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Sex Differences in the Development of an Opioid Addiction–Like Phenotype: A Focus on the Telescoping Effect

Eleanor Blair Towers, Kyle A. Hsu, Emaan I. Qillawala, Shaniece D. Fraser, and Wendy J. Lynch

ABSTRACT

BACKGROUND: Women develop addiction and drug-related health consequences after fewer years of drug use than men; this accelerated time course, or telescoping effect, has been observed clinically for multiple drugs, including opioids. Preclinical studies indicate that this is a biologically based phenomenon; however, these studies have focused exclusively on cocaine, and none have considered health effects.

METHODS: In this study, we used a rat (Sprague Dawley) model to determine sex differences in the time course for the development of an opioid addiction–like phenotype, as defined by the development of physical dependence (withdrawal-induced weight loss) and an increase in motivation for fentanyl (under a progressive-ratio schedule). Effects were determined following either 10 days (optimized, experiment 1) or 3 days (threshold, experiment 2) of extended-access fentanyl self-administration (24 hours/day, fixed ratio 1, 2- to 5-minute trials/hour) or following short-access fentanyl self-administration (subthreshold, experiment 3; fixed ratio 1, up to 40 infusions/day). Opioid-related adverse health effects were also determined (experiment 4).

RESULTS: Motivation for fentanyl was similarly increased in males and females following 10 days of extended-access self-administration (experiment 1), was transiently increased in females, but not males, following 3 days of extended-access self-administration (experiment 2) and was not increased in either sex following short-access self-administration (experiment 3). Females developed fentanyl-associated adverse health effects more readily than males (experiment 4), with particularly robust differences during extended-access self-administration and withdrawal.

CONCLUSIONS: As with findings in humans, female rats developed opioid addiction–like features and adverse health consequences more readily than male rats. These data provide support for a biologically based telescoping effect in females for opioids, particularly for opioid-related adverse health consequences.

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Historically, research on opioid use disorder (OUD) has overlooked females (1–3), which has led to significant gaps in our understanding of the disease in this demographic. While overall rates of OUD and opioid-related overdose deaths are still higher in men than in women (4), it is notable that the proportion of females who initiate opioid use and develop OUD has risen (5). Moreover, studies indicate an enhanced vulnerability to addiction among females (6), as seen in the "telescoping effect," a quicker escalation from initial opioid use to seeking treatment for OUD in women than men [(7–13), but see (14,15)]. This pattern extends beyond opioids to other substances such as alcohol and cocaine [for a review of evidence for and against, see (16)], and for the development of drug-associated health consequences such as cirrhosis and hepatitis C (16–24).

Preclinical studies have similarly shown that addiction-like features emerge after less drug exposure or sooner during withdrawal in females than males (25). These studies have shown that 3 key features of addiction in humans, enhanced motivation, compulsive use, and increased relapse vulnerability, emerge/peak sooner during withdrawal following extended-access (ExA) self-administration in females than males (25–28). These features develop following ExA, but not following short-access (ShA) self-administration (29), and they increase in magnitude over a period of protracted withdrawal (25). Seven days of withdrawal appears to be the threshold for triggering heightened drug motivation in females, but not males, whereas 14 days appears to be optimal and induces the phenotype in both sexes (25,26,29). Moreover, following a prolonged period of ShA cocaine self-administration, females are also more likely than males to develop a preference for drug over a competing reward, such as food (~3–5 weeks; ~50 vs. 17%, respectively) (30–32), another key characteristic of substance use disorders in humans. While these findings strongly support the biological basis of the telescoping effect observed in humans, all the preclinical work reported to date has focused on cocaine rather than opioids.

In this study, we aimed to bridge this gap by exploring whether a similar telescoping effect would appear in a rat model of OUD using fentanyl. We examined 2 principal

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addiction-like features: physical dependence, indicated by withdrawal-induced weight loss, and heightened motivation for fentanyl, assessed through a progressive-ratio (PR) schedule before and after fentanyl self-administration and 14 days of withdrawal. We tested the effects under 3 conditions: after 10 days of ExA fentanyl self-administration (optimized), hypothesizing an addiction-like phenotype in both sexes (25,26,29); after 3 days of ExA (threshold), predicting a phenotype in females only (25,27); and after ShA administration (subthreshold), anticipating no phenotype in either sex (29). We also evaluated changes in motivation for fentanyl immediately and 24 hours post-ExA under optimized conditions to delineate the motivation trajectory from early to later withdrawal periods. Based on previous results with cocaine where motivation did not increase immediately post-ExA but rose significantly after prolonged withdrawal (25,33), we hypothesized that motivation for fentanyl would be minimal immediately post-ExA and peak following 14 days. Furthermore, we predicted that females would develop an addiction-like phenotype following less fentanyl exposure and/or after less withdrawal than males.

