Caroline Jones[1] and Hao Chen[2]

[1]Department of Genetics, Genomics and Informatics, College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee 38163; [2]Department of Pharmacology, Addiction Science and Toxicology, University of Tennessee Health Science Center, Memphis, Tennessee 38163

Correspondence: to B.M.S. (labsharp1@gmail.com) and H.C. (hchen@uthsc.edu)

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Abstract

Most individuals affected in the national epidemic of oxycodone abuse began taking oral oxycodone by prescription. We studied vulnerability to oxycodone intake in a rat model of oral drug self-administration (SA), since pharmacokinetics affect abuse potential. Females (33 inbred strains) and males (26) obtained oxycodone at increasing concentrations in operant sessions (FR5; 1-16-h) followed by extinction and reinstatement. Active spout licks were greater in females than males during 4-h and 16-h sessions ($p < 0.001$ for all). Across all stages of oxycodone SA, intake/session was greater in females ($p<0.001$). Both sexes escalated intake during 16-h extended access vs 4-h sessions ($p<2e-16$). Intake and active licks varied greatly by strain. The heritability (h^2) of active licks/4-h at increasing oxycodone dose was larger in males (h^2 females: 0.30-0.39 vs. males: 0.41-0.53). Under a progressive ratio schedule, breakpoints differed by strain ($p<2e-16$) and by sex in some strains ($p=0.018$). For cue-induced reinstatement, active licks were greater in females than males ($p<0.001$). Behavior in naive rats was assessed using elevated plus maze (EPM), open field (OF) and novel object interaction. (NOI) tests. We correlated these behaviors with 28 parameters of oxycodone SA. EPM-defining traits were most commonly associated with SA in both sexes, whereas more OF and NOI traits were SA-associated in males. Overall, sex and heredity are major determinants of the motivation to take and seek oxycodone, which escalates during extended access. The correlation of EPM, a measure of anxiety, with multiple SA parameters indicates the influence of pleiotropic genes.

Introduction

Opioids such as morphine and *oxycodone* have long been prescribed for effective analgesia. However, the rising prevalence of prescriptions for opioids such as oxycodone, in the U.S.A., has led to widespread abuse and deaths. Approximately 12 million people misused opioids in 2016 (NSDUH 2018). Compared to 2015 (Vadivelu et al. 2018), by 2017 deaths from opioid overdoses rose sharply to approximately 12 million individuals, and in 2022, approximately 80,000 opioid-involved overdose deaths were reported (National Center for Health Statistics 2007).

Most commonly, individuals initiate their habitual intake of oxycodone with prescription oral oxycodone (Cerdá et al. 2015; Compton and Volkow 2016). Since pharmacokinetic parameters are important determinants of abuse potential, we designed a rat oral operant self-administration (SA) model. Rats, which are commonly used to study addictive substances (Parker et al. 2013; Jaramillo and Zador 2014; Ellenbroek and Young 2016), were used to model the oral pattern of drug intake by most humans who use and abuse oxycodone. Previous reports of oral oxycodone SA (Shaham 1993; Enga et al. 2016; Jimenez et al. 2017) required water deprivation (Enga et al. 2016) or home cage drug consumption (Shaham 1993; Jimenez et al. 2017) to initiate drug SA, yet both procedures alter the motivation to obtain drugs. The model we developed utilizes operant licking for oxycodone that does not require water restriction or prior drug exposure. Rats have limited initial drug intake followed by intermittent access, i.e., alternating days with extended 4-h sessions. Cyclical drug taking and withdrawal under a schedule of intermittent access engenders addictive behavior.

The transition from deliberately controlled drug intake to compulsive drug seeking and taking, characteristic of addiction (Edwards and Koob 2013), is often accompanied by a sharp rise in drug use. The marked variation in amount of drug consumed and the extent of addictive behavior observed amongst individuals depends on multiple factors. Human twin studies have shown that approximately half of the vulnerability to develop an opiate use disorder (OUD) is heritable (Tsuang et al. 1998; Kendler et al. 2003).

Complex, sex-dependent patterns are also a major factor determining opiate use. Male rats self-administer more oxycodone than females at early stages, while females are more susceptible to opioid reward (Mavrikaki et al. 2017).

Based on the known impact of genetics and sex on the vulnerability to opiate use disorder, we studied the patterns of drug intake in both sexes during 4-h and 16-h extended sessions; a total of 36 fully inbred rat strains were evaluated, 23 were common to both sexes. We also correlated oxycodone SA parameters to exploratory behaviors and locomotion in drug naive rats from many of these strains. These strains are part of the hybrid rat diversity panel (HRDP) (Tabakoff et al. 2019), which combines two panels of recombinant inbred strains and 30 classic inbred strains to gain power and precision for genetic mapping studies.

Some strains manifest greater than a 100-fold *increase in oxycodone intake* during our 65-day protocol. We also found significant sex and strain differences that affect multiple parameters of oxycodone SA, including the sex and strain-specific escalation of intake with increased drug availability during extended access sessions and the correlation of intake between 4 vs 16-h sessions in both sexes. We confirmed the significant heredity (h^2) for multiple parameters of oxycodone SA including the motivation to take the drug, the amount consumed, the behavior emitted (i.e., active licks) to obtain drug, and the reinstatement of drug seeking behavior. These findings are similar to those reported in seven inbred rat strains (Sharp et al. 2021) and in studies of human twins. Lastly, we found significant sex and strain-dependent correlations between specific behavioral tests (e.g., elevated plus maze, EPM) conducted in drug naive rats and oxycodone SA. Overall, this oral model of oxycodone SA captures multiple behavioral parameters involved in the strong abuse potential of oral oxycodone and demonstrates the existence of pleiotropic genes shared between these behavioral parameters of oxycodone SA and independent behaviors elicited in paradigms such as EPM.

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 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 participated in oxycodone self-administration experiments. A separate group of adolescent rats (PND38-44) underwent behavioral tests. 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.

Drugs

Oxycodone HCl was a kind gift by Noramco (Wilmington, DE).

Open Field Test

OFT was carried out as previously reported (Gunturkun et al. 2022). Two OFT arenas were constructed using black acrylic glass, measuring 100 cm (L) × 100 cm (W) × 50 cm (H), which were placed side by side. The floors were covered by wood boards painted with either black or white acrylic paint (ART-Alternatives, ASTM D-4236, Emeryville, CA, USA) to contrast the coat of the animals (i.e., a black board was used for rats with white fur). The test chambers were illuminated by a long-range, 850-nm infrared light (LIR850-70, LDP LLC, Carlstadt, NJ) located 160 cm above the center of the two test chambers. No source of visible light was present during behavioral testing, with the exception of a flat panel monitor (Dell 1908FP). A digital camera (Panasonic WV-BP334) fitted with an 830 nm infrared filter (X-Nite830-M37, LTP LLC, Carlstadt, NJ) and located next to the infrared light source was used to record the behavior of the rats.

All rats were released at the same corner of the test chamber, and data were collected for 20 min.

Novel Object Interaction (NOI) Test

This test was conducted the day after the OFT in the same arena. A cylindrical rat cage constructed using 24 aluminum rods (30 cm in length) spaced 1.7 cm apart was used as the novel object (Wang et al. 2014). The bottom and top of the cage (15 cm in diameter) were manufactured using a 3D printer from polylactic acid. The novel object was placed in the center of the arena before testing. The test duration was 20 min and was recorded using the same camera as that used in the OFT.

Elevated Plus Maze

The maze was constructed using black acrylic glass. The floors of the maze were covered by wood boards painted with black or white acrylic paint. The platform was 60 cm above the floor, with all four arms measuring 12 cm (W) × 50 cm (L). The two opposing closed arms had walls measuring 30 cm (H). Rats were placed into the center of the maze facing the closed arm. The behavior of the rat was recorded for 6 min using the digital video system described above.

Analysis of Video Data

Ethovision XT video tracking system (RRID:SCR_000441, Version 15.0, Noldus Information Technology, The Netherlands) was used to analyze the videos recorded in all behavioral tests. After identifying the arena and calibrating the size of the arena, specific zones in the arena were outlined. For OFT and NOI, one center zone, which was a circular region with a diameter of 20 cm, was used. The extracted data included the total distance traveled in the arena, the duration and the frequency the test rat was present in specific zones, and the distance of the subject to the zones. The center of the subject rat was used for all calculations.

Schedule	FR5													PR	FR5			Extinction			FR5
Length (hours)	1			4											16			1			
Dose (mg/ml)	0.025					0.05		0.1													
Session	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20+	21+

Figure 1. Protocol and schedule of operant oxycodone self-administration. The first three sessions were conducted daily and on alternate days, thereafter. The final scheduled FR5 session was reinstatement of extinguished oxycodone seeking.

