Priority Communication

Biological Psychiatry

Mitofusin-2 in the Nucleus Accumbens Regulates Anxiety and Depression-like Behaviors Through Mitochondrial and Neuronal Actions

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ABSTRACT

BACKGROUND: Emerging evidence points to a central role of mitochondria in psychiatric disorders. However, little is known about the molecular players that regulate mitochondria in neural circuits regulating anxiety and depression and about how they impact neuronal structure and function. Here, we investigated the role of molecules involved in mitochondrial dynamics in medium spiny neurons (MSNs) from the nucleus accumbens (NAc), a hub of the brain's motivation system.

METHODS: We assessed how individual differences in anxiety-like (measured via the elevated plus maze and open field tests) and depression-like (measured via the forced swim and saccharin preference tests) behaviors in outbred rats relate to mitochondrial morphology (electron microscopy and 3-dimensional reconstructions) and function (mitochondrial respirometry). Mitochondrial molecules were measured for protein (Western blot) and messenger RNA (quantitative reverse transcriptase polymerase chain reaction, RNAscope) content. Dendritic arborization (Golgi Sholl analyses), spine morphology, and MSN excitatory inputs (patch-clamp electrophysiology) were characterized. *MFN2* overexpression in the NAc was induced through an AAV9-syn1-MFN2.

RESULTS: Highly anxious animals showed increased depression-like behaviors, as well as reduced expression of the mitochondrial GTPase MFN2 in the NAc. They also showed alterations in mitochondria (i.e., respiration, volume, and interactions with the endoplasmic reticulum) and MSNs (i.e., dendritic complexity, spine density and typology, and excitatory inputs). Viral *MFN2* overexpression in the NAc reversed all of these behavioral, mitochondrial, and neuronal phenotypes.

CONCLUSIONS: Our results implicate a causal role for accumbal MFN2 on the regulation of anxiety and depression-like behaviors through actions on mitochondrial and MSN structure and function. MFN2 is posited as a promising therapeutic target to treat anxiety and associated behavioral disturbances.

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High anxiety (HA), as a behavioral predisposition to perceive events as threatening, is associated with negative mood (1,2) and confers vulnerability to develop depression (2,3). This comorbidity is in line with evidence for shared genetic factors between neuroticism, a personality factor for which trait anxiety is one of the main facets, and depression (4,5). However, little is known about the neurobiological underpinnings underlying comorbidity between high trait anxiety with depression.

Motivational deficits, including anhedonia and reduced drive to exert effort, are core alterations in depression (6–8). The nucleus accumbens (NAc), a critical element of the brain's reward and motivation systems, is a key node in the pathophysiology of depression (9,10) and is involved in the distributed network of brain regions implicated in anxiety (11–14). Depressive patients show NAc volume reductions (15) and blunted activation in response to positive stimuli (16,17). NAc deep brain stimulation leads to decreased depression and anxiety ratings in treatment-resistant depression [(18); for a review, see (19)]. In addition, individuals high in trait anxiety show alterations in effort exertion (20) and reduced social competitiveness (21,22), as well as structural changes in the NAc (23). Furthermore, anxiety-like behaviors can be regulated by targeting the NAc with pharmacological (22,24,25) or genetic (26–28) manipulations.

Medium spiny neurons (MSNs), the major cell type (~95% of cells) in the NAc and its primary projection neurons, have been implicated in motivational deficits in animal models of depression (29–31). In particular, reduced excitatory input (32,33) and dendritic complexity (9,34) in dopamine D_1 receptor–expressing NAc MSNs were shown to causally contribute to stress-induced depressive-like behaviors. Given that neuronal morphology is a strong determinant of synaptic connectivity and strength (35), understanding the factors that control MSN dendritic regulation may help in developing treatments to ameliorate motivational deficits.

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Mitochondria appear to be ideally suited to contribute. Increasing evidence points at a central role of mitochondria in the etiology of psychiatric disorders (36), including anxiety and depression (37). Importantly, variations in mitochondrial function (22,25) and metabolism (1,38,39) in the NAc are related to differences in trait anxiety and depression phenotypes. In neurons, mitochondria support metabolic demands through energy supply and contribute to Ca^{2+} buffering (40,41). Cell cultures and neurodevelopmental studies have implicated mitochondria in the regulation of both dendritic arborization (42–46) and spine and synapse formation (47,48). However, whether mitochondria control individual differences in NAc MSN dendritic structure and thereby regulate motivational alterations linked to anxiety and depression remains unknown.

Here, by exploting natural phenotypic variation in anxiety-like behaviors in outbred rats, we identify alterations in mitochondrial morphology and function in the NAc in highly anxious animals. Mitochondrial alterations underlie impoverishment of MSN dendritic arborization, spine density, and excitatory inputs. Importantly, highly anxious animals show, as well, reduced contacts between mitochondria and the endoplasmic reticulum (ER), a critical interaction for a myriad of cellular functions including mitochondrial function and dynamics as well as Ca^{2+} homeostasis (49). Accordingly, we find that highly anxious animals show reduced levels of mitofusin MFN2—a mitochondria outer member GTPase that plays a major role in sustaining mitochondria-ER contacts (50)—in NAc D_1 and D_2 receptor–expressing MSNs. Importantly, we show that overexpression (OE) of MFN2 in the NAc of highly anxious animals restores alterations in mitochondrial and neuronal structure and function, as well as anxiety-like behavior and motivational deficits associated with depression, to levels observed in low anxious rats. Our data point at NAc MFN2 as a key target for the treatment of anxiety and depression phenotypes.

METHODS AND MATERIALS

A detailed description of all experimental procedures, including animals, viral-mediated NAc *Mfn2* OE, behavioral testing, mitochondrial respirometry, electrophysiology, immunohistochemistry, morphological analyses, electron microscopy, and statistics, is provided in the Supplement.

Animals

Adult male Wistar rats weighing ∼275 g at arrival at the facility were individually housed in a temperature- and light-controlled room.

Viral-Mediated NAc *MFN2* OE

Plasmid containing Myc-Human *MFN2* was generously provided by Dr. Darren Moore (Van Andel Institute, Grand Rapids, MI). The Myc-hMFN2 or GFP (green fluorescent protein) coding sequence was subcloned in the pAAV-hSyn1-MCS-WPRE vector [described in (51)] for transgene expression under the control of the human synapsin promoter (52). To target both the core and the shell of the NAc, two injection sites were used with the following coordinates (53): 1.3 and 2.5 mm posterior to bregma, 1.0 and 1.5 mm from midline, 7.0 mm ventral from skull. A volume of 500 nL pAAV9-hSyn1-Myc-hMFN2 or AAV9-hSyn1-GFP-WPRE (injected at a titer of 10^9 VG/μL) was bilaterally injected in the NAc. Behavioral experiments commenced 4 to 5 weeks following surgery.

