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Selective Mitochondrial Targeting Exerts Anxiolytic Effects *In Vivo*

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Current treatment strategies for anxiety disorders are predominantly symptom-based. However, a third of anxiety patients remain unresponsive to anxiolytics highlighting the need for more effective, mechanism-based therapeutic approaches. We have previously compared high *vs* low anxiety mice and identified changes in mitochondrial pathways, including oxidative phosphorylation and oxidative stress. In this work, we show that selective pharmacological targeting of these mitochondrial pathways exerts anxiolytic effects *in vivo*. We treated high anxiety-related behavior (HAB) mice with MitoQ, an antioxidant that selectively targets mitochondria. MitoQ administration resulted in decreased anxiety-related behavior in HAB mice. This anxiolytic effect was specific for high anxiety as MitoQ treatment did not affect the anxiety phenotype of C57BL/6N and DBA/2J mouse strains. We furthermore investigated the molecular underpinnings of the MitoQ-driven anxiolytic effect and found that MitoQ treatment alters the brain metabolome and that the response to MitoQ treatment is characterized by distinct molecular signatures. These results indicate that a mechanism-driven approach based on selective mitochondrial targeting has the potential to attenuate the high anxiety phenotype *in vivo*, thus paving the way for translational implementation as long-term MitoQ administration is well-tolerated with no reported side effects in mice and humans.

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INTRODUCTION

High anxiety levels are responsible for a significant health-related life quality loss in the general population (Saarni *et al*, 2007). Anxiety disorders, the most common psychiatric conditions, affect approximately one out of five people worldwide, are highly co-morbid with other major psychiatric diseases (Achim *et al*, 2011), and pose an additional burden to patients with a negative impact on recovery and disease outcome (Braga *et al*, 2005). Owing to the lack of in-depth molecular understanding of anxiety pathobiology, current therapeutic approaches are mainly symptom- rather than mechanism-driven with a third of patients being unresponsive to anxiolytic treatment (Bystritsky, 2006).

To elucidate the molecular underpinnings of anxiety traits, we have compared mice selectively inbred for ∼45 generations for high (HAB) or low anxiety-related behavior (LAB) according to their performance on the elevated plus maze (Kromer *et al*, 2005). Using a hypothesis-free systems biology platform based on proteomics, metabolomics, and bioinformatics (Filiou and Turck, 2012; Frank *et al*, 2009; Haegler *et al*, 2009; Zhang *et al*, 2009), we identified alterations in multiple mitochondrial pathways in high anxiety. The most predominant change was the increased expression of more than 60 proteins across all electron transport chain subunits of oxidative phosphorylation resulting in increased oxidative stress and decreased antioxidant capacity in HAB mice (Filiou *et al*, 2011, 2014; Iris *et al*, 2014).

In this work, we examined whether we can manipulate the high anxiety phenotype by pharmacologically targeting the identified mitochondrial changes *in vivo*. We treated HAB mice with MitoQ, a mitochondria-targeted compound that enhances the mitochondrial protection against oxidative damage (Cocheme *et al*, 2007). MitoQ consists of a lipophilic cation moiety that allows mitochondria-specific accumulation and a ubiquinone that is converted to the antioxidant ubiquinol by the activity of complex II of the electron transport chain (Smith *et al*, 2003). MitoQ was selected based on a series of criteria. First of all, long-term MitoQ administration has been reported to be non-toxic and well-tolerated in mice (Smith and Murphy, 2010). MitoQ is water soluble and can be administered orally through the drinking water, thus circumventing invasive and more stressful ways of administration (eg injections) that may interfere with the anxiety phenotype. Furthermore, MitoQ can cross the blood–brain barrier, albeit slowly, as it is detected in the mouse brain after treatment (Rodriguez-Cuenca *et al*, 2010). MitoQ administration was shown to have protective effects in animal models of diseases characterized by mitochondrial oxidative damage (Adlam *et al*, 2005). In brain disorders,

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MitoQ administration prevented cognitive decline and neuropathological correlates of Alzheimer's disease in a transgenic mouse model (McManus *et al*, 2011).

