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Behavioral predictors of alcohol drinking in a neurodevelopmental rat model of schizophrenia and co-occurring alcohol use disorder

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ABSTRACT

Alcohol use disorder commonly occurs in patients with schizophrenia and contributes greatly to its morbidity. Unfortunately, the neural and behavioral underpinnings of alcohol drinking in these patients are not well understood. In order to begin to understand the cognitive and reward-related changes that may contribute to alcohol drinking, this study was designed to address: 1) latent inhibition; 2) conditioning; and 3) extinction of autoshaping in a neurodevelopmental rat model with relevance to co-occurring schizophrenia and alcohol use disorders, the neonatal ventral hippocampal lesioned (NVHL) rat. NVHL lesions (or sham surgeries) were performed on post-natal day 7 (PND7) and animals were given brief exposure to alcohol during adolescent (PND 28–42). Latent inhibition of autoshaping, conditioning and extinction were assessed between PND 72–90. On PND90 animals were given alcohol again and allowed to establish stable drinking. Latent inhibition of autoshaping was found to be prolonged in the NVHL rats; the NVHL rats pre-exposed to the lever stimulus were slower to acquire autoshaping than sham pre-exposed rats. NVHL rats that were not pre-exposed to the lever stimulus did not differ during conditioning, but were slower to extinguish conditioned responding compared to sham controls. Finally, the NVHL rats from both groups drank significantly more alcohol than sham rats, and the extent of latent inhibition predicted future alcohol intake in the pre-exposed animals. These findings suggest that the latent inhibition of autoshaping procedure can be used to model cognitive- and reward-related dysfunctions in schizophrenia, and these dysfunctions may contribute to the development of co-occurring alcohol use.

1. Introduction

Substance and alcohol use disorders (AUD) commonly occur in patients with schizophrenia; 30% of patients drink regularly, and this use significantly worsens the course of schizophrenia (Regier et al., 1990). AUD in these patients is associated with poor treatment response, treatment non-compliance (Owen et al., 1996), relapse (Drake and Mueser, 1996; Gupta et al., 1996), violence (Bartels et al., 1991; Swanson et al., 1990) and suicide (Allebeck et al., 1987; Harkavy-Friedman and Nelson, 1997). Unfortunately, there is a lack of studies describing the neural and behavioral underpinnings of AUD in patients with schizophrenia. While a variety of hypotheses have been presented regarding the prevalence of substance use in schizophrenia (Chambers et al., 2001; Green et al., 1999; Ng et al., 2013), there is a great need for further understanding the cognitive and reward-related underpinnings of this substance use (Duijkers et al., 2016).

In this regard, we employed a neurodevelopmental model of schizophrenia and co-occurring substance use disorder, namely the neonatal ventral hippocampal lesioned (NVHL) rat. The NVHL rat is one of the best-described animal models of SCZ (Lipska and Weinberger, 2002) and displays behavioral and neurobiological dysfunctions analogous to those seen in patients with schizophrenia. The NVHL rat, experimentally created by producing an excitotoxic lesion in the ventral hippocampus in a 7-day old rat pup (analogous to a third trimester insult), has strong construct, face, and predictive validity for schizophrenia (Tseng et al., 2009). For example, schizophrenia is a neurodevelopmental disorder involving a prefrontal-limbic network disconnection, which is modeled in the NVHL rat (i.e., construct validity). Further, as adults, NVHL rats exhibit a variety of behavioral, neurochemical, and molecular alterations associated with the clinical symptoms of schizophrenia (i.e., face validity (Tseng et al., 2009)). Finally, in the NVHL rat, many of the behavioral and biological analogs of schizophrenia can be reversed by antipsychotic medications (i.e., predictive validity (Tseng et al., 2009)).

Modeling the rates of substance and alcohol use disorders in schizophrenia (Volkow, 2009), NVHL rats display enhanced sensitivity to, and increased use of, cocaine, nicotine, and methamphetamine (Berg et al., 2014; Brady et al., 2008; Chambers and Self, 2002; Chambers and

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Taylor, 2004; Karlsson et al., 2013), as well as altered reactivity to the rewarding effects of cannabinoids (Gallo et al., 2014a; Gallo et al., 2014b). Importantly, a recent investigation demonstrated that brief exposure of NVHL rats to alcohol in adolescence (between post-natal days [PND] 28–42) produced a loss of control over alcohol drinking in adulthood, potentially suggesting the NVHL rat as a valid model of AUD and schizophrenia (Jeanblanc et al., 2015). These animals also lever-pressed more for alcohol, required more sessions to extinguish behavior, and showed greater relapse than shams.

To further assess the underlying cognitive and reward-related dysfunctions underlying alcohol drinking in this rat model of AUD in schizophrenia, the current study assessed the performance of NVHL rats on latent inhibition, conditioning and extinction of appetitive autoshaping. We were interested in a behavioral assay that would capture multiple facets of psychopathology of schizophrenia including both cognitive (captured by latent inhibition (Almey et al., 2013; Weiner, 2003)) and reward-related deficits (captured by the conditioning and extinction of autoshaping (Day and Carelli, 2007)). Latent inhibition refers to the observation that the rate of association between a conditioned stimulus (CS) and an unconditioned stimulus (US) is decreased if the subject is first exposed to the to-be-conditioned CS in the absence of the US (i.e., stimulus takes longer to acquire meaning). Autoshaping refers to a procedure in which a cue (CS) repeatedly paired with a reward (US) elicits a conditioned response to the cue itself ("sign-tracking"), even if the reward is not contingent upon the action toward the cue. Thus, the current study was designed to address: 1) latent inhibition, 2) conditioning (learning of cue-reward association) and 3) extinction (cue responding in absence of reward) of autoshaping, in the NVHL rat model of schizophrenia and co-occurring alcohol use disorder (Jeanblanc et al., 2015).

