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# Na,K-ATPase and the role of α isoforms in behavior

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**Abstract** The Na,K-ATPase is composed of multiple isoforms and the isoform distribution varies with the tissue and during development. The α1 isoform for example, is the major isoform in the kidney and many other tissues, while the α2 isoform is the predominate one in skeletal muscle. All three isoforms are found in the brain although in adult rodent brain, the α3 isoform is located essentially in neurons while the α2 isoform is found in astrocytes and some limited neuronal populations. Interestingly the α4 isoform is found exclusively in the mid region of the sperm tail. The distribution of the isoforms of the Na,K-ATPase has been extensively studied in many tissues and during development. The examples cited above provide some indication to the diversity of Na,K-ATPase isoform expression. In order to understand the significance of this distribution, we have developed animals which lack the α1, α2, and α3 isoforms. It is anticipated that these studies will provide insight into the role that these isoforms play in driving various biological processes in specific tissues. Here we describe some of our studies which deal with the behavioral aspects of the α1, α2, and α3 deficient mice, particularly those that are haploinsufficient in one isoform i.e. lacking one functional gene for the α1, α2, or α3 isoforms. Such studies are important as two human diseases are associated with deficiency in the α2 and α3 isoforms. These are Familial Hemiplegic Migraine type 2 and Rapid-Onset Dystonia Parkinsonism, these diseases result from α2 and α3 isoform haploinsufficiency, respectively. We find that the haploinsufficiency of both α2 and α3 isoforms result in behavioral defects.

**Keywords** Na,K-ATPase · α isoforms · Rapid-Onset Dystonia Parkinsonism · Familial Hemiplegic migraine type 2 headache

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## Introduction

The Na,K-ATPase is composed of three subunits α, β, and a FXYD protein (Kaplan 2002). Isoforms for each subunit exist. The α isoform is the catalytic subunit and is responsible for binding and transporting $Na^+$ out of the cell and $K^+$ in and contains the binding site for ATP. The β subunit is required for maturation of the Na,K-ATPase and movement to the plasmid membrane. This isoform can alter the activity of the Na,K-ATPase and is absolutely required for activity. One member of the FXYD protein family, which includes the gamma subunit and phospholemman, appears to be always associated with the Na,K-ATPase and again while not absolutely required for activity, does influence enzymatic activity such as $Na^+$ and $K^+$ affinities.

Our laboratory has been particularly interested in the role that the various α isoforms play. While the Na,K-ATPase transports $Na^+$ out of cells and $K^+$ in, utilizing ATP, the gradient formed is coupled to various biological processes and therefore it is possible that the α isoforms as well as the β and FXYD proteins are tailored to the specific biological requirements of the organ and cell type it is expressed. For example, the Na,K-ATPase in the kidney is largely involved