We analyzed the behavioral and molecular effects of this selective mitochondrial targeting and investigated differences between MitoQ treatment responders and non-responders. We focused our analyses on cortical and hippocampal areas due to their involvement in emotional processing (Tovote *et al*, 2015) and on plasma as it is the specimen of choice for translational implementation. To determine whether our findings are specific for high anxiety, we also assessed the behavioral effects of MitoQ treatment in DBA/2J and C57BL/6N inbred mouse strains. Here we report on a molecular mechanism-driven anxiolytic effect of selective mitochondrial targeting which is specific for high anxiety.

MATERIALS AND METHODS

Animals

Mouse experiments were approved by local authorities and conducted according to current regulations for animal experimentation in Germany and the European Union (European Communities Council Directive 2010/63/EU). HAB (6 weeks old), C57BL/6N (Martinsried, Germany), and DBA/2J (Charles River, Sulzfeld, Germany) male mice were group housed under standard conditions in the animal facility of the Max Planck Institute of Psychiatry as previously described (Frank *et al*, 2009).

Total Antioxidant Capacity

Prior to the assignment to the MitoQ treatment or control group, tail blood was collected in 1.5 ml EDTA-coated microcentrifuge tubes (Kabe Labortechnik, Nümbrecht-Elsenroth, Germany) without anesthesia by a small incision in the dorsal tail vein using a scalpel blade. Plasma was obtained by centrifugation for 10 min, at 1300 *g*, 4 °C. Tail blood sampling took place between 0900 and 1100 hours. Plasma total antioxidant capacity (TAC) representing the amount of small molecule and protein antioxidants was assessed by a TAC kit (K274-100; Biovision, Mountain View, CA) according to the manufacturer's instructions.

MitoQ Treatment

After a week of habituation, HAB mice were randomly assigned to MitoQ treatment and control groups. The treatment group of HAB mice ($n = 13$) received 400 μM MitoQ for 10 weeks (MitoQ was provided in the form of MS010 (1.2 g/l), a β-cyclodextrin complex of MitoQuinone mesylate, corresponding to 400 μM of MitoQ). MitoQ was dissolved in drinking water and was prepared fresh twice a week. The control group of HAB mice ($n = 13$) received only drinking water. Drinking behavior was monitored twice a week and animal weight once a week during the treatment period. C57BL/6N and DBA/2J mice ($n = 15$ for the treatment group and $n = 15$ for the control group for each strain) were treated with MitoQ following the same protocol as used for HAB mice.

Behavioral Phenotyping

During the tenth week of MitoQ treatment a behavioral battery assessing anxiety-related (dark–light box (DL), open field (OF)) and depression-like (tail suspension test (TST)) behavior was performed. Behavioral tests were kept to a minimum to avoid learning/memory effects and reduce the burden of continuous behavioral testing that may hamper behavioral readouts in mice. Experimenters were blind to animal treatment for all behavioral tests.

Dark–light box. The set up for DL was as previously described (Kromer *et al*, 2005) using 10 and 300–400lux for the dark and illuminated areas, respectively. Each mouse was placed in the corner of the darkened compartment facing away from the aperture. During the 5 min test, data were scored using Eventlog 1.0 (EMCO Software, Reykjavik, Iceland). A mouse was defined to have entered the lit box when both front paws and shoulders were inside the lit compartment.

Open field. OF was performed 2 days after DL as previously described (Bunck *et al*, 2009) illuminating the arena with dim light of 50 lux.

Tail suspension test. TST was performed 2 days after OF as previously described (Kromer *et al*, 2005).

Animal Sampling

Two days after TST, animals were killed, blood was collected by cardiac puncture, and plasma was obtained by centrifugation for 10 min, at 1300 *g*, 4 °C. Mice were perfused with 0.9% saline. The prefrontal cortex, hippocampus, and cerebellum were dissected according to the mouse brain atlas (Paxinos and Franklin, 2001) and stored at − 80 °C. Hippocampi and plasma were used for metabolite-based analyses and cortices were used for protein-based analyses.