An additional aim of this study was to investigate whether the telescoping effect observed in females extends to adverse health consequences associated with fentanyl use. Based on clinical findings and observations from our laboratory suggesting that females are less likely to complete fentanyl self-administration studies due to adverse effects, we conducted a survival analysis on all gonad-intact adult male and female rats involved in our fentanyl (and saline) studies from February 2020 to September 2023. We hypothesized that female rats are more susceptible to opioid-related adverse health effects, leading to a higher likelihood of removal from studies due to these adverse outcomes.

METHODS AND MATERIALS

Animals

Sexually mature male (n = 53) and female (n = 55) Sprague Dawley rats (Charles River) were used as subjects. Rats were individually housed in operant test chambers (Med Associates) and maintained on a 12-hour light/dark cycle (lights on at 7 AM) with ad libitum access to water and food (Teklad LM-485 7912; except briefly when necessary during fentanyl self-administration training). After acclimating to the chambers (2 days) and lever pretraining (25), each rat was implanted with a jugular catheter (34). The health status of each rat was monitored daily. Body weights were determined a minimum of 3 times a week throughout the study and used as an indicator of overall health and a measure of physical dependence during withdrawal (25,35). All procedures were conducted within animal care guidelines set by the National Institutes of Health and were approved by the University of Virginia Animal Care and Use Committee.

Procedure

Fentanyl Self-Administration Training. Rats were trained to self-administer fentanyl (0.25 μg/kg/infusion) under a fixed ratio 1 schedule with a maximum of 40 infusions/day (36). Sessions were conducted daily until acquisition occurred (5 consecutive days wherein all 40 infusions were obtained, typically within the first 5–7 days). Catheters were flushed with heparinized saline 3 days a week, and when necessary, patency was verified using methohexital (1.5 mg/kg). Any catheter that was no longer patent was replaced with a new catheter in the left jugular vein, with testing resuming following recovery.

Baseline Motivation for Fentanyl. After acquisition, a baseline level of motivation for fentanyl (0.25 μg/kg/infusion) was established using a PR schedule (pre-PR) (36) wherein the response requirement to obtain an infusion increased progressively throughout the session (i.e., 1, 2, 4, 6, 9, 12, 15, 20). Sessions were run for 3 consecutive days. Rats were then randomly tested in 1 of 3 experiments as detailed below.

Experiment 1: Time Course of Changes Following 10 Days of ExA Self-Administration (Optimized). Rats were given ExA (24 hours/day) to fentanyl (0.25 μg/kg/infusion) under an intermittent-access procedure (two 5-minute trials/hour) for 10 days (Figure 1A) (36). We have previously shown that these conditions induce multiple addiction-like features, including binge-abstinent patterns, physical dependence, and enhanced motivation and vulnerability to relapse in both males and females when assessed following 14 days of withdrawal (26,35,36).

After the final ExA session, rats were retested for fentanyl motivation following 0, 1, or 14 days of withdrawal (post-ExA PR) using the same PR conditions as were used for initial testing. Body weights, which are indicative of physical dependence, were recorded immediately after the last ExA session and again 24 hours later, with continued assessments at least 3 times weekly during withdrawal and testing phases. One male in the 1-day withdrawal group and 3 females in the 14-day withdrawal group were removed from the study due to health complications, and 1 male in the 1-day withdrawal group was removed due to patency issues, which resulted in final group sizes of 12 females and 11 males for the 0-day withdrawal group, 9 females and 13 males for the 1-day withdrawal group, and 9 females and 11 males for the 14-day day withdrawal group.

Experiment 2: Phenotypic Changes Following 3 Days of ExA Self-Administration (Threshold). This experiment mirrored the procedure for experiment 1, but with rats given ExA to fentanyl (0.25 μg/kg/infusion) for only 3 days (Figure 2A), and motivation for fentanyl was reassessed after 14 days of withdrawal only. Five females were removed from the study due to health complications, and 2 males and 1 female were removed due to patency or technical issues, which resulted in a final group size of 9 for females and males.

Experiment 3: Phenotypic Changes Following ShA Self-Administration (Subthreshold). Motivational testing for fentanyl was conducted following the third PR test session (pre-PR) and 14 days of withdrawal (post-PR) using the same conditions as before (Figure 3A). This experiment also included additional male and female rats trained and maintained on a higher fentanyl dose (1.5 μg/kg/infusion) to explore