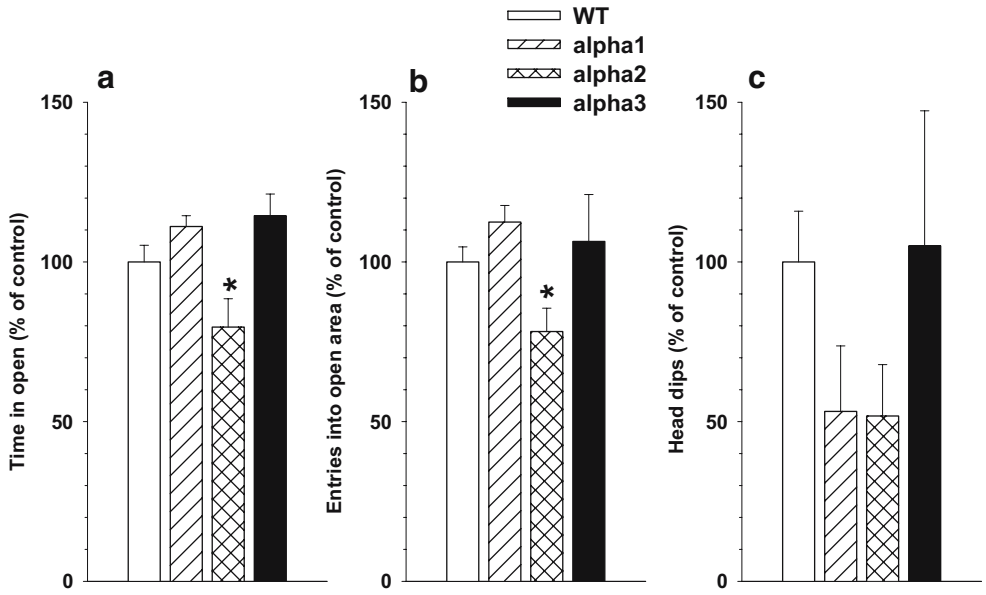


**Fig. 1** Elevated zero maze. α2 haploinsufficient mice displayed increased anxiety compared to wild-type. **a** α2 haploinsufficient mice spent significantly less time in the open than wild-type mice. **b** α2 haploinsufficient mice spent significantly less time entering open areas than wild-type mice. **c** α2 haploinsufficient mice did not differ in looking over the edge of the platform (head-dips) than wild-type mice. Alpha 1, alpha 2 and alpha 3 mice tested were haploinsufficient adult males and wild-type mice tested were adult male mice from each of the three Na,K-ATPase haploinsufficient mouse colonies, respectively. *$p<0.5$ ANOVA. $n>11$ per group (this figure is modified from figure 4 of Moseley et al. 2007)

in reabsorbing $Na^+$ from the glomerular filtrate. In other organs, the sodium and potassium ion gradients generated from enzyme activity of the Na,K-ATPase gradient are coupled to the transport of nutrients such as glucose. The ion gradients generated by the Na,K-ATPase also regulate the concentration of ions such as $Ca^{2+}$. In neural tissue, the α3 isoform is found mainly in neurons and therefore plays an important role in neurotransmission while the α2 isoform is found largely in astrocytes and appears to be important in maintaining appropriate levels of $K^+$ in the extracellular space. The α4 isoform is found exclusively in sperm and its role is to help remove $H^+$ from the sperm tail. $H^+$ ions inhibit motility and the $Na^+$ gradient produced by the Na,K-ATPase drives the $Na^+$,$H^+$ exchanger to remove protons from sperm (Woo et al. 2000, 2002). It is unknown from these studies whether each isoform has a unique function contributing to these biological processes through the ion gradient each Na,K-ATPase isoform generates. Alternatively, regulatory mechanisms may have changed over the course of evolution resulting in specific isoform expression in certain tissues related to the regulation of expression of the gene coding for that isoform.

Our approach has been to develop mice lacking various α isoforms and determine the consequence of such loss. Animals lacking the α1 isoform die at the blastocyst stage indicating that this isoform is absolutely required for early development (James et al. 1999; Barcroft et al. 2004). Animals lacking the α2 isoform are born but die upon birth from a lack of synchronized firing of the neurons in the breathing center of the brain (Moseley et al. 2003). Animals lacking the α3 isoform also die at birth but the cause of this is unknown at present (Moseley et al. 2007). Animals lacking one copy of the α isoform gene i.e. a haploinsufficiency, are viable but subtle effects are observed. For example, in the α2 isoform haploinsufficient mice, there is an increase in contraction of heart (James et al. 1999) and skeletal muscle (He et al. 2001) and an increase in vascular tone (Zhang et al. 2005). This is in line with earlier studies that showed that inhibition of the Na,K-ATPase by ouabain caused an increase in contractile force which is thought to be due to the increase in $Ca^{2+}$ levels through the Na,$Ca^{2+}$ exchanger. When cellular $Na^+$ increases, this causes the Na/Ca Exchanger to act in reverse and transport $Ca^{2+}$ into the cell. The increased calcium ions then act to enhance muscle contraction.

Recently there have been two diseases identified in humans which result from haploinsufficiency of the α2 and α3 isoforms. These are Familial Hemiplegic Migraine Headache type 2 (DeFusco et al. 2003; Spadaro et al. 2004; Kaunisto et al. 2004; Riant et al. 2005; Vanmolkot et al. 2006; Pierelli et al. 2006) caused by an α2 haploinsufficiency, and Rapid-Onset Dystonia Parkinsonism (deCarvalho Aguiar et al. 2004) resulting from an α3 haploinsufficiency. Haploinsufficiency, i.e. the loss of one of the two copies of a α2 or α3 isoform gene of the Na,K-ATPase is associated with Familial Hemiplegic Migraine