MitoQ Quantification in the Brain

Brain tissue from all MitoQ-treated HAB treated animals (∼50 mg wet weight per animal) was used to estimate the amount of MitoQ in the brain as previously described (Rodriguez-Cuenca *et al*, 2010). Briefly, the tissue was homogenized, spiked with a deuterated MitoQ internal standard, and then extracted into acetonitrile/formic acid. The extract was then analyzed for MitoQ content by LC-MS/MS relative to a MitoQ standard curve and MitoQ levels between responder and non-responder groups were compared by unpaired Student's *t*-test.

Metabolomics Analysis

Hippocampi and plasma from MitoQ-treated and untreated HAB mice were prepared for LC-MS/MS-based targeted metabolite analysis as previously described (Filiou *et al*, 2014). LC-MS/MS metabolomics measurements were performed at the Mass Spectrometry Core of Beth Israel Deaconess Medical Center (Boston, MA) using a 5500 QTRAP triple quadrupole mass spectrometer (AB/SCIEX, Framingham, MA) coupled to a Prominence UFCL HPLC system (Shimadzu, Columbia,

MD). A targeted single reaction monitoring (SRM)-based platform that quantifies the levels of 286 metabolites was used (Yuan et al, 2012). Following normal phase chromatography using a 4.6-mm i.d. × 10 cm Amide Xbridge HILIC column (Waters Corp., Milford, MA) samples were introduced to the mass spectrometer. Selected metabolites were targeted in both positive and negative modes by positive/negative polarity switching. All other measurement parameters were as previously described (Webhofer et al, 2013).

Catalase Activity Determination

Catalase activity in the cortex of MitoQ-treated HAB mice was measured as previously described (Filiou et al, 2012) using a catalase assay kit (707002; Cayman, Ann Arbor, MI).

Western Blot

Western blot analysis in the cortex of MitoQ-treated HAB mice was performed as previously described (Filiou et al, 2010) using the following primary antibodies: Slc25a22 (1:2000, ab60332; Abcam, Cambridge, UK), malate dehydrogenase 1 (1:1000, ARP48284_T100; Aviva Systems Biology, San Diego, CA), HK1 (1:600, HPA007044; Sigma Aldrich, Darmstadt, Germany), and Prdx3 (1:2000, ab16751; Abcam) (10 µg per lane). Equal total protein loading was ensured by staining of the transfer membrane with Coomasie Brilliant Blue R-250 (BioRad, Hercules, CA) and signal intensity comparison.

Statistical Analysis

Unless otherwise specified, animal data were analyzed by the non-parametric Mann–Whitney test to address non-normal data distributions and are presented as mean ± SEM. Metabolite data were statistically analyzed using MetaboAnalyst (Xia et al, 2009). For metabolites measured both in positive and negative modes, only one of the two measurements was included for further analysis. Metabolites with > 10% missing values were not considered for statistical analysis. Data were median-normalized (row-wise) and pareto-scaled (column-wise). To identify metabolite level alterations the significance analysis of microarrays (SAM) was used. SAM also follows non-parametric statistics and uses permutation-based analysis to determine statistically significant changes in metabolite levels, thus avoiding parametric assumptions about the distribution of individual metabolites unlike other approaches, which assume equal variance and/or independence of tests (Tusher et al, 2001). For SAM, significant differences with FDR < 0.1 and $q < 0.1$ were accepted. Metabolite data are presented in box and whisker plots of median normalized peak intensities. For metabolite pathway identification the KEGG database was used (http://www.genome.jp/kegg/genes.html).

RESULTS

MitoQ Treatment Exerts Anxiolytic Effects in HAB Mice

HAB mice were treated with MitoQ for 10 weeks (Figure 1). To address potential effects of intra-line heterogeneity, we compared TAC in tail plasma prior to the beginning of the
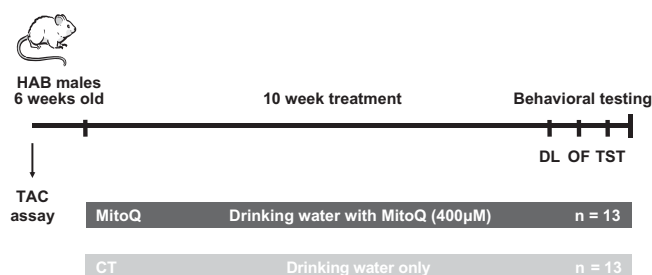

Figure 1 Experimental set up of MitoQ treatment in HAB mice. DL, dark–light box; HAB, high anxiety-related behavior; OF, open field; TAC, total antioxidant capacity; TST, tail suspension test.