Oral Operant Oxycodone Self-Administration

We employed the same methodology as previously reported (Sharp et al. 2021), with minor adjustments. 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 (0.025–0.1 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. 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. A final reinstatement session was carried out where active spout licks triggered only visual cue delivery without oxycodone. We recorded both the number and timing of oxycodone deliveries as well as licks on active and inactive spouts. The procedure is summarized in Figure 1. Full strain names and sexes of rats involved in oxycodone self-administration, along with the number of rats per strain and sex are listed in Table 1.

Table 1. The number of rats per strain for each sex.

Strain	F	M
ACI/EurMcwi	9	6
BDIX/NemOda	4	
BN-Lx/CubMcwi	5	3
BN/NHsd	6	
BN/NHsdMcwi	4	3
BUF/Mna	3	
BXH2/CubMcwi	7	7
BXH6/CubMcwi	10	5
F344/DuCrl	6	4
F344/NHsd	6	
F344/StmMcwi	8	7
FHH/EurMcwi	5	4
FXLE15/StmMcwi	7	8
FXLE19/StmMcwi	5	
HXB10/IpcvMcwi	10	10
HXB10XWMI	7	7
HXB2/IpcvMcwi	0	7
HXB23/IpcvMcwi	5	6
HXB31/IpcvMcwi	11	9
HXB4/IpcvMcwi		5
LE/StmMcwi	5	4
Lew/NHsd	8	8
LEXF2B/StmMcwi	3	
LEXF5/StmMcwi		5
M520/NMcwi	6	3
MWF/Mcwi	5	4
SHR/NCrl	6	
SHR/OlaIpcvMcwi	6	4
SHR/OlaIpcvxBN/NHsdMcwi	4	

SR/JrHsd	3	
SS/JrHsdMcwi	8	12
SS/JrHsdMcwiCrl	6	
SS/JrHsdMcwixSHR/Olalpcv	9	7
WAG/RijCrl	4	7
WLI/Eer	6	3
WMI/Eer	6	5

Estimate of heritability

The between-strain variance provides a measure of additive genetic variation (VA), while within-strain variance represents environment variability (VE). An estimate of narrow-sense heritability (i.e. the proportion of total phenotypic variation that is due to the additive effects of genes, h^2) for nicotine or food reward was obtained using the formula: h2=½*VA/(½ *VA+VE) (Hegmann and Possidente 1981; Mogil et al. 1999).

Statistical analysis

The number of licks on active and inactive spouts were transformed into log scale to fit a normal distribution. Licks, reward, and intake during acquisition were analyzed by repeated measures ANOVA, where session and spouts were treated as within subject variables. Phenotypic correlations were determined using the Pearson test. Data were expressed as mean ± SEM. Statistical significance was assigned at $p< 0.05$. All analyses were conducted using R statistical language. Data are available on request from the authors.

Results

Oxycodone SA in Males and Females Across the HRDP

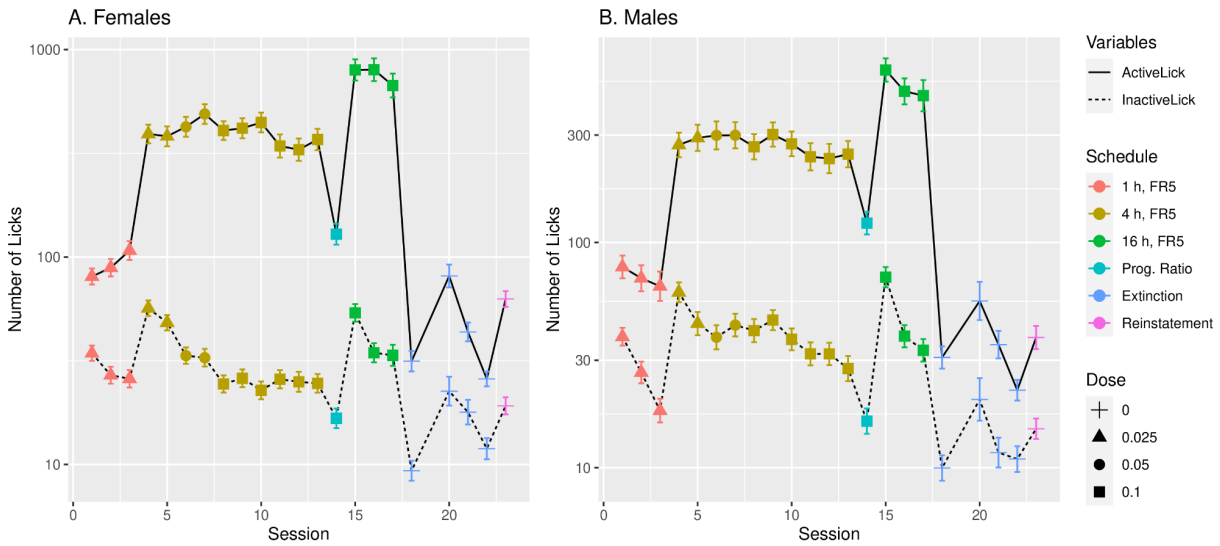

Figure 2. Number of licks during oxycodone SA across HRDP in females (A) from 33 strains and males (B) from 26 strains. Rats showed strong preference for the active over inactive spout in both sexes. Number of licks on the active spout were greater in females (n=204) than in males (n=154) for both 4-h and 16-h sessions ($p< 0.001$ for all). Number of licks on the inactive spout was greater in males than in females ($p<0.001$) during 4-h but not different during 16-h sessions. The Y-axis is in log scale.

Oral operant oxycodone SA was studied in females from 33 HRDP strains (Figure 2A) and in males from 26 strains (Figure 2B). The number of active (mean±sem) and inactive licks emitted by rats across all strains were shown for operant sessions that increased in duration and dose from 1 to 4-h at oxycodone 0.025 mg/ml per 5 licks, then to 4-h at oxycodone 0.05 mg/kg to 0.1 mg/kg, and then to 16-h of extended access to oxycodone 0.1 mg/ml, followed by 4 extinction sessions on alternating days and reinstatement of extinguished drug seeking behavior. A progressive ratio schedule of oxycodone 0.1 mg/kg was conducted on the day prior to the first 16-h session. In both sexes (Figure 2), a strong preference for the active vs. inactive spout was observed across the HRDP at all doses and session durations. The number of licks emitted on the

active spout were significantly greater in females than males during both 4-h and 16-h sessions (4-h, $F_{1,316}=12.04$, $p<0.001$; 16-h, $F_{1,264}=6.14$, $p<0.01$). Licks on the inactive spout were not different between sexes during 4-h and 16-h sessions (4-h, $F_{1,314}=1.10$, $p>0.05$; 16-h, $F_{1,264}=0.08$, $p>0.05$).

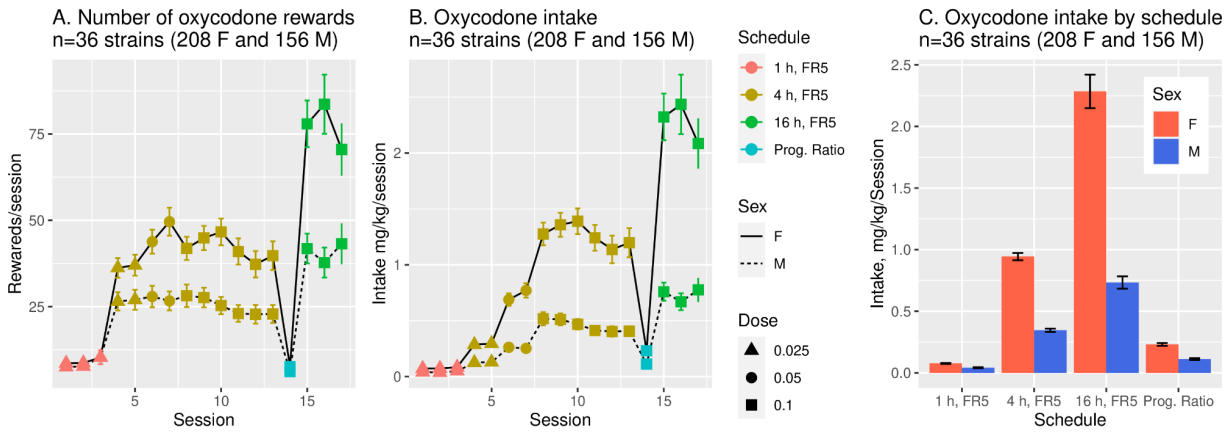

Figure 3. Sex differences in oxycodone rewards (A) and intake (B) across HRDP. Both parameters are means for all rats during each session. C) Intake was significantly greater in females than in males across different stages of SA ($p<0.001$ for all). In both sexes, the greatest intake occurred during 16-h sessions.

