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Differential behavioral functioning in the offspring of rats with high vs. low self-administration of the opioid agonist remifentanil

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ABSTRACT

Opioid use disorder (OUD) has a variety of adverse effects on both the users and their offspring. In the current study, a random group of Sprague-Dawley rats (25 females and 15 males) were tested for intravenous self-administration of the opioid agonist remifentanil to determine the range of acquisition for opioid. One-month after the end of self-administration of remifentanil, rats with the highest intake were mated together and rats with lowest intake were mated together. Then, the offspring of the two groups were tested for anxiety-like behavior, locomotor activity, nociception and intravenous remifentanil self-administration. The parents showed a range of remifentanil self-administration, especially in the female rats. The offspring of the parents with low and high remifentanil self-administration showed significant differences in specific behavioral functions. On the hotplate test of nociception, the female offspring parents with high remifentanil self-administration had significantly longer hotplate latencies, indicating reduced nociception, than the female offspring of parents with low remifentanil-self-administration, whereas there was no difference in the male offspring of low and high responding parents. In the elevated plus maze test of anxiety-like behavior, the offspring of the parents with high remifentanil intake showed more anxiety-like behavior than the offspring of the parents with low remifentanil intake regardless of sex. Locomotor activity was not significantly different. Interestingly, no significant differences in remifentanil self-administration in the offspring of parents with low and high remifentanil self-administration were detected. Overall, our data suggest a considerable range in remifentanil self-administration in rats and the offspring of rats with high opioid self-administration exhibit different behaviors vs offspring of rats with low opioid self-administration.

1. Introduction

Opioid addiction in the US has become a major health problem with devastating social impacts. According to the Center for Disease Control (CDC), from 1999 to 2016, more than 350,000 people died from an overdose involving any prescription or illicit opioids (http://wonder.cdc.gov). Substantially, there are more people who continue using opioids and many go on to have children. They pass on to their children both the genes that may contribute to opioid abuse as well as the effects of the drug abuse itself. This combination of genes that predispose to drug abuse and the drug effects themselves can significantly alter behavioral development in the offspring of drug abusers (Byrnes et al. 2011, 2013; Crist et al., 2019). Drug addiction, similar to other mental illnesses, is a complex disease involving a variety of biological factors and neuronal systems (Koob and Volkow, 2016; Jia et al., 2011; Vereczkei et al., 2013; Rezvani et al. 2010, 2016, 2018, 2019; Levin et al., 2018, 2019; Scavone et al., 2011).

Similar to some other mental illnesses such as alcoholism and depression, genetic factors play an important role in the manifestation of opioid use disorder (OUD). Both family and twin studies indicate that there is a clear genetic contribution to OUD (Crist et al., 2019; Berrettini, 2017; Tsuang et al., 1998; Kendler et al., 2013). These studies suggest that approximately 23–54% of risk to OUD is inherited (Tsuang et al., 1998; Kendler et al., 2013). Tsuang et al. (1998) have shown that 54% of the liability for opioid addiction was due to genetic variance and that 38% of the liability can be explained by genetic variance specific to opioid addiction. It has also been shown that in male-male twin pairs the genetic liability for opioid addiction is 48%. Several genome-wide

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association studies indicate that genetic risk for opioid addiction is complex and conveyed by multiple genes (Kendler et al., 2013; Crowley et al., 2003; Gelenter et al., 2014; Laveran et al., 2008; Nishizawa et al., 2014; Tan et al., 2003). These genetic-behavioral links suggest the potential for heritable phenotypes which may directly or indirectly enhance opioid abuse risk across subsequent populations. However, these associations in human populations come with a host of potential confounders, including social and familial circumstances, life stress, nutrition, and co-use to other drugs of abuse.

While it is often assumed that familial characteristics are by default genetic, parents who use opioids may influence the neurodevelopment of their offspring through other means as well. Both animal studies and human epidemiological studies have demonstrated a significant association between maternal opioid drug use during pregnancy and behavioral impairment in their offspring (Johnson et al., 2011; Sithisarn et al., 2012; Vathy, 2002). Additionally, epigenetic mechanisms modify and restrict the expression of various genes, and allow preconception opioid exposures to alter offspring behavior (Goldberg and Gould, 2019). Given the number of potential contributors to opioid abuse, controlled laboratory models are needed to separate out and examine how parental factors influence abuse liability in the next generation.