MitoQ administration between treatment and control HAB groups and found no difference (Supplementary Figure S1A). MitoQ treatment did not result in morphological or body weight alterations in HAB mice (Supplementary Figure S1B). During the last week of treatment, mice were subjected to a behavioral battery of anxiety-related (OF, DL) and depression-like (TST) tests. We observed an anxiolytic effect in DL with MitoQ-treated HAB mice spending significantly more time in the lit compartment compared with untreated HAB mice (Figure 2a). No significant differences were observed in OF or TST (Supplementary Figure S1C and D).

MitoQ Treatment Alters the Hippocampal Metabolome

To identify molecular correlates of the MitoQ-driven anxiolytic effect in DL, we compared the hippocampal metabolomes of MitoQ-treated vs untreated HAB mice by an SRM-based, targeted metabolomics platform (Yuan et al, 2012). After application of exclusion criteria, we quantified 222 metabolites in the hippocampus (Supplementary Table S1). SAM analysis revealed decreased levels of sarcosine, AMP, and ascorbic acid in MitoQ-treated compared with untreated HAB mice (Figure 2b).

MitoQ Anxiolytic Effects are Present Only in High Anxiety

To investigate whether the MitoQ-driven anxiolytic effects are specific for high anxiety, 400 µM MitoQ were administered for 10 weeks to mice from C57BL/6N and DBA/2J strains that are not inbred for anxiety traits following the same protocol for treatment and behavioral testing that was used for HAB mice. For both strains, no behavioral differences were observed in anxiety-related (DL, OF) and depression-like (TST) behavior between treated and untreated mice (Supplementary Figure S2). These results indicate that the anxiolytic effects of MitoQ are specific for HAB mice.

MitoQ DL Responders Show Altered Brain Metabolite and Protein Signatures Compared with Non-Responders

