Exploring the epigenetics of cocaine resistance

Drug addiction is known to have a heritable component and to run in families. However, a recent study in rats by Vassoler *et al.*[1] shows an unexpected result—that the sons of males who had self-administered cocaine had a reduced propensity to take this drug and a delay in their acquisition of drug-seeking behavior. The authors linked these behavioral changes to epigenetic changes in the sperm from cocaine-exposed males and in the brains of their male offspring. We asked four experts to comment on the results of this study and their implications for understanding how addictive phenotypes are inherited.

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Cocaine self-administration induces hyperacetylation of the *Bdnf* promoter and increases expression of brain-derived neurotrophic factor (BDNF) protein in the reward pathway from the ventral tegmental area (VTA) to the nucleus accumbens and the medial prefrontal cortex (mPFC) of rats[2]. Now Vassoler *et al.*[1] have shown that cocaine self-administration in male rats induces hyperacetylation of the *Bdnf* promoter not only in their sperm but also in the mPFC of their drug-naive male (but not female) progeny. Similar to previous studies[3], increased BDNF expression in the mPFC of the sons suppressed their responses to cocaine and cocaine cues, and this effect was prevented by systemic injections of an antagonist of TrkB, the BDNF receptor. These findings lead to the unexpected (paradoxical) conclusion that a father's cocaine addiction epigenetically protects his son from addiction.

This study has potential implications for human addiction because BDNF concentrations are increased in the serum of cocaine addicts and predict how quickly they relapse to drug use during abstinence[4]. Thus, it will be interesting to determine in whether male cocaine addicts also pass a similar effect onto their sons. Additionally, serum BDNF concentrations are lower in people with depression, eating disorders and other cognitive disorders[5]. If acetylation of the *BDNF* promoter is similarly decreased in these individuals and if this decrease is passed to their sons, then the findings from Vassoler *et al.*[1] may be part of a larger story of *BDNF* epigenetic alterations that are passed from generation to generation. Finally, an important question for future research is whether the transgenerational effect of increased *Bdnf* acetylation in the mPFC is also observed in other brain reward areas such as the VTA and nucleus accumbens in which increased concentrations of BDNF are linked to an increased vulnerability to relapse after prolonged abstinence from an addictive drug[2].

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The recent report by Vassoler *et al.*[1] provides the provocative observation in rats that a father's ingestion of cocaine can alter the epigenetic programming of his sperm, which then allows the epigenetic inheritance of cocaine resistance in male but not female offspring. Epigenetic alterations in the *Bdnf* promoter were identified in fathers' sperm and in brains of male offspring. This is a good example of epigenetic inheritance through alterations in the sperm due to direct exposures to an environmental factor.

Direct exposure to environmental factors has been shown to promote epigenetic alterations in sperm that can influence disease and phenotypes in subsequent generations[6]. As the germline is directly exposed to the environmental factor to alter epigenetic programming, this information can be transmitted to the F_1 offspring. Generally, epigenetic marks get erased and reset after fertilization and embryonic development[7], but some epigenetic marks such as DNA methylation can become permanently programmed to promote the epigenetic inheritance of phenotypes[8]. In the current study, because the F_0 father is directly exposed to cocaine, as are the sperm that will generate the F_1 offspring, this does not constitute an epigenetic transgenerational inheritance phenotype (which would require transmission to the F_2 generation grandchild). A crucial future study will be to determine the potential

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The notion that acquired habits such as drug-taking behavior are heritable has long been controversial[9]. Skepticism was based largely on the apparent lack of a mechanism by which learned behaviors could alter the genetic code of germ cells for their subsequent transmission to offspring. However, over recent years the concept of heritability has 'evolved' beyond the central dogma of modifications in genomic sequence to an appreciation that epigenetic modifications of DNA or histone proteins may also have a contributory role[10].

> "However, caution should be exercised when interpreting decreased cocaine intake as a reduction in addiction vulnerability *per se*."

