Mitochondria dysfunction occurs in the aging brain as well as in several neurodegenerative disorders and predisposes neuronal cells to enhanced sensitivity to neurotoxins. In particular, defects in any of the mitochondria respiratory chain complexes lead to impaired adenosine triphosphate production resulting in diseases that often affect the central nervous system. For instance, innate deficits in succinate dehydrogenase (SDH) mitochondria respiratory chain complex II deficiency are associated with variable levels of complex II deficiency in the central nervous system (Tünez et al., 2010). Also, chemically induced complex II deficiency leads to neurodegeneration.

Livestock and human poisoning by plants or fungi containing 3-nitropropionic acid (3-NP), a naturally occurring neurotoxin that irreversibly inhibits complex II SDH activity, leads to impaired mitochondrial bioenergetics, oxidative stress, and loss of adenosine triphosphate, triggering a cascade of intracellular events that ultimately result in neuronal cell death (Tünez et al., 2010). Interestingly, in all cases, whether genetic or chemically induced, despite similar reduction in SDH activity throughout the central nervous system, neuronal degeneration is restricted to the basal ganglia, with the striatum being particularly susceptible (Tünez et al., 2010; Jain-Ghai et al., 2013).

Even more intriguing, in rodents, 3-NP-induced striatal neurodegeneration depends on the strain background, suggesting that specific genetic variants can prevent 3-NP-induced neuronal cell death (Tünez et al., 2010). The likelihood that genetic factors play an essential role in disease susceptibility and resistance to 3-NP-induced striatal lesions is also suggested by the well-documented outbreaks of human poisonings with 3-NP that resulted in variable neurological involvement, with bilateral basal ganglia lesions observed in only about 50% of the cases (Perica et al., 1999). While differences in metabolism of 3-NP leading to variable basal ganglia toxicity could potentially explain the variable responses, the observation that 3-NP exposure in vitro leads to differential neuronal transcriptional responses depending on the strain (Teunissen et al., 2002) indicates that strain-specific genetic variants determine neuronal response despite similar levels of SDH inhibition. Likewise, the age of onset and range of neurological abnormalities in genetically inherited complex II deficiency vary extensively between individuals despite similar reductions in SDH complex II activity, with most patients displaying lesions in the basal ganglia in infancy while others display adult-onset cerebellar atrophy with no signs of damage to the basal ganglia (Jain-Ghai et al., 2013). Significantly, although the neurological findings vary between individuals, they are strikingly similar between family members (Jain-Ghai et al., 2013), implying that genetic factors modulate the age of onset and pathology caused by complex II deficiency.

Altogether, these observations raise two important questions that are still elusive: 1) why are striatal neurons particularly susceptible to complex II deficiency-induced neurodegeneration, and 2) what are the genetic mechanisms that confer striatal neuroprotection against complex II deficiency. In an effort to address these questions, we performed unbiased forward genetics analyses in mice and we found that striatal-specific alternative processing of Ccnd1 (the gene encoding cyclin D1) mRNA 3′ untranslated region (UTR) underlies sensitivity to 3-NP induced neurodegeneration (Dietrich et al., 2022).