Animal models have been an informative method for examining risk factors for addiction in the absence of societal and environmental confounds that impact human populations. Of particular importance to the present work are selectively-bred models, where high- and low-phenotype individuals are stratified and bred together over a series of generations to create distinct lines with distinct genotypic and phenotypic features. This has been most famously done for alcohol-preferring and non-preferring rats (i.e. P and NP rats, HAD and LAD rats), which differ from one another on not only their alcohol intake, but a variety of linked behavioral and neurochemical characteristics (Bell et al., 2012; McBride et al., 2014). Such models allow potentially heritable traits and biomarkers to be proposed, tested and elaborated on, and so may provide valuable insights into the heritability of opioid abuse as well.

The current study was conducted to provide an initial indication of the influence of parental opioid intake characteristics on addiction-related behaviors in their offspring. To do this, groups of outbred male and female rats were tested for acquisition of intravenous (i.v.) self-administration of the short-acting opioid agonist remifentanil, and then subdivided into high and low-responder categories based on their opioid intake. Remifentanil is not a commonplace opioid of abuse due to its short half-life, but it is ideal for this study as it leads to robust and distributed patterns of self-administration behavior in rats, which can then be used to generate high and low liability groups. Male and female members of each group were then mated together to produce the first generation offspring (F1), as would be performed when developing a selectively bred line. Behavioral testing of the offspring included the figure-8 maze locomotor test, elevated plus maze anxiety test, the hotplate test of nociception, and finally, remifentanil i.v. self-administration. Based on the high heritability of opioid addiction, it was hypothesized that some specific behavioral characteristics would differ between the offspring of high- and low-responding rats.

2. Materials and methods

2.1. Subjects

Young adult male and female Sprague-Dawley rats were used. They were housed in climate controlled conditions with a 12:12 h reverse light:dark cycle with lights on at 7:00 p.m. The rats had *ad lib* access to water and were fed (5001 Rodent Chow, Lab Diet, Brentwood, MO, USA) daily 20–30 min after testing with sufficient food to keep their body weight at a healthy lean weight approximately 85% of *ad lib* levels. All remifentanil i.v. self-administration, elevated plus maze, hotplate test and locomotor activity test sessions were conducted between 9 a.m. and 4 p.m., when rats were on their active phase of their diurnal cycle. All studies were performed in accordance with the rules and regulations outlined by the Animal Care and Use Committee of Duke University. All female rats included as breeders were drawn from the control groups of an ongoing series of studies aimed at developing pharmacotherapies for opioid self-administration and relapse. Over the course of that experiment, these female breeders completed 25 remifentanil self-administration sessions, of which, the first 5 were used to generate "high-" and "low-responder" categories. All male breeders were separately tested over 15 remifentanil self-administration sessions and categorized using identical methods to those used for female breeders. All procedures were conducted according to the animal protocol A246-17-11 approved by the Institutional Animal Care & Use Committee of Duke University.

2.2. Remifentanil preparation

Remifentanil hydrochloride is a potent and fast acting selective μ opioid receptor agonist. Remifentanil has an onset of action of about 1 min. It also has a short elimination half-life of less than 10 min (Kapila et al., 1995). Solutions of remifentanil hydrochloride (NIDA Drug Supply, RTI International, Raleigh, NC, USA) were prepared in pyrogen-free glassware in sterilized isotonic saline and then vacuum filtrated using a Nalgene filter (Nalgene Nunc International, Rochester, NY, USA) for sterilization. When not in use, solutions were refrigerated and stored in the dark to prevent decomposition of remifentanil. For self-administration sessions, the dose of remifentanil infused was 0.9 μg/kg/infusion.

2.3. Experimental design

First, a random group of outbred male and female Sprague-Dawley rats were tested for voluntary i.v. self-administration of remifentanil for 15–25 sessions (25 for females and 15 for males). When given multiple weeks, all rats acquired substantial remifentanil self-administration, but intake over the initial 5 consecutive days varied considerably among the rats. Therefore, the average intake across the initial 5 days of testing was used as the measure of high- or low-drug abuse liability. One month post-testing, rats with the highest initial remifentanil intake were mated together and rats with lowest initial remifentanil intake were mated together. Then, adult offspring (F1 generation) of the two groups were tested for anxiety-like behavior on the elevated plus maze, locomotor activity using the Figure-8 maze, nociception using the standard hotplate test and finally intravenous remifentanil self-administration.

2.4. Behavioral training and intravenous remifentanil self-administration

The method for remifentanil is the same as we have used in previous studies (Levin et al., 2019, 2020, 2020; Blair et al., 2020; Rezvani et al., 2018), and has been shown to be sensitive to the diversity of remifentanil acquisition in an outbred strain of rats (Sprague -Dawley). Before starting the remifentanil i.v. self-administration, the rats were trained daily with autoshaping sessions, lasting 30 min, to press the levers for food pellet reinforcers. Food restricted rats were placed in dual lever operant test chambers (Med Associates, Georgia, VT, USA). Only the cue light over the correct lever was illuminated while the light over the incorrect lever was off. Pressing on the correct lever was reinforced by immediate delivery of one 45-mg food pellet and activation of the feedback tone for 0.5 s. There was no timeout period in the tutor sessions.

