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Animal Models for Schizophrenia: The *chakragati* Mouse Mutant

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Neuroscience continues the search for novel and more clinically effective therapies to treat the broad spectrum of psychopathologies associated with schizophrenia. To facilitate this search, the need for animal models which have face, construct and predictive validity with respect to etiology, symptomatology, biochemistry and response to treatment is of paramount importance. Thus, an animal model of schizophrenia should accurately recapitulate a number of falsifiable features observed in the corresponding human disorder. The most direct and simple approach to study certain manifestations of schizophrenia is to deconstruct the brain disease into specific endophenotypes. An endophenotype is a specific anatomical, behavioral or cognitive biomarker that provides insight into possible candidate genes as well as validation criteria for any preclinical model of the disease (O'Tuathaigh et al., 2006). For instance, enlargement of the lateral cerebral ventricles, social withdrawal and pre-pulse inhibition are some of the earliest reported abnormalities found in schizophrenic patients, as well as some of the most stable biomarkers in clinical studies (Gaser et al., 2004; Ross et al., 2006). Accordingly, the aforementioned endophenotypes can also be introduced in mice strains often by deleting, adding or mutating candidate susceptibility genes. This experimental approach obviously does not recreate all features of the corresponding disease, particularly uniquely human features such as thought disorder, hallucinations and paranoia psychosis. Nevertheless, generating core symptoms of schizophrenia in mice advances our knowledge of disease pathogenesis and provides rational rather than serendipitous approaches to drug development and treatment design.

Several mouse models of schizophrenia based on targeted disruption of genes implicated in neurodevelopment have been established and summarized in recent reviews (Gainetdinov et al., 2001; Chen et al., 2006; O'Tuathaigh et al., 2006). As part of this ex-
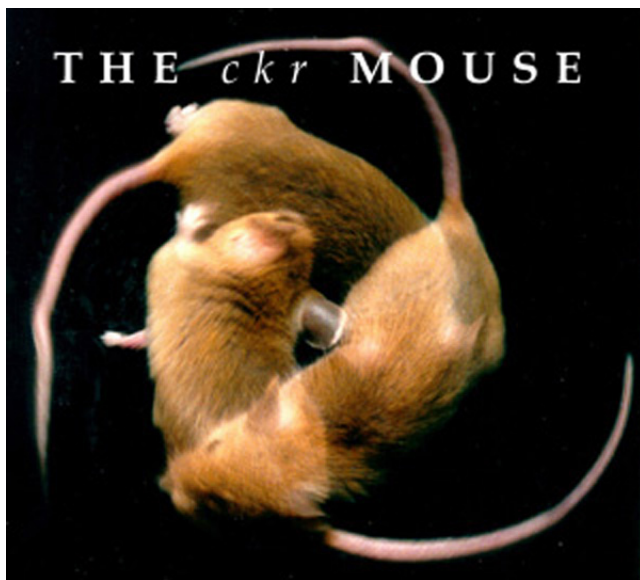
Figure 1

perimental approach, we have undertaken a multidisciplinary scale screening of a mutant mouse that displays several schizophrenia-like endophenotypes as a result of a transgenic insertional mutation (Torres et al., 2004; Torres et al., 2005; Torres et al., 2005; Torres et al., 2008). These mutant mice (referred to as *chakragati,* or *ckr*) were generated by microinjection of a 24-kb genomic fragment containing the mouse Ren-2^d renin gene into BCF (C57BL/10Ros^{pd} X C3H/HeRos) fertilized oocytes (Ratty et al., 1990). Genetic and physical analyses of this insertion revealed that 2.5 copies of the transgene, comprising 65-70-kb, had integrated, duplicated and inverted portions of a particular locus within chromosome 16 of the mouse genome (Smiraglia et al., 1997; Smiraglia et al., 1997). This suspected loss-of-function mutation is expressed only when bred to homozygosity and mating studies indicate an autosomal recessive mode of inheritance (Ratty et al., 1990). Thus, *ckr* mice homozygous for the transgene insertion show a constellation of anatomical, biochemical and behavioral deficits which resemble those often reported in schizophrenic patients (Table 1). The validity of the *ckr* mouse mutant for understanding the molecular pathogenesis of schizophrenia is further supported in its ability to respond to antipsychotic drug treatment, a prelude to exploring new therapeutic avenues (Torres et al., 2004). In this context, there is a severe drought of new drug targets in schizophrenia, particularly drugs that improve cognitive deficits and social role performance (i.e., negative symptoms). Developing newer genetic mouse models of schizophrenia to model these more complex behavioral processes could shed light on more clinically effective therapies.