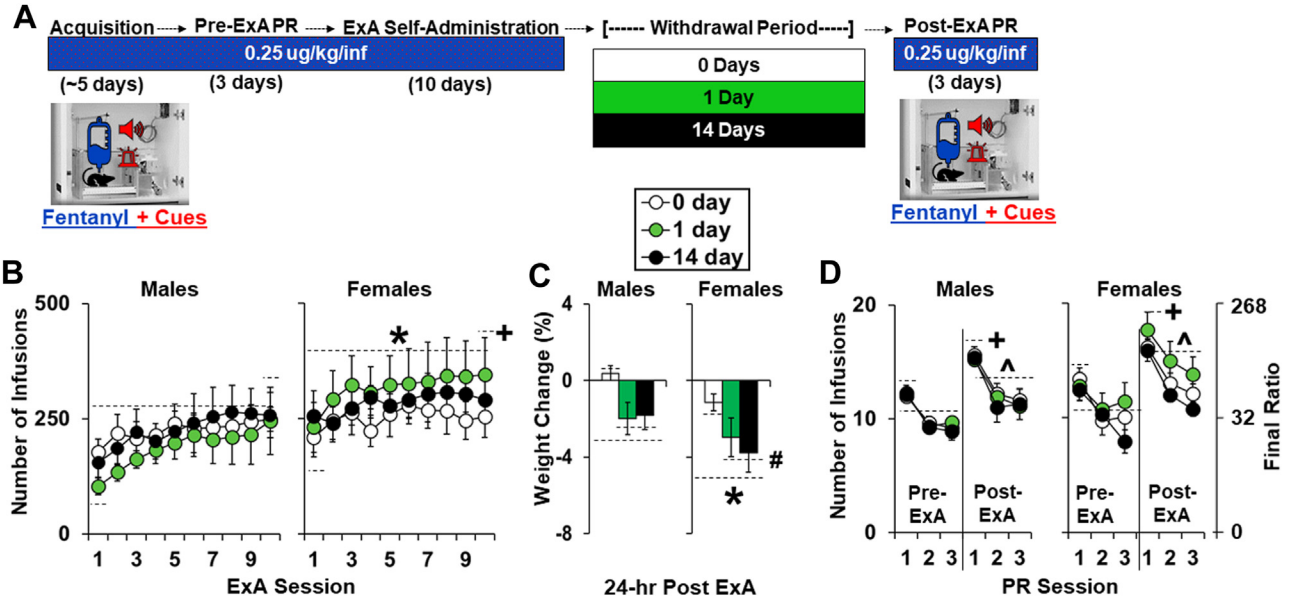

Figure 1. Effect of sex and length of withdrawal on the development of an enhanced motivation for fentanyl following 10 days of ExA self-administration (optimized). Male and female rats were trained to self-administer fentanyl (0.25 μg/kg/infusion) under a fixed ratio 1 schedule (acquisition), and once they acquired (5 days, 40 infusions), a baseline level of motivation for fentanyl was established under a PR schedule for 3 sessions (pre-ExA PR). Then, rats were given ExA (24 hours/day) to fentanyl under an intermittent-access procedure for 10 days. Following the last day of ExA self-administration, physical dependence was measured using percentage change in body weight 24 hours post-ExA compared to immediately prior to withdrawal. Motivation for fentanyl was then reassessed following a 0, 1, or 14-day period of withdrawal (post-PR). Data are plotted as mean (± SEM) number of infusions obtained during each of the 10 ExA sessions **(B)**, percentage change in body weight 24 hours following the last ExA session relative to body weight immediately following the last ExA session **(C)**, and number of infusions obtained and corresponding final ratios reached during the initial 3 PR sessions vs. the 3 retest PR sessions **(D)** for males and females in the 0-day (n = 11 males/12 females), 1-day (n = 13 males/9 females), and 14-day (n = 11 males/9 females) withdrawal groups. *significant effect of sex **(B, C)**; +significant difference from session 1 **(B)** and sessions 2 and 3 **(D)**; #significant difference from baseline (0) and the 0-day group **(C)**; ^significant difference from pre-ExA **(D)**. ExA, extended access; inf, infusion; PR, progressive ratio.

whether moderate to high doses could induce addiction-like features under ShA conditions. Two males and 1 female in the 0.25-μg/kg dose group were removed from the study due to patency issues, which resulted in final group sizes of 8 for males and females (collapsed across dose) and 8 for the 0.25 and 1.5-μg/kg groups (collapsed across sex).

Experiment 4: Fentanyl-Associated Adverse Health Effects. Rates of severe fentanyl-associated adverse health effects, defined as removal from a study due to a severe health issue, were determined in all adult, gonad-intact males and females tested under the ExA procedure in our laboratory over the past 3 years (from February 2020 to September 2023). We focused on 4 discrete phases: training/ShA self-administration, ExA self-administration, withdrawal, and an additional ExA-withdrawal cycle. Training/ShA self-administration included the initial training phase and baseline PR testing and 46 of the 53 males and 47 of the 55 females from this study plus an additional 121 males and 147 females tested previously (25,26,36). ExA self-administration included 153 males and 162 females that were given ExA to fentanyl (0.25–3.0 μg/kg/infusion) for 3 or 10 days. Withdrawal included both withdrawal and postwithdrawal testing and 148 males and 146 females. An additional ExA-withdrawal cycle included a period of re-exposure to ExA fentanyl self-administration, withdrawal, and phenotype testing and 62 males and 48 females. We also examined rates of adverse effects during these same discrete study phases in saline control male (n = 24) and female (n = 19) rats tested during this time frame.