After the pellet sessions training, animals had catheters surgically implanted into their jugular vein for remifentanil self-administration by intravenous infusion. A combination of ketamine (60 mg/kg i.p.) and dexmedetomidine (0.15 mg/kg i.p.) was used to anesthetize the animal. Once the animal was sufficiently anesthetized, an incision slightly lateral to the midline was made, the jugular vein was exposed and a

small incision was made in the vein to allow the insertion of the catheter. The catheter was then inserted into the vein until the tip was just outside the heart. Then, the catheter was sutured to deep muscle, and the remaining portion was routed subcutaneously around the back of the animal to emerge between the scapulae. The catheter was then attached to an infusion harness (SAI Infusion Technologies, Libertyville, IL, USA) that was fitted around the animal. Each animal was given ketoprofen (5 mg/kg, s.c.) for postoperative pain. After surgery and for the remainder of the studies, all animals' catheters were flushed daily with a 0.25 ml solution containing 100 U/ml heparinized saline. After each self-administration testing session, the remifentanil solution contained in the animals' harness ports was removed and replaced with a sterile lock solution that contained heparinized saline and the antibiotic gentamicin (8 mg/ml, Butler Schein Animal Health, Dublin, OH, USA) (Blair et al., 2020; Hall et al., 2015).

Following the recovery from the surgery, rats were placed in dual lever operant test chambers (Med Associates, Georgia, VT, USA) for remifentanil self-administration. Each chamber was equipped with a tone generator, house light, cue light above each lever, and a metal tether to cover the drug delivery line. Each catheter was connected to a Micro Liter Syringe Pump filled with remifentanil, and tethers made of polyethylene tubing with huber needles for access to ports and catheters. During each self-administration session, the rats wore infusion harnesses to connect them to the tethers. A computer programmed with MED-PC software controlled experimental events and data collection. A plastic SoloPort was attached intraoperatively to a polyurethane catheter, inserted into a subcutaneous interscapular pocket, and sutured to underlying fascia. 2–4 days after the surgery, the rats began self-administration sessions with remifentanil (0.9 μg/kg/infusion, i.v.) as the reinforcer for 5 consecutive days.

A lever press on the active side resulted in the activation of the feedback tone for 0.5 s, and the immediate delivery of one 50-μl infusion of remifentanil in less than 1 s. Each infusion was immediately followed by a 20-sec timeout in which the house light was illuminated and cue lights were extinguished, and responses were recorded but not reinforced. The infusion dose of remifentanil was set at 0.9-mg/kg/infusion, and the fixed ratio (FR) requirement was set at FR1. Each remifentanil infusion sessions lasted 1-h (Rezvani et al., 2019). Upon the completion of the study the patency of the catheters was verified by injection of barbiturate into the catheter. Only data from rats with patent catheters were included in the data analysis.

2.5. Selective breeding

First, a random group of adult male and female Sprague-Dawley rats (50 females and 12 males) were tested for voluntary intravenous self-administration of remifentanil for 5 consecutive days. One-month post-testing, rats with the highest remifentanil intake (8 males and 8 females) were mated together and rats with lowest remifentanil intake (7 males and 7 females) were mated together. This mating resulted in a total of 6 pups of each group. Then, some of the offspring of the two groups (20 rats from the high intake parents and 17 rats from the low intake parents) were tested for locomotor activity, anxiety-like behavior and nociception at the age of 6–7 weeks. Following these tests, these rats were tested for the self-administration of remifentanil for 10 days when they were 8–9 weeks old.

2.6. Elevated plus maze test of anxiety-like behavior

To assess the anxiety-like behavior vs. risk-taking behavior, rats were tested on a standard elevated plus maze (Med Associates, St Albans, VT, USA). The maze measured 142 cm × 104 cm x 76 cm high and consisted of two arms with 15 cm high, enclosed walls and two open arms with 2 cm railings. The maze was painted black and the rats were tested under dim light. Each rat was assessed individually on the elevated plus maze for a single 5 min session. The percentage of time the rat spent in the open vs. enclosed arms of the maze was calculated as an index of anxiety vs. risk taking. The number of center crossings was also counted as a measure of activity (Levin et al., 2019). The maze was cleaned after each trial.

