Impaired Reality Testing in an Animal Model of Schizophrenia

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Background: Schizophrenia is a chronic and devastating brain disorder characterized by hallucinations and delusions, symptoms reflecting impaired reality testing. Although animal models have captured negative symptoms and cognitive deficits associated with schizophrenia, none have addressed these defining, positive symptoms.

Methods: Here we tested the performance of adults given neonatal ventral hippocampal lesions (NVHL), a neurodevelopmental model of schizophrenia, in two taste aversion procedures.

Results: Normal and NVHL rats formed aversions to a palatable food when the food was directly paired with nausea, but only NVHL rats formed a food aversion when the cue predicting that food was paired with nausea. The failure of NVHL rats to discriminate fully real from imagined food parallels the failure of people with schizophrenia to differentiate internal thoughts and beliefs from reality.

Conclusions: These results further validate the NVHL model of schizophrenia and provide a means to assess impaired reality testing in variety of animal models.

Key Words: Hallucination, mediated devaluation, neonatal ventral hippocampal lesion, psychosis, reality testing, schizophrenia

Schizophrenia is a chronic brain disorder affecting on average 1% of the U.S. adult population. Among the more devastating symptoms are hallucinations and delusions. These symptoms are thought to reflect impaired reality testing, with *reality testing* referring to "the capacity to distinguish internal fantasy from external reality" (1). Although these symptoms form a core and defining feature of schizophrenia, existing animal models used to study the disease typically do not address them. Indeed, these aspects of schizophrenia have been described as difficult or impossible to capture in animal models (2).

As a result, animal research has focused primarily on capturing the so-called negative symptoms and cognitive deficits. This approach has been remarkably successful. For example, in one neurodevelopmental model in which neonatal rats receive ventral hippocampal lesions, adult rats later exhibit a number of symptoms paralleling those observed in schizophrenic patients, including impaired in sensory-motor gating (3), hyperactivity in response to stimulants (4), comorbidity with drug use (5), and abnormal social behaviors (6). Despite the success of the neonatal ventral hippocampal lesion (NVHL) and other models in recreating some aspects of schizophrenia (7,8), our understanding of the disorder—and our ability to design effective treatments—might progress more rapidly if impaired reality testing could be assessed (9).

Here we used a simple taste aversion procedure combined with Pavlovian conditioning to assess the ability of rats to distinguish between a real and internal representation of a palatable food. We found that normal rats were readily able to distinguish the two. As a result, they appropriately reduced consumption of the food (and learned behaviors directed toward obtaining the food) when the food was directly paired with induced nausea but not when only the representation of the food was paired with induced nausea. In contrast, NVHL rats reduced food consumption in both settings. We suggest that this approach provides a tool with which to assess rudimentary reality testing in animal models of schizophrenia.

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Methods and Materials

Subjects

Timed pregnant Long-Evans females were obtained at embryonic Days 15–18 from Charles River (Wilmington, Massachusetts) and were individually housed with free access to food and water in a temperature- and humidity-controlled environment with a 12-hour light/dark cycle (lights on at 7:00 AM). Neonatal pups were left undisturbed until postnatal day (PD) 6 or 7, when they were weighed and sex was determined. Following surgery (see next section) at approximately PD21, animals were weaned and housed in pairs of like lesion status. Upon reaching adulthood (PD56), animals were single-housed. Before training, rats were reduced to 85% of their baseline weights. All procedures adhered to guidelines set forth by the University of Maryland School of Medicine Animal Care and Use Committee and the National Institutes of Health.

Surgical Procedures

Between PD6 and PD8, male pups (15–20 g) received either an excitotoxic lesion of the ventral hippocampus (NVHL; $n = 17$) or a control procedure ($n = 15$). Pups were anesthetized via hypothermia; NVHL pups received bilateral infusions (.3 μL per side; .15 μL/min) of ibotenic acid (10 μg/μL in artificial cerebrospinal fluid [aCSF]) into the ventral hippocampus, at coordinates of 3 mm posterior to bregma, 3.5 mm lateral to bregma, and 5 mm from the surface of the skull. Control rats received bilateral infusions of aCSF into the ventral hippocampus at the same coordinates. Following recovery, pups were returned to their mothers.

Apparatus

Testing was conducted in 16 standard-sized behavioral boxes (12 × 10 × 12 inches) using equipment modules purchased from Coulbourn Instruments (Allentown, Pennsylvania). A recessed food cup was located in the center of the right wall approximately 2 cm

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above the floor. The food cup was connected to a feeder mounted outside of the chamber to deliver 45-mg sucrose pellets (plain sugar or cocoa-flavored, Research Diets, New Brunswick, New Jersey). For conditioned taste aversion (CTA), an external speaker approximately 20 cm from the chamber was connected to a white noise (72-dB) generator. For representation-mediated taste aversion (RMTA), a cue light was placed directly above the food cup approximately 10 cm from the floor. Data were collected by Graphic State behavioral software from Coulbourn Instruments.

Behavioral Measures

The primary measure of conditioning for both CTA and RMTA was food cup rate—specifically, the number of food cup entries per minute during the white noise or cue light. The primary measure of eating was number of pellets consumed. For CTA, raw consumption numbers are shown. For RMTA, the change in pellet consumption [(number of pellets consumed prenausea) – (number of pellets consumed postnausea)] was calculated. Positive numbers would indicate a taste aversion was formed.

