A GENETIC MOUSE MODEL OF EPILEPSY WITH INTERNEURONOPATHY AND AUTISTIC-LIKE FEATURES

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INTRODUCTION

X-linked Infantile Spasms Syndrome (ISSX), or West Syndrome, is a catastrophic epilepsy of infancy, characterized by hypsarrhythmia, seizures and myoclonic spasms in the first year of life. Mental retardation with autistic features are the most common developmental consequences of ISSX[1]. Although the myoclonic spasms may resolve spontaneously, the cognitive and behavioral deficits do not improve with age. Early ACTH treatment leads to only limited success in treating patients of ISSX. A large proportion of autism spectrum disorder (ASD) patients also suffer from seizures or epilepsy[2]. Therefore, common or overlapping mechanisms of epilepsy and autism are likely to exist, but remain poorly understood.

The X-linked *Aristaless*-Related homeoboX gene (ARX) is a transcription factor that plays important roles in the neuronal differentiation, migration and maturation of interneurons[3,4,5]. An expansion in the first poly-Alanine tract of the ARX gene is a common genetic cause of ISSX in humans[1]. Our lab recently developed a knock-in mouse model of ISSX that contains the human poly-Ala expansion in the ARX gene and replicates many features of the human syndrome, including seizures and infantile spasms[6]. Immunohistochemistry experiments showed a migration deficit of calbindin$^+$ cortical inhibitory interneurons, as well as CHAT$^+$ striatal interneurons, and NPY$^+$ interneurons in the dentate gyrus. Deficits in specific interneuron subpopulations have been associated with both cortical hyperexcitability and behavioral abnormalities[3].

Behavioral experiments in ARX$^{(CGC)10+7}$ mice show they are less anxious/fearful, are cognitively impaired, and less dominant than wild-type mice, suggesting an autistic-like phenotype[6]. In order to further characterize the extent of autistic-like features of the ARX$^{(CGC)10+7}$ mutant, a modified tube test and a 3-chamber social novelty test were performed. Establishing the ARX$^{(CGC)10+7}$ mouse as a valid neurobehavioral model of autism, allows the study of cellular mechanisms that produce autistic-features in the context of a known genetic cause of epilepsy, leading to a better understanding of shared mechanisms between two distinct neurological disorders.

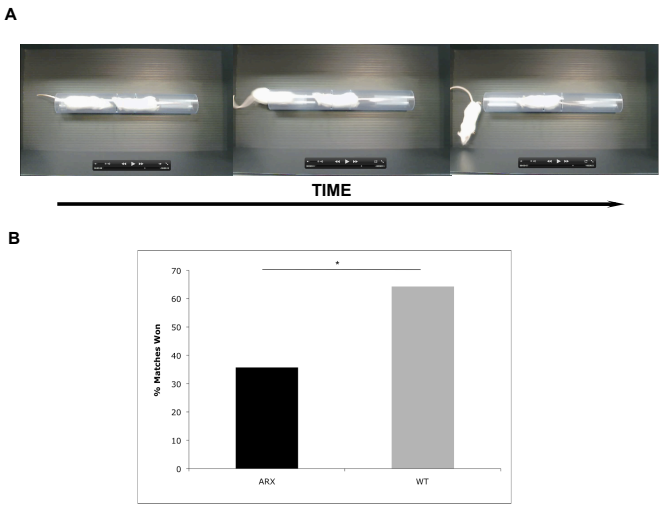
METHODS

Mice: Adult male ARX$^{(CGC)10+7}$ (75% C57 albino : 25% 129svc) and adult male C57BL/6 mice were used.

Tube Test: Two non-littermate adult male mice were simultaneously introduced to both ends of a 30-cm long, 3-cm wide clear PVC tube. The first mouse to be pushed out of the tube received a score of zero. The mouse that remained received a score of 1. The percentage of wins was computed for each group and a chi-square test was used to determine statistical significance.

3-Chamber Social Approach Test: Apparatus and habituation—A rectangular box made of clear polycarbonate divided in 3 chambers was used. The partner mice were kept in a smaller subdivision of the side chambers, which allowed only for visual and olfactory cues to be transmitted. All partner mice were habituated to the apparatus for 5 days. During the first 5 minutes, the test mouse was put in the middle with the doors closed. Then the doors were lifted and the test mouse was allowed to explore the apparatus freely with no nothing inside either subdivision. Time spent in each chamber and time spent at the partitions were measured over 10-minute period. Social Recognition—Partner mouse #1 was placed in one of the side chambers' sub-compartments and a black plastic box in the other. Time spent in each chamber and time spent directly at the partition were measured over 10 minutes. Social Novelty Preference—Partner mouse #2 was placed where the plastic box was and the same parameters were measured as before. Adult male C57BL/6 mice were used as partners.