Fig. 1. The most salient endophenotype in the *ckr* mouse is its circling behavior. Under conditions of subjective stress, the mutant mouse shows consistent circling behavior with individual turning rates ranging from 10 to 80 full body turns per minute. This behavior syndrome is also characterized by lateralized circling behavior (i.e., a left-preference population bias), postural asymmetry and hyperactivity to sensory stimuli. In this context, drugs that block the N-Methyl-D-Aspartate (NMDA) subtype of the glutamate receptor, such as phencyclidine (PCP, also known as angel dust) and ketamine (a dissociate anesthetic), usually elicit a psychotic-like-state that resembles schizophrenia in preclinical models of the disease. This psychotic-like state includes aberrant behavior syndromes (i.e., positive symptoms) similar to those listed for the *ckr* mouse. It should be noted that atypical neuroleptics such as clozapine and olanzapine (antipsychotic agents that selectively alleviate symptoms of schizophrenia) also alleviate the lateralized circling behavior and aberrant postural asymmetry exhibited by the *ckr* mouse.

Fig. 2. Neuropathological features of the *ckr* mouse brain. A microscopic lesion reliably observed in the mutant brain is enlargement of the lateral ventricles (right panel. For comparison, wild-type mouse brain: left panel). This ventricular pathology parallels the enlargement of the lateral ventricular space in the schizophrenic brain. Large ventricles do not correlate with drug treatment history. It is hypothesized that enlargement of the lateral ventricular system in both patients and *ckr*
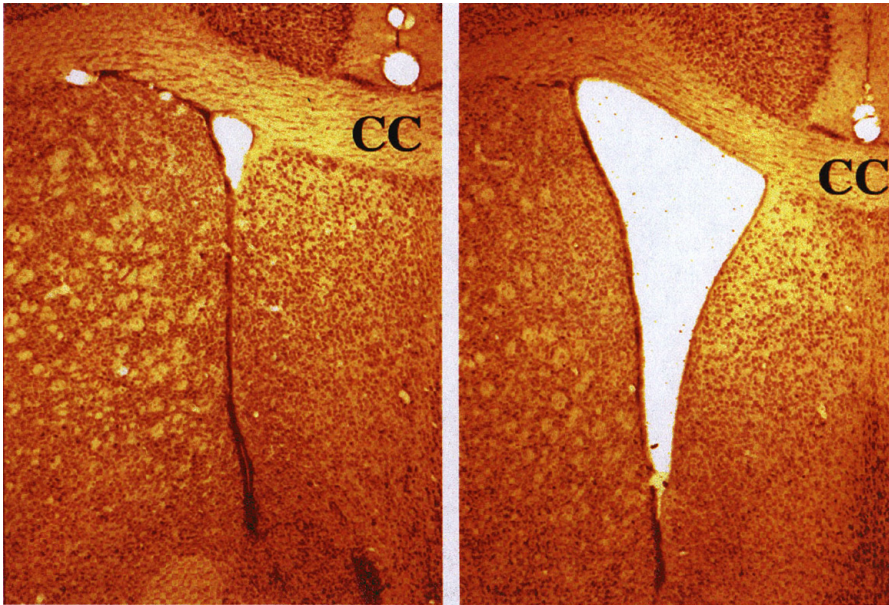
Figure 2

mice is related to specific shrinkage of white matter rather than diffuse brain atrophy (Gaser et al., 2004; Torres et al., 2008). When lateral ventricles become pathologically enlarged, severe cognitive and motor difficulties can result. Postmortem brain sections (60 µm thick) counterstained with Neutral Red. CC = corpus callosum. Reprinted from: A neurobehavioral screening of the *ckr* mouse mutant: implications for an animal model of schizophrenia, Vol 62, Torres et al., 315-326 (2005), with permission from Elsevier.