Conditioned Taste Aversion

All rats (control, $n = 14$; NVHL, $n = 17$) received 8 days of conditioning. Each day consisted of 12 pairings of 30-sec white noise with delivery of three cocoa-flavored pellets (time between trials was 7–10 min). Over the next 6 days, conditioned taste aversion training and probe testing was conducted. Rats in each lesion group were divided into paired (control, $n = 7$; NVHL, $n = 9$) and unpaired groups (control, $n = 7$; NVHL, $n = 8$). On Days 1 and 3, Paired rats were given access to 100 cocoa pellets, and unpaired rats were placed in the chambers with no access to pellets. Following 10 min all rats were removed and received intraperitoneal injections of .3 M lithium chloride [LiCl—5 mL/kg]. This dose and delivery of LiCl naturally induces nausea/gastric irritation and when given following food consumption results in a conditioned taste aversion (10,11). On Days 2 and 4, unpaired rats were given 10-min access to 100 cocoa-flavored pellets, whereas paired rats placed in the chambers with no access to pellets. Previous studies have shown that spacing LiCl-induced nausea and food consumption by 24 hours prevents the acquisition of a CTA (12). Thus, whereas both paired and unpaired rats experienced the food and became nauseas, only paired rats should form a CTA. On Day 5, an extinction probe was given in which all rats received 12 presentations (30-sec each) of white noise in the absence of any pellets (time between trials was 7–10 min). On Day 6, a consumption test was given in which all rats had access to 100 pellets.

Representation-Mediated Taste Aversion

Over 3 days, rats (control, $n = 11$; NVHL, $n = 11$) received 6 or 12 pairings of 30-sec cue light illumination with delivery of three plain sugar pellets (time between trials was 7–10 min). Following conditioning to the cue light, rats were given a 10-min consumption test, in which 100 sugar pellets were placed in the recessed food cup. This would serve as a baseline for measuring subsequent taste aversion. Next, in two consecutive 60-min sessions; nausea was induced by intraperitoneal injection of .3 M LiCl (5 mL/kg), and rats were placed in the experimental chamber. Five minutes into each session, the cue light was illuminated for 30-sec. In this way, cue light illumination (and presumably any representation of sugar elicited by the cue light) was paired with nausea. The following day, a consumption test identical to the first was given to determine whether pairing the cue light and nausea resulted in any aversion to the actual sugar pellets.

Statistical Analyses

Data were acquired using Coulbourn GS2 software. Raw data were processed in Matlab to extract food cup rate during cue and baseline periods. To be included in consumption analysis for either RMTA or CTA, rats must have eaten at least 40 of 100 pellets in the baseline consumption test. Data were analyzed with analysis of variance (ANOVA) using Statistica. Post hoc comparisons were made with Tukey's honestly significant difference. In all cases, $p < .05$ was considered significant.

Results

Histological Results

Nissl-stained hippocampal sections from NVHL animals exhibited varying degrees of cell loss, cavitation, enlarged ventricles, and cellular disorganization restricted to the ventral subiculum, ventral CA1, and/or CA3 region. Control-treated animals showed no evidence of damage to either the hippocampus or adjacent areas. A representative photomicrograph from a control rat (Figure 1A) and NVHL rat (Figure 1B) is shown.

Conditioned Taste Aversion

Experimental timeline and training procedures are shown in Figure 2A and 2B. Control and NVHL rats were trained that a white noise predicted cocoa-flavored pellets (Figure 3A). ANOVA [(lesion – control, NVHL) × (group – paired, unpaired)] for mean food cup rate during the white noise on the final day of conditioning found no significant main effects or any interactions with lesion or group (all $ps > .1$). Control and NVHL rats were then divided into two groups: paired and unpaired. Paired rats received the pellets with nausea induced by lithium chloride injection. Unpaired rats received the pellets at the same time as paired rats, but nausea was induced 24 hours later. As expected, only paired rats formed an aversion to the pellets (Figure 3B). ANOVA for cocoa pellet consumption [(day – taste aversion Day 1 (TA1), TA2, test) × (lesion – control, NVHL) × (group – paired, unpaired)] revealed significant effects of day [$F(2,54) = 68.10$, $p < .01$], group [$F(1,27) = 33.62$, $p < .01$], and a day × group interaction [$F(2,54) = 62.11$, $p < .01$). Post hoc comparisons found significant differences between unpaired and paired consumption on TA2 and test ($ps < .01$). Importantly, how-

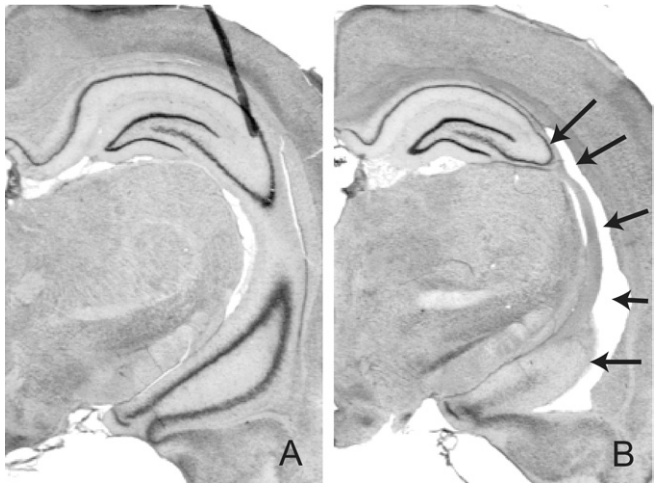

Figure 1. Representative control and neonatal ventral hippocampal lesions. Photomicrographs from Nissl-stained **(A)** control and **(B)** neonatal ventral hippocampal lesioned rats are shown. Black arrows point to regions of cellular loss and disorganization and ventricular enlargement.