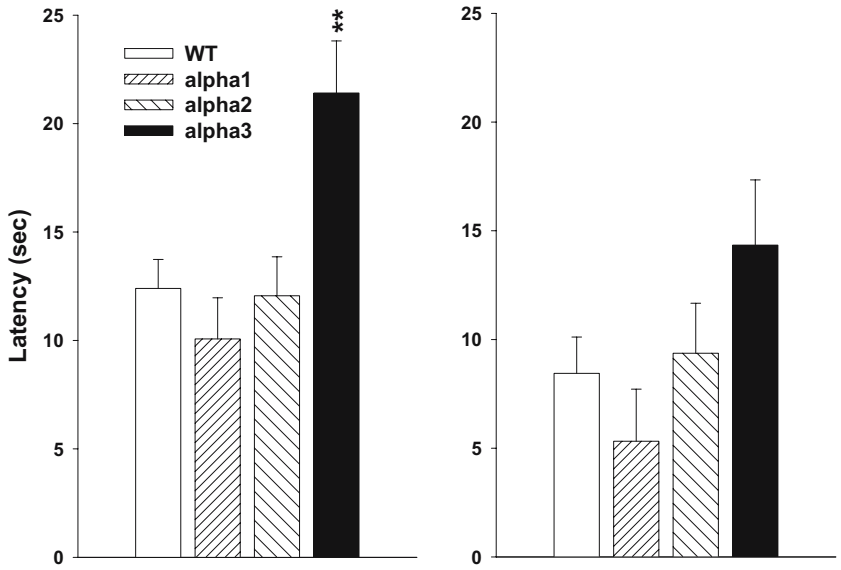


**Fig. 2** Morris water maze performance on cued trials. Average latency to reach a visibly marked platform was tested and averaged across 6 test days (6 days, 4 trial/day). *Left panel*: days 2–5, α3 haploinsufficient mice took significantly longer to find the platform compared to wild-type mice. *Right panel*: day 6, performance of α3 haploinsufficient mice were similar to that of wild-type mice. Alpha 1, alpha 2 and alpha 3 mice tested were haploinsufficient adult males and wild-type mice tested were adult male mice from each of the three Na, K-ATPase haploinsufficient mouse colonies, respectively. $^{**}p<0.01$ ANOVA, $n>11$ per group (this figure is from Moseley et al. 2007)

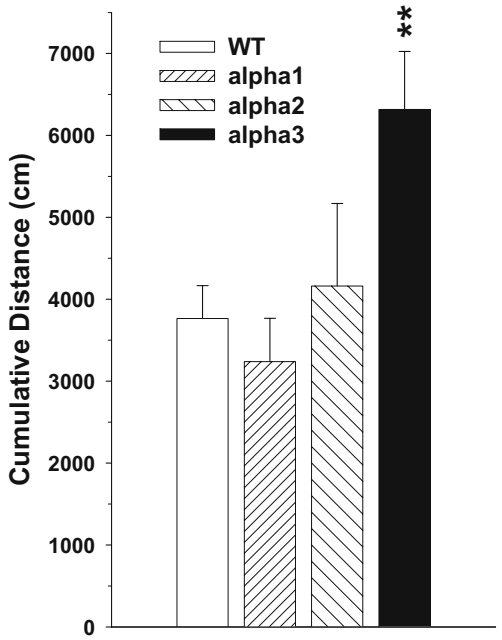


**Fig. 3** Morris water maze performance on hidden platform trials. α3 haploinsufficient mice swam significantly farther from the submerged platform than wild-type mice, averaged across days and trials (6 days, 4 trial/day). Alpha 1, alpha 2 and alpha 3 haploinsufficient mice tested were adult males and wild-type mice tested were adult male mice from each of the three Na,K-ATPase haploinsufficient mouse colonies, respectively. $^{**}p<0.01$ ANOVA, $n>10$ per group (this figure is modified from figure 7 of Moseley et al. 2007)

type 2 headache and Rapid-Onset Dystonia Parkinsonism in humans, respectively. Some individuals simply lack one functional gene while others express α2 isoforms from both copies of the gene and the resulting protein expressed from one gene having altered enzyme activity. It is unclear whether the disease is caused simply by the loss of one functional isoform gene or whether the altered protein products in some cases contribute to the disease. Since either reducing total Na,K-ATPase content or altered enzyme function could lead to neural defects, we studied the behavior of α1, α2 and α3 haploinsufficient mice to identify neurological deficits in these animals.