There was a strong sex difference in oxycodone intake across the HRDP (Figure 3). Across all stages of oxycodone SA at FR5, mean intake per session was significantly greater in females than males (Figure 3B, $F_{1,327}=64.4$, $p=1.88e-14$ for all stages). In both sexes, the greatest intake occurred during extended access sessions (Figure 3C): given extended access to the same dose of oxycodone (i.e.,0.1 mg/ml) available in 16-h sessions, both males and females significantly escalated their intake (Females: $F_{1,901}=162.9$, $p=2e-16$; Males: $F_{1,753}=83.6$, $p=2e-16$). Across the HRDP, intake escalated during our 65-day protocol, from the initial 1-h sessions at 0.025mg/ml to the final 16-h sessions at 0.1 mg/ml. In males, the escalation range was 4.67 (BN-Lx/CubMcwi) to 126.0-fold (LE/StmMcwi); in females, it was 7.2 (BN/NHsdMcwi) to 167.2-fold (SR/JrHsdMcwi).

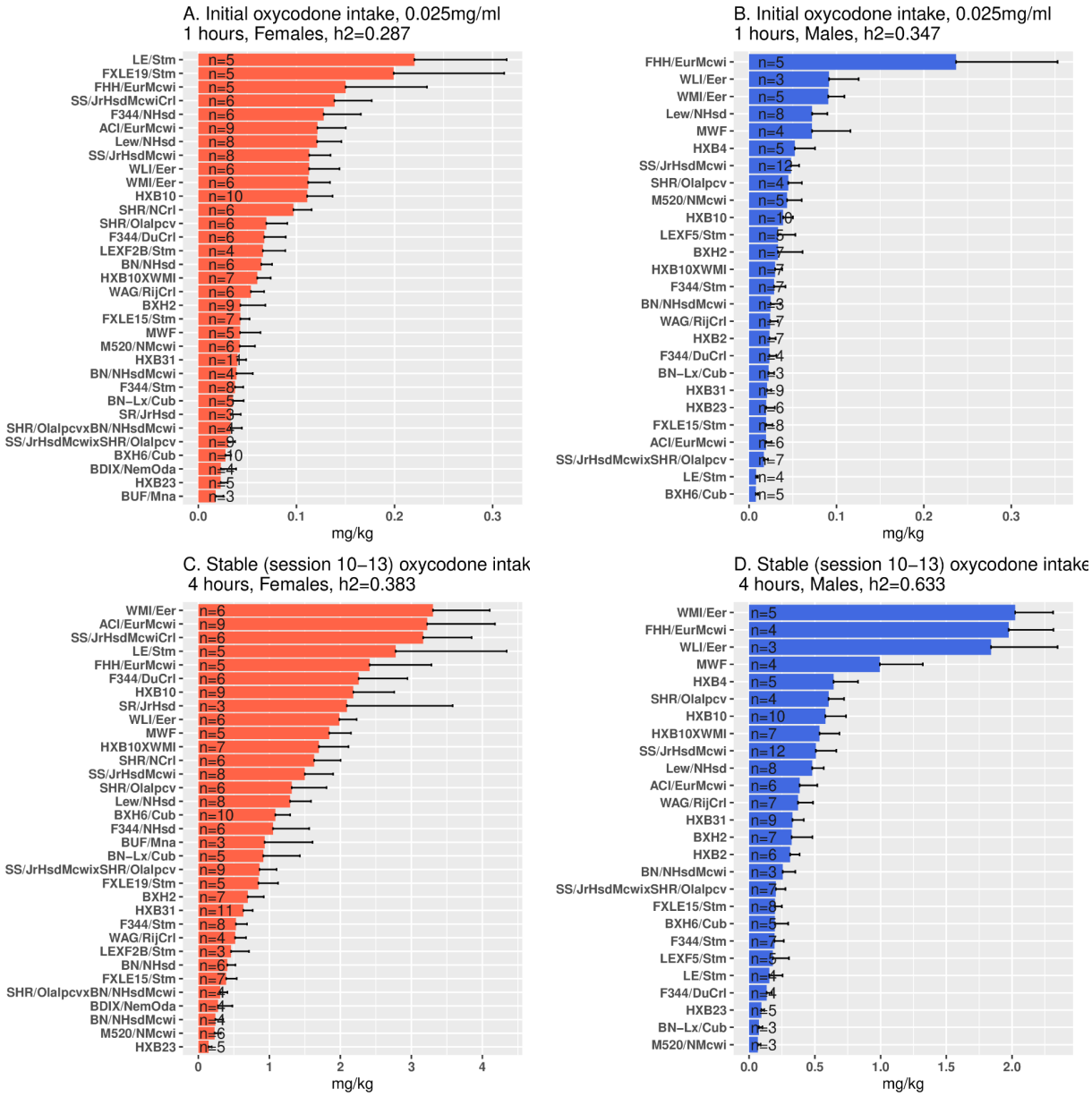

Figure 4. Initial and stable oxycodone intake by strain across the HRDP. Mean intake from the first three sessions (top 2 panels) showed large strain differences in both sexes. Similarly, mean intake during the last three 4-h sessions (stable intake; lower 2 panels) varied across the HRDP in both sexes.

Oxycodone intake was strain-dependent in both sexes. Mean intake during the first three 1-h sessions, when SA of oxycodone (0.025 mg/ml) was acquired (Figure 4A and 4B), varied greatly by strain ($F_{25,432}$=9.07, p=9.5e-27 for males and $F_{32,570}$=7.45, p=5.3e-27 in females). Similarly, mean intake during the final three 4-h sessions, when

stable oxycodone (0.1 mg/ml) intake was attained (Figure 4C, 4D), varied greatly by strain in both sexes ($F_{25,506}$=25.74, p=3.8e-74 in males and $F_{32,620}$=9.59, p=1.30e-36). Substantially greater intake by females than males was evident across most strains in both the initial and stable stages of oxycodone SA (note: the difference in X axis scales).

Stable Oxycodone Intake during 4-h Limited Access vs.16-h Extended Access Sessions

We compared and correlated stable oxycodone intake (0.1 mg/ml) during limited 4-h vs. extended 16-h sessions in both sexes across all HRDP strains. Oxycodone intake in 16-h sessions exceeded 4-h sessions in many strains (Figure 5A, 5B). This change was sex-specific in that specific strains showed increased 16-h consumption in only one sex. In addition, a greater number of strains throughout HRDP showed increased 16-h oxycodone intake in males compared to females, and rat strains in the lowest quartile and lower 50% of 4-h oxycodone intake showed a considerably greater number of strains with increased intake during 16-h sessions in males compared to females. Figures 5C and 5D show the correlations in females and males, respectively, between oxycodone intake in 4-h vs. 16-h sessions across all strains (female, r=0.832, p<0.0001; male, r=0.835, p<0.0001). In summary, males escalated their oxycodone intake during extended access sessions in a greater number of strains than females; this was particularly evident in the males of strains with relatively low 4-h oxycodone intake. However, in both sexes across the entire HRDP, oxycodone intake during 4-h sessions was predictive of intake during 16-h sessions.