Fig. 3. Representative magnetic resonance imaging (MRI) scans of the lateral cerebral ventricles (yellow banding) obtained from mice at different stages of development. At day 5 of postnatal life a conspicuous enlargement of the lateral ventricular system is readily reconstructed by RF pulse forces in the *ckr* mouse brain (homozygous; -/-). Notice that lateral ventricular enlargement is progressively accelerated as a function of age. T2-weighted structural images of the ventricular system demonstrate that mice carrying a single copy of the mutated gene (heterozygous; +/-) also show increase in ventricular size when compared with that of wild-type cohorts (+/+). These results may be reconciled with MRI studies that point to the presence of large lateral ventricles in obligate carriers of schizophrenia (i.e., relatives of schizophrenia patients who are unaffected by the disease). In this regard, it is thought that the key to unlocking the etiology of schizophrenia may lie not in the patients themselves but in their unaffected relatives. The homozygous *ckr* mouse with its heterozygous kin offers the opportunity to further test this hypothesis. In general, our findings support a prominent role of genetic factors toward changes in brain anatomy that manifest as lateral ventricular enlargement in animal models of psychosis. Reprinted from: Ventricular size mapping in a transgenic model of schizophrenia, Vol 154, Torres et al., 35-44 (2005), with permission from Elsevier.

Fig. 4. T2-weighted brain sections of adult mouse genotypes selected for ^{1}H magnetic resonance spectroscopy (^{1}H-MRS, see below). Note enlargement of the lateral ventricles (yellow banding) in heterozygous and homozygous (*ckr*) mice relative to a wild-type cohort. Ventricular areas range from ~0.150 mm^2 (in wild-type mice) to ventricular areas of ~0.900 mm^2 (in heterozygous and homozygous mice). ^{1}H-MRS studies indicate abnor-
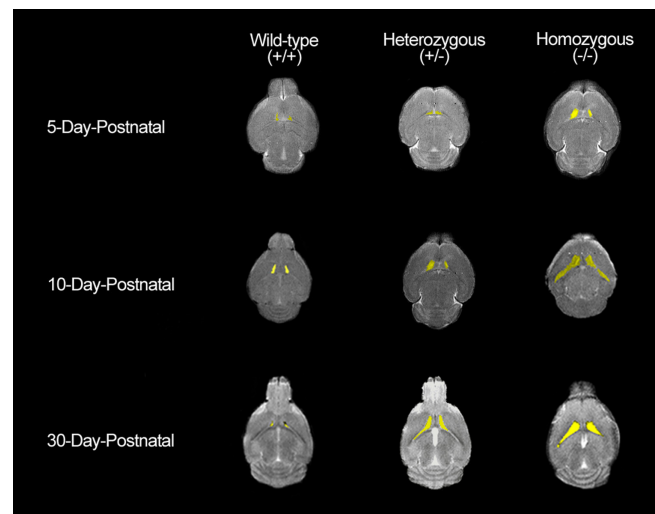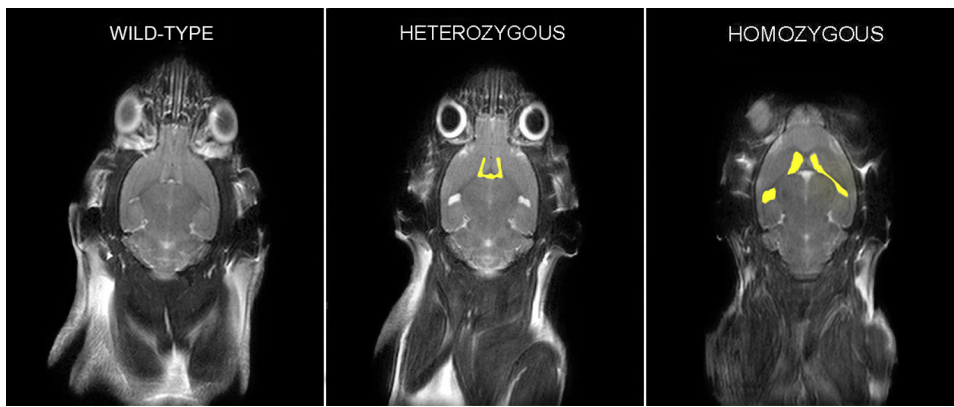