## Results and discussion

In our mice there is a deficiency of one of the α isoforms which makes these animals a reasonable model for the two inherited diseases which involve the α2 and α3 isoforms of the Na,K-ATPase. In the case of the α2 haploinsufficiency we cannot visibly determine whether these animals suffer from headache. In the case of the α3 haploinsufficiency, it is possible that with the right stimulus, these animals could initiate a Rapid-Onset Dystonia Parkinsonism phenotype but this has not yet been accomplished. Therefore, we have examined these animals in terms of behavioral characteristics to learn more about the function of the α2 and α3 isoforms of the Na,K-ATPase that may help understand Familial Hemiplegic migraine type 2 and Rapid-Onset Dystonia Parkinsonism in humans.

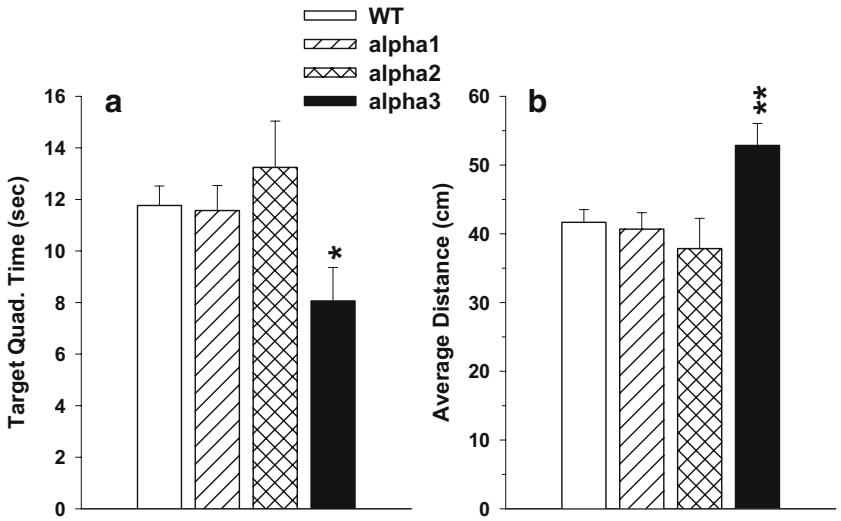


**Fig. 4** Morris water maze performance during probe trials. Probe trials test memory for ability to locate the platform where it had been placed during the Test Trials. **a** α3 haploinsufficient mice were significantly impaired as observed by the reduced time in the target quadrant. **b** α3 haploinsufficient mice were significantly impaired as observed by the increased distance in finding platform location compared to wild-type mice. Alpha 1, alpha 2 and alpha 3 mice tested were haploinsufficient adult males and wild-type mice tested were adult male mice from each of the three Na,K-ATPase haploinsufficient mouse colonies, respectively. *$p<0.5$ ANOVA. **$p<0.01$ ANOVA, $n>10$ per group (this figure is from Moseley et al. 2007)

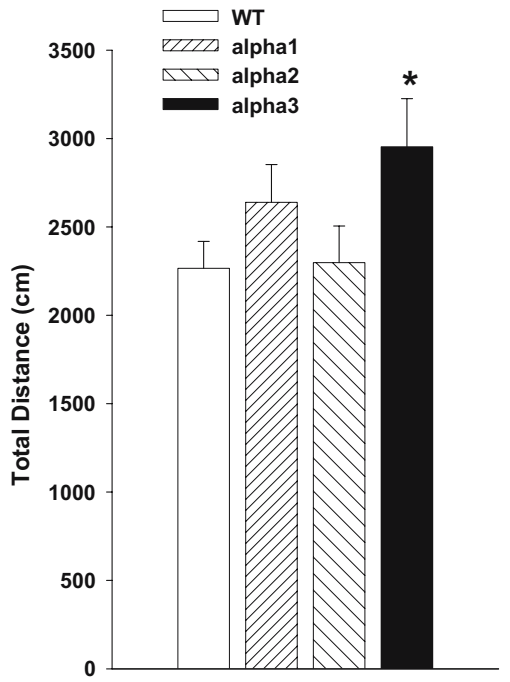


**Fig. 5** Locomotor activity after methamphetamine challenge. Mice were tested for 1 h, removed from locomotor activity box, given an acute subcutaneous injection of methamphetamine (1 mg/kg) and returned to the test for 2 h. α3 haploinsufficient mice were significantly more active than wild-type mice. Alpha 1, alpha 2 and alpha 3 mice tested were haploinsufficient adult males and wild-type mice tested were adult male mice from each of the three Na,K-ATPase haploinsufficient mouse colonies, respectively. *$p<0.5$ ANOVA. $n>11$ per group (this figure is modified from figure 8 of Moseley et al. 2007)

