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Research report

Preliminary evidence for reduced social interactions in *Chakragati* mutants modeling certain symptoms of schizophrenia

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Abstract

Rodent models of schizophrenia provide powerful experimental tools for elucidating certain manifestations of the brain disease. The *chakragati* (*ckr*) mouse mutant, for instance, reproduces aberrant neuroanatomical and behavioral phenotypes observed in the corresponding human condition. To further investigate the utility of this mouse in the context of social behavior, we compared spontaneous behavioral activity and social interactions recorded during the subjective night among wild-type, heterozygous, and homozygous *ckr* mice. We found that both heterozygous and homozygous *ckr* animals failed to show appropriate norms of social behavior, including proximity, approach, huddling, and anogenital investigation in response to novel conspecifics. We further found that the anatomical distribution, topography, and connectivity of the neuropeptides oxytocin and vasopressin in the anterior hypothalamus did not differ among wild-type, heterozygous, or homozygous *ckr* animals. These latter findings suggest that although oxytocin and vasopressin influence social behavior, connectivity of such cells may not be phenotypically relevant for the observed social deficits seen in heterozygous and homozygous *ckr* mice. Collectively, *ckr* mice and their heterozygote kin are valuable experimental tools for pre-clinical studies involving disruptions of social behavior (e.g., social withdrawal).

Theme: Disorders of the nervous system
Topic: Neuropsychiatric disorders

Keywords: Behavioral activity; Brain; Circling behavior; *Ckr* mice; Oxytocin; Social withdrawal; Vasopressin

1. Introduction

Schizophrenia is a brain disease with variable deficit manifestations in behavior. Deficit symptoms (also commonly referred to as negative symptoms) include blunted affect, social ambivalence, and social withdrawal [2,17]. The observation that schizophrenic patients have deficits in social interaction suggests that certain neural circuits processing social information have been pathologically modified by the disease. To achieve a better understanding of brain–behavior relationships, particularly those that are symptomatic of schizophrenia, genetically engineered mouse mutants might be relevant for investigating aberrant social behavior. Currently there are several genetic animal models of schizophrenia that reproduce certain aspects of the human disease phenotype [3,13,19,20,22]. The *chakragati* (*ckr*) mouse, for instance, shows behavioral abnormalities, including novelty-driven hyperactivity that is manifested as increased circling activity in the open field. Importantly, this schizophrenia-related behavior is attenuated by treatment with the antipsychotics clozapine and olanzapine [19]. Further, *ckr* mice show asymmetric elevation of dopamine D_2-like receptors in the striatum, and high-resolution structural MRIs of these

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mutants indicate selective enlargement of the lateral ventricles, changes that are reminiscent of schizophrenia [14,20].

We have previously reported that the phenotype of the *ckr* mouse is transmitted as an autosomal recessive trait, suggesting that it may result from a loss of function at a particular locus (e.g., D16Ros1) of chromosome 16 [14,16]. Determination of the disrupted gene(s) responsible for this phenotype may offer insight into the control of complex motor behaviors. Thus, *ckr* mutants are not only amenable to pre-clinical interventional trials but are also useful to characterize the behavioral effects of experimentally induced mutations. Against this background and to further investigate the behavioral significance of the Ren-2^d renin gene insertion in the mouse genome, male *ckr* mice were paired with unfamiliar (female) conspecifics and several behavioral features of social behavior were videotaped during the subjective night. In addition, as the neurohypophyseal neuropeptides oxytocin (OT) and arginine vasopressin (AVP) have been shown to influence a number of forms of social behavior, including affiliation and reproduction [1,24], we mapped the anatomical distribution of OT and AVP nerve cells in brains of *ckr* (*ckr*/*ckr*), heterozygous (+/*ckr*), and wild-type (+/+; C57BL/10Ros^{pd} × C3H/HeRos) mice. In general our data suggest that the *ckr* mouse mutant mimics certain behavioral symptoms of schizophrenia, namely deficits in social interactions.

2. Materials and methods

2.1. Animals and experimental design

The *ckr* mouse was generated as described previously [14]. All transgenic mice used for the experiments below were adult (6–9 months; 30–40 g) male and female F_2 animals of the mixed genetic background of BCF_1 (C57BL/10Ros^{pd} × C3H/HeRos). Homozygous, heterozygous, and wild-type adult mice were kept in (same-sex/same-genotype) groups of 3–4/cage and maintained on a light–dark cycle of 12:12 h (lights on at 0700) with free access to food and water. Mice (from different litters) were never handled or isolated before the social interaction test paradigm (see below). Classification of genotype for both *ckr* and heterozygous mice was conducted by restriction fragment-length polymorphism analysis of biopsied tail DNA taken during the first week of postnatal life [14]. All behavioral procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and with approval from the Roswell Park Cancer Institute IACUC. All efforts were made to minimize animal stress and to reduce the number of mice used for these experiments.

2.2. Behavioral testing: general behavioral activity

Adult wild-type, heterozygous, and homozygous *ckr* mice housed in their respective home-cages were videotaped (under an infrared light emitting wavelengths greater than 680 nm) for 60 min during the subjective night (subjective night denotes the onset of daily activity). In brief, spontaneous behavioral activity was recorded (4 h after subjective night onset) on a Sony Model TRV 900 videotape recorder equipped with a standard 35 mm Sony mini-DV tape. Overall behavioral activity, including circling behavior, was digitized for 1 min and the number of complete, full (left or right) 360° circles was scored by two investigators with no knowledge of the genotype identity. In addition, the percentage of time spent on walking, rearing, or time spent on stationary activities (e.g., grooming, sniffing, or nibbling) was quantified from the video recordings (for further methodological details see Ref. [19]).

2.3. Behavioral testing: social interactions in neutral cages

To assess social behavior between two mice of the opposite sex in a neutral environment, first we videotaped genetically identical mice that had been housed separately by sex and then placed in a home-cage for one night. Testing began when a stimulus female mouse was introduced into the neutral environment of a male animal habituated to that environment 30 min prior to the social confrontation. The neutral home-cage was a rectangular Plexiglas cage (30 cm × 15 cm × 20 cm) with food and water, containing the above infrared video camera. Recorded spontaneous social interactions were digitized for each mouse using a Peak Motus Program. This was achieved by marking several constant points on every animal recorded: the nose, occiput, left and right shoulders, left and right hips, and the base of the tail. The aforementioned coordinates were followed for 3 s to generate a spatial diagram of motor activities by plotting the range of circular motion angles (for further methodological details see Ref. [6]). Second, duration of social interactions (i.e., proximity, approach, and huddling) and investigative behaviors (i.e., olfactory exploration and anogenital investigation) were scored and quantified from the video recordings for a 60-min period by investigators with no knowledge of the mouse genotype(s). Recordings began 4 h after the onset of the subjective night. After the testing procedures, animals (N = 4 mice/genotype) were returned to their original, respective home-cages.

2.4. Olfactory test

To determine olfactory performance, wild-type, heterozygous, and homozygous *ckr* animals (N = 4 mice/genotype) were placed individually in a neutral cage 4 h after the onset of the subjective night. An investigator blind to the genotype of the mouse tested hid a miniature Oreo cookie in the cage bedding and immediately recorded the latency (in seconds) of each individual mouse to locate and retrieve the cookie. Two successive trials were performed for each mouse genotype under an infrared light emitting wavelengths greater than 680 nm.