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Research report

Early avoidance of a heroin-paired taste-cue and subsequent addiction-like behavior in rats

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ABSTRACT

The ability to predict individual vulnerability to substance abuse would allow for a better understanding of the progression of the disease and development of better methods for prevention and/or early intervention. Here we use drug-induced devaluation of a saccharin cue in an effort to predict later addiction-like behavior in a model akin to that used by Deroche-Gamonet et al. (2004) and seek to link such vulnerability to changes in expression of various mu opioid receptor and D2 receptor-interacting proteins in brain. The results show that the greatest heroin-induced suppression of intake of a saccharin cue is associated with the greatest vulnerability to later addiction-like behavior and to differences in the expression of WLS, β-catenin, and NCS-1 in brain compared to rats that exhibited the least suppression of intake of the heroin-paired cue and/or saline controls. Finally, because the self-administration model employed produced no significant differences in drug intake between groups, overall, the resultant changes in protein expression can be more closely linked to individual differences in motivation for drug.

1. Introduction

More than 15.3 million people worldwide have drug use disorders (World Health Organization, 2015a) and approximately 69,000 people die each year from opioid overdose (World Health Organization, 2015b). However, not everyone who takes a drug of abuse becomes addicted. Roughly 15% of drug users will eventually become drug abusers (Anthony et al., 1994). What contributes to the vulnerability of the 15% and what protects the other 85% of users from becoming addicted? Are there early behavioral or molecular indicators that can help identify those individuals that are vulnerable to addiction? The ability to identify such vulnerability could make early detection and intervention a reality.

Several rodent models have been developed to simulate behaviors that are exhibited by human addicts as described in the Diagnostic and Statistical Manual of Mental Disorders (DSM). Several measures of drug taking (escalation), seeking (signaled non-availability), working (progressive ratio), persistence in the face of adverse consequences (punishment), and relapse (reinstatement) are commonly used to study addiction-like behaviors in rat models. However, the self-administration paradigm itself can lead to differences in how animals perform on these measures. For example, rats that are given extended access (6 h) to drugs of abuse escalate the amount of drug intake over time (Ahmed and Koob, 1998). They also take large quantities at the beginning of each session (load up) in order to achieve a baseline high. In contrast, Piazza and colleagues have developed an intermittent access model (three 40 min drug access periods alternated with two 15 min periods of drug non-availability) for cocaine addiction (Deroche-Gamonet et al., 2004). This intermittent schedule of drug access does not lead to significant differences in the amount of drug taken across trials, but does

Abbreviations: ALB, addiction-like behavior; CS, conditioned stimulus; CTA, conditioned taste aversion; D2R, D2 dopamine receptor; NCS-1, neuronal calcium sensor-1; NAc, nucleus accumbens; PFC, prefrontal cortex; PR, progressive ratio; SNA, signaled non-availability; US, unconditioned stimulus; VTA, ventral tegmental area; WLS, Wntless.

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result in differences in several other measures of addiction-like behavior including an increase in responding for drug during periods of signaled non-availability, an increase in the willingness to work for drug, and an increase in responding for drug in the face of adverse consequences (e.g., foot shock).

Difficulties may arise when choosing between these models when looking for differences in protein or epigenetic markers of vulnerability. In the extended access model, changes in gene and protein expression could be the result of differences in drug exposure and/or differences in motivation for drug. With the intermittent access model there is more confidence that molecular differences are not the result of differential drug exposure, but rather more directly linked to observed addictive behavior. That said, even with this model, several weeks of drug exposure are required before individual differences become evident in addiction-like behavior. Ideally, it would be advantageous to have an early indicator of future addiction-like behavior for drug, prior to a great deal of drug access.

Our laboratory has been using drug-induced avoidance of a natural reward (i.e., a saccharin solution) as an early indicator of vulnerability to addiction. As demonstrated by Huhn et al. (this issue), humans recovering from opioid addiction are less responsive to natural rewards and this is thought to predict increased vulnerability to relapse. A similar pattern is evidenced in a preclinical rodent model. Specifically, rats suppress intake of an otherwise palatable saccharin cue when paired with a drug of abuse. There are, however, robust individual differences whereby some rats, referred to as large suppressors, exhibit greater avoidance of the drug-paired cue than do others, referred to as small suppressors. This is true when the taste cue predicts access to cocaine or heroin (Grigson and Twining, 2002; Imperio and Grigson, 2015; Wise et al., 1976). Interestingly, in the saccharin-heroin extended access paradigm, this split occurs early (after only a few taste-drug pairings), before differences in drug-taking become evident. Further, with repeated taste-drug pairings, the large suppressors ultimately exhibited greater load up for drug, greater drug-taking, escalated intake over trials, marked willingness to work for drug, and greater seeking during extinction and during drug-induced reinstatement (see (Imperio and Grigson, 2015) and Imperio et al. (this issue). This finding suggests that vulnerability for addiction, as evidenced by greater devaluation of a natural reward cue, links to many other measures of addiction, including escalated drug intake (Edwards and Koob, 2013). Here, we aimed to test whether conditioned avoidance of a heroin-paired saccharin cue following just three taste-drug pairings would predict addiction-like behavior in a variation of the intermittent access model employed by Deroche-Gamonet et al. (2004).