Statistics

Statistical analyses were performed in Prism v.7.0 (GraphPad Software, San Diego, CA). All p values $<.05$ were considered to be significant. In graphs, individual points represent single subjects in all behavioral experiments, cells in electrophysiological experiments, cells in morphology experiments, sections in electron microscopy analyses. All data are presented as mean ± SEM. The statistical details can be found in Table S1.

RESULTS

High-Trait-Anxiety Rats Showed Reduced Motivation to Exert Effort in a Depression-Related Test

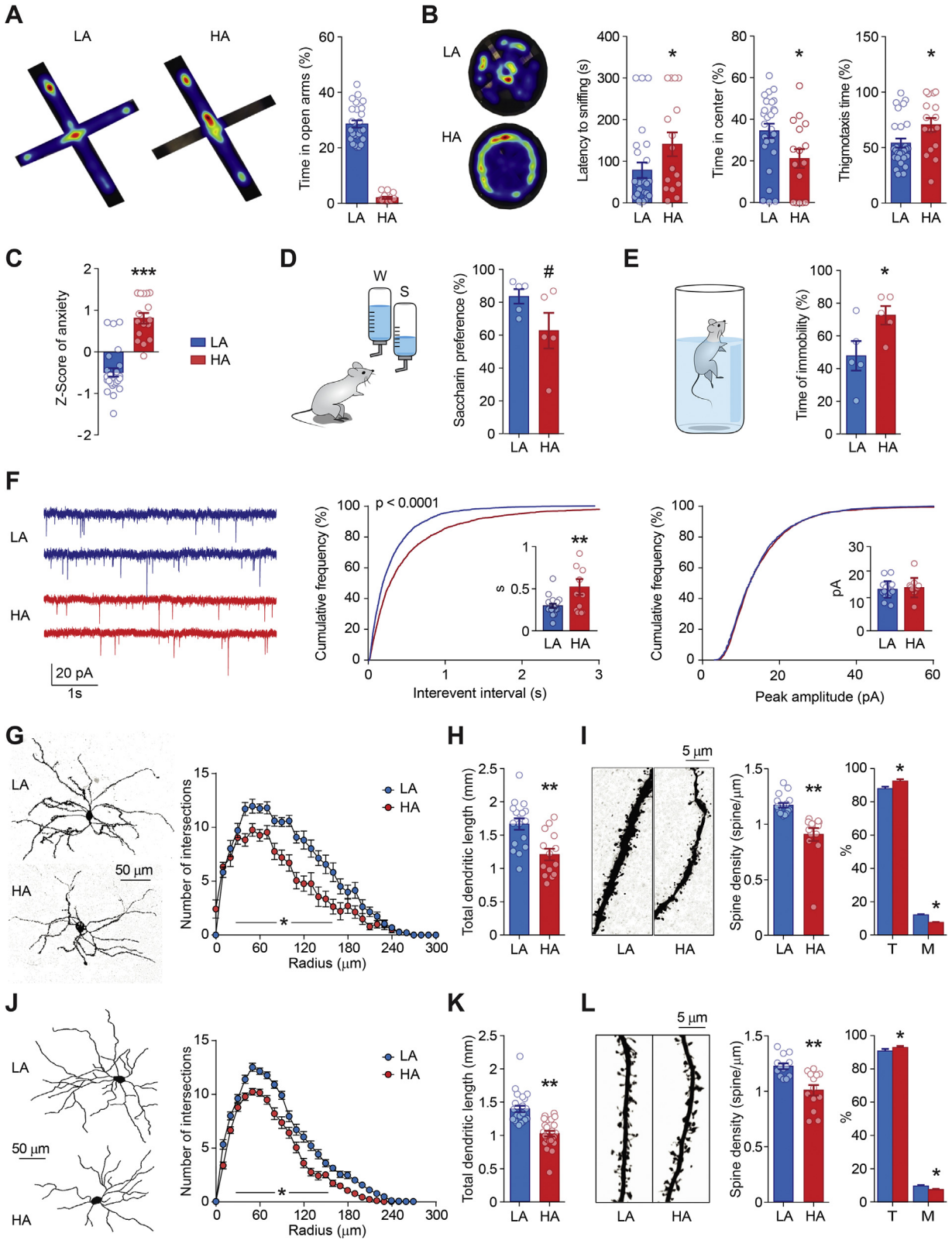
First, male rats were classified according to the time spent in the open arms of the elevated plus maze, a measure indicating anxiety-like behavior (Figure 1A), as either HA (≤5% open arm duration) or low anxiety (LA) (≥20% open arm duration). These criteria are kept constant across studies in our lab [e.g., (22,54)], yielding ∼8% of HA and ∼20% of LA rats from each cohort. The two anxiety groups also differed in their exploratory behavior in the novel object test, in which HA rats showed higher latency to sniff the object, spent less time in the center of the arena, and displayed more thigmotactic behavior (Figure 1B). Notably, there were no group differences in general locomotor activity in any of these tests (for the elevated plus maze, see Figure S1A; for the novel object test, see Figure S1B). We further performed a z score with behavioral data from these two tests (see Supplement) that confirmed consistent differences in anxiety-like behaviors across tests between HA and LA rats (Figure 1C).

We then assessed whether the two groups differ in depression-like behaviors. In a test for anhedonia, HA rats exhibited a tendency to show lower saccharin preference than LA rats (Figure 1D). In the forced swim test, HA rats displayed higher immobility levels (Figure 1E), indicating increased passive coping responses and lower motivation to exert effort under adversity.

Collectively, these results indicate that when considering natural variation in trait anxiety observed in outbred Wistar rats, HA rats display motivational deficits related to depression.