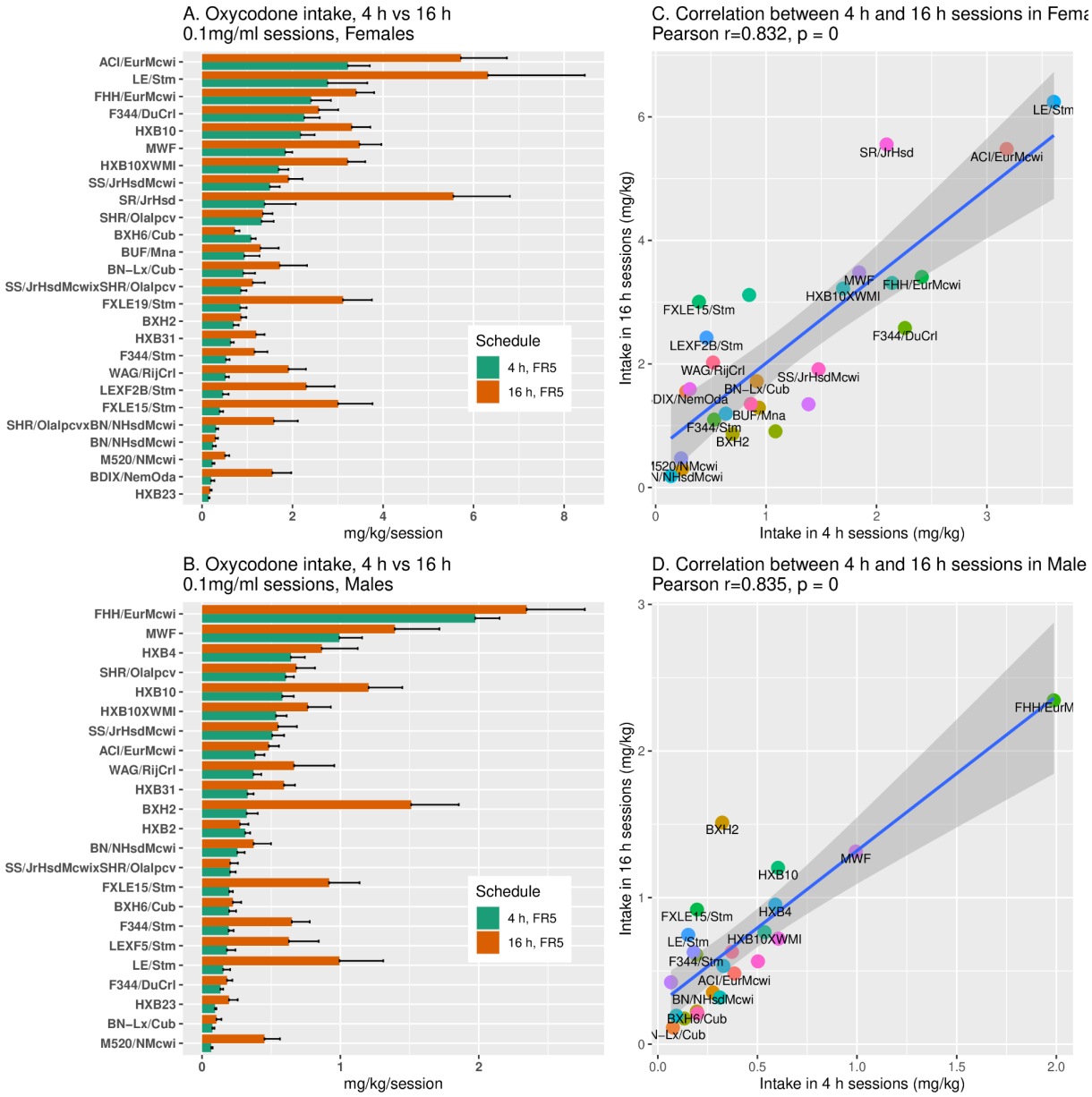

Figure 5. Oxycodone (0.1 mg/kg) intake in 4-h and 16-h sessions by sex. A, B: Extending access from 4-h to 16-h increased total drug intake/session in many strains and both sexes. Within specific strains, increased 16-h intake was sex-specific. C, D: The correlation of oxycodone intake between 4 h and 16 h sessions was significant in both females ($r=0.832$, $p< 0.0001$) and males ($r=0.835$, $p<0.0001$).

Progressive Ratio Schedule and Reinstatement of Extinguished Oxycodone Seeking

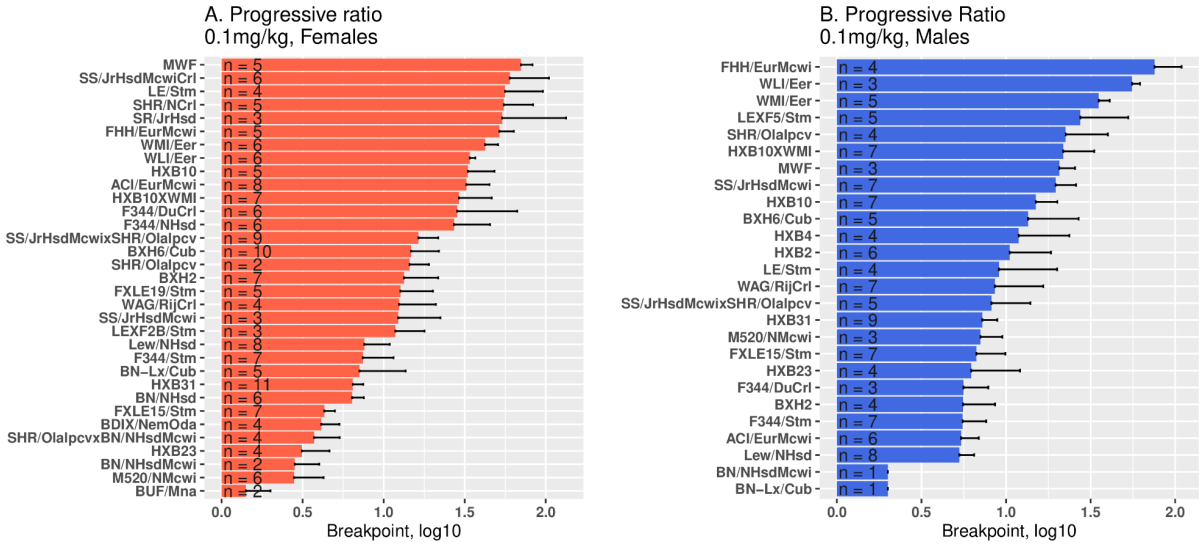

Figure 6. **Breakpoints during progressive ratio schedule of increasing active licks per oxycodone dose (0.1 mg/kg).** In both sexes, the breakpoints reached were significantly strain-dependent (2-way ANOVA: strain, $F_{35,273}$=5.48, p<2e-16; sex, $F_{1,273}$=5.65, p=0.018) and greater in females than males. X-axis is in log scale.

In both sexes, the breakpoints reached were significantly strain-dependent (Figure 6A, 6B) and greater in females than in males (2-way ANOVA: strain, $F_{35,273}$=5.48, p<2e-16; sex, $F_{1,273}$=5.65, p=0.018). Therefore, in both sexes, the motivation to obtain oral oxycodone is strain- and sex-dependent.

Figure 7 shows cue-induced reinstatement of extinguished oxycodone seeking. The number of active licks elicited during this protocol was strain- ($F_{35,257}$=3.31, p=2.2e-8) and sex-dependent ($F_{1,257}$=17.5, p=4.1e-5). This suggests that oxycodone-seeking elicited by cue is stronger in females across the HRDP than males.

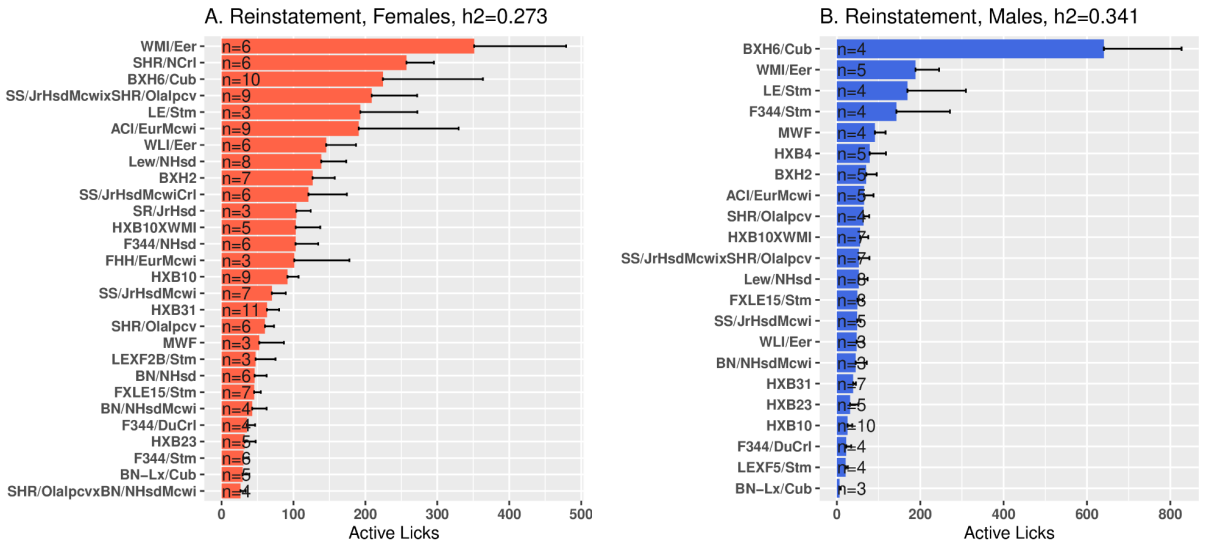

Figure 7. **Cue-induced reinstatement of extinguished oxycodone seeking.** Reinstatement was conducted after the number of licks on the active spout was less than 100 during two consecutive extinction sessions. In strains that manifest strong drug seeking, the behavior were most commonly elicited in both males and females.

The Correlation between Male (M) vs. Female (F) Oxycodone Intake

We found significant correlations between male vs. female oxycodone (0.1 mg/kg) intake during 4-h and 16-h sessions across the HRDP. During 4-h sessions with stable oxycodone intake, male vs. female intake was highly correlated across the HRDP (Figure 8A, Pearson r=0.569, p=0.005). However, during 16-h sessions, intake between sexes was not correlated across the HRDP (Figure 8B, r=0.374, p=0.104). The heritability of oxycodone intake declined in males from 4-h to 16-h sessions (Table 2; h^2 0.633 to 0.222) and this also occurred, to some extent, in females (h^2 0.383 to 0.299); this decrease would increase intra-strain variability in intake and reduce the correlation between sexes (Figure 8B).