It has been recently reported that responders to psychiatric drug treatment show distinct molecular signatures compared with non-responders (Belzeaux et al, 2012). Based on these findings, we examined whether a similar phenomenon can also be observed for the MitoQ treatment response. It should be noted that although HAB mice are inbred for anxiety
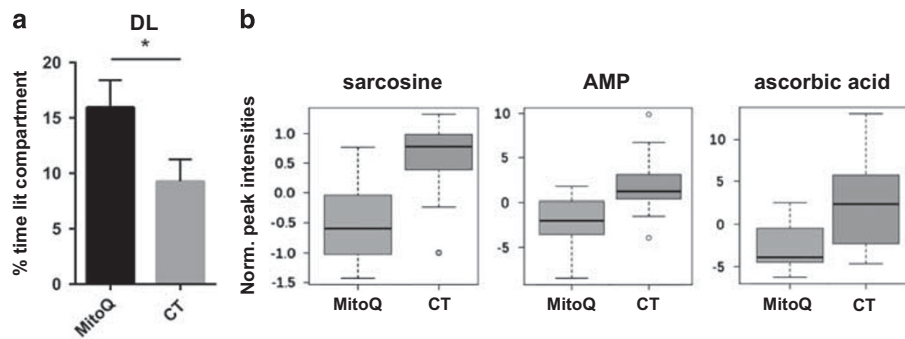
Figure 2 MitoQ treatment exerts anxiolytic effects in HAB mice. (a) HAB mice treated with 400 μM MitoQ ($n = 13$) spent more time in the lit compartment of DL compared with untreated HAB mice ($n = 13$) ($p = 0.0455$). (b) Decreased levels of sarcosine ($p = 0.0009$), AMP ($p = 0.001$), and ascorbic acid ($p = 0.002$) in hippocampi of MitoQ-treated compared with untreated HAB mice. CT, untreated HAB mice; DL, dark–light box; MitoQ, MitoQ-treated HAB mice. *$p < 0.05$.
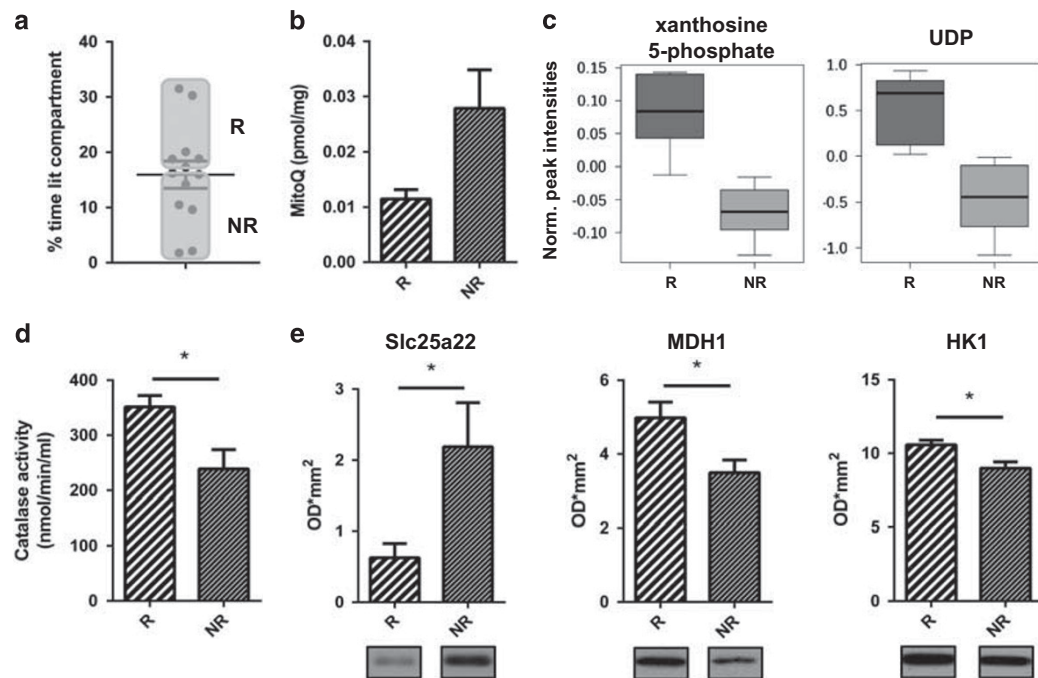

Figure 3 Altered molecular profiles between MitoQ responders and non-responders in DL. (a) Mice were classified into MitoQ responder and non-responder groups based on the median value of the % time spent in DL lit compartment. (b) No differences in brain MitoQ amount (measured as pmol/mg brain tissue) between MitoQ responders and non-responders in DL ($p > 0.05$). (c) Increased levels of xanthosine 5-phosphate ($p = 0.0003$) and UDP ($p = 0.0006$) in MitoQ responders compared with non-responders. (d) Increased catalase activity ($p = 0.0221$) in MitoQ responder compared with non-responder cortices. (e) Decreased expression levels of Slc25a22 ($p = 0.0303$) and increased expression levels of MDH1 ($p = 0.0177$) and HK1 ($p = 0.0101$) in MitoQ responder vs non-responder cortices. HK1, hexokinase 1; MDH1, malate dehydrogenase 1; NR, non-responders; R, responders; UDP, uridine diphosphate. *$p < 0.05$.

traits, variability of inbred strains in behavioral testing readouts is comparable to that of outbred strains. Based on DL behavioral data, MitoQ-treated mice were median separated to high responders ($n = 6$) and low responders ($n = 7$) according to their % time spent in the DL lit compartment (Figure 3a). For simplification purposes, these two groups will be referred to as responders and non-responders from now on. The group of DL MitoQ responders showed a significant increase in % time spent in the lit compartment compared with untreated HAB mice while DL MitoQ non-responders showed no difference in the % time spent in the lit compartment compared with untreated HAB mice (Supplementary Figure S3).