High-Trait-Anxiety Rats Showed Reduced Dendritic Complexity and Connectivity in MSNs of the NAc

To explore whether differences in trait anxiety are associated with structural and functional differences in MSNs of the NAc, as previously shown for mouse models of depression (9,34), we performed whole-cell recordings of miniature excitatory postsynaptic currents in NAc shell MSNs in acute slices from HA and LA rats. HA rats exhibited a lower occurrence of excitatory inputs, as indicated by the rightward shift in the cumulative frequency plot of the interevent interval (Figure 1F, middle panel). There were no differences in the peak amplitude of miniature excitatory postsynaptic currents (Figure 1F, right


A
LA
HA
Time in open arms (%)
B
Latency to sniffing (s)
Time in center (%)
Thigmotaxis time (%)
C
Z-Score of anxiety
D
W
S
Saccharin preference (%)
E
Time of immobility (%)
F
p < 0.0001
Cumulative frequency (%)
Interevent interval (s)
Peak amplitude (pA)
20 pA
1s
G
50 µm
Number of intersections
Radius (µm)
H
Total dendritic length (mm)
I
5 µm
Spine density (spine/µm)
T
M
J
K
L

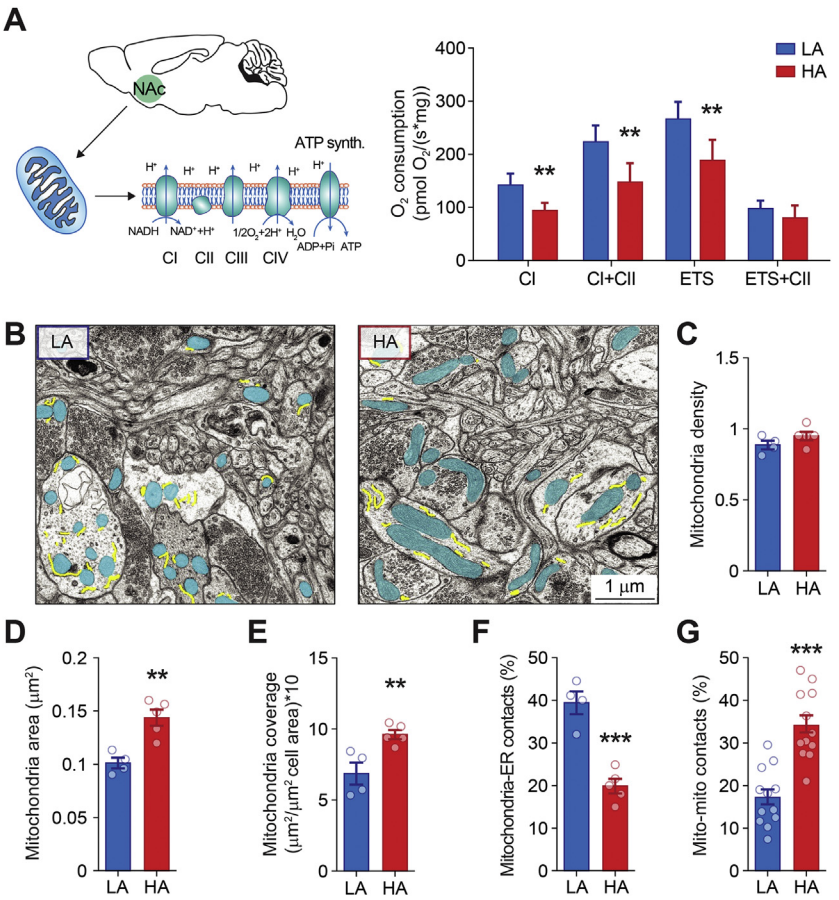


Figure 2. Divergent trait anxiety is associated with different mitochondrial function. **(A)** Mitochondrial respiration in the NAc of LA (n = 12) and HA (n = 12) rats. **(B)** Representative electron micrographs from the NAc of LA and HA rats with tracing of mitochondria (light blue) and ER (yellow). **(C)** Mitochondria density was comparable, whereas **(D)** area, **(E)** coverage, and **(F)** percentage of mitochondria-ER contacts differed between LA rats (n = 4) and HA rats (n = 5). **(G)** Percentage of mitochondria-mitochondria contacts was higher in HA rats (LA: n = 12 sections; HA: n = 12 sections). Data are mean ± SEM. Circles in the bar graphs represent single observations. $^{**}p < .01$, $^{***}p < .001$. Statistics are presented in Table S1. ADP, adenosine diphosphate; ATP, adenosine triphosphate; ATP synth., ATP synthase; C, complex; ER, endoplasmic reticulum; ETS, maximal electron transport system capacity; HA, high anxiety; LA, low anxiety; NAc, nucleus accumbens: NAD, nicotinamide adenine dinucleotide, oxidized form; NADH, nicotinamide adenine dinucleotide, reduced form.

panel), suggesting a comparable postsynaptic response between HA and LA rats.

In line with the electrophysiological data, confocal analysis of biocytin-filled MSNs obtained from the electrophysiological recordings revealed less complex dendritic arborization (Figure 1G) and shorter total dendritic length (Figure 1H) in HA rats as compared with LA rats. Furthermore, HA rats exhibited lower spine density (Figure 1I). Dendritic spine diameter increases with neuronal maturation (55,56) and reflects synaptic strength (57). To examine how anxiety relates to dendritic spine maturation, we classified dendritic spines in two categories, based on the maximal diameter of the spine head (thin spines <0.45 µm and mushroom spines >0.45 µm) (58). Interestingly, HA rats displayed more thin spines and less mushroom spines

Figure 1. Distinct behavioral and accumbal neuronal phenotypes are observed in rats with divergent trait anxiety. **(A)** (Left panel) Heatmap representations of the elevated plus maze exploratory behavior of rats selected for low and high trait anxiety. Bar graphs show the time spent in the open arms of the elevated plus maze (LA: n = 26; HA: n = 16). **(B)** (Left panel) Heatmap representations of the novel object exploratory behavior of rats selected for low and high trait anxiety. Bar graphs show the latency to sniffing the object, the percentage of time spent in the center, and the percentage of time in thigmotactic behavior. **(C)** The z score of anxiety, based on the elevated plus maze and novel object test (LA: n = 26; HA: n = 16). **(D)** Saccharin preference indicates a tendency toward anhedonia in HA rats (LA: n = 5; HA: n = 5). **(E)** Time spent immobile during the forced swim test indicates higher passive coping behavior in HA rats (LA: n = 5; HA: n = 5). **(F)** (Left panel) Example traces of miniature excitatory postsynaptic currents in nucleus accumbens MSNs from LA and HA rats. Cumulative frequency plots indicate larger interevent interval in HA rats (middle panel), with no change in miniature excitatory postsynaptic current peak amplitude (right panel). Insets with bar graphs represent mean values of interevent interval and peak amplitude per cell (LA: n = 19; HA: n = 14). **(G)** (Left panel) Representative confocal images of biocytin-filled MSNs in LA and HA rats. (Right panel) Sholl profile reporting less complex neuronal dendritic arborization in MSNs from HA rats (LA: n = 19; HA: n = 14). **(H)** Total dendritic length of reconstructed biocytin-filled MSNs. **(I)** Confocal micrographs of MSN dendrites from LA and HA rats and bar graphs with quantification of spine density and spine morphology (LA: n = 17; HA: n = 14). **(J)** (Left panel) Reconstructions of Golgi-stained MSNs in LA and HA rats. (Right panel) Sholl profile reporting less complex neuronal dendritic arborization in HA rats (LA: n = 24; HA: n = 24). **(K)** Total dendritic length of reconstructed Golgi-stained MSNs. **(L)** Confocal micrographs of MSN dendrites from LA and HA rats and bar graphs with quantification of spine density (LA: n = 13; HA: n = 13) and spine morphology (LA: n = 18; HA: n = 18). Data are mean ± SEM. Circles in the bar graphs represent single observations. This figure is complemented by Figure S1. $^{\#}p = .1152$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. Statistics are presented in Table S1. HA, high anxiety; LA, low anxiety; M, mushroom; MSN, medium spiny neuron; S, saccharin; T, thin; W, water.