The work of Vassoler *et al.*[1] now shows that epigenetic mechanisms do indeed play a part in the transmission of a cocaine-taking phenotype from sires to their male offspring, findings that have important scientific and social implications. Surprisingly, the addiction liability of cocaine was decreased in the offspring, reflected in lower intake of the drug, which seems counterintuitive. Extrapolating this result to humans, paternal drug use would be expected to protect male offspring against addiction, an outcome opposite to common experience[11]. Perhaps more extensive cocaine exposure triggering addiction-like responses in the sires would instead increase the motivational properties of cocaine in offspring. However, caution should be exercised when interpreting decreased cocaine intake as a reduction in addiction vulnerability *per se*. Addiction-like cocaine behaviors can emerge in vulnerable rats even when they consume similar drug amounts as rats that do not show these behaviors[12]. This suggests that absolute amounts of cocaine consumed can be dissociated from development of addiction-like behaviors.

The findings raise other intrigu-

transgenerational impacts of cocaine by extending the observations to the F_2 generation.

This study also showed that histone acetylation at the *Bdnf* promoter was altered in the sperm of cocaine-exposed males[1]. Most histones are replaced by protamines during spermatogenesis in the testis, which are then removed at fertilization. It has been speculated that histones are maintained at specific regions of the sperm genome, but this remains to be thoroughly investigated. A more well-established epigenetic mark mediating epigenetic inheritance is DNA methylation[7], and thus future studies on the role of DNA methylation in the epigenetic inheritance phenotype observed by Vassoler *et al.*[1] are required. The observation that a male's environmental exposure to an addictive compound such as cocaine may affect his offspring's brain development and cocaine resistance has important implications in the field of neuroscience and also highlights the key role of epigenetic inheritance.

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ing issues. The increased cortical BDNF in the male offspring may explain their resistance to cocaine, but cortical BDNF regulates other complex behaviors such as decision making, learning and memory. Are these behaviors also affected? Also, is BDNF abundance increased only in the cortex or in other brain regions as well?

Finally, crucial mechanistic challenges remain, such as understanding how cocaine can induce chromatin remodeling in the promoter of *Bdnf* in sperm, if and how such chromatin remodeling is restricted to a single gene and why only male offspring are affected.

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According to some estimates, up to 4% of the human genome in germ cells maintains organization of nucleosomes, the fundamental units of chromatin, and also carries epigenetic marks associated with nucleosomes such as DNA cytosine methylation and various types of post-translational histone modification[13]. Interestingly, these DNA sequences—which in total outnumber the entire coding portion of the genome by at least twofold and are obvious candidates for conveying epigenetic heritability in humans—include not only loci of fundamental importance for early development (for example, *HOX* genes) but also regulatory sequences of the gene encoding BDNF[13]. BDNF is of pivotal importance for brain differentiation and plasticity, and it has a central role in the pathophysiology of a wide range of neurological and psychiatric diseases. Therefore, the results of Vassoler *et al.*[1] showing that both the sperm of cocaine-exposed rats and the frontal cortex of their drug-naive offspring have increased histone acetylation at the *Bdnf* promoter is of potential clinical interest far beyond the field of addiction research.

"This is surprising because, intuitively, one would expect drug-induced epigenetic inheritance to vary between offspring."

The effects of paternal cocaine exposure on the behavior of their offspring seem to be powerful, and cohort sizes of only 10–15 rats were sufficient to detect statistically significant effects[1]. This is surprising because, intuitively, one would expect drug-induced epigenetic inheritance to vary between offspring. Perhaps the reported global histone hyperacetylation after cocaine use, affecting not only specific *Bdnf* gene sequences but also bulk chromatin in the testicular tissue, could explain the robust behavioral effects in the offspring of cocaine-exposed male rats[1].

Although this study, like most other studies exploring epigenetic inheritance, focused on male germline epigenomic regulation, another pressing issue is our current lack of knowledge on epigenome regulation in the female germ line, owing to the difficulties in conducting genome-wide chromatin assays (which typically require an input of $>10^5$ cells) in pools of oocytes. Given the emerging evidence that epigenetic inheritance, which can be mediated by drugs of abuse[1] or species-specific regulation[14], leaves a strong footprint in the chromatin of our brain cells, these are exciting times in psychiatric epigenomics.

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