2.7. Figure-8 maze test of locomotor activity and its habituation

Locomotor activity of low and high preferring rats were tested in a standard Figure-8 maze over the course of a 1-h session in both male and female rats. The mazes had continuous enclosed alleys 10 × 10 cm in the shape of a figure-8. The overall dimensions of the apparatus were 70 cm long and 42 cm wide, with a 21 × 16 cm central arena, a 20-cm high ceiling and two blind alleys extending 20 cm from either side. Eight infrared photobeams, which crossed the alleys, indexed locomotor activity. One photobeam was located on each of the two blind alleys and three were located on each of two loops of the figure-8. The number of photobeam breaks was tallied during the 1-h session. The linear and quadratic trends across twelve 5-min blocks in each session were calculated to determine the habituation of locomotor activity over the course of the session.

2.8. Hotplate test of nociception

To determine if the offspring of low remifentanil-infusing rats respond differently to hot plate test vs high remifentanil-infusing rats, we tested both groups for analgesia using the standard hotplate test. The test consists of placing a rat on an enclosed hot plate (55 °C) and measuring its paw withdrawal latency. Twenty seconds after placing the rat on the hot plate or the second the rat licked its paws, indicating that the paws are feeling the aversive heat pain, the animal was removed from the hot plate. The latency in removing their paws from the hot plate was recorded (Rezvani et al., 2019).

2.9. Remifentanil self-administration in the offspring

After the other behavioral tests, the male and female offspring of the low and high remifentanil preferring rats were tested for remifentanil self-administration with the same methods as used for their parents as described above.

2.10. Statistical analysis

The data were evaluated with analysis of variance for within-and between-subjects factors. Alpha of $p < 0.05$ (two-tailed) was used as the threshold for statistical significance. The analysis in the chronic self-administration study included a between-subjects factor of rats that quickly or slowly took up remifentanil self-administration based on a median split of the remifentanil infusions per session for the initial five sessions. Interactions $p < 0.10$ were followed up with tests of the simple main effects of group at each level of the interacting factor as recommended by (Snedecor and Cochran, 1967). The data from rats with IV catheters which were not patent were excluded from the data analysis.

3. Results

3.1. Remifentanil self-administration of the parents

The data from the parents showed a considerable range in acquisition for remifentanil self-administration, especially in female rats. Some rats quickly took up remifentanil self-administration and significantly infused more remifentanil in each session (33.71 ± 4.30 in females and 38.50 ± 6.15 in males) while some other rats took up remifentanil more slowly and infused significantly less remifentanil in each session (9.34 ± 1.73 in females and 23.3 ± 2.02 in males) during the first five consecutive sessions of access (Fig. 1). The group sizes with the median split of low and high responders based on remifentanil self-administration/

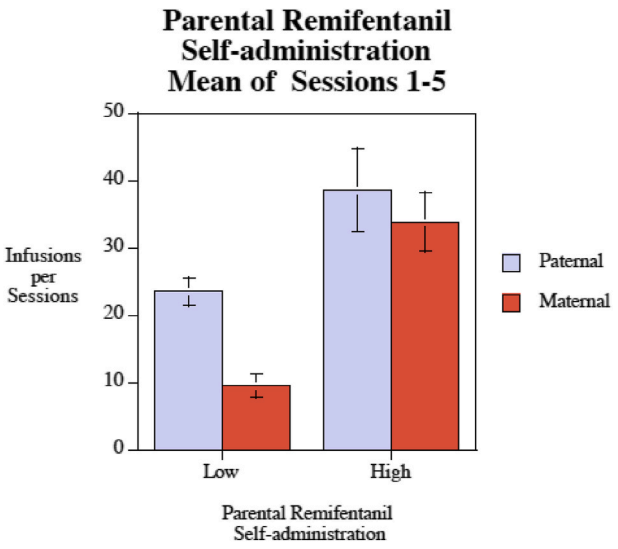

Fig. 1. Parental remifentanil self-administration. Means of 5 consecutive sessions in 25 females and 15 males.

session were: Female low = 7 rats, Female high = 7 rats, Male low = 4 rats, Male high = 4 rats. Thus, these data display the range of opioid intake is wide and exists in both male and female rats.

3.2. Elevated plus maze test of anxiety-like behavior

The offspring of the parents with high remifentanil intake had significantly ($F(1,68) = 6.36$, $p < 0.05$) less time spent on the open arms of the elevated plus maze than the offspring of the parents with low remifentanil intake. As shown in Fig. 2, the offspring of parents with low remifentanil intake had 41.3 ± 1.9% open arm time whereas the offspring of the parents with high remifentanil intake had 33.8 ± 2.3% open arm time on the elevated plus maze. This is indicative of greater anxiety-like behavior in the offspring of the parents with high remifentanil intake. There was also a significant ($F(1,68) = 4.87$, $p < 0.05$) main effect of sex, with the male offspring showing 34.3 ± 2.2% open arm time and the female offspring showing 41.0 ± 2.1%. The interaction of parental remifentanil intake x sex was not significant. No significant effects were seen in the number of center crossing on the maze. The offspring of the parents with high remifentanil self-administration averaged 6.1 ± 0.5 center crossings per session, while the offspring of the parents with low remifentanil self-administration averaged 5.6 ± 0.5 center crossings per session.