Drugs

Fentanyl hydrochloride was obtained from the National Institute on Drug Abuse, dissolved in sterile saline (32), and sterile filtered (0.22 μm; Millipore) prior to use. To ensure that the mg/kg were consistent throughout the study, the duration of the infusions was adjusted for changes in body weight 3 times a week.

Analysis

For experiment 1, differences in fentanyl intake over the 10-day ExA period were examined using a mixed-effects model with sex, withdrawal group, and day as fixed factors. Univariate analysis of variance was used to determine sex and group differences in withdrawal-induced changes in body weight, which was defined by the change 24 hours post-ExA versus immediately following the last ExA session. Sex differences were assessed as the percentage change from immediately following ExA given the large sex difference in body weights. We also conducted a within-sex analysis based on body weights at these time points (see Figure S1). Effects of withdrawal length on motivation for fentanyl were determined by comparing the number of infusions obtained during the 3 PR sessions prior to versus following ExA self-administration and 0, 1, or 14 days of withdrawal. To limit the number of

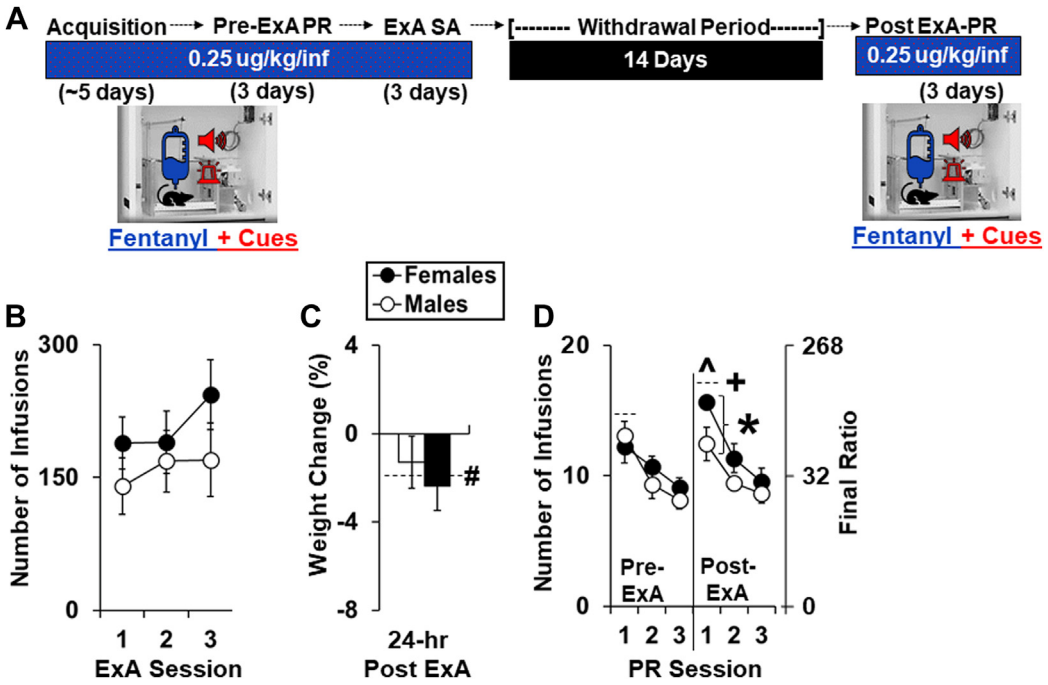

Figure 2. Effect of sex on the development of an enhanced motivation for fentanyl following 3 days of ExA self-administration (threshold). Male and female rats were trained to self-administer fentanyl (0.25 μg/kg/infusion) under a fixed ratio 1 schedule (acquisition), and once they acquired (5 days, 40 infusions), a baseline level of motivation for fentanyl was established under a PR schedule for 3 sessions (pre-ExA PR). Then, rats were given ExA (24 hours/day) to fentanyl under an intermittent-access procedure for 3 days. Following the last day of ExA self-administration, physical dependence was measured using percentage change in body weight 24 hours post-ExA relative to immediately prior to withdrawal. Motivation for fentanyl was then reassessed following a 14-day period of withdrawal (post-PR). Data are plotted as mean (± SEM) number of infusions obtained during each of the 3 ExA sessions **(B)**, percentage change in body weight 24 hours following the last ExA session relative to body weight immediately following the last ExA session **(C)**, and number of infusions obtained and corresponding final ratios reached during the initial 3 PR sessions vs. the 3 retest PR sessions **(D)** for males (n = 9) and females (n = 9). #Significant difference from baseline (0) **(C)**; *significant effect of sex **(D)**; +significant difference from sessions 2 and 3 **(D)**; ^significant difference from pre-ExA **(D)**. ExA, extended access; inf, infusion; PR, progressive ratio.

