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Transgenerational attenuation of opioid self-administration as a consequence of adolescent morphine exposure

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ABSTRACT

The United States is in the midst of an opiate epidemic, with abuse of prescription and illegal opioids increasing steadily over the past decade. While it is clear that there is a genetic component to opioid addiction, there is a significant portion of heritability that cannot be explained by genetics alone. The current study was designed to test the hypothesis that maternal exposure to opioids prior to pregnancy alters abuse liability in subsequent generations. Female adolescent Sprague Dawley rats were administered morphine at increasing doses (5–25 mg/kg, s.c.) or saline for 10 days (P30-39). During adulthood, animals were bred with drug-naïve colony males. Male and female adult offspring (F1 animals) were tested for morphine self-administration acquisition, progressive ratio, extinction, and reinstatement at three doses of morphine (0.25, 0.75, 1.25 mg/kg/infusion). Grandoffspring (F2 animals, from the maternal line) were also examined. Additionally, gene expression changes within the nucleus accumbens were examined with RNA deep sequencing (PacBio) and qPCR. There were dose- and sex-dependent effects on all phases of the self-administration paradigm that indicate decreased morphine reinforcement and attenuated relapse-like behavior. Additionally, genes related to synaptic plasticity, as well as myelin basic protein (MBP), were dysregulated. Some, but not all, effects persisted into the subsequent (F2) generation. The results demonstrate that even limited opioid exposure during adolescence can have lasting effects across multiple generations, which has implications for mechanisms of the transmission of drug abuse liability in humans.

1. Introduction

The cost of opiate addiction is exceedingly high to individuals and society (Li and Burmeister, 2009); however, the effect of widespread exposure to opiates on future generations is unknown. There is a growing body of evidence demonstrating that experiences of one generation can have lasting effects on subsequent generations. For example, inter-generational effects have been documented in animal models following variations in stress, diet, as well as toxin and drug exposures (Skinner, 2015). Following exposure to drugs of abuse animal studies have found both increases and decreases in offspring propensity towards addiction-like behaviors (Szutorisz et al., 2014; Vassoler et al., 2013, 2016) that indicate alterations within the reward pathway that may increase vulnerability in certain populations. There remains a paucity of studies examining transgenerational effects following exposure to drugs of abuse.

Transgenerational epigenetics refers to the transmission of a phenotype across multiple generations of a species in the absence of changes in the DNA sequence (Bohacek et al., 2013; Franklin and Mansuy, 2010; Gapp et al., 2014; Skinner, 2015; Yohn et al., 2015). The term is limited to effects that extend to a generation that was not exposed to the initial environmental manipulation. Thus, in the absence of *in utero* exposure, grandoffspring of female animals that show an effect are demonstrating transgenerational epigenetic inheritance. There is mounting evidence that supports the hypothesis that preconception drug use has effects on multiple generations (Vassoler et al., 2014a; Yohn et al., 2015). However, this distinction should not downplay the significance of preconception drug exposure on the F1 generation, as these also have significant implications for public health.

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In the current set of studies we tested the hypothesis that female adolescent morphine exposure, terminating several weeks prior to pregnancy, would increase opiate abuse liability in offspring and grandoffspring. Further, based on other studies examining sex differences in opiate self-administration, we hypothesized that females will take more morphine than males (Cicero et al., 2003). Finally, we hypothesized that the effect would be diminished in the F2 generation as there was no direct exposure in F2 animals (Dunn and Bale, 2011). Voluntary responding of male and female F1 and F2 animals was measured during morphine self-administration acquisition, PR, extinction and drug-primed reinstatement, as these model distinct phases of substance use (i.e. addiction/reinforcement, withdrawal/rehabilitation, and relapse). We then used deep sequencing to identify pathways of gene expression changes in the nucleus accumbens in F1 animals. The nucleus accumbens was chosen based on its role in addiction and on our previous work demonstrating transgenerational effects on receptor expression within the accumbens using this model (Byrnes et al., 2013). Based on sequencing data, high level targets related to neuroplasticity were identified. The genes were examined using qPCR in the nucleus accumbens of both F1 and F2 animals of both sexes.

The data revealed significant decreases in the acquisition, extinction, and reinstatement of morphine self-administration that were sex- and dose-dependent. A number of effects were observed in both F1 and F2 animals demonstrating transgenerational epigenetic inheritance as a function of maternal drug history. Taken together, we show that female adolescent morphine exposure, in the absence of any direct fetal exposure, induces sex-specific transgenerational epigenetic effects that span at least two generations. These findings suggest the inheritance of homotypic drug resistant phenotypes following parental exposure to drugs of abuse.