While latent inhibition of autoshaping procedures have not been tested in the NVHL rat, previous studies have suggested that NVHL rats displayed lower sign-tracking compared to NVHL rats (Lopez et al., 2015). Moreover, both reduced as well as no changes in latent inhibition have been observed in these animals compared to sham animals depending on the behavioral assays tested (Angst et al., 2007; Grecksch et al., 1999). Despite these variable findings, we hypothesized that, consistent with clinical investigations of latent inhibition in patients with schizophrenia (Yogev et al., 2004), NVHL rats would show decreased latent inhibition compared to sham animals.

We also hypothesized that these deficits would be present prior to the development of excessive alcohol drinking. Furthermore, by correlating measures from this task with future alcohol drinking, we assessed the utility of these measures to predict alcohol drinking as a potential biomarker that can then be targeted for the development of therapeutic strategies to decrease (or prevent [if captured prior to development of AUD]) alcohol use in schizophrenia. A recent study used a similar approach to suggest that impairments in radial arm maze performance could predict nicotine seeking in the NVHL rat (Rao et al., 2016), further supporting the utility of translational behavioral measures as biomarkers.

2. Methods

2.1. Subjects

Timed pregnant Sprague-Dawley dams were ordered from Charles River (Wilmington, MA) to arrive at gestational day 13 and were singly housed with ad libitum access to food and water. Male rat pups used in the study were individually housed in a colony room maintained on a 14:10 h light-dark cycle. Experimentation took place during the light period of the cycle. Two weeks before the beginning of test procedures the rats were placed on a food deprivation schedule to maintain their weights between 80% and 85% of their free feeding weights. They were monitored and cared for in compliance with the Association for Assessment and Accreditation of Laboratory Care guidelines and the IACUC of Dartmouth College.

2.2. NVHL preparation and surgery

Male Sprague-Dawley rat pups ($n = 40$) on post-natal day 7 (PND 7, 15–20 g) were anesthetized using hypothermia and then placed on a Styrofoam platform attached to a stereotactic apparatus (Kopf Instruments, Tujunga). Half of the pups (NVHL) were bilaterally injected with excitotoxic ibotenic acid (3.0 μg ibotenic acid [Tocris, Minneapolis] dissolved in 0.3 μl of artificial cerebrospinal fluid (aCSF); $n = 20$) into their ventral hippocampi (AP −3.0 mm, ML ± 3.5 mm, VD ± 5.0 mm relative to bregma). The remaining pups ($n = 20$) were injected with aCSF at the same co-ordinates (Sham, unlesioned). After the surgery, wounds were closed using tissue glue, and when activity level had returned to normal, pups were returned to their dams. Rats were weaned on PND 21 and housed individually for the duration of the study.

2.3. Alcohol drinking in adolescence and adulthood

We followed the protocol of Jeanblanc et al. (2015), in which the rats were given access to alcohol in a free-access 2-bottle (water and 10% alcohol) design between PND 28 and 42. This was done to ensure that the NVHL rats would consume alcohol preferentially in adulthood; NVHL rats not exposed to alcohol during adolescence do not display increased drinking in adulthood (Jeanblanc et al., 2015). At the end of this period, the alcohol bottle was removed, and the animals only had access to water until adulthood. Alcohol, water and food intake as well as body weight were measured daily during PND 28–42 and then again in adulthood upon resuming alcohol drinking. All animals went through the latent inhibition of autoshaping procedure (described below) between PND 56 and PND 90. Alcohol was then reintroduced to the rats in adulthood (PND 90) in a continuous-access 2-bottle choice (water and 20% alcohol) design. The position of the two bottles was switched daily to prevent positional preference, consistent with our previous investigations (Khokhar and Green, 2016). The animals' performance on the behavioral assay was correlated with their alcohol drinking between PND 90 and PND 150 to assess the predictive ability of these measures toward future alcohol drinking.

2.4. Behavioral apparatus

Latent inhibition, conditioning and extinction of autoshaping were carried out in eight identical standard conditioning chambers (24 × 30.5 × 29 cm: Med Associates) enclosed in sound-attenuating chambers (62 × 56 × 56 cm) with background noise (68 dB SPL) provided by an exhaust fan. The conditioning chambers (Med Associates, ENV-007) consisted of aluminum front and back walls and clear acrylic sides and top. The grid floor was stainless steel rods (5 mm diameter) spaced 1.5 cm apart (center to center). The chambers were illuminated by one 6-W bulb, with a red cover, mounted on the ceiling of the sound attenuation chamber. Each chamber contained a food cup, recessed in the center of the front wall, mid-way between two retractable levers (Med Associates model: ENV-112CM). A 10 second insertion of the left lever served as the CS throughout the experiment. (The right lever was not used and remained retracted throughout the experiment.) Lever presses were counted throughout the session, and a photocell recorded head entries into the food cup. The apparatus was controlled by computer equipment located in an adjacent room. The reinforcer was a 45-mg grain-based rodent food pellet (Bioserv).

2.5. Behavioral procedures

Prior to the start of the pre-exposure (PE) phase, each rat was fed 1 g of the 45-mg reward pellets in its home cage for two consecutive days.

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