factors to 3, these data were first analyzed in males and females using separate mixed-effects models and using phase, session, and group as fixed factors. Sex differences were then determined within each day of PR testing (session 1, 2, or 3) given the large overall effect of session in the overall analyses and consistently higher infusions in the first PR session relative to sessions 2 and 3. Similar univariate and mixed-effects analyses were used in experiment 2 to determine sex differences in changes in body weight during early withdrawal, fentanyl intake over the 3-day ExA period, and phase × sex interaction effects on motivation for fentanyl. For experiment 3, we tested for differences between males and females in changes in body weight and motivation for fentanyl (collapsed across dose) and between dose groups (collapsed across sex) using separate univariate (body weight) and mixed-effects models (motivation for fentanyl). For experiment 4, sex differences in adverse health effects were plotted as survival probability as a function of study phase and analyzed using a Kaplan-Meier survival analysis and the log-rank (Mantel-Cox) statistic. All post hoc comparisons were Bonferroni corrected. Statistical analyses were performed using SPSS (version 26; IBM Corp.). Alpha was set at 0.05. Data are presented as the mean ± SEM.

RESULTS

Experiment 1: Optimized Conditions—10 Days of ExA Fentanyl Self-Administration

Females self-administered more fentanyl than males during the ExA period (main effect of sex, $F_{1,80}$ = 6.9, $p < .05$) (Figure 1B), but both sexes similarly escalated their intake of fentanyl over the 10 ExA sessions (main effect of session, $F_{1,579}$ = 44.6, $p < .001$; session 1 vs. 10, $p < .01$). There were no effects of withdrawal group, indicating that within males and females, intake was similar between groups prior to withdrawal and subsequent PR testing.

Females lost a greater percentage of body weight following ExA fentanyl self-administration (main effect of sex, $F_{1,59}$ = 4.7, $p < .05$) (Figure 1C). There was also a significant main effect of group ($F_{2,59}$ = 5.1, $p < .05$), but this was due to the 0-day withdrawal group (vs. the 1- and 10-day groups, ps < .05), which had undergone 1 PR test session following ExA self-administration. In contrast to the other 2 groups (ps < .001), weight loss in the 0-day group did not reach statistical significance (p = .051), indicating that even low levels of fentanyl (~16 infusions in the first PR retest session) prevented physical dependence (weight loss) 24 hours post-ExA.