2. Materials and methods

2.1. Animals and housing

For all experiments, post-natal day 23 (PND23) female Sprague-Dawley rats [Crl:CD(SD)BR] were purchased from Charles River Breeding Laboratories. All animals were housed in standard acrylic laboratory cages at Cummings School for Veterinary Medicine at Tufts University. Animals were maintained on a 12-hour light/dark cycle with lights on at 7:00 a.m. and all procedures were performed during the light phase. Food and water was available *ad libitum*, unless otherwise stated. All procedures were approved by the Institutional Animal Care and Use Committee of Tufts University, and were carried out in accordance with the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals.

2.2. Generation of F0, F1, and F2 animals

All animals were housed 3–4 per cage. The adolescent exposure is described in Vassoler et al., 2015. Briefly, beginning at PND30 females (n = 36) were injected (s.c.) once daily with morphine sulfate (MS) for a total of 10 days using an increasing dosing regimen with doses increased every other day (5, 5, 10, 10, 15, 15, 20, 20, 25, 25 mg/kg). Age-matched control animals (n = 36) received saline injections (s.c) with volumes adjusted to match those of drug-treated females. On PND 60–80 (3–6 weeks after their final injection), females were mated with drug-naïve colony males. Each male was placed with 4 age-matched females (2 morphine treated and 2 saline treated). Once an animal was visibly pregnant (approximately E16-E20) she was housed singly. On PND1 (day of birth = PND0) all litters were culled to ten pups (5 male, 5 female) (Kosten et al., 2014). The weight of the pups and the dam was recorded. All litters were weighed and weaned on PND21 and housed with same-sex littermates. No differences in bodyweights were observed at either time point (data not shown). To generate F2 animals (grandoffspring), naïve, adult, female F1 animals were mated with drug-naïve colony males in the same way as F0 animals (ie. 4 females per male, two MOR-F1 and two SAL-F1). Offspring were culled to ten pups (5 male, 5 female) on PND1. All litters were weighed and weaned on PND21 and housed with same sex littermates. It is important to note that F1 female animals used to generate F2 grandoffspring did not receive any drug or behavioral manipulations. Offspring of morphine-exposed females are designated Mor-F1. Offspring of saline controls are designated Sal-F1. Grandoffspring are designated Mor-F2 and Sal-F2, respectively. All testing was conducted once F1 and F2 animals were at least 60 days of age. In all of the reported findings only one offspring per litter was used in any individual treatment group. Table 1 lists the sample size for each experimental group.

2.3. Morphine self-administration

2.3.1. Catheterization surgery

Animals were anesthetized using a ketamine/xylazine cocktail (80 mg/kg and 8 mg/kg, respectively). The catheter (CamCaths, Cambridge, UK) was composed of silastic tubing that was fed into the right external jugular vein and routed to a mesh backmount platform secured subcutaneously between the shoulder blades. Catheters were flushed daily with an antibiotic (Cefazolin, 0.02 mg/ml) dissolved in heparinized saline and sealed with plastic obturators when not in use.

2.3.2. Apparatus

Self-administration was conducted in operant chambers housed within sound-attenuating cubicles (MedAssociates, St. Albans, VT). Chambers were equipped with house lights, cue lights, and two retractable levers (one active; one inactive). Active lever pressing initiated the activation of the syringe pump (MedAssociates) to deliver a drug infusion (rate 60 μl in 5 s, 15 s post-infusion timeout). Drug delivery and data collection were controlled by MedAssociates software (MedPCIV). Following catheterization surgery, animals were recovered for 1 week prior to initiation of self-administration.

2.3.3. Acquisition/progressive ratio

Animals were allowed to self-administer morphine (0.25, 0.75, or 1.25 mg/kg/infusion; the dose was a between subjects variable, thus different animals were used for each dose) for 15 days on a fixed ratio 1 (FR1; one lever press = one infusion) schedule of reinforcement. Each session was 2 h long. A cue light was illuminated over the active lever during the infusion for FR1 only, there were no cues associated with FR5, PR, extinction, or reinstatement. There was a 15 s time out following an infusion. Responding that is presented is from the active phase and does not include the time out period. Following 15 days of FR1, the animals were switched to a FR5 (five lever presses = one infusion) schedule for 5 days. This allowed for a greater response output in the animals. After the fifth day on FR5 animals were tested on a progressive ratio schedule of reinforcement. Under a PR schedule the response requirement for each subsequent drug delivery was increased until the subject failed to meet a requirement. The response requirement for the ith reinforcement was given by $R(i) = [5e^{0.2i}-5]$ and the session expired when an animal took more than 60 min to receive an injection. The breakpoint was operationally defined as the number of responses prior to the termination of the session. The following day, the animals entered into the extinction phase.