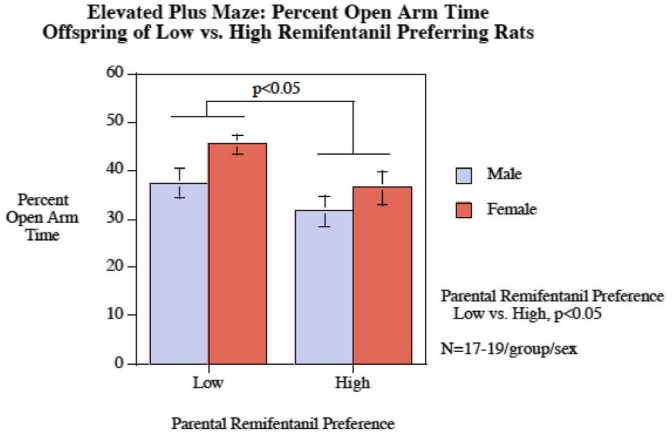

Fig. 2. Percent open arm time in the elevated plus maze (mean ± sem).

3.3. Figure-8 maze test of locomotor activity and its habituation

The main effects of parental remifentanil intake and sex were not significant with regard to locomotor activity scores on the Figure-8 maze test. There was a significant ($F(11,748) = 78.91$, $p < 0.005$) main effect of 5-min time block within the hour-long test session showing habituation of activity. There was also a significant ($F(11,748) = 1.92$, $p < 0.05$) parental remifentanil intake x time block interaction. As shown in Fig. 3, there was a difference in the habituation curve between the offspring of the parents with low and high remifentanil intake. The analysis of trends across the test gave insight into the nature of this interaction. The linear trend did not differ between the offspring of low and high groups suggesting that the groups did not significantly differ in their overall decrease in motor activity from the start to finish of the session. The quadratic trend over the time blocks did significantly ($F(1,68) = 6.47$, $p < 0.05$) differ between the offspring of the parents with low and high remifentanil intake. This indicates that the progression in activity change during the intermediate parts of the session did differ.

3.4. Hotplate test of nociception

The main effects of parental remifentanil intake and sex were not significant with regard to scores on the hotplate test of nociception. However, there was an interaction of parental remifentanil intake x sex ($F(1,68) = 3.65$, $p < 0.07$) that warranted tests of the simple main effects of parental remifentanil intake with each sex of the offspring. The simple main effects tests showed that female offspring of the high remifentanil preferring parents had significantly ($F(1,68) = 5.31$, $p < 0.05$) longer latencies in removing their paws from the hot plate than female offspring of the low remifentanil preferring parents (Fig. 4) indicating less nociception in the female offspring of the parents with high

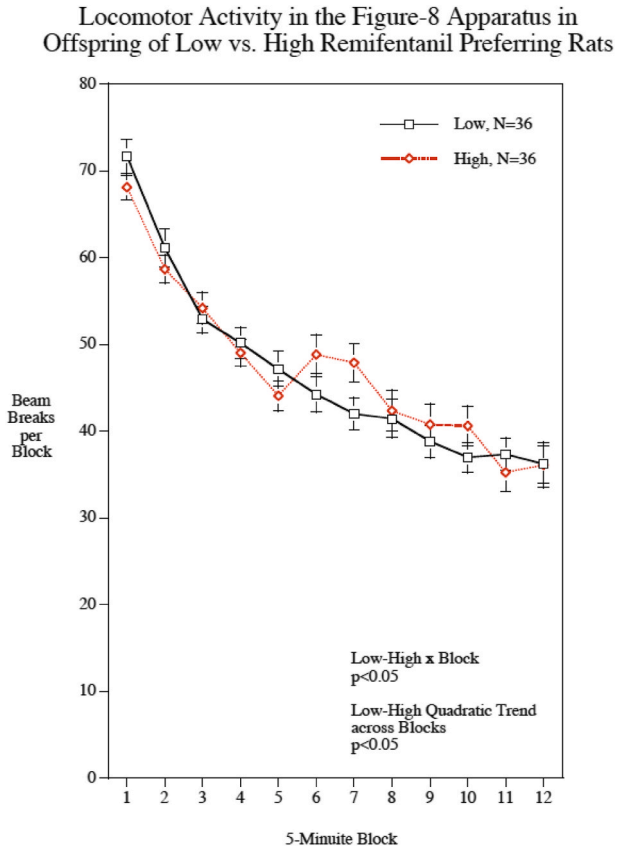

Fig. 3. Locomotor activity (beam breaks per 5-min block) on the Figure-8 maze over the course of the 1-h session (mean ± sem).