Another aim of this study was to determine whether changes in the expression of several protein mediators of synapse function could act as molecular markers of addiction vulnerability. We have previously performed protein interaction screens to identify novel modulators of mu-opioid receptor and D2 dopamine receptor (D2R) function that may contribute to the addiction phenotype. We demonstrated that opioids alter Wnt secretion, presumably through an interaction between the mu opioid receptor (the primary mediator of opioid reward) and WNTless (WLS), a regulator of Wnt release (Jin et al., 2010). Wnts are secreted molecules that act through cell surface receptors to stabilize β-catenin which, in turn, activates transcription of genes involved in embryonic development, synapse stability, and cancer. Also, we identified neuronal calcium sensor-1 (NCS-1) as a D2R interactor that serves to inhibit internalization and desensitization of the D2 receptor (Kabbani et al., 2002). If these proteins are altered as a function of vulnerability for addiction-like behavior, then they may serve as novel therapeutic targets for the prevention and/or treatment of addiction.

2. Materials and methods

2.1. Animals

The subjects were 48 naïve, male Sprague-Dawley rats (Charles River, Wilmington, MA). Data from rats with catheters that did not remain patent for the entire study were eliminated. This study was conducted in 2 replications with final ns = 23 and 19, respectively. The rats were housed singly in suspended, wire mesh cages in a humidity-controlled environment under a 12/12 h light/dark cycle. Food (Teklad 2018, Harlan Industries, US) and water were available ad libitum, except where otherwise noted. Experiments were approved by the Penn State College of Medicine, Institutional Animal Care and Use Committee and were performed in accordance with National Institutes of Health specifications as outlined in the Guide for the Care and Use of Laboratory Animals.

2.2. Catheters

In-dwelling intra-jugular catheters were custom made in our laboratory and implanted into rats as described previously (Grigson and Twining, 2002). After three days' recovery, patency was maintained by daily flushing of catheters with heparinized saline (0.2 ml of 30 IU/ml heparin) and verified, when necessary, by using 0.2 ml of 1% propofol (Diprivan, APP Pharmaceuticals, Schaumburg, IL) administered through the catheter. After surgeries, rats were given one week to recover before the start of testing.

2.3. Apparatus

2.3.1. Self-administration chambers

Each rat was trained in one of 12 identical operant chambers (Med Associates, St. Albans, VT) measuring 30.5 × 24.0 × 29.0 cm and housed in light and sound attenuating cubicles as previously described (Puhl et al., 2012). All chambers have clear Plexiglas tops, fronts, and backs. Sidewalls are aluminum. The floors consist of 19 stainless steel rods (4.8 mm) spaced 1.6 cm apart center to center. Each chamber is equipped with three retractable operant sipper tubes (spouts) that enter the left side of the chamber through 1.3 cm diameter holes spaced at 8.0 cm center to center. A stimulus light is located 6 cm above each spout. A lickometer circuit is used to record spout licks (and contacts such as nose pokes). Each chamber is equipped with a house light (25 W), a tone generator (Sonalert Time Generator, 2900 Hz), and a white noise speaker (75 dB). Self-administration is controlled by an electronic circuit operating a syringe pump (Med Associates). Collection of the data and control of chamber events are performed on-line using a Windows-based computer running programs written in Medstate notation language (Med Associates). The lickometer circuit was used to monitor licking on the leftmost spout (saccharin), the middle spout ("inactive", i.e. the spout upon which responding was measured but elicited no consequence), and the rightmost spout ("active", i.e. the spout upon which responding typically elicited an iv infusion of heroin).

2.3.2. Coupling assembly

Before the start of each self-administration trial, a coupling assembly is attached to the cannula exiting through the rat's back. The coupling assembly consists of a metal spring attached to a metal spacer with Tygon tubing inserted down the center to protect the tubing from interference by the rat. The tubing is attached to a counterbalanced swivel (Instech, Plymouth Meeting, PA) that in turn is attached to the syringe pump outside of the experimental chambers.