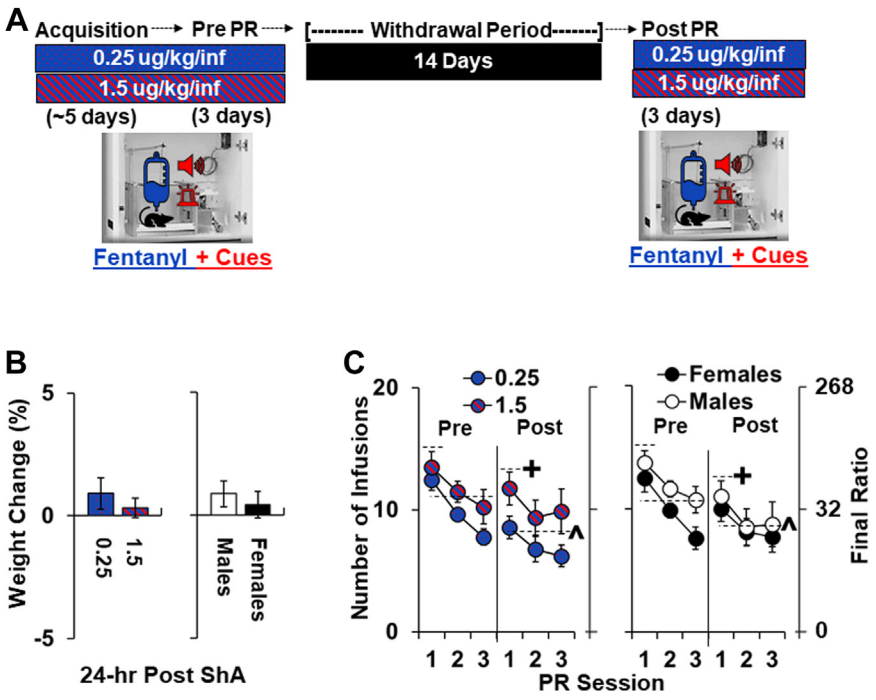

Figure 3. Effect of sex on the development of an enhanced motivation for fentanyl following ShA self-administration (subthreshold). Male and female rats were trained to self-administer fentanyl at either our standard dose (0.25 μg/kg/infusion) or a higher one (1.5 μg/kg/infusion) under a fixed ratio 1 schedule (acquisition), and once they acquired (5 days, 40 infusions), a baseline level of motivation for fentanyl was established under a PR schedule for 3 sessions (pre-PR). Following the last PR session, physical dependence was measured using percentage change in body weight 24 hours post self-administration relative to immediately following the last session. Motivation for fentanyl was then reassessed following a 14-day period of withdrawal (post-PR). Data are plotted as mean (± SEM) percentage change in body weight 24 hours following the last PR session relative to body weight immediately following the last PR session **(B)** and number of infusions obtained and corresponding final ratios reached during the initial 3 PR sessions vs. the 3 retest PR sessions **(C)** for male (n = 8) and female (n = 8) rats in the 0.25 μg/kg/infusion (n = 8) and 1.5 μg/kg/infusion (n = 8) dose groups. +significant difference from sessions 2 and 3 **(C)**; ^significant difference from preextended access **(C)**. inf, infusion; PR, progressive ratio; ShA, short access.

Surprisingly, motivation for fentanyl was similarly increased following ExA fentanyl self-administration and 0, 1, and 14 days of withdrawal in both males and females (Figure 1D). Within-sex analyses revealed significant effects of phase (pre- vs. post-ExA) in both males ($F_{1,166}$ = 22.0, $p < .001$) and females ($F_{1,141}$ = 19.8, $p < .001$), and there was a trend for a phase × group interaction in females (p = .085), which appears to have been driven by a greater increase in motivation in the 1-day group (mean increase of ∼43%), but not the 14-day group (mean increase of ∼38%). There was also a significant effect of session in both males ($F_{1,166}$ = 89.1, $p < .001$) and females ($F_{1,141}$ = 75.8, $p < .001$), with motivation being highest in both sexes on the first day of PR testing within both phases (vs. days 2 and 3, $ps < .05$). Analyses of sex differences within each day of PR testing revealed nonsignificant interactions between sex and phase for each of the 3 PR test sessions. The results from these analyses also mirrored effects observed in the within-sex analyses, with significant effects of phase ($ps < .05$) and nonsignificant effects of group within each session.

Thus, females took more fentanyl over the 10-day ExA period and had lost a greater percentage of body weight at 24 hours post-ExA, but then showed a similar increase in motivation for fentanyl. Surprisingly, withdrawal length did not impact the increase in motivation.

Experiment 2: Threshold Conditions—3 Days of ExA Fentanyl Self-Administration

Males and females self-administered similar amounts of fentanyl over the abbreviated 3-day ExA period (Figure 2B), and both tended to escalate their intake over time (effect of session, p = .078). Males and females also showed a similar decrease in body weight during early withdrawal (nonsignificant effect of sex; >0 for weight loss, $p < .05$) (Figure 2C). There was a sex difference in the development of an enhanced motivation for fentanyl (Figure 2D), with females, but not males, taking more infusions during post-ExA PR testing than during pre-ExA PR testing (sex × phase interaction, $F_{1,84}$ = 6.6, $p < .05$); this was particularly apparent during the first post-ExA PR session (sex × phase × day interaction, $F_{1,84}$ = 4.2, $p < .05$). There was also a significant overall effect of session ($F_{1,84}$ = 75.8, $p < .001$) that reflects higher infusions during the first PR test session than during sessions 2 and 3 during both phases ($ps < .05$). Analysis within each day similarly revealed a significant interaction of sex and phase on day 1 ($F_{1,16}$ = 10.3, $p < .01$) and higher infusions in females than males during post-ExA testing on day 1 ($p < .01$), but no difference during pre-ExA testing. There were no sex effects on days 2 and 3. The within-sex analyses similarly revealed significant effects of day ($F_{1,42}$ = 39.0, $p < .001$), phase ($F_{1,42}$ = 8.0, $p < .01$), and phase × day interaction ($F_{1,42}$ = 4.1, $p < .05$) in females, as well as higher infusions during post-ExA testing than pre-ExA on day 1 ($p < .01$) but not on days 2 or 3; in contrast, in males, only the effect of day was significant ($F_{1,42}$ = 36.8, $p < .001$).

Thus, males and females self-administered similar levels of fentanyl over the 3 days of ExA and lost a similar percentage of body weight during early withdrawal. Notably, females, but not males, showed an enhanced motivation for fentanyl following ExA and withdrawal, but this effect was restricted to the first PR retest session, indicating that these abbreviated ExA conditions only transiently induced this addiction-like feature in females.

Experiment 3: Subthreshold Conditions—ShA Fentanyl Self-Administration

There were no sex differences in fentanyl intake during ShA self-administration because all rats in both dose conditions (0.25 and 1.5 μg/kg/infusion) obtained the maximum number of infusions (40 infusions) on each of the 5 sessions. In contrast to experiments 1 and 2, none of the groups lost weight following ShA fentanyl self-administration (24 hours after the last session) (Figure 3B). Percentage change in body weight also did not differ between males and females or the 0.25 and 1.5-μg/kg dose conditions. Additionally, in contrast to experiments 1 and 2, motivation for fentanyl decreased following withdrawal from ShA fentanyl self-administration (Figure 3C). The decrease was similar between dose conditions (main effect of phase only, $F_{1,74} = 8.8$, $p < .01$) and sexes (main effect of phase only, $F_{1,74} = 8.9$, $p < .01$). All groups also showed the highest motivation for fentanyl on day 1 of testing during both phases (effect of day, ps $< .001$). Thus, ShA self-administration at a low or high dose of fentanyl was not sufficient to induce physical dependence (weight loss) or an enhanced motivation for fentanyl in males or females.