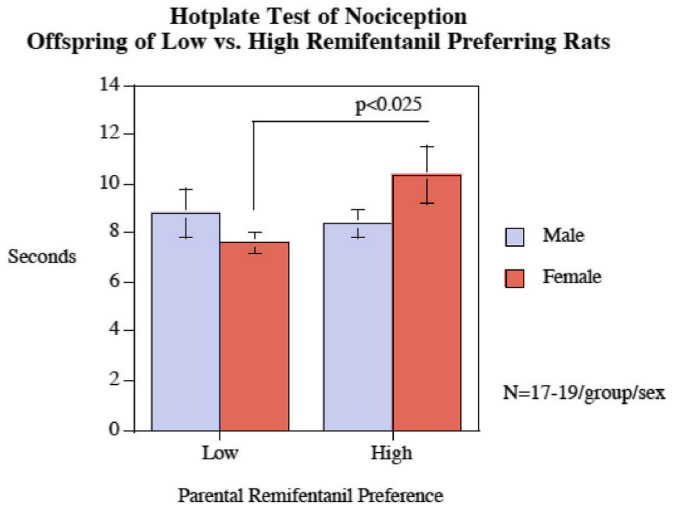

Fig. 4. Latency for paw withdraw on the hot plate test (mean ± sem).

remifentanil intake. No significant effect on hotplate response was seen in the male offspring.

3.5. *Remifentanil self-administration of the offspring*

There were no significant differences in remifentanil self-administration between the offspring (F1) of parents with low and high remifentanil self-administration. Nor were there significant main effects of sex or interaction of parental remifentanil intake and sex of the offspring. The offspring of the parents with low remifentanil intake averaged 46.9 ± 4.6 (mean ± sem) infusions per session over ten sessions of testing, while the offspring of the parents with high remifentanil intake averaged 36.7 ± 3.9 infusions per session during the same period. Fig. 5 shows remifentanil self-administration for each of the ten sessions for male and female offspring. There was a significant main effect of session ($F(9,297) = 11.90$, $p < 0.0005$) and a significant interaction of sex x session ($F(9,297) = 2.23$, $p < 0.05$). Tests of the simple main effects of sex at each of the sessions showed that the females self-administered significantly less remifentanil than males for sessions 1 ($p < 0.05$) and 2 ($p < 0.005$) but not for the subsequent sessions. No significant interactions of parental remifentanil acquisition x session or parental remifentanil acquisition x sex x session were seen.

4. Discussion

As hypothesized, our data demonstrate a range of initial i.v. self-administration of remifentanil, a fast-acting opioid, among outbred male and female Sprague-Dawley rats. Furthermore, there were significant differences in some behavioral functions of the offspring (F1 generation) of the parents with low vs. high remifentanil intake. This included significant differences in anxiety-like behavior and nociception, however we did not detect differences in remifentanil self-administration in the offspring (F1) of the low vs. high remifentanil self-administering parents. This suggests that patterns of opioid acquisition in one generation are related to some of the addiction-related behavioral characteristics in the next generation. However, this did not result in a full replication of the difference used for selective breeding, as would be expected in a fully developed selectively-bred line. Together, this indicates that inheritance of substance abuse characteristics may be more subtle from one generation to the next than is often assumed. Although both male and female rats in the high group self-administered similar amount of remifentanil, the male rats (F0) in the low group self-administered significantly more remifentanil than the female rats. Although the mechanisms underlying this difference are currently unknown, we can speculate that the hormonal factors may play an important role in females of this group (See Maurais-Jarvis et al., 2021 for review). Additional studies are needed to fully explore the mechanisms underlying this difference in remifentanil self-administration in males vs. females.