Despite strong sex differences in oxycodone intake (Figures 4-7), motivation to obtain oxycodone, seeking behavior during reinstatement, and 4-h intake were highly correlated between males and females across strains. This demonstrates the strong strain-dependent genetic control of oxycodone intake that co-exists with sex-dependent differences in oxycodone behaviors. The data in Table 2 underscores this strong strain-

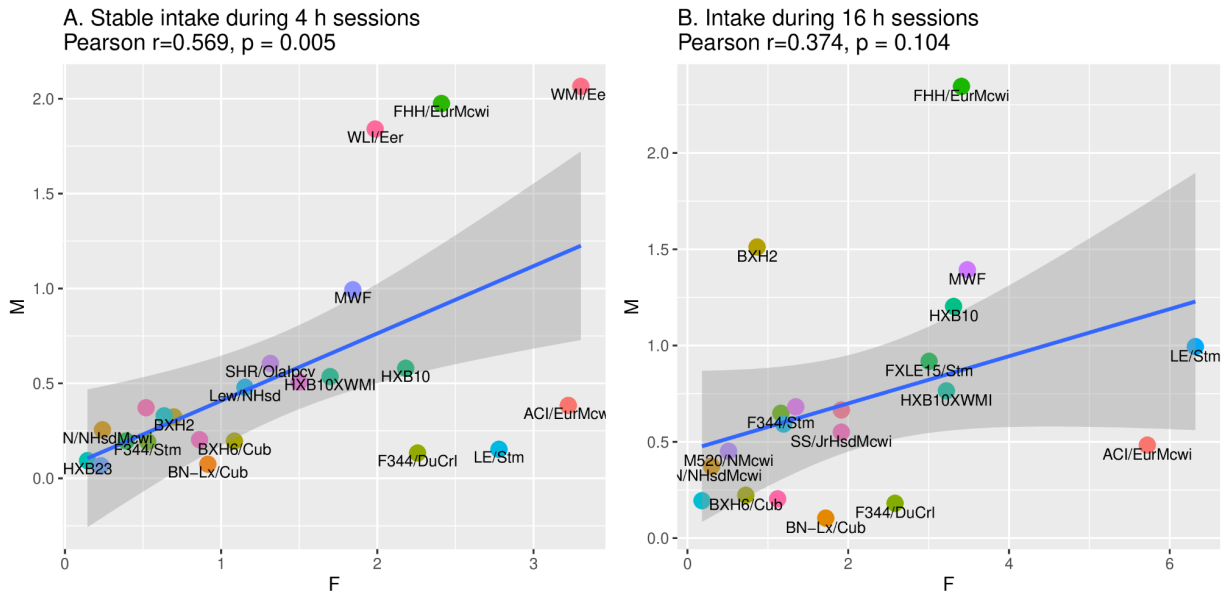

Figure 8. **Correlation in male (M) vs. female (F) oxycodone (0.1 mg/kg) intake/session during 4-h and 16-h sessions across the HRDP.** A. During 4-h sessions with stable oxycodone intake, M vs F intake was highly correlated across the HRDP (Pearson r=0.54, p=0.008). B. However, during 16-h sessions, intake between sexes was not significantly correlated (r=0.347, p=0.104).

dependent genetic control: we found that multiple parameters of oxycodone self-administration are significantly correlated between males vs. females across the HRDP. These are: oxycodone intake during 1-h, 4-h and 16-h sessions, effort to obtain reward (i.e., breakpoint and rewards obtained during PR trial), and active licks during reinstatement of oxycodone seeking.

Table 2. Correlation between males and females in oxycodone self-administration.

Stage	Phenotype	pearson r	p
FR5, 0.025mg, 1h	ActiveLick	0.185	0.3986
	InactiveLick	0.563	0.0051
	Reward	0.514	0.0120
	Intake	0.402	0.0573
FR5, 0.025mg, 4h	ActiveLick	0.298	0.1666
	InactiveLick	0.331	0.1227
	Reward	0.674	0.0004

	Intake	0.589	0.0031
FR5, 0.05mg, 4h	ActiveLick	0.419	0.0464
	InactiveLick	0.188	0.3901
	Reward	0.713	0.0001
	Intake	0.629	0.0013
FR5, 0.1mg, 4h	ActiveLick	0.435	0.0380
	InactiveLick	0.254	0.2422
	Reward	0.768	0.0000
	Intake	0.690	0.0003
FR5, 0.1mg, 4h Stable	ActiveLick	0.439	0.0362
	InactiveLick	-0.090	0.6831
	Reward	0.653	0.0007
	Intake	0.567	0.0047
FR5, 0.1mg, 16h	ActiveLick	0.328	0.1578
	InactiveLick	0.361	0.1182
	Reward	0.441	0.0513
	Intake	0.374	0.1044
Progressvie Ratio	ActiveLick	0.426	0.0429
	InactiveLick	0.169	0.4405
	BreakPoint	0.649	0.0008
	Intake	0.493	0.0168
Extinction Day1	ActiveLick	0.239	0.3112
	InactiveLick	0.125	0.6009
Extinction Last Day	ActiveLick	0.300	0.1858
	InactiveLick	0.045	0.8478
Reinstatement	ActiveLick	0.517	0.0138
	InactiveLick	0.217	0.3313
Total	ActiveLick	0.302	0.1613
	InactiveLick	0.314	0.1439
	Reward	0.587	0.0032
	Intake	0.402	0.0574

The Heritability (h^2) of Oxycodone Behavioral Phenotypes

Table 3 shows the heritability (h^2) of oxycodone SA parameters by sex. In males, the h^2 for oxycodone intake in 4-h sessions (mg/kg b.wt./session) was consistently greater than in females. For example, in 4-h sessions at oxycodone 0.025, 0.05 and 0.10 mg/ml/dose, the h^2 in males were 0.44, 0.65 and 0.63, respectively, compared to 0. 30, 0.41 and 0.37, respectively, in females. Hence, h^2 was approximately 50% higher in males than females. In contrast, h^2 for active licks in 4-h sessions at the same three doses of oxycodone was consistently higher in females than males (females: 0.41, 0.47, and 0.51 vs. males: 0.3, 0.3, 0.39). In 16-h sessions at oxycodone 0.10 mg/ml/dose, the h^2 for both intake and active licks was greater in females than males: intake, 0.30 vs 0.22, respectively; active licks, 0.40 and 0.25, respectively. Additionally, the female h^2 for intake under a. progressive ratio schedule was 0.41 vs. 0.31 in males; active licks under this schedule showed a greater divergence in h^2 between female and male: 0.46 and 0.15, respectively. Overall, the relative degree of oxycodone heritability in males vs. females was specific for each behavioral phenotype. Oxycodone intake in 4-h sessions at three doses showed the highest heritability and some of the largest differences between sexes, with males considerably greater than females. However, active licks in 4-h sessions and both intake and active licks in 16-h sessions and under a PR schedule were greater in females than males.

Table 3. Heritability and ANOVA results for oxycodone SA parameters

		Males				Females			
	Phenotype	**h2**	**Df**	**F**	**p**	**h2**	**Df**	**F**	**p**
FR5, 0.025mg, 1h	Active Lick	0.302	(25,441)	7.77	1.3E-22	0.368	(32,588)	10.87	2.2E-41
	Inactive Lick	0.216	(25,436)	5.20	8.4E-14	0.227	(32,579)	5.89	3.0E-20
	Reward	0.341	(25,441)	9.13	4.9E-27	0.261	(32,588)	6.99	4.0E-25
	Intake	0.347	(25,432)	9.07	9.5E-27	0.287	(32,570)	7.45	5.3E-27
FR5, 0.025mg, 4h	Active Lick	0.297	(25,277)	5.34	3.3E-13	0.411	(32,376)	8.71	2.9E-29
	Inactive Lick	0.081	(25,272)	1.86	9.0E-03	0.252	(32,366)	4.63	1.2E-13