To ensure that the response to treatment was not a consequence of different MitoQ amounts in the brain, we determined MitoQ brain levels in all MitoQ-treated HAB mice by LC-MS/MS. MitoQ was present in all treated HAB brains, and no difference in MitoQ brain amounts was observed between DL responders and non-responders (Figure 3b). We then compared the brain metabolomes of the two groups. Of the 226 metabolites quantified in hippocampi (Supplementary Table S2), we found increased levels of xanthosine 5-phosphate and uridine diphosphate in DL responders compared with non-responders (Figure 3c). Based on identified differences in mitochondrial pathways between high and low anxiety, which included oxidative stress,
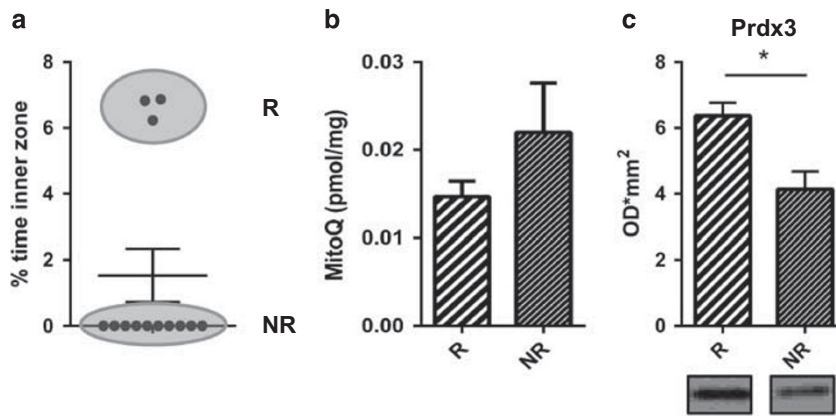
Figure 4 Molecular changes between MitoQ responders and non-responders in OF. (a) Mice were classified into MitoQ responder and non-responder groups based on whether they entered the inner zone in OF. (b) No differences in brain MitoQ amount (measured as pmol/mg brain tissue) between MitoQ responders and non-responders in OF ($p > 0.05$). (c) Increased Prdx3 protein expression in MitoQ responders compared with non-responder cortices ($p = 0.0467$). NR, non-responders; OF, open field; Prdx3, peroxiredoxin 3; R, responders. $*p < 0.05$.

metabolism, and mitochondrial import/transport (Filiou et al, 2011), we investigated the expression of a selected number of mitochondrial/energy metabolism proteins and oxidative stress markers in MitoQ-responder vs non-responder cortices. We observed an increased activity of the antioxidant catalase, which detoxifies reactive oxygen species (Figure 3d). In addition, we found decreased expression of the mitochondrial glutamate carrier Slc25a22 and increased expression of the enzyme malate dehydrogenase 1 and the glycolysis enzyme hexokinase (HK1) in DL MitoQ responders compared with non-responders (Figure 3e).

MitoQ OF Responders Show a Distinct Metabolite Signature in Plasma Compared with Non-Responders

To get further insights into the molecular correlates of MitoQ treatment response we also classified MitoQ-treated HAB mice according to their performance in OF. HAB mice that entered the OF inner zone ($n = 3$) were classified as responders. HAB mice that did not enter the OF inner zone were classified as non-responders ($n = 10$) (Figure 4a). MitoQ brain levels were not significantly different between the two groups (Figure 4b). We then quantified the levels of 246 metabolites in plasma (Supplementary Table S3) and found altered levels of 11 metabolites between MitoQ OF responders and non-responders (Table 1). These metabolites are primarily involved in secondary metabolite and amino acid metabolism. We also observed increased expression of peroxiredoxin-3 (Prdx3), a mitochondrial protein antioxidant, in MitoQ-responder compared with non-responder cortices (Figure 4c).