First, we examined anxiety in the α1, α2, and α3 haploinsufficient animals (Moseley et al. 2007). Ikeda and coworkers (Ikeda et al. 2003) had already shown that animals deficient in the α2 isoform are more anxious than wild type animals. We have compared these animals to those with α1 and α3 haploinsufficiency, Fig. 1. α2 haploinsufficient mice displayed reduced time in the open (Fig. 1a) and reduced number of entries into the open area (Fig. 1b) compared to wild-type animals. However, no difference was observed in the number of head dips for α2 haploinsufficient mice compared to wild-type (Fig. 1c). While the α2 haploinsufficiency animals exhibit an anxious phenotype as measured by the Elevated Zero Maze test, the α1 and α3 haploinsufficient animals do not exhibit an anxious phenotype.

Using the Morris water maze, non-spatial learning was measured using a submerged platform with a cue mounted over the platform, Fig. 2. The cued Morris water maze measures non-hippocampal dependent learning (Moseley et al. 2007). The α3 haploinsufficient animals were slower on days 2–5 during the test to learn the location of the platform (Fig. 2, left panel), but by day 6 at the end of the test they performed as well as wild-type mice (Fig. 2, right panel), The α1 and α2 haploinsufficient animals showed no difference in learning behavior with the cued platform compared to wild type animals.

Hippocampal learning was measured in the hidden platform water maze but in this case there was no cue over the submerged platform (Moseley et al. 2007). but instead the animals use distal objects on the wall to navigate their

way to the submerged platform. Shown in Fig. 3, both α2 and α3 haploinsufficient animals took a longer time to find the platform than wild type animals. This was not because the animals were slower, they swam at the same speed as wild-type, but were cumulatively further away from the platform when measured every 0.2 s than were wild-type mice. Interestingly the α1 haploinsufficient animals were not different from wild type animals in these studies Fig. 3. In addition, the α2 and α3 haploinsufficient animals had more difficulty re-finding where the platform had been after it was removed (data not shown), indicating an impairment of reference (long-term) memory.

From these studies it is clear that the α2 and α3 haploinsufficient animals show differences from wild type in learning behavior. Furthermore, the α2 and α3 haploinsufficient animals while both showing differences in hippocampal dependent learning, differ in non-hippocampal learning in that only the α3 haploinsufficient animals are initially slower to find the platform when it is visibly marked but later during the test are able to find the platform.

After the learning phase in the Morris water maze, a probe trial was given with the platform removed, and the animals were started from a novel position and assessed for their ability to remember the position of the platform (Moseley et al. 2007). The α3 haploinsufficient mice were significantly impaired as observed by their reduced time in the target quadrant, Fig. 4a. In addition, α3 haploinsufficient mice swam an increased distance compared to wild-type mice from the exact location where the platform had previously been placed, Fig. 4b.

The α3 haploinsufficient mice also explored more in a novel environment, whereas the α1 haploinsufficient mice explored less initially than wild-type mice (Moseley et al. 2007). In addition, α3 haploinsufficient mice showed greater locomotor movement in response to an injection of the dopaminergic agonist, (+)-methamphetamine, than wild-type mice (Fig. 5).

While it is difficult to equate these studies with the two human diseases, namely Familial Hemiplegic migraine type 2 headache and Rapid-Onset Dystonia Parkinsonism, these findings should be useful in future studies of these conditions since each haploinsufficient genotype showed a distinct pattern of behavioral changes and the α3 haploinsufficient animals showed two features characteristic of Parkinsonism, namely, altered response to a dopaminergic challenge and impaired learning and memory. These characteristics may prove useful in further understanding of the function of each of the Na,K-ATPase α isoforms and their function in the central nervous system.

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