	Reward	0.443	(25,277)	9.15	3.8E-24	0.342	(32,376)	6.75	5.2E-22
	Intake	0.439	(25,275)	8.95	1.4E-23	0.297	(32,374)	5.65	9.8E-18
FR5, 0.05mg, 4h	Active Lick	0.298	(25,276)	5.31	4.2E-13	0.469	(32,374)	10.82	1.9E-36
	Inactive Lick	0.156	(25,276)	2.84	1.6E-05	0.228	(32,358)	4.11	1.5E-11
	Reward	0.655	(25,278)	20.38	5.2E-49	0.417	(32,374)	8.95	4.5E-30
	Intake	0.652	(25,278)	20.12	1.6E-48	0.406	(32,374)	8.59	9.0E-29
FR5, 0.1mg, 4h	Active Lick	0.295	(25,277)	5.23	7.0E-13	0.531	(32,372)	13.40	1.7E-44
	Inactive Lick	0.072	(25,275)	1.78	1.4E-02	0.158	(32,363)	2.96	4.5E-07
	Reward	0.663	(25,277)	20.93	6.3E-50	0.378	(32,372)	7.65	2.5E-25
	Intake	0.654	(25,277)	20.17	1.5E-48	0.367	(32,372)	7.36	2.8E-24
FR5, 0.1mg, 4h Stable	Active Lick	0.39	(25,506)	10.17	2.9E-31	0.509	(32,620)	15.33	4.4E-59
	Inactive Lick	0.206	(25,505)	4.65	4.2E-12	0.252	(32,615)	5.53	7.9E-19
	Reward	0.644	(25,506)	26.86	1.1E-76	0.369	(32,620)	9.10	1.5E-34
	Intake	0.633	(25,506)	25.74	3.8E-74	0.383	(32,620)	9.59	1.3E-36
FR5, 0.1mg, 16h	Active Lick	0.25	(22,352)	5.60	2.0E-13	0.396	(25,426)	10.92	2.1E-32
	Inactive Lick	0.149	(22,350)	3.41	7.0E-07	0.122	(25,425)	3.10	1.4E-06
	Reward	0.252	(22,352)	5.65	1.4E-13	0.292	(25,426)	7.25	1.0E-20
	Intake	0.222	(22,352)	4.94	1.9E-11	0.299	(25,426)	7.44	2.3E-21
Progressvie Ratio	Active Lick	0.147	(23,103)	1.82	2.2E-02	0.458	(29,145)	5.42	2.7E-12
	Inactive Lick	0.083	(23,101)	1.41	1.2E-01	0.212	(29,145)	2.41	3.4E-04
	BreakPoint	0.256	(23,103)	2.64	4.6E-04	0.44	(29,145)	5.10	1.7E-11
	Intake	0.311	(23,103)	3.14	3.8E-05	0.408	(29,145)	4.60	3.6E-10
Extinction Day1	Active Lick	0.316	(21,99)	3.24	4.4E-05	0.299	(25,125)	3.20	1.0E-05
	Inactive Lick	0.142	(21,99)	1.80	2.9E-02	0.106	(25,125)	1.61	4.6E-02
Extinction Last Day	Active Lick	0.22	(20,81)	2.26	5.4E-03	0.275	(25,127)	2.95	3.9E-05
	Inactive Lick	0.036	(20,81)	1.17	3.1E-01	0.221	(25,127)	2.46	5.9E-04
Reinstatement	Active Lick	0.341	(21,92)	3.41	2.5E-05	0.273	(27,140)	2.94	2.0E-05
	Inactive Lick	0.07	(21,92)	1.35	1.7E-01	0.255	(27,140)	2.76	5.9E-05
Total	Active Lick	0.217	(25,2395)	22.84	7.3E-93	0.305	(32,3097)	37.86	3.0E-195
	Inactive Lick	0.122	(25,2380)	11.63	4.5E-44	0.142	(32,3047)	14.40	2.0E-71
	Reward	0.326	(25,2397)	39.14	8.6E-158	0.181	(32,3097)	19.50	5.1E-100
	Intake	0.196	(25,2386)	20.14	2.4E-81	0.146	(32,3077)	15.21	4.8E-76

Correlations between Behavioral Tests and Oxycodone SA

We assessed anxiety-like and response to novelty using elevated plus maze (EPM), open field test (OFT) and novel object interaction (NOI) tests in naive rats (strain mean by sex is provided in supplementary Table 1). Table 4 shows that all traits defining each behavioral test (i.e., 5 EPM traits, 4 OF, 4 NOI) differed significantly across a subset of HRDP strains (n=19 female and 17 male) that we evaluated in both sexes. Additionally, Table 4 shows the h^2 values for each trait by sex. In general, the greatest h^2 values in both sexes were found for total distance in all three behavioral tests (range across tests by sex: female, 0.51-0.58; male, 0.24-0.49).

Table 4. Heritability and ANOVA for comparison across HRDP of anxiety-like and novelty response traits in naive inbred rats.

Behavior Test	Trait	Males				Females			
		h2	Df	F	p	h2	Df	F	p
OFT	totalDistance	0.493	(15,96)	6.41	3.19E-09	0.585	(15,105)	10.06	2.19E-14
OFT	dist2CtrMean	0.553	(15,96)	7.87	3.26E-11	0.437	(15,105)	5.98	7.96E-09
OFT	cntrDurat	0.326	(15,96)	3.69	4.44E-05	0.123	(15,105)	1.90	3.07E-02
OFT	cntrFreq	0.334	(15,96)	3.79	3.09E-05	0.395	(15,105)	5.19	1.32E-07
NOI	totalDistance	0.398	(15,100)	4.89	4.74E-07	0.562	(16,114)	9.06	5.71E-14
NOI	dist2CtrMean	0.276	(15,100)	3.25	2.13E-04	0.248	(16,114)	3.07	2.54E-04
NOI	cntrDurat	0.105	(15,100)	1.69	6.40E-02	0.152	(16,114)	2.13	1.15E-02
NOI	cntrFreq	0.272	(15,100)	3.20	2.58E-04	0.489	(16,114)	7.03	5.35E-11
EPM	totalDistance	0.237	(16,88)	2.75	1.31E-03	0.510	(18,101)	6.69	1.12E-10
EPM	openDurat	0.191	(16,88)	2.34	6.27E-03	0.216	(18,101)	2.50	2.04E-03

EPM	openFreq	0.295	(16,88)	3.37	1.29E-04	0.241	(18,101)	2.74	7.68E-04
EPM	closedDurat	0.300	(16,88)	3.42	1.08E-04	0.118	(18,101)	1.73	4.60E-02
EPM	closedFreq	0.225	(16,88)	2.64	2.01E-03	0.339	(18,101)	3.80	8.58E-06

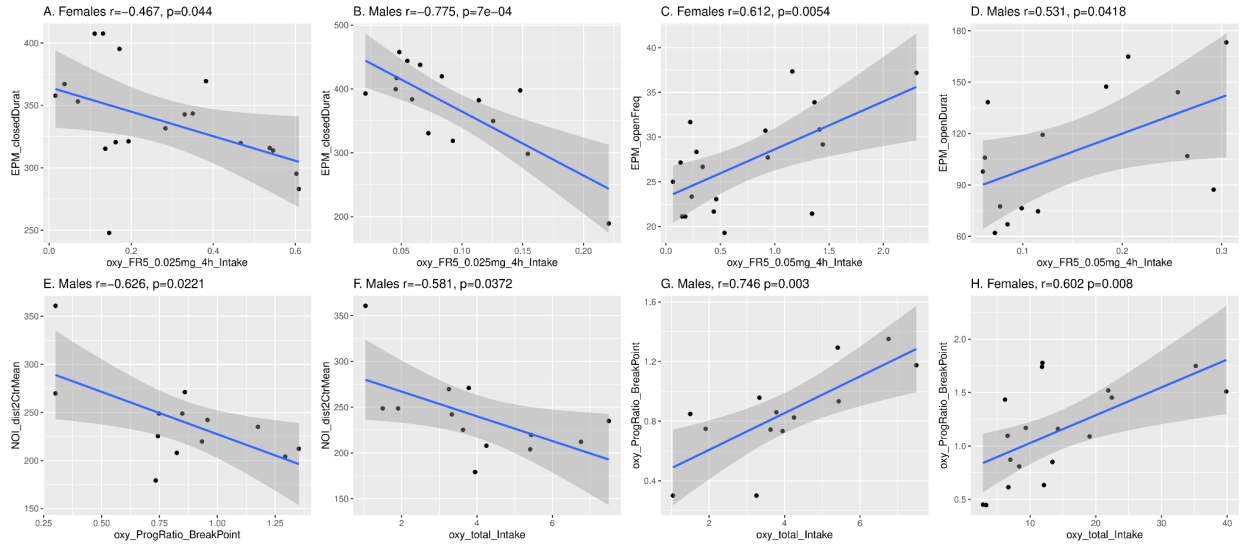

Figure 9. Representative correlations between oxycodone SA parameters and behavioral traits. Panels A-F show significant correlations for EPM vs. oxycodone intake in 4-h SA sessions at 0.25 and 0.5 mg/ml in female (panels A, C) and male (B, D) HRDP strains. Significant correlations in male HRDP strains between NOI (distance to center) and PR (breakpoint) or total oxycodone intake are in panels E and F, respectively. Correlations between NOI and PR or total intake were not significant in females (see supplementary Figure S1). Panels G and H, show significant correlations between two SA parameters, PR and total intake, in males and females, respectively.