In terms of the anxiety-like behavior, the offspring of the parents with high remifentanil intake exhibited a significantly higher anxiety-

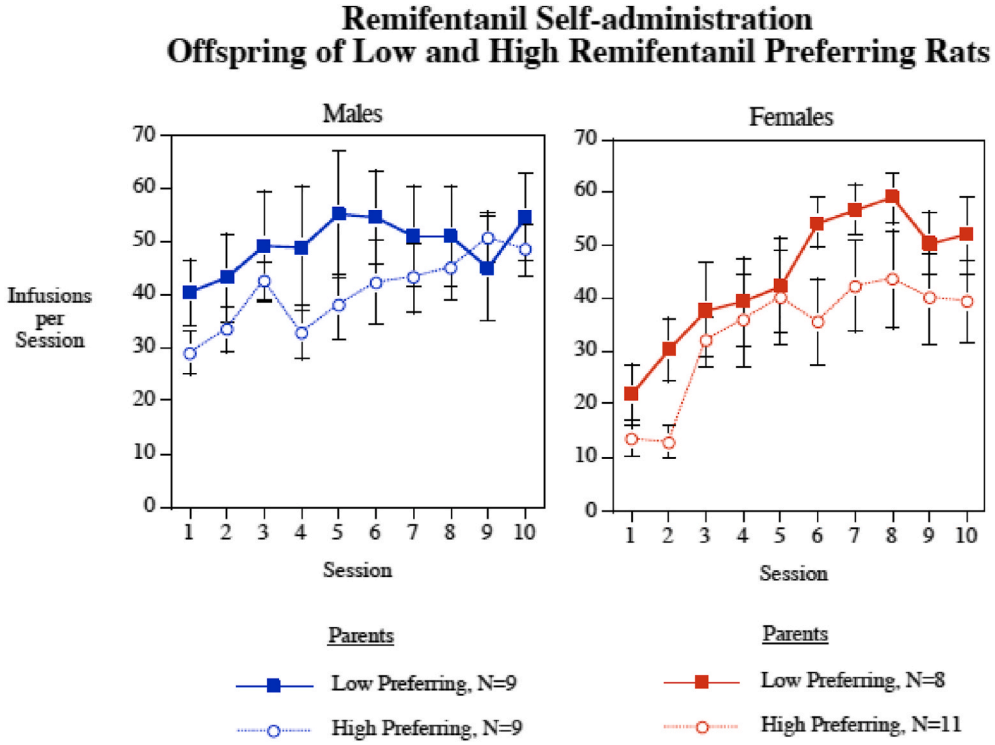

Fig. 5. Remifentanil self-administration in the adult offspring of Low vs. High remifentanil preferring parents: sessions 1–10 (mean ± sem).

like behavior compared with the offspring of the parents with lower remifentanil intake by spending less time in the open arms of the elevated plus maze. Interestingly, it has been shown that maternal morphine exposure in rats, followed by a drug-free period of several weeks, still induced anxiety-like behavior in the offspring (Byrnes et al. 2011, 2013; Vassoler et al., 2014). The difference in anxiety-like behavior seen between these two groups was not seen to be related to the motor activity of the rats. The measure of activity on the elevated plus maze test, center crossings, was not found to be at all affected by parental remifentanil intake. Also, as shown in the Figure-8 maze test, the main effects of parental remifentanil intake were not significant with regard to locomotor activity scores (Fig. 3). The level of motor activity in the offspring of parents with low remifentanil intake was not significantly different from the level of activity of offspring of parents with high remifentanil intake. The mechanisms underlying this difference in anxiety-like behavior are currently unknown and additional studies are needed to fully understand the possible mechanisms underlying this difference in anxiety-like behavior.

In terms of nociception, we found that the female offspring of the parents with higher remifentanil-intake showed a longer latency in removing their paws from the hot plate suggesting higher threshold for pain sensation compared with the offspring of the parents with lower remifentanil intake. Although preliminary, the result of this study suggests the influence of parental opioid use on the nociceptive response to thermal stimuli. Both preclinical and some clinical studies suggest that genotype affects individual responsiveness to painful stimuli (Elmer et al., 1998). It is possible that these offspring inherited a particular gene or genes related to pain sensitivity. Of additional relevance, several studies have examined the behavior of the offspring of rats exposed to morphine or heroin prior to conception, indicating epigenetic factors. As in the current study, multiple studies have found that males and females were differentially affected by these exposures (Cicero et al., 1995) and that parental opioid use led to changes in tests of nociception (Pachenari et al., 2018) and anxiety-like behaviors (Farah Naquiah et al., 2016; Li et al., 2014).

Twin and family studies provide strong evidence for the involvement of genetic in the manifestation of OUD (Berrettini, 2017; Byrens, 2005; Crist et al., 2019; Scavone et al., 2011,). However, it needs to be mentioned that the genetic factor is not the only factor and other factors, such as environmental and social factors, definitely play a major role in addiction to opioids and other drugs of abuse.