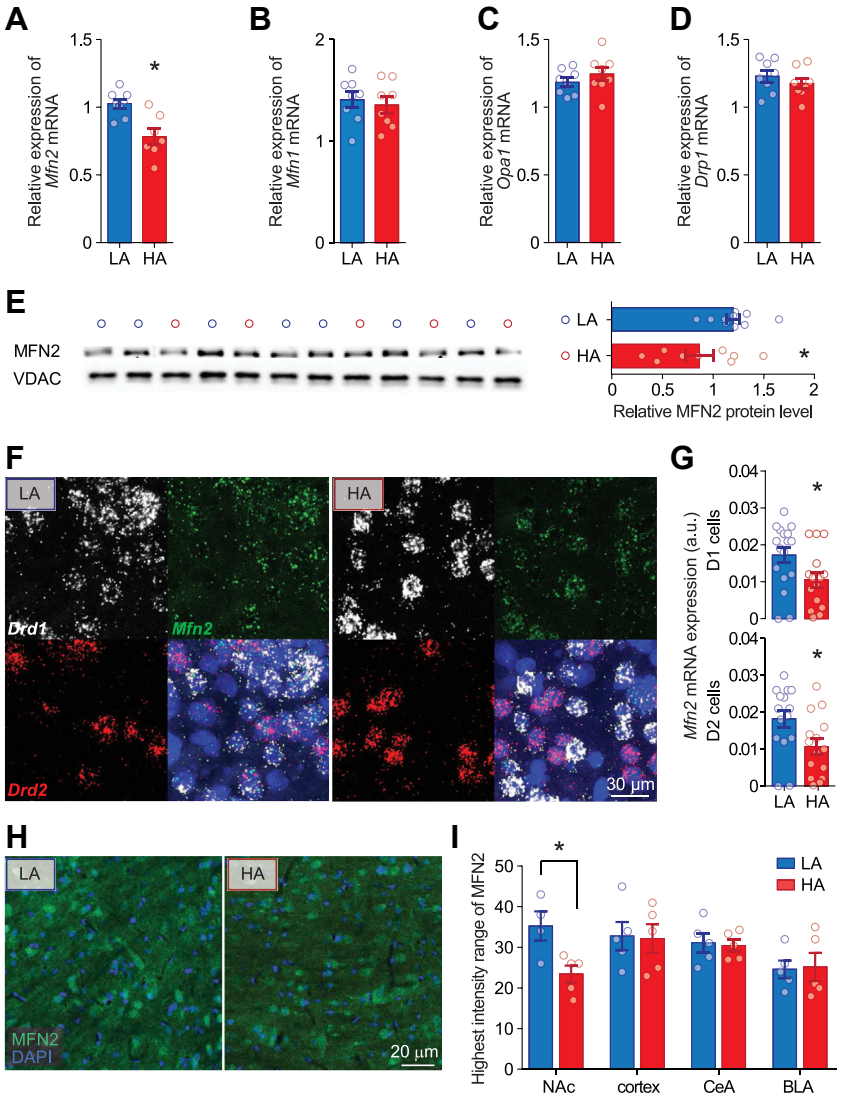


Figure 3. Divergent trait anxiety is associated with different mitochondrial dynamics and reduced MFN2. **(A–D)** mRNA expression (relative to *EEF1* mRNA levels) of mitochondria-related genes in the NAc punches from LA and HA rats (LA: $n = 8$; HA: $n = 8$). **(E)** Western blot and quantification of MFN2 protein level (LA: $n = 10$; HA: $n = 9$). **(F)** Confocal micrographs of RNAscope for *Drd1*, *Drd2*, and *Mfn2* mRNA (color-coded) in the NAc shell from LA and HA rats. Merged images include DAPI staining (blue). **(G)** Quantification of *Mfn2* mRNA in D_1 and D_2 receptor–expressing medium spiny neurons (D1 cells and D2 cells) indicating lower expression in HA rats (for D1 cells, LA: $n = 17$; HA: $n = 15$, for D2 cells, LA: $n = 15$; HA: $n = 15$). **(H)** Confocal micrographs of MFN2 immunostaining and DAPI (color-coded) in NAc shell. **(I)** Bar graph showing the highest intensity range of MFN2 in the NAc, cortex (S1), CeA, and BLA (for the NAc, LA: $n = 4$; HA: $n = 5$; for the other panels, LA: $n = 5$; HA: $n = 5$). Data are mean ± SEM. Circles in the bar graphs represent single observations. This figure is complemented by Figure S2. *$p < .05$. Statistics are presented in Table S1. a.u., arbitrary units; BLA, basolateral amygdala; CeA, central amygdala; HA, high anxiety; LA, low anxiety; mRNA, messenger RNA; NAc, nucleus accumbens; S1, primary somatosensory cortex.

than LA rats (Figure 1I). We corroborated these findings with morphometric analysis of Golgi-impregnated neurons in another set of animals, which confirmed features of dendritic atrophy in MSNs of HA rats (Figure 1J), including significantly shorter dendritic lengths (Figure 1K) and lower spine density (Figure 1L) than LA rats. Further, the relative abundance of thin spines was higher, whereas abundance of mushroom spines was lower in HA than in LA rats (Figure 1L, right panel). To investigate whether the observed structural differences were general across the brain, we measured dendritic arborization in the basolateral amygdala, where the two groups showed similar arborization and total dendritic length (Figure S1C, D). In summary, HA rats show reduced dendritic arborization and spine density, as well as reduced excitatory inputs onto NAc MSNs.

High-Trait-Anxiety Rats Displayed Lower Mitochondria-ER Contacts in the NAc

Mitochondria have been implicated in dendritic maturation (45) and synapse formation and maintenance (59). We previously showed that, as compared with LA rats, HA rats have impaired mitochondrial function (i.e., decreased respiration, reduced protein content for the electron transfer chains I and II, and reduced ATP [adenosine triphosphate]) in the NAc (22,25). We further showed that local inhibition of mitochondrial function in the NAc reduces social competitiveness (22,25), a behavioral risk factor for depression (60). Here, using ex vivo high-resolution respirometry, we confirmed under our current experimental conditions the impairment in mitochondrial function in HA rats NAc, as showed by their lower complex I activity and maximal mitochondrial respiration (Figure 2A).