Experiment 4: Fentanyl-Associated Health Consequences

Females had a higher probability than males of developing a fentanyl-associated adverse health event that resulted in their removal from the study ($\chi^2 = 18.5$, $p < .001$) (Figure 4). Further analysis within each phase of the study revealed that this effect was driven by sex differences during fentanyl self-administration training/ShA, the first cycle of fentanyl self-administration, and the subsequent withdrawal/phenotype testing phase ($p < .001$), when the risk of having an adverse health effect was 2.8, 7.0, and 2.8 times higher in females than males, respectively. In contrast, in rats that underwent an additional cycle of fentanyl self-administration, withdrawal, and phenotype testing, the likelihood of developing a fentanyl-associated adverse health event was similarly increased in females and males (14 vs. 16%). Notably, there were no serious adverse health effects in saline self-administering controls during any of the phases of the study, and each of the 24 males and 19 females completed self-administration training, ExA self-administration, and subsequent withdrawal/phenotype testing, indicating that the health effects observed in fentanyl self-administering animals were specific to fentanyl and not self-administration more generally. Thus, females had a greater chance of developing fentanyl-associated adverse health events than males, particularly during early phases of the study, when the addiction-like phenotype was developing.

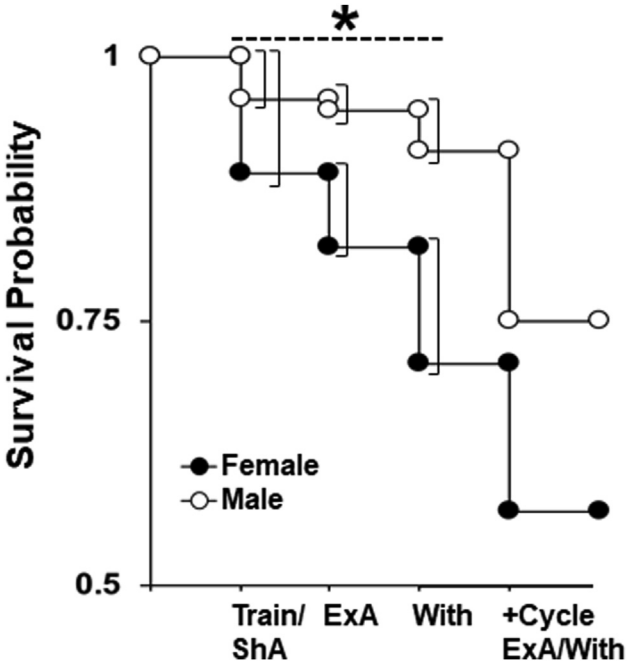

Figure 4. Effect of sex on the risk of developing a severe fentanyl-associated health consequence. Data are plotted as probability of survival (removal from study due to the development of a severe health effect) during fentanyl self-administration training/ShA self-administration (train/ShA), ExA self-administration, withdrawal/phenotype testing (With), and following an additional cycle of fentanyl self-administration, withdrawal, and phenotype testing (+Cycle ExA/With) in males ($n = 181$) and females ($n = 208$). *Significant effect of sex. ExA, extended access; ShA, short access.

DISCUSSION

The goal of this study was to investigate sex differences in the development of opioid addiction–like phenotypes and opioid-related adverse health effects. Our results indicate that, similar to findings in humans, females develop adverse health consequences more readily following opioid self-administration than males. Females had a 2.8-fold or greater risk of developing serious adverse health effects than males, which was evident from the initial training phase and persisted through withdrawal. We also showed that females self-administered more fentanyl and showed greater weight loss under the optimized 10-day ExA condition. Furthermore, females but not males developed an enhanced motivation for fentanyl under the threshold 3-day ExA condition, although this effect was transient and only apparent on the first day of post-ExA PR testing. Together, these findings provide the first evidence for telescoping effects with opioids and indicate that females have an enhanced biological vulnerability compared with males, particularly for opioid-related health consequences.

The most important finding is the higher risk in females for developing fentanyl-associated adverse health events, consistent with sex differences reported in humans (16). These effects appear to be specific to fentanyl given that no serious adverse health effects were observed in saline self-administering male and female rats. The vulnerability in females was particularly robust during early study phases, suggesting that sex differences in opioid-related health consequences may occur even before addiction has developed. Future research is needed to understand the mechanisms that underlie this enhanced vulnerability in females, especially because most studies on chronic opioid self-administration have been conducted using males.