DISCUSSION

In this work we report that pharmacological manipulation of mitochondria exerts anxiolytic effects in vivo. This approach is based on previous, hypothesis-free data that revealed altered mitochondrial pathways in a high anxiety mouse strain (Filiou et al, 2011). Following up on these findings, we hypothesized that selective pharmacological targeting of

Table 1 Altered Plasma Metabolite Levels Between MitoQ Responders and Non-Responders in OF

Metabolite	d.value	SD	p-value	KEGG ID
Higher levels in MitoQ OF responders				
2-keto-isovalerate	3.51	1.19	0.005	C00141
Alanine	3.59	2.65	0.005	C00041
dCMP	3.54	0.04	0.005	C00239
Fumarate	4.09	1.12	0.003	C00122
Maleic acid	3.57	1.20	0.005	C01384
Myo-inositol	4.80	1.35	0.001	C00137
Proline	3.61	3.45	0.005	C00148
Lower levels in MitoQ OF responders				
Fructose-6-phosphate	− 5.04	0.10	0.001	C00085
Glutathione disulfide	− 3.65	1.41	0.005	C00127
Hexose-phosphate	− 3.76	0.49	0.004	C05345
Methylmalonic acid	− 3.44	3.33	0.006	C02170

these mitochondrial pathways may result in a mitigation of the high anxiety phenotype. HAB mice, which were treated with the mitochondria-specific antioxidant MitoQ, showed a decreased anxiety-related behavior in the DL, a test assessing the aversion of mice to lit environments (Bourin and Hascoet, 2003). Owing to selective inbreeding for the high anxiety traits for ∼ 45 generations, the HAB phenotype is extremely robust and barely amenable to any kind of pharmacological manipulation (Landgraf et al, 2007). Intriguingly, HAB mice, which largely remain unresponsive to chronic treatment with conventional anxiolytics or widely used non-specific antioxidants (Sah et al, 2012), responded to MitoQ that specifically targets mitochondria.

At the molecular level, MitoQ treatment resulted in altered levels of sarcosine, AMP, and ascorbic acid in the HAB hippocampus. Sarcosine (N-methylglycine) has been discussed in the context of psychiatric disorders as an inhibitor

of the glycine transporter. Inhibition of glycine transporters has been suggested to both induce and attenuate anxiety-related behavior (Depoortere *et al*, 2005; Labrie *et al*, 2009). AMP is involved in purine metabolism, components of which have been associated with symptoms of major psychiatric disorders (Yao *et al*, 2012) whereas ascorbic acid is a known antioxidant.

In addition to comparing treated *vs* untreated HAB mice, we performed a molecular comparison between MitoQ responders *vs* non-responders in DL and OF anxiety-related tests. Ideally, anxiolytic response to MitoQ administration can be assessed at the level of individual animals by performing the anxiety-related tests before and after treatment. However, repeating the same behavioral test more than one time has been shown to compromise its validity due to strong memory effects of mice (Holmes *et al*, 2001). Therefore, we assessed the effect of treatment in responders *vs* the untreated control group and showed that responders have significantly lower anxiety-related behavior in the DL, unlike the non-responders, *vs* the control group (Supplementary Figure 3).

In our analysis, comparison of MitoQ responders *vs* non-responders revealed altered protein expression in the brain and a distinct metabolite signature in plasma of HAB mice that responded to treatment. Glucose metabolism was one of the predominantly implicated pathways as altered levels of glycolysis (HK1, fructose-6-phosphate) and glyconeogenesis (uridine diphosphate, hexose-phosphate) enzymes and metabolites were observed upon MitoQ treatment. Increased levels of citric acid cycle components (fumarate) implicate mitochondrial energy metabolism in response to MitoQ whereas several antioxidant-related readouts were affected, including increased catalase activity as well as altered Prdx3 and glutathione disulfide levels.