Given the close coupling between mitochondrial morphology and function (61), we conducted electron microscopy analyses in the NAc of LA and HA rats to assess diverse parameters of mitochondrial morphology and interorganellar interactions (Figure 2B). As expected, and in agreement with our previous observations (22), the two groups did not differ in total mitochondria number (Figure 2C). Importantly, HA rats presented larger mitochondria area (Figure 2D) and mitochondria tissue coverage (Figure 2E), with their morphology suggestive of mild mitochondria swelling. We then analyzed mitochondria-ER contacts, given their essential role in mitochondrial function, homeostasis, and dynamics (49,62). Strikingly, HA rats displayed considerably lower number of mitochondria-ER contacts than LA rats (Figure 2F). Moreover, HA rats showed a higher number of mitochondria-mitochondria contacts (Figure 2G). Although the exact function of mitochondria-mitochondria contacts remains poorly described, they may promote synergy of intrinsic mitochondrial functions (63). Together, these results indicate that HA and LA rats differ in several parameters related to mitochondrial function, shape, and their association with ER in NAc neurons.

High-Trait-Anxiety Rats Displayed Lower MFN2 Levels in the NAc MSNs

Both mitochondrial shape and their interactions with the ER are under strong regulation by molecules involved in mitochondrial dynamics (i.e., fission and fusion). In particular, the mitochondria fusion protein MFN2 has been critically implicated in the establishment of contacts with the ER (50). Thus, we hypothesized a reduction of accumbal MFN2 expression in HA rats. Indeed, we measured messenger RNA (mRNA) level of *Mfn2* in NAc tissue punches and found lower expression of *Mfn2* mRNA in HA than in LA rats (Figure 3A). Relative expression levels of other fusion (i.e., *Mfn1*, *Opa1*) (Figure 3B, C) and fission (i.e., *Drp1*) (Figure 3D) genes were comparable between both groups. Furthermore, Western blot assays further validated that MFN2 protein expression is lower in NAc tissue of HA rats than in LA rats (Figure 3E; Figure S2A), while expression levels for OPA1 and DRP1 were equivalent for both groups (Figure S2A–C).

To quantify the levels of *Mfn2* in specific NAc cell types, we conducted RNAscope analysis. Previous studies indicated that the NAc shell is a key node in the circuit for anxiety (64), anhedonia, and passive coping (65,66). HA rats presented lower *Mfn2* expression in both D_1 and D_2 receptor–expressing neurons in the NAc shell than LA rats (Figure 3F, G). Immunohistochemical analyses with an MFN2 antibody confirmed lower levels of this protein for HA rats in the NAc shell (Figure 3H, I), while no differences were found in several other brain regions analyzed, including the cortex and the central and basolateral subdivisions of the amygdala (Figure 3I). In contrast, in the NAc core, there were only nonsignificant trends toward lower expression of *Mfn2* in HA than in LA rats in each of the two cell types analyzed (Figure S2D, E). Moreover, no group differences in *Mfn2* expression in cells lacking D_1 and D_2 receptors were observed in either the NAc core or NAc shell subdivisions (Figure S2F). These results support NAc shell MSN specificity for the link between reduced MFN2 levels and HA.

OE of *MFN2* in the NAc of HA Rats Normalized Mitochondrial Parameters and Mitochondria-ER Contacts

Next, we sought to normalize cellular and behavioral alterations in HA rats by manipulating accumbal MFN2 levels. To this aim, we injected an AAV for *MFN2* OE in the NAc of HA rats. We then compared their cellular and behavioral phenotypes with those of sham-operated LA and HA rats. *MFN2* OE led to a significant increase in *Mfn2* expression in the NAc of HA rats as compared with HA-sham rats, at both the mRNA and protein levels (Figure 4A, B; Figure S3A, B), and restored HA mitochondrial respiratory capacity to LA-sham rats' levels (Figure 4C).

We then examined the effect of *MFN2* OE on mitochondrial morphology (Figure 4D). Mitochondrial density was not affected by anxiety or *MFN2* OE (Figure 4E). We confirmed, again, anxiety-related differences in mitochondria area (Figure 4F) and coverage (Figure 4G) as well as in mitochondria-ER contacts (Figure 4H). Strikingly, *MFN2* OE decreased mitochondria area (Figure 4F) and coverage (Figure 4G) and increased mitochondria-ER contacts (Figure 4H) in HA rats, thus normalizing the mitochondrial characteristics of HA rats toward the profile of LA rats. Moreover, in order to obtain a better understanding of mitochondrial structure, we performed a 3-dimensional reconstruction of the mitochondrial network in the NAc shell, which allowed us to exactly calculate the volume of a single mitochondrion. We were able to reconstruct the mitochondrial network in a cube of tissue of 1000 μm^3 per rat in a total of 8 rats (i.e., summing up to 8000 μm^3 of accumbal reconstructed tissue). These analyses revealed that HA rats display a larger overall volume of mitochondria than LA rats, which was normalized by *MFN2* OE (Figure 4I, L). Notably, the increase of volume was evident when specifically analyzed in dendritic mitochondria (Figure 4M, N). On the contrary, mitochondrial number and length were affected neither by anxiety nor by *MFN2* OE (Figure 4K, L). We verified that the changes in mitochondrial function and neuronal morphology were not caused by a cellular immune response to the AAV (adeno-associated virus) transfection. In rats injected with a pAAV9-hSyn1-GFP-WPRE, we did not find any alteration in mitochondrial respiration, key mitochondrial gene expression, or neuronal morphometric parameters when compared with sham-operated rats (Figure S4).

OE of *MFN2* in the NAc of HA Rats Normalized Neuronal and Behavioral Phenotypes

We next probed the cellular and behavioral consequences of *MFN2* OE. The reduced frequency of miniature excitatory postsynaptic currents in NAc MSNs observed in HA rats (Figure 1) was replicated here, and restored by *MFN2* OE in HA rats, to levels comparable of those in LA rats (Figure 5A). In addition, we observed that currents recorded in *MFN2* OE rats presented on average a smaller peak amplitude, which is likely due to a reduced postsynaptic response in newly generated synapses. *MFN2* OE in HA rats was able to normalize the poorer dendritic arborization and spine morphometry observed in the HA rats to the levels of LA rats. This was consistently observed both in biocytin-filled neurons obtained from the