We correlated multiple traits measured in each behavioral paradigm with 28 parameters of oxycodone SA. Figure 9 shows examples of two significant correlations for EPM by sex vs. intake of oxycodone 0.025 (panels A, B) and 0.05 (panels E, F) mg/ml in 4-h sessions. Figure 10 shows that significant EPM-defining traits (i.e., female, 13; male, 7) were associated with multiple SA parameters in both sexes, and a large fraction of these associations were unique to a sex (e.g., only in females, 7/13). In females, Figure 10 shows that three EPM traits were significantly correlated with total 4-h oxycodone intake, whereas only one trait correlated with 4-h intake in males. Many more OFT traits

were SA-associated in males than females (i.e., 8 in males vs 2 in females) and specific SA associations were unique to each sex (Figure 10). NOI traits were associated with four SA parameters in males, but none in females. Amongst these male NOI associations, NOI was significantly correlated with total oxycodone intake (i.e., sum of

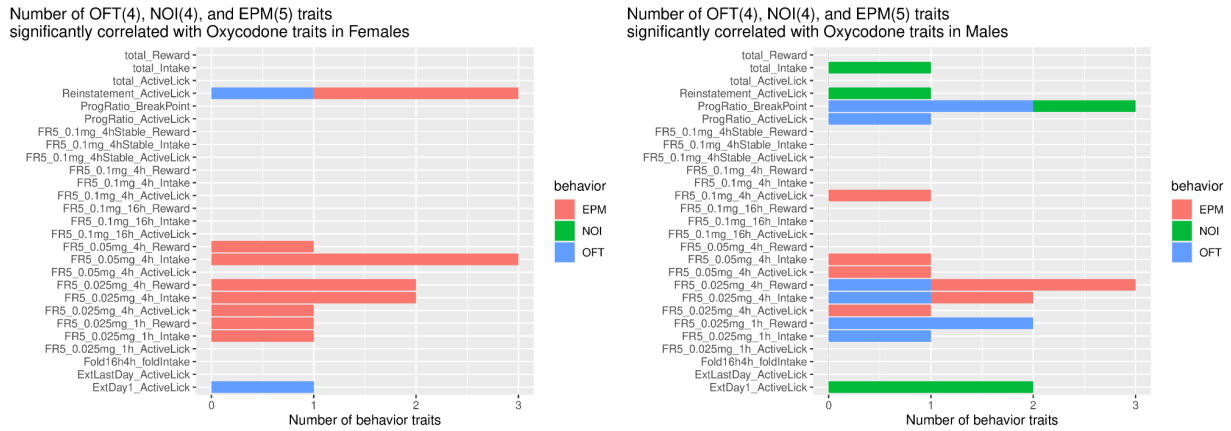

Figure 10. Behavioral traits that significantly correlate with oxycodone SA parameters. This bar graph groups all the behavioral traits measured in EPM, OFT, and NOI trials that were associated with each SA parameter; this grouping is based on the significant correlations ($p<0.05$) identified between a single behavioral trait and an SA parameter (supplementary data, figures S1 and S2).

all doses at all time intervals in males/strain) and with active licks during reinstatement compared to OFT and EPM in reinstating females. Figure 9 D shows the significant correlation by strain for NOI (distance to center) and total oxycodone intake. With respect to oxycodone SA at the highest dose (0.1 mg/ml), no behavioral traits were correlated with intake, reward or active licks at 4-h and 16-h in either sex, barring one exception - active licks in males during 4-h sessions correlated with EPM. This is the same oxycodone dose consumed in the PR study that correlated with NOI and OF only in males.

Correlation in Males between PR Breakpoint, NOI (distance to center), and Total Oxycodone Intake

NOI (distance to center) was significantly correlated with both PR breakpoint and total oxycodone intake in males, but not females (Figure 10); indeed, no behavioral traits

were associated with either of these two SA parameters in females. Figure 9C shows this significant correlation by strain for NOI and PR breakpoint in males. In Figure 9 (panels G, H), PR breakpoint was also significantly correlated across strains with total oxycodone intake in both males and females. In summary, the following correlations were identified in males across strains: PR breakpoint x NOI (distance to center); total oxycodone intake x NOI; PR breakpoint x total oxycodone intake. Hence, these three independent measures are all significantly inter-correlated across strains in males, but not in females. This strongly suggests that CNS mechanisms governing novelty seeking and motivation to take oxycodone interact in males to regulate the total consumption of oxycodone.

Discussion

Inter-individual variation amongst humans in the susceptibility to chronic drug abuse is characteristic of addiction to opioids and other drugs of abuse. As in humans, outbred animal models demonstrate significant inter-individual variation in behavioral responses to testing paradigms with face validity for important dimensions of human addiction (Deroche-Gamonet et al. 2004). We took advantage of the highly replicable behaviors observed amongst individuals within inbred rat strains to identify behavioral parameters of oxycodone SA that consistently varied across a large panel of inbred strains (i.e. hybrid rat diversity panel, HRDP) -bred, raised and tested in the same vivarium. Under these conditions, consistent inter-strain variation in oxycodone SA should reflect significant differences in the genetic regulation of CNS functions controlling SA behavioral parameters. Indeed, we identified multiple heritable parameters of oxycodone SA that also showed significant sex-dependency. Many of these SA parameters were significantly correlated with the responses in naive HRDP rats to independent behavioral testing in EPM, NOI and OFT.

Oxycodone intake, both during initiation of SA and stable intake in 4-h sessions, was significantly strain-dependent in both sexes (Figure 4) and heritable (Table 3). Considering all strains, the mean amount consumed across all sessions (Figure 3) of increasing duration and dose was significantly greater in females than males ($p<0.001$). Additionally, the mean number of licks (Figure 2) emitted on the active spout was significantly greater in females than males during both 4-h and 16-h sessions ($p< 0.001$ for all). . Given extended access to drug in 16-h sessions (Figure 3), both sexes consumed approximately twice as much compared to 4-h sessions at the same dose. Similar to 4-h sessions, oxycodone intake in 16-h sessions was heritable in both sexes (Table 2). On a strain basis, escalation of intake was more prominent in males, occurring in many more strains (Figure 5 A, B). However, within both sexes across the HRDP, oxycodone intake was correlated in 4-h vs. 16-h sessions (Figure 5C, 5D). Therefore, in individual female and male strains, intake in 4-h sessions predicted intake during extended access sessions. This within-strain correlation of oxycodone intake at the same dose (0.1 mg/ml) in 4-h and 16-h sessions across the HRDP is most probably controlled by the heritability of genes that regulate intake in both short and extended access sessions.

In general, females across all strains manifest greater oxycodone intake in both short and extended access sessions and greater numbers of active licks. This strongly suggests the existence of a basic sex difference in the amount of oxycodone required to establish stable oral oxycodone reinforcement within the CNS. This sex difference in SA responses to oxycodone may reflect brain differences in interaction between oxycodone and the opioid circuitry expressed in brain reward centers. An extensive literature on sex differences in the sensitivity to opiate reinforcement and analgesia supports this hypothesis.

Morphine has been reported to induce a more pronounced place preference in female Wistar rats at similar doses (Karami and Zarrindast 2008). In self-administering female Sprague Dawley rats, more intravenous (i.v.) morphine and heroin were consumed and a broader range of doses were reinforcing than in males (Cicero et al. 2003). Moreover,

at doses in the upper end of the dose-response range, morphine induced place preference in females , but was no longer effective in males (Cicero et al. 2000). Both estrogen and progesterone receptors have been detected in dopamine terminals and median spiny neurons within nucleus accumbens (NAc), which is involved in reward-associated learning and motivation to goal-oriented behaviors (Yoest et al. 2018); (Kalivas and Volkow 2005). Estradiol rapidly enhanced NAc dopamine release and modulated dopamine binding, effects also observed in cycling females (Yoest et al. 2018). In mice, basal dopamine neuron activity and dopamine release in NAc were similar in both sexes, except during estrus when both increased (Calipari et al. 2017). Additionally, both systemic and intrastriatal estradiol rapidly amplified amphetamine-induced dopamine release in rat dorsal striatum (Shams et al. 2016). In summary, rat models of opiate preference and operant self-administration demonstrate that opiates are more reinforcing in females, and at a broader and higher dose range. This is likely due, in part, to enhanced responsiveness to opiate-induced dopamine release under the influence of ovarian steroids - particularly during estrus. These findings cohere with our observations that female rats show greater oxycodone intake and greater numbers of active licks in both short and extended access sessions across a range of doses. Hence, oral oxycodone appears to be more reinforcing in females across the HRDP, which drives greater licking behavior.