Figure 3

Figure 4

malities in relative levels of choline (Cho) 3.20 ppm and N-acetylaspartate (NAA) 2.01 ppm in the *ckr* mouse brain. These findings are consistent with previous studies reporting decreases of Cho and NAA ratios in some psychotic patients, an effect also seen in brains of individuals diagnosed with bipolar depression (Tsai et al., 1995; Winberg et al., 2000). ppm = parts per million. Reprinted from: Magnetic resonance imaging and spectroscopy in a mouse model of schizophrenia, Vol 75, Torres et al., 556-561 (2005), with permission from Elsevier.

Fig. 5. Digitized video-images depicting mouse social interactions in neutral cages. For details see Torres et al (2005). Male (green line) and female (purple line) wild-type animals show several proximity interactions during each of four 3 sec digitized trials. In contrast, spatial proximity interactions between male and female con-specifics are decreased in heterozygous and homozygous (*ckr*) mice. Note the range of circular motions shown by homozygous (*ckr*) mice. The observation that schizophrenia patients have deficits in social interaction suggests that certain neural circuits processing social information have been pathologically modified by the disease. Reprinted from: Preliminary evidence for reduced social interactions in *chakragati* mutants modeling certain symptoms of schizophrenia, Vol 1046, Torres et al., 180-186 (2005), with permission from Elsevier.

In conclusion, the *ckr* mouse mutant might be relevant for understanding the biochemical underpinnings of certain manifestations of schizophrenia. It should be noted, however, that like many other genetically complex brain disorders, the etiology of schizophrenia is likely to prove heterogeneous. Moreover, schizophrenia may turn out to be clusters of several psychotic conditions that bear a strong resemblance to each other, although differing in etiology, symptoms, trajectory and drug response. The *ckr* mouse mutant only addresses a limited number of characteristics of the human disease phenotype. Developing newer genetic mouse models based on susceptibility, etiology and treatment response will undoubtedly help find new antipsychotic drugs at last.
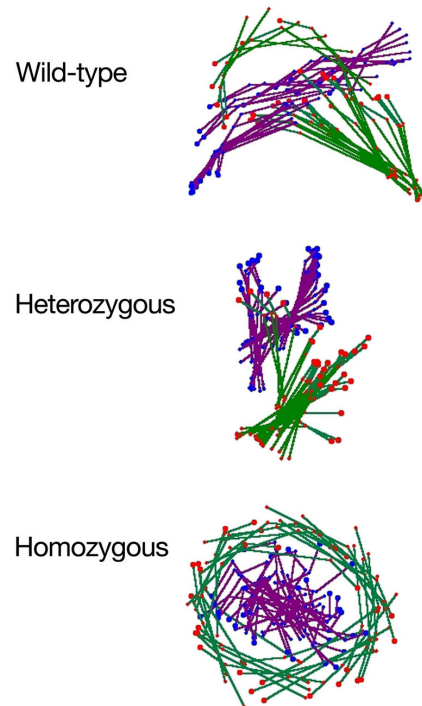

Figure 5

Table 1. Summary of well-characterized deficits observed in the human condition and the *ckr* mouse mutant*

Condition	Schizo-phrenia	*Ckr* Mice
Aberrant Behaviors		
Circling Behavior & Hyperactivity	Yes	Yes
Sensorimotor Gating Deficits	Yes	Yes
Brain Pathologies		
Ventricular Enlargement	Yes	Yes
Myelination Abnormalities	Yes	Yes
Metabolic Deficits	Yes	Yes
Response to Antipsychotics		
Clozapine and Olanzapine	Yes	Yes
Neurochemical Correlates		
Alterations in Dopamine Systems	Yes	Yes
Deficit is in Choline and N-acetylaspartate	Yes	Yes
Typical Age of Onset Pathology		
Early Adulthood	Yes	Yes**
Sex-Dependent Prevalence of Disease		
Males	Yes	Yes

*Human and mice share important genomic, anatomical and physiological similarities. These similarities, particularly in the genes involved in brain development, might provide insight into disease pathogenesis. For detailed descriptions of some of the above deficits see Figs. 1-5.