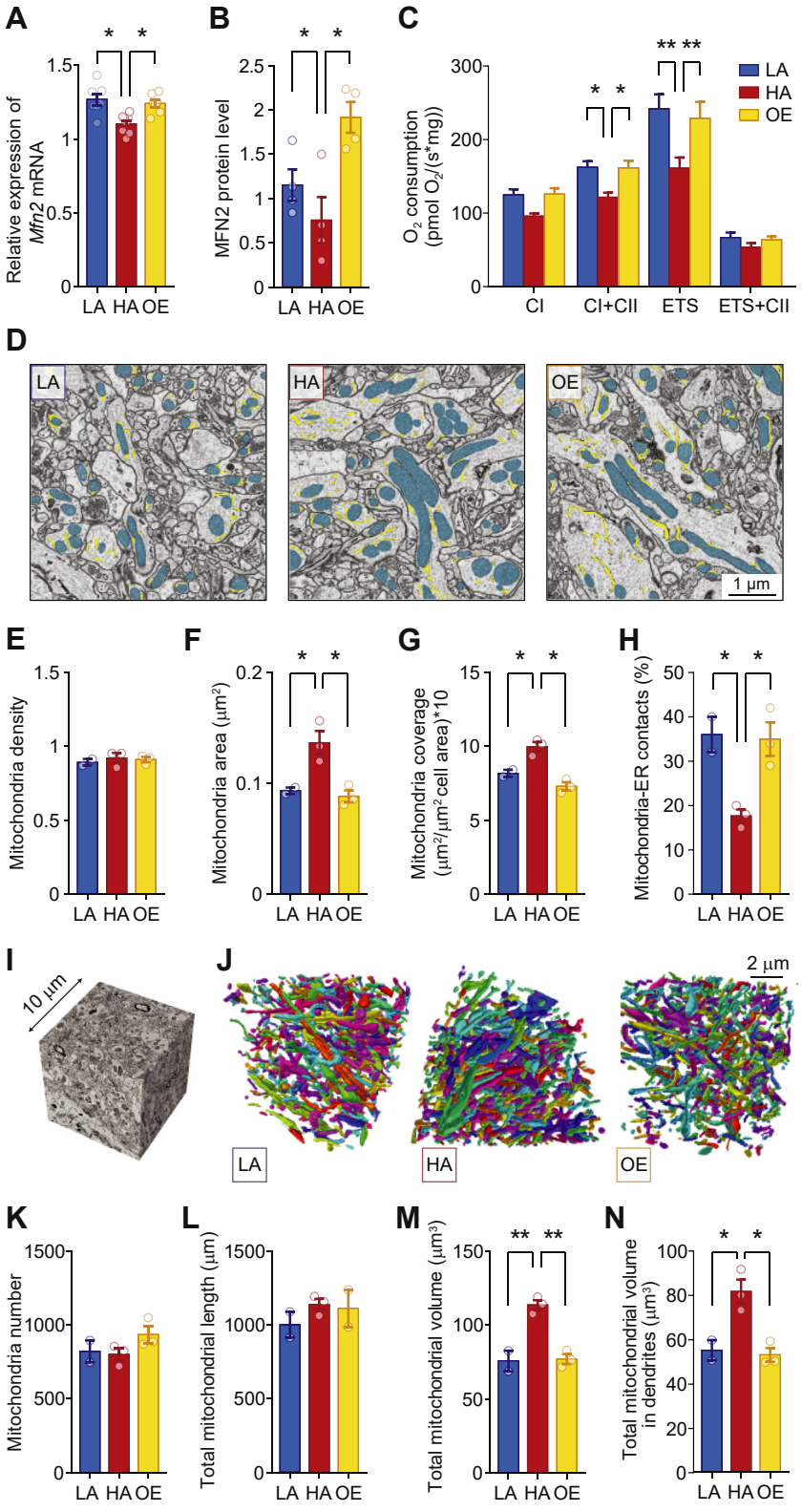


Figure 4. OE of *MFN2* in the NAc normalizes mitochondrial function. **(A)** Relative expression of *Mfn2* mRNA in LA rats, HA rats, and HA rats with *MFN2* OE (LA: $n = 7$; HA: $n = 8$; OE: $n = 7$). **(B)** OE restores MFN2 protein levels (LA: $n = 4$; HA: $n = 4$; OE: $n = 4$). **(C)** Mitochondrial respiration in the NAc of LA, HA, and OE rats (LA: $n = 4$; HA: $n = 4$; OE: $n = 4$). **(D)** Representative electron micrographs of the NAc with tracing of mitochondria (light blue) and ER (yellow) in LA, HA, and OE rats. **(E)** Mitochondria density is comparable in the three groups (LA: $n = 2$; HA: $n = 3$; OE: $n = 3$). **(F–H)** Mitochondria area, mitochondria coverage, and percentage of mitochondria-ER contacts were normalized by MFN2 OE. **(I)** Example image of an electron microscopy block for serial 3-dimensional reconstruction. **(J)** Three-dimensional reconstruction of mitochondria in the NAc of LA, HA, and OE rats. **(K, L)** Mitochondria number and total length are comparable in the three groups (LA: $n = 2$; HA: $n = 3$; OE: $n = 3$). **(M, N)** Total volume was larger in HA rats and normalized by MFN2 OE. This is also verified when mitochondrial analysis is restricted to dendrites. Data are mean ± SEM. Circles in the bar graphs represent single observations. This figure is complemented by Figure S3. $^{*}p < .05$, $^{**}p < .01$. Statistics are presented in Table S1. C, complex; ER, endoplasmic reticulum; ETS, maximal electron transport system capacity; HA, high anxiety; LA, low anxiety; mRNA, messenger RNA; NAc, nucleus accumbens; OE, overexpression.