Although different metabolites were affected between MitoQ-treated *vs* untreated HAB mice and between MitoQ responders *vs* non-responders, the overall affected pathways are partially overlapping, with oxidative stress being the common denominator of the observed changes. Response to MitoQ may depend on the antioxidant status of each animal upon treatment, which is shaped by different antioxidants. Variations in the basal levels of these antioxidants across individual animals may interfere with the MitoQ-driven antioxidant effect and result into differences in MitoQ response. Based on our results, we suggest that by acting as a regulator of oxidative stress in mitochondria, MitoQ affects on the one hand the equilibrium of antioxidant defense and on the other mitochondrial energy metabolism. Oxidative stress has been linked to increased anxiety (Rammal *et al*, 2008) and MitoQ may complement the action of more generalized antioxidants (eg ascorbic acid). Alterations in the abundance of antioxidants such as catalase, the activity of which was found to be increased in MitoQ-responders, have been also shown to exert beneficial effects in anxiety-related behavior (Olsen *et al*, 2013), in accordance to our findings. Furthermore, prevention of oxidative damage to the mitochondria by MitoQ may alter mitochondrial energy metabolism pathways that have been also implicated in high anxiety (Szego *et al*, 2010). In a future set of experiments, tissue cultures from MitoQ-responder mice will be established to investigate in detail the effect of MitoQ on protein expression. Future work will also include assessment of

MitoQ effects in a larger number of HAB mice as well as in other anxiety mouse models.

Interestingly, there is extensive overlap between the MitoQ-driven molecular alterations in HAB mice reported here and the molecular differences previously described between HAB and LAB mice (Filiou *et al*, 2011, 2014; Zhang *et al*, 2011). AMP levels were found to be altered both in MitoQ-treated *vs* untreated HAB brain and in HAB *vs* LAB brain (Filiou *et al*, 2014). We also observed a considerable overlap between the molecular alterations of MitoQ responders *vs* non-responders and HAB *vs* LAB mice. Both MitoQ responders and LAB mice (Zhang *et al*, 2011) have higher plasma myo-inositol levels. At the protein level, Prdx3 expression in the brain was higher in responders *vs* non-responders. Members of the peroxiredoxin protein family were also expressed at higher levels in LAB compared with HAB mice (Filiou *et al*, 2011). This was also the case for Slc25a22, and HK1 with altered expression levels in MitoQ responders *vs* non-responders as well as in HAB *vs* LAB mice. At the pathway level, amino acid metabolism was differentially affected in MitoQ responder *vs* non-responder plasma as well as in HAB *vs* LAB mouse plasma.

Our findings suggest that MitoQ treatment exerts its anxiolytic effects through molecular pathways, which are affected in anxiety-related behavior. The resulting anxiolytic response of mitochondrial pathways to MitoQ treatment indicates that a mechanism-driven pharmacological strategy to manipulate the high anxiety phenotype is viable.

Mitochondrial targeting has emerged as a therapeutic approach for several disorders involving mitochondrial oxidative stress (Edeas and Weissig, 2013; Fulda *et al*, 2010; Smith *et al*, 2012). Although accumulating evidence points towards mitochondrial dysfunction in psychiatric disorders (Manji *et al*, 2012), the therapeutic potential of mitochondrial targeting has only been recently highlighted for bipolar disorder (de Sousa *et al*, 2014) and autism (Ghanizadeh *et al*, 2013) and has never been tested *in vivo*. Our study is the first *in vivo* application of selective mitochondrial targeting in the context of psychiatric disorders. The anxiolytic effect of MitoQ in mice selectively bred for an extreme genetic predisposition towards high anxiety is indicative of the potential that selective mitochondrial targeting holds to manipulate behavioral phenotypes related to trait anxiety. The fact that the anxiolytic effect was only present in HAB mice and not in other mouse strains which are not inbred for high anxiety, underlines a specificity that could be of use for translational applications in patients with anxiety disorders. Importantly, long-term treatment with MitoQ is also well-tolerated in human cohorts and was administered in clinical trials for Parkinson's disease (Snow *et al*, 2010) and chronic hepatitis C (Gane *et al*, 2010) with no adverse side effects.

In summary, we show that mitochondrial targeting exerts beneficial effects in high anxiety *in vivo*. This is a mechanism-driven approach to manipulate disease symptoms and is based on reported metabolic and mitochondrial alterations which have been linked to anxiety pathogenesis (Einat *et al*, 2005; Filiou *et al*, 2011). As advanced methodologies are now available for selective compound transport to mitochondria (Pathak *et al*, 2014; Smith *et al*, 2011), pharmacological manipulation of mitochondrial pathways may serve as the basis for a novel therapeutic path for

translational applications in high anxiety and stress-related psychiatric disorders.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)