**(Postnatal Day 10 for onset of Aberrant Behaviors)

Biographies of the Authors

Dr. German Torres (torresg@nyit.edu) received a Ph.D. in Neuroscience from the University of California at Santa Barbara and is currently an Associate Professor in the Department of Neuroscience and Histology at the New York College of Medicine of New York Institute of Technology. His specific research interests are centered on the biological basis of mental disorders. Dr. German Torres is supported by grants from the National Institutes of Health and The Johns Hopkins University.

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Dr. Brian Hallas (bhallas@nyit.edu) received his Ph.D. in neurobiology from Purdue University in 1979. He also holds an M.S. in biology from Purdue University, a B.A. in Psychology, and a B.S. in Biology from University of Hartford. He has been awarded numerous research grants and has authored many articles in scientific journals. He is the recipient of various honors and awards, most recently the prestigious Presidential Service Award from New York Institute of Technology in 2007. In 2004, he was awarded a fellowship by the Heritage Foundation Health Policy for Research in Health Policy based on stem cell research. From 2003 to 2007, he was Associate Dean of Research at the New York College of Osteopathic Medicine of New York Institute of Technology, where he is currently Chair and Professor of the Department of Neuroscience. He has published over 70 peer reviewed articles, several book chapters and 225 abstracts.

Dr. Judith Horowitz (jhorowitz@medaille.edu) received a Ph.D. in biopsychology from the State University of New York at Buffalo, and has continued studying the relationship between brain and behavior. She has published over two dozen articles in the areas of depression, Parkinson's Disease, schizophrenia, and drug addiction. She has received extramural funding from the National Institutes of Health and the National Institute of Mental Health, the National Association for Research on Schizophrenia and Depression, and the Parkinson's Foundation. She has taught in Medaille's Psychology Program since 1997,

and now serves the College as the Dean of the School of Adult and Graduate Education (SAGE).

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Editor's Column

As this is being written, it is springtime in the Midwest. I am in Michigan just north of Detroit visiting our son and his family for almost 2 weeks. It is beautiful weather here as it is in Florida. Actually in Florida just now there is a heat wave for this time of year. We are dry and the temperature is up to about 93°F (33.8°C). This is about 10°F above normal. The hurricane season starts at the beginning of June and we certainly hope it is a quiet one this year. In addition to the heat, the dry conditions have resulted in numerous wild fires in our area on the East Coast and in the Everglades. An interesting point is that fires in the Everglades are generally a good thing as they burn off excess foliage and allow new growth. Not so in populated areas though, so we hope the rains start soon, as they usually do in late May.

This issue of the *Carrier*, number 66, was written by German Torres, Brian H. Hallas and Judith M. Horowitz from the College of Osteopathic Medicine at the New York Institute of Technology. Brian has contributed to the *Carrier* before and we welcome him back. The article details a mouse model of schizophrenia that is very interesting. As we in the neurosciences know, the mouse is becoming very popular as a subject for many areas of study. The availability of genetic engineering techniques such as gene transfer and knockouts have greatly increased the usefulness of the mouse in areas like the one they describe here. The drawback of the mouse in brain research is, of course, the small size of the brain and the fragility of the skull that makes mounting in the stereotaxic holder. These drawbacks are being mitigated by the new Kopf instrument system, the 1900 series. This instrument system provides unparalleled stability and accuracy for investigating the mouse brain. It is a simply superb system.

As the school year winds down now, many of us are looking forward to a time without teaching or other academic activities. It is a time for doing more research, writing, or going on a vacation. As I reach this time, I am looking forward to actual retirement from academia, although not from activities in the professional area, such as working with Kopf Instruments and an occasional invited lecture. In fact, I will be in St. Petersburg, Russia lecturing early in July, in Germany in October and in Italy in November. Then a cruise in January. I will also continue to attend the Society for Neuroscience meetings and remain active in other professional affairs.

Should anyone reading this want to write an article for the *Carrier*, please contact me or David Kopf Instruments for any help. We will publish articles on various topics, including history of neuroscience, neuroethics, neuroscience techniques as well as interesting data based articles. David Kopf Instruments has generously supported the *Carrier* for 36 years and continues to make the articles available to the entire community through their web site. All of us in the Neuroscience community are grateful for this support.

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