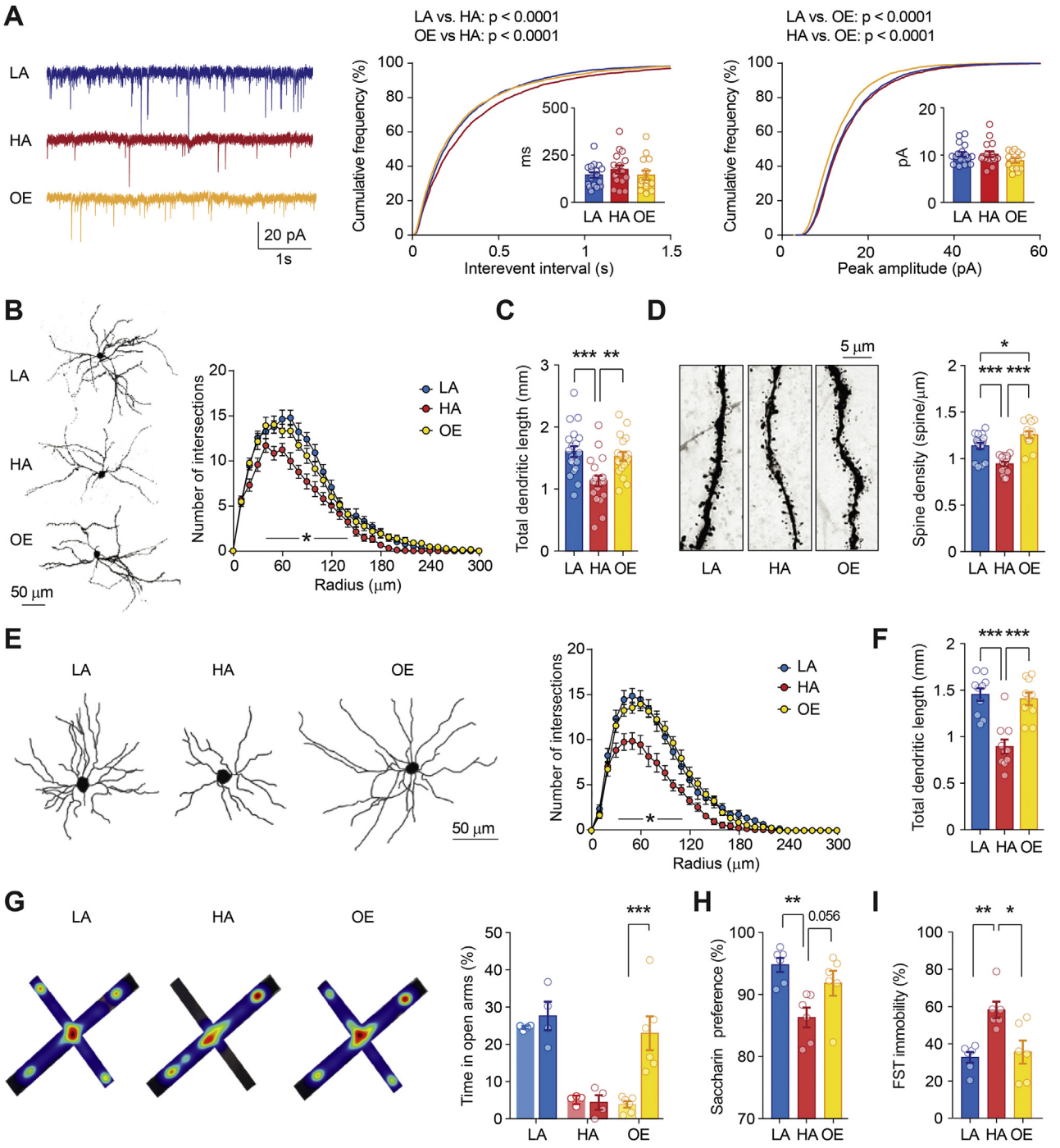


Figure 5. *MFN2* OE in the NAc of HA rats normalized cellular and behavioral phenotypes. **(A)** (Left panel) Example traces of miniature excitatory postsynaptic currents in NAc MSNs from LA, HA, and OE rats. (Middle panel) Cumulative frequency plots indicate that *MFN2* OE normalized the larger interevent interval in HA rats toward LA values. (Right panel) OE rats displayed decreased miniature excitatory postsynaptic current peak amplitude. Insets with bar graphs represent mean values of interevent interval and peak amplitude per cell (LA: n = 17; HA: n = 16; OE: n = 14). **(B)** (Left panel) Representative confocal images of biocytin-filled MSNs in LA, HA, and OE rats. (Right panel) Sholl profile showing normalized neuronal dendritic arborization in MSNs from HA rats upon *MFN2* OE (LA: n = 20; HA: n = 20; OE: n = 19). *$p < .05$, HA group compared with LA and OE groups. **(C)** Total dendritic length of reconstructed biocytin-filled MSNs. **(D)** Confocal micrographs of MSN dendrites from LA, HA, and OE rats and bar graphs with quantification of spine density and spine morphology (LA: n = 17; HA: n = 16; OE: n = 14). **(E)** (Left panel) Reconstructions of Golgi-stained MSNs in LA, HA, and OE rats. (Right panel) Sholl profile showing normalized neuronal dendritic arborization in MSNs from HA rats upon *MFN2* OE (LA: n = 10; HA: n = 10; OE: n = 10). *$p < .05$, HA group compared with LA and OE groups. **(F)** Total dendritic length of reconstructed Golgi-stained MSNs. **(G)** (Left panel) Representative heat maps of elevated plus maze exploratory behavior for LA, HA, and OE rats. (Right panel) Quantification of anxiety levels (% exploration of the elevated plus maze open arms) before (shaded colors) and after (full color) sham operation in LA and HA rats and *MFN2* OE viral delivery in OE rats (LA: n = 4; HA: n = 4; OE: n = 6). **(H)** Saccharin preference indicates that *MFN2* OE ameliorated the anhedonic phenotype of HA rats (LA: n = 6; HA: n = 6; OE: n = 6). **(I)** *MFN2* OE normalized the passive coping behavior of HA rats, as indicated by the time spent immobile during the forced swim test (LA: n = 6; HA: n = 6; OE: n = 6). Data are mean ± SEM. Circles in the bar graphs represent single observations. *$p < .05$, **$p < .01$, ***$p < .001$. Statistics are presented in Table S1. HA, high anxiety; LA, low anxiety; M, mushroom; MSN, medium spiny neuron; NAc, nucleus accumbens; OE, overexpression; T, thin.

electrophysiological recordings (Figure 5B–D) and in Golgi-stained cells (Figure 5E, F).

Finally, in addition to replicating anxiety-related differences reported above, we confirmed that *MFN2* OE in the NAc decreased measures of anxiety- and depression-related behaviors in HA animals to levels comparable to those in LA rats. HA rats with *MFN2* OE spent more time in the open arms of the elevated plus maze than HA rats (Figure 5G), indicating an anxiolytic-like effect. Moreover, *MFN2* OE rats showed increased saccharin preference (Figure 5H) and spent less time immobile in the forced swim test (Figure 5I) than HA rats, indicating a depression-resilient phenotype. Collectively, these results show that a differential level of *Mfn2* in NAc MSNs is causally implicated in anxiety-related differences in mitochondrial, neuronal, and behavioral phenotypes.

DISCUSSION

Here, we identify a novel role for MFN2—a mitochondrial GTPase localized as well on the ER—in the regulation of interindividual differences in anxiety phenotypes. Specifically, we establish a mechanistic link for MFN2 in accumbal MSNs on the regulation of behaviors that implicate accumbal circuits. Importantly, we show that this connection from molecule to behavior is mediated by regulatory roles of MFN2 in key structural and functional features of both mitochondria and neurons. Specifically, we causally implicate MFN2 deficiency in MSNs from the NAc shell—observed in naturally highly anxious rats—in the alterations found in 1) mitochondria, which range from reduced maximal respiratory capacity to increased volume, and reduced interactions with the ER; 2) MSNs, which comprise a poorer dendritic complexity, a reduced spine density and typology, and reduced excitatory inputs; and 3) behaviors, which include anxiety-like behaviors, reduced motivation to exert effort under adversity, and reduced saccharin preference.