PR breakpoints were higher in female strains across the HRDP (2-way ANOVA: sex, $F_{1,273}$=5.65, p=0.018). Similar differences have been reported for i.v. opiate SA in outbred rats (Cicero et al. 2003). Since PR breakpoint is a heritable SA parameter in both sexes (h^2, F=0.44 ; M=0.256), it is reasonable to expect significant inter-strain variability in the effect of sex if the overall effect of sex is small to moderate and the interaction between sex and heredity in each strain depends on the specific subsets of genes modulating PR in each strain.

Multiple traits measured during independent behavioral tests, conducted in naive rats across a subset of HRDP strains, varied significantly across strains (Table 4) and were heritable (Table 4). These traits, which define key dimensions of each behavioral test,

were significantly correlated with specific parameters of oxycodone SA, depending on sex (Figure 10 and supplementary Figure S1). EPM traits were associated with a common subset of SA parameters in both sexes, whereas NOI traits were associated with SA only in males. In both 4-h and 16-h sessions at high dose oxycodone (0.1 mg/kg), with one exception (Figure 10: male, EPM vs. active licks, 4-h), no SA parameters were correlated with behavior in either sex. At high dose oxycodone, intake and active licks in short and long access sessions were not correlated with behavioral measures of anxiety (i.e., EPM and OFT) and novelty-seeking (i.e., NOI). These findings suggest that the reinforcing efficacy of high dose oxycodone is unaffected by the intrinsic, strain-dependent level of anxiety or novelty-seeking associated with oxycodone intake at lower doses.

The NOI trait of distance to the center was inversely associated with both PR breakpoint and total oxycodone intake in males across HRDP (Figure 9, 10). In contrast, no correlations were found between PR parameters or total oxycodone intake and any behavioral traits in females (Figure 10). Overall, the heritable, sex-specific correlation of particular SA parameters with specific behavioral traits indicates the operation of pleiotropic genes with functional effects on both parameters of oxycodone SA and specific behavioral traits that are modulated by sex, Significant 3-way correlations of PR breakpoint x NOI-distance to center, PR breakpoint x total oxycodone intake, and total oxycodone intake x NOI-distance to center strongly suggest that one set of pleiotropic genes may underlie these correlations in males. It is most likely that these genes directly modulate oxycodone and novelty seeking behavior, because the behavioral tests we conducted were conducted in drug naive individuals. Novelty-seeking and motivation to take oxycodone may interact and regulate oxycodone intake depending on the strain-specific expression of these common pleiotropic genes in males.

These studies demonstrate the strong strain-dependent inheritance of multiple oxycodone SA parameters and their correlations with independent measures of anxiety and novelty in established behavioral tests (i.e., EPM, OFT, NOI). Overall, active licks and oxycodone intake in multiple phases of our experimental protocol, including stable

intake in 4-h sessions and in 16-h extended access sessions, were significantly greater in females of most strains across the HRDP. In both sexes, intake during 16-h sessions showed escalation compared to 4-h, although more male strains escalated their intake compared to females. Yet, 4-h intake predicted 16-h intake in both sexes. The genome-wide search for quantitative trait loci (QTLs) based on mapping these strongly heritable SA parameters and behavioral traits is likely to yield positive results, especially when these parameters and traits (e.g., PR-breakpoint, total oxycodone intake, NOI-distance to center) involve pleiotropic genes. Such genes increase the confidence of finding strong gene candidates in QTLs because they are likely to be found as a result of overlapping single nucleotide polymorphisms (SNPs) discovered by independently mapping of several different behaviors.

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Conflict of Interest Statement

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Supplementary Figures

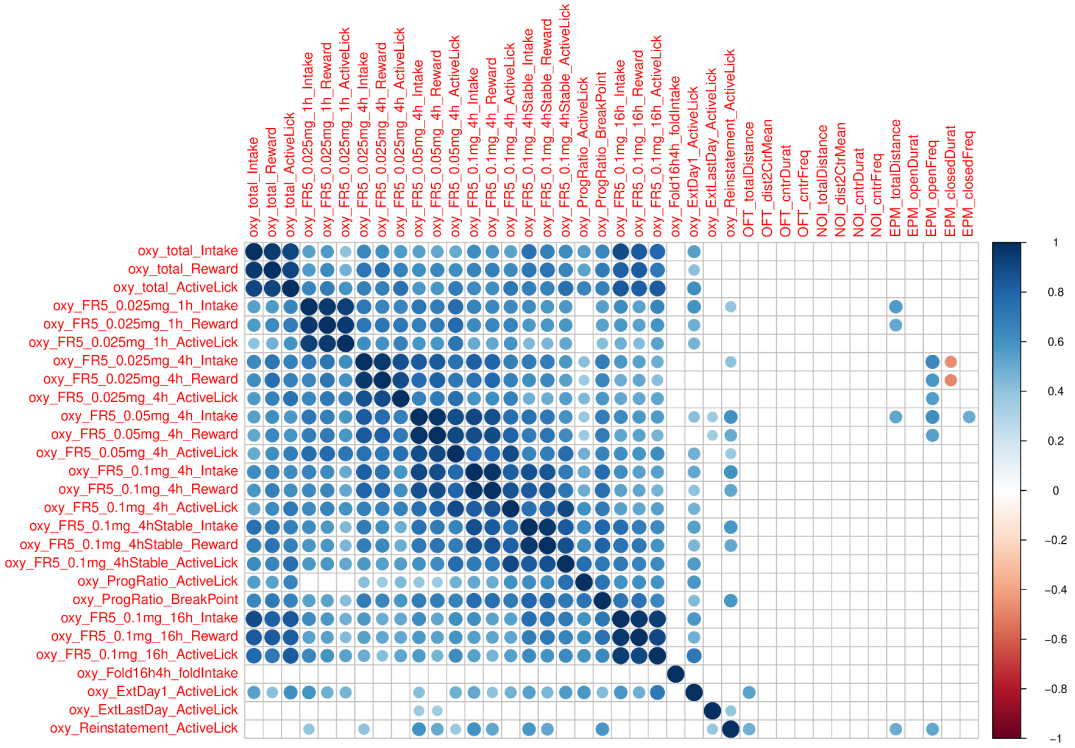

Figure S1-A. Correlation between oxycodone and behavioral traits in females

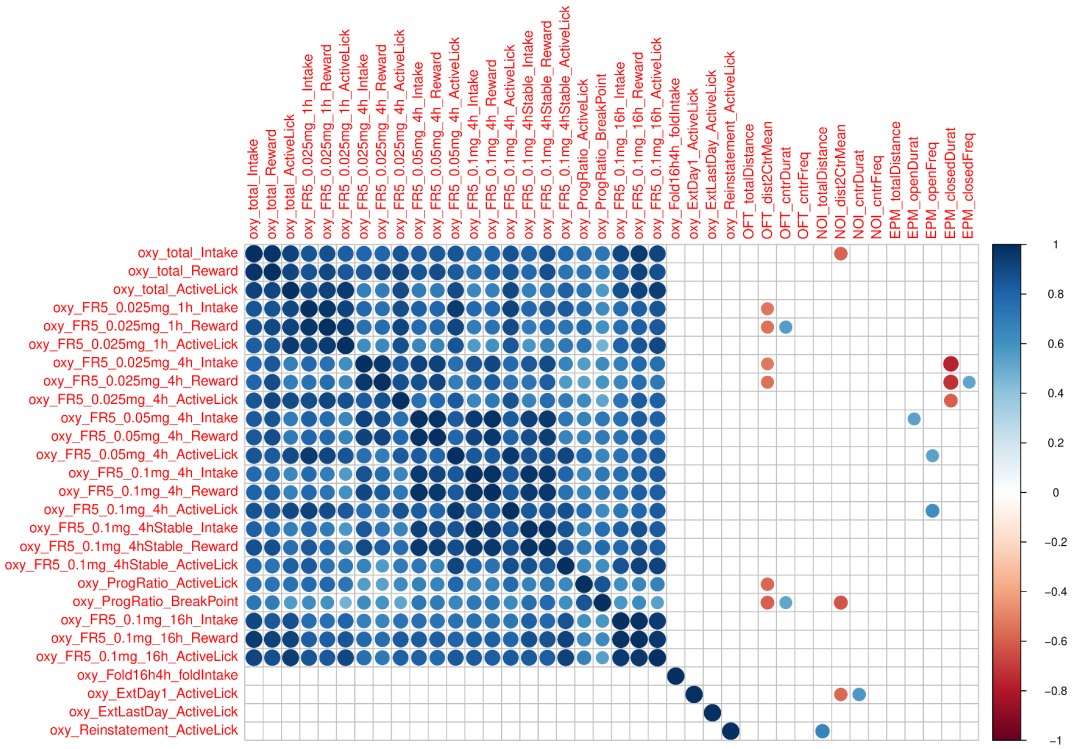

Figure S1-B. Correlation between oxycodone and behavioral traits in males