Another notable finding is that females, but not males, developed an enhanced motivation for fentanyl following an abbreviated period of ExA self-administration, which we

predicted would be the threshold for inducing this phenotype. While these data are consistent with a telescoping effect, the effect was transient, indicating that 3 days of ExA self-administration was subthreshold for inducing a persistent phenotypic change. Surprisingly, no sex differences were observed following 10 days of ExA self-administration, which we predicted would be optimal for inducing an enhanced motivation for fentanyl when assessed following 14 days of withdrawal but threshold when assessed following shorter periods of withdrawal. Instead, we found that motivation for fentanyl was similarly increased in both females and males immediately following 10 days of ExA self-administration (withdrawal day 0); these high levels of motivation were similarly maintained throughout protracted withdrawal (day 14), indicating that our ExA procedures induced a severe addiction-like phenotype that is not further enhanced by withdrawal. There were also no sex differences in motivation for fentanyl following ShA self-administration, and neither male nor female rats showed an increase in motivation for fentanyl. Together, these findings reveal experimental conditions that are subthreshold (ShA), threshold (3 days of ExA), and optimal (10 days of ExA) for inducing an opioid addiction–like phenotype. Furthermore, they predict that the use of ExA access between 3 and 10 days is necessary to induce a lasting addiction-like phenotype, similar to our previous findings with cocaine (26); however, additional work is needed to confirm this.

It is surprising that motivation for fentanyl did not incubate, or increase, with longer withdrawal periods following 10 days of ExA self-administration. This contrasts with our findings with motivation for cocaine (25,27) and the broader literature showing that drug seeking, including opioid seeking, incubates over withdrawal (37). Most studies on incubation have examined effects following continuous, fixed ratio 1 access to the drug (38–43) rather than intermittent-access self-administration (44–46). While some intermittent-access studies with cocaine have shown incubation of drug seeking (44–46), it is notable that these studies have used shorter intermittent-access sessions (6 hours/day) than we used here; these studies also showed that drug seeking on withdrawal day 1 was higher following intermittent-access self-administration than following continuous access. Together, these findings suggest that the extended, intermittent-access conditions used here likely induced a severe opioid addiction–like phenotype that does not require withdrawal for expression. This idea is also consistent with our recent findings showing that fentanyl seeking does not incubate over protracted withdrawal, with both males and females responding at high levels for fentanyl-associated cues during early (withdrawal day 0 and 1) and protracted withdrawal (day 14). This is also consistent with clinical reports indicating persistently high opioid craving in individuals with OUD, including treatment-refractory active users and treatment-seeking individuals (47–49).

It is also notable that physical dependence/withdrawal was not necessary for, or predictive of, the subsequent development of an enhanced motivation for fentanyl. Specifically, we found that motivation for fentanyl increased similarly in males and females tested immediately after 10 days of ExA self-administration versus after 1 day of withdrawal despite differing levels of withdrawal severity/physical dependence. Females also lost a greater percentage of body weight following 10 days of ExA self-administration but then showed the same level of enhancement for fentanyl as males during subsequent PR testing. Additionally, both sexes exhibited signs of physical dependence (weight loss) following 3 days of ExA fentanyl self-administration, but only females showed increased motivation for fentanyl thereafter. This is consistent with our previous findings on relapse vulnerability and clinical findings showing that a subgroup of people with OUD have low levels of physical dependence (50). Thus, physical dependence alone should not define an addiction-like phenotype in animals, as in humans, where physical dependence is just 1 criterion for OUD, with 2 to 6 criteria being required for a diagnosis based on DSM-5 criteria.

Conclusions

In summary, our findings show that female rats developed an increased motivation for fentanyl and fentanyl-related health consequences more readily than male rats, indicating that the telescoping effect observed with cocaine also applies to opioids. The accelerated disease progression in females is concerning and warrants further study to understand the underlying mechanisms. Our findings also highlight the need for early intervention, especially in females, consistent with recent efforts to address addiction early because treatment penetration in severe stages is $<$20% (51,52). Our data also identified experimental conditions that are subthreshold (ShA), threshold (3 days of ExA), and optimal (10 days of ExA) for inducing an addiction-like phenotype in males and females. This can be used to advance our understanding of sex differences in neuroadaptations at different disease stages.

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EBT and WJL designed the study, performed the statistical analysis, and wrote the manuscript. EBT, EIQ, KAH, and SDF collected the data. All authors contributed to manuscript revision and read and approved the submitted version.

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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ARTICLE INFORMATION

From the Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, Virginia (EBT, KAH, EIQ, SDF, WJL) and Medical Scientist Training Program, University of Virginia, Charlottesville, Virginia (EBT).

Address correspondence to Wendy J. Lynch, Ph.D., at wjl6w@virginia.edu.

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