A striking aspect of our study is the implication of a mitochondrial and ER molecule, MFN2, in natural variation in anxiety-related behaviors with the inclusion of a mechanistic account of the neurobiological intermediaries from the organelle to the cellular and synaptic levels. Although a central role for mitochondria in neuronal function is clearly emerging (48,67), to our knowledge, all studies that have so far related components of the brain mitochondrial machinery with behavioral manifestations lay within the “disease” domain. Indeed, increasing evidence implicates mitochondrial alterations in the pathophysiology of major psychiatric disorders, such as major depressive disorder, bipolar disorder, and schizophrenia, as well as of neurodegenerative disorders (36,68–70). Chronic stress leads to a myriad of behavioral changes (71,72) and results in alterations in mitochondria gene expression and metabolism in different brain regions [(71,72); for a review, see (73)]. However, the causal implication of specific mitochondria mechanisms in stress-induced behavioral changes is lacking. This link has notwithstanding been established for cocaine addiction. Specifically, increased Drp1 levels in NAc D1-MSNs, observed along with increased mitochondria fission, following cocaine consumption and abstinence were causally implicated in drug-seeking behavior (74). In the mitochondrial disorders domain, a mouse model of Leigh syndrome—one of the most common childhood mitochondrial diseases—lacking the mitochondrial complex I subunit Ndufs4 selectively in striatal MSNs exhibits nonfatal progressive motor impairment without concomitant neuronal loss (75). Therefore, a distinct and novel contribution of our study is to identify a novel role for a mitochondrial molecule (i.e., accumbal MFN2 levels) on the regulation of natural (i.e., not disease-induced) variation in behavior.

The involvement of mitochondrial function and mitochondria-ER interactions in dendritic and spine complexity has been previously acknowledged for early development (42,43,46–48) and neurodegeneration (76–78). In our study, from the mitochondrial dynamics (i.e., fusion and fission) machinery, only MFN2 mRNA and protein are reduced in the NAc shell MSNs of HA as compared with LA animals, an observation consistent with the lower number of mitochondria-ER contacts in HA animals [(50,79); but see (80)]. This finding fits with recent reports implicating MFN2 in cortical synaptic development (81) and implicating an MFN2 deficit in impaired hippocampal dendritic maturation in a mouse model of fragile X syndrome (45).

Given that MFN2 is a mitochondrial fusion molecule (82), it may seem counterintuitive that, with lower MFN2 levels, mitochondria in the NAc of HA rats are not smaller and more numerous than in LA rats. In fact, they are more rounded and voluminous in HA rats, while mitochondrial density is equivalent for the two groups. However, the impact of MFN2 deficiency has been shown to be cell autonomous; for example, its downregulation leads to very different phenotypes, with some cell types showing no alterations, others displaying swollen mitochondria, and others displaying more fragmented mitochondria. Similar to our findings in HA rats showing a natural low MFN2 levels, several studies involving MFN2 downregulation in different types of mature neurons have reported no differences in number but more rounded mitochondria than the elongated mitochondria found in wild-type animals (83–85). Similar to HA rats, MFN2 deletion in these studies led as well to reduced electron transport chain activity. This is in line with observations that more rounded mitochondria are often associated with less efficient mitochondria bioenergetics (86–88) and ATP levels (89). Interestingly, the same features of more rounded mitochondria, with a larger coverage per tissue area, and lower mitochondria-ER contacts were reported in hypothalamic POMC (pro-opiomelanocortin) neurons for both a mouse model of high-fat diet–induced obesity that leads to reduced MFN2 expression and a mouse model harboring a deletion of MFN2 in POMC neurons (85). A possible mechanism for not observing smaller mitochondria could be secondary to the lower mitochondria-ER contacts, such as an impairment of fission processes. Indeed, mitochondria-ER contacts contain the machinery to trigger mitochondrial division subsequently implemented by fission molecules (90,91). Furthermore, although in some cell types, such as cerebellar granule cells, MFN2 deletion can lead to neurodegeneration and cell death (92), this seems to be as well a cell-autonomous effect, because, similar to in our study, MFN2 deletion in different hypothalamic neuronal types was found not to affect neuronal function (83,85).

Similar to our findings in HA rats, reduced dendritic length in NAc MSNs has also been found in a chronic social defeat model of depression (34,93)—but note that, in contrast to our findings, other studies reported increased spine density in MSNs following chronic social defeat stress (94,95)—and following chronic corticosterone treatment (96). In agreement with their role in dendritic structure and plasticity (97), previous studies have established a role for Rho GTPases, such as RhoA, in this context (93,98). Given that RhoA activation can regulate mitochondrial distribution and dynamics (99,100), future studies are warranted to explore the potential link between RhoA and MFN2 in the NAc in the context of anxiety. Dendritic spines of MSNs are the targets of convergent mesolimbic dopamine and cortical and limbic glutamate axons. In addition to the poorer dendritic arborization of accumbal MSNs, we find that HA rats show reduced excitatory inputs in these neurons. This is in line with lower activation of MSNs—specifically, the D_1 receptor–containing subtype—observed in the NAc of HA animals during a social confrontation (25). Reduced excitatory input in D1-MSNs in mice has also been reported in chronic stress models of depression (33,34,98) and causally implicated in the motivational deficits induced by stress (32). Conversely, activation of excitatory projections to the NAc, such as the prefrontal cortex (101) or hippocampus (102), ameliorates the expression of depressive-like behaviors. However, in contrast to several studies in mice in which cell type–specific changes in dendritic morphology and molecular processes have been described (see above), reductions in MFN2 in our study were observed in both D1- and D2-MSNs. Future studies are warranted to investigate the contribution of MFN2 to neuronal structure and function in a cell type–specific manner. Additionally, it will be important to perform loss-of-function experiments to assess whether reducing MFN2 expression in either D1- or D2-MSNs mimics the behavioral and neuronal phenotypes observed in HA rats.

In conclusion, our findings demonstrate that MFN2-mediated mitochondrial and neuronal features are paramount in regulating anxiety and motivated behaviors, thus identifying novel roles of mitochondria in the regulation of brain function and behavior. Personality traits are behavioral predispositions that exist on a phenotypic continuum with psychiatric disorders (103). Thus, HA confers heightened risk for a broad spectrum of behavioral dysfunctions, including motivational deficits (20), competitiveness (21), and more broadly, depression (104), all of them relying on the NAc. Our study highlights accumbal MFN2 as a promising target to tackle anxiety manifestations and associated motivational deficits.

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ARTICLE INFORMATION

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