Based on work with other selectively-bred lines, such as alcohol-preferring rats, it was hypothesized that selective breeding of rats with high- and low-intake of remifentanil would result in heritable differences in drug intake in their offspring, along with a variety of genetically-linked neurological and behavioral characteristics. It is acknowledged that selectively-bred lines are cultivated over many generations (McBride et al., 2014), and that the first breeding generation (F1), without the benefit of extensive inbreeding, may not have the same rich genotype/phenotype that we would expect from a fully developed breeding line. It may be the case that subtle behavioral changes such as anxiety and nociception are more reliably inherited over a single generation than more complex functions like motivated behavior such as drug taking. Additional data will be needed to verify this hypothesis. Follow-up studies could investigate whether parental opioid intake corresponds with more basic features of opioid sensitivity, such as opioid pharmacokinetics, pharmacodynamics, and psychopharmacological effects like opioid sedation, analgesia and conditioned-place preference. More information could also be gained by prospectively measuring the parent's nociception and anxiety prior to opioid self-administration. This would allow drug- and non-drug-related behaviors to be correlated with one another within each generation and between-generations.

The present study was able to control for a number of confounds which influence human familial patterns of opioid abuse, including differences in socialization, culture, life stress, nutrition, and the continuation of maternal opioid abuse into pregnancy. However, the more precise mechanisms of what has been inherited remains to be thoroughly investigated. In any selective breeding model, it is presumed that inheritance is primarily moderated by the presence or absence of certain genes. However, epigenetic programming is also a likely mechanism that could allow one generation influencing the next, even without substantial inbreeding. Studies examining maternal or paternal opioid use have also found multigenerational effects on non-drug behaviors, even in models where the exposure did not overlap with pregnancy (Goldberg and Gould, 2019). This includes effects on social and anxiety-like behaviors, as well as nociception and neurochemical effects (Byrnes et al., 2013; Goldberg and Gould, 2019; Johnson et al., 2011; Pachenari et al., 2018). Unsurprisingly, this can also influence the sensitivity of offspring to the parent's drug of abuse, potentially enhancing their response (Cicero et al., 1995).

One limitation of the current study design is a confound between the parents' genes driving low or high remifentanil self-administration and the persisting effects of the parents' exposure to remifentanil which could have effects on offspring neurodevelopment. This study was designed as a selective breeding study rather than a parental toxicology study, so the parents of both offspring groups were exposed to some remifentanil, albeit at differing levels of exposure. However, this confound is present to some degree in any selective breeding study involving pharmacological testing, and in humans whose parents were using drugs. Future studies could potentially perform breeding before behavioral testing in order to avoid this confound. Also, it would be interesting to look at the acquisition of a natural reinforcer such as sucrose in the offspring.

5. Conclusions

The current study found that the offspring (F1 generation) of rats with lower vs. higher remifentanil self-administration were significantly different in anxiety-like behavior and pain perception but not in remifentanil self-administration. This provides evidence that differences in opioid intake in parents may correspond to heritable differences in behavioral function of offspring beyond simple opioid reward or opioid-motivated behavior. Although these effects were observed in offspring in the absence of the drug, both effects are related to systems that are acutely affected by opioids and so may mediate the risk for opioid abuse and dependence across generations. Mechanistic studies will be necessary to elaborate on what exactly is inherited by the offspring of parents with higher vs lower opioid intake.

Ethical statement

All authors acknowledge that they have exercised due care in ensuing the integrity of the work and none of the original material contained in the manuscript has been submitted for consideration nor will any of it will be published elsewhere except in abstract form in connection with scientific meetings. All authors are entirely responsible for the scientific content of the manuscript. All procedures were conducted according to the protocol A246-17-11 approved by the Institutional Animal Care & Use Committee of Duke University.

CRediT authorship contribution statement

Amir H. Rezvani: conceptualized, designed the study, analyzed the data, interpreted the results and wrote the paper. **Corinne Wells:** conducted all experiments and data collection. All authors contributed and have approved the final revision of the manuscript. **Andrew Hawkey:** revised the manuscript. **Graham Blair:** conducted all experiments and data collection. All authors contributed and have approved the final revision of the manuscript. **Reese Koburov:** conducted all experiments and data collection. All authors contributed and have approved the final revision of the manuscript. **Ashley Ko:** conducted all experiments and

data collection. All authors contributed and have approved the final revision of the manuscript. **Andrea Schwartz:** conducted all experiments and data collection. All authors contributed and have approved the final revision of the manuscript. **Veronica J. Kim:** conducted all experiments and data collection. All authors contributed and have approved the final revision of the manuscript. **Edward D. Levin:** conceptualized, designed the study, analyzed the data, interpreted the results and wrote the paper.

Declaration of competing interest

The authors have no conflicts of interests to report.

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