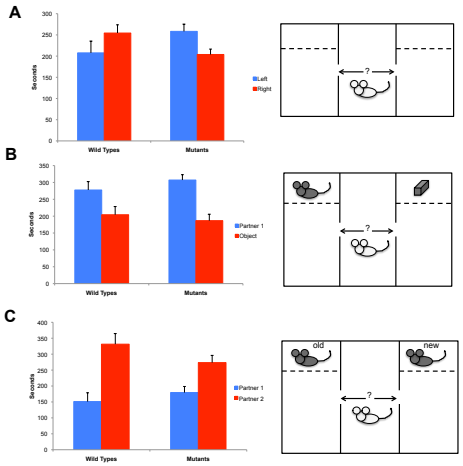
Fig. 1: ARX$^{(CGC)10+7}$ mice are more subordinate than WTs



(A) Sample frames from a match between a ARX$^{(CGC)10+7}$ mutant (left) and a wild-type mouse (right).

(B) ARX$^{(CGC)10+7}$ mutants "won" only 35.7% of matches with wild-type mice ($p < 0.01$, $n = 12$).

Fig. 2: ARX$^{(CGC)10+7}$ mice show normal exploration

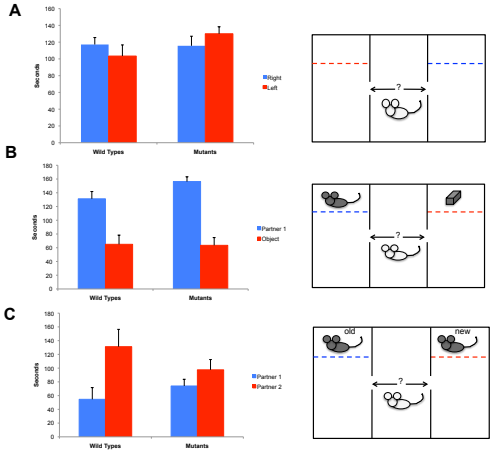


(A) Control for side preference. Test mice were habituated for 10 min with both right and left cages empty. Total time spent in left and right sides were measured. N = 5 (WTs) and N = 6 (Mutants)

(B) Social recognition. Test mice were allowed to explore freely in the presence of a unfamiliar (partner) mouse and a inanimate object for 10 minutes. Total time in Partner 1 and Object side were measured.

(C) Social Novelty Preference. Test mice were allowed to explore in presence of a familiar (Partner 1) and an unfamiliar (Partner 2) mouse for 10 minutes. Total time spent in either side was measured.

Fig. 3: Mutants spent less time with unfamiliar mice



(A) Control for side preference: Time at partition. Same experiment as in Fig. 2 but only time at the partitions was measured. No side preference is apparent.

(B) Social recognition: Time at partition. Both mutants and WTs spent more time at the partition with the partner mouse than at partition with plastic box.

(C) Social novelty preference: Time at partition. Mutant mice did not spend more time at partition with unfamiliar mouse (Partner 2).

SUMMARY

1. ARX$^{(CGC)10+7}$ mutant male mice were significantly more subordinate to wild-type mice in the tube test. This behavior is suggestive of an autistic-like phenotype.
2. In the 3-chamber social approach test, ARX mutants dedicated approximately the same amount of time to the unfamiliar partner mice, while wild-type mice spent considerably more time exploring the socially novel stimulus. A larger sample size is needed for adequate statistical analysis of the 3-chamber data.
3. Other behavioral tests to be implemented include: T-maze learning/ unlearning test and Partition Test
4. Together, the results reinforce the validity of ARX$^{(CGC)10+7}$ mice as a neurobehavioral model of autism.
5. Due to the specific pattern of interneuronopathy, the ARX$^{(CGC)10+7}$ mouse is a valuable model to examine the role of defined neuronal deficits in cortical and subcortical microcircuits relevant to the neurobiology of autism, and may provide important clues about possible shared mechanisms between epilepsy and autism

References:
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