Forward genetics using linkage analyses provides an unbiased strategy to screen for relevant susceptibility and resistance genes in vivo. Recombinant inbred strains, developed by crossing two inbred parental strains and sequentially mating the resulting siblings until they are 99% inbred, have been an invaluable resource for genetic mapping of Mendelian quantitative traits in the mouse over the past several decades (Ashbrook et al., 2021). Using a sub-acute regimen of 3-NP administration, we have found that while the widely used C57Bl/6J (B6) mouse strain is highly sensitive to 3-NP-induced striatal neuronal cell death, the DBA/2J (D2) strain is resistant, although striatal SDH activity is equally reduced in the brain in both strains. These observations indicated that genetic variants in D2 and B6 strains determined the differential neuronal response to SDH inhibition. To map the potential loci that confer resistance, we, therefore, used recombinant inbred strains derived from a cross (X) between the parental B6 and D2 strains, referred to as BXDs. By analyzing 18 randomly selected BXD strains, we have found that resistance to 3-NP is a highly heritable trait, that is, BXD strains would either be equally sensitive as their parental B6 strain or resistant as the parental D2 strain. This allowed us to map the resistance locus to the end of Chromosome 7, which contains 12 known genes, including Ccnd1, the gene that encodes cyclin D1. Haplotype analyses (gn2.genetwork.org) revealed that all the BXD strains carrying the D2 Ccnd1 variant were resistant to 3-NP-induced striatal neurodegeneration, while the BXD strains sensitive to 3-NP all carried the B6 Ccnd2 variant. Up-regulation of cyclin D1 is often observed in neurodegenerative disorders and is associated with aberrant neuronal cell cycle re-entry. In several neurodegenerative conditions, expression of cell cycle proteins has been shown to predict neuronal cell death, while inhibition of cell cycle re-entry conferred neuroprotection (Marlier et al., 2018; Nandakumar et al., 2021). With this in mind, we investigated cyclin D1 expression in both strains with or without 3-NP administration. Our analyses showed that although basal levels of Ccnd1 do not vary significantly between the two strains, 3-NP administration induced cyclin D1 in B6 striatal neurons but not in D2. Further analyses identified that cis-elements present in the B6 Ccnd1 allele lead to striatal-specific post-transcriptional alternative processing of the mid-to-distal part of the Ccnd1 mRNA regulatory 3′UTR. In silico analyses and literature search revealed that the portion of the 3′UTR that is specifically deleted in the striatum of the B6 strain contains highly conserved neuronal transcription factor binding elements. Alternative processing of this particular negative regulatory region has been well documented in the context of cancer where it has been shown that aberrant deletion of this region occurs in cancer cells and leads to increased Ccnd1 mRNA stability, increased cyclin D1 translation, and high rates of cell proliferation (Wang et al., 2018). Hence, it is likely that the predominant deletion of the mid-to-distal 3′UTR of Ccnd1 transcripts in B6 striatal neuronal cells underlies the increased cyclin D1 expression in response to 3-NP; and consequent cell cycle re-entry and cell death, while in D2 retention of this region prevents cyclin D1 induction and cell cycle re-entry, resulting in neuronal cell survival (Figure 1).
What triggers cell cycle re-entry in neurons and why cell cycle re-entry leads to neuronal apoptosis are still open questions in need of further investigation. One appealing hypothesis is that post-mitotic neurons re-enter the cell cycle as part of their DNA repair response. However, due to the low levels of DNA repair proteins in neurons, cell cycle responses are hindered and because DNA is not repaired the apoptotic pathway is activated (Marlier et al., 2020). Since one of the first and main intracellular effects of 3-NP is the generation of reactive oxygen species (ROS), which are known to cause oxidative DNA damage (Tünez et al., 2010), it is likely that the up-regulation of cyclin D1 observed in B6 striatal neurons represents the first step towards cell cycle re-entry in the attempt to repair DNA damage. However, due to low levels of DNA repair proteins in B6 neurons, neuronal apoptosis ensues (Figure 1). What happens then in D2, where cyclin D1 up-regulation is blunted? Although we did not detect any signs of neuronal cell death at day 5 after 3-NP administration, it is possible that not entering cell cycle leads to the accumulation of DNA damage and potentially delayed necrotic cell death (Figure 1), something that remains to be verified. The availability of BXD lines may also allow to answer another open question: is cell cycle re-entry necessarily bad for neurons? While endocycling (e.g. DNA replication without mitosis resulting in polyploid neurons) occur in many cell species, including humans, whether polyploidy confers neuroprotection or leads to neuronal senescence is still controversial (Marlier et al., 2020; Nandakumar et al., 2021). This controversy can potentially be addressed using BXD lines. So far, we have identified one BXD line in which cyclin D1 is upregulated (Ccnd1 derived from B6 parental strain) but there are no signs of neuronal cell death (Dietrich and Dragatsis, unpublished). The analyses of additional BXD lines harboring the B6 parental Ccnd1 allele will allow us to investigate the characteristics of surviving neurons, and to determine the mechanisms and genetic variant(s) that ensure their survival (Figure 1).

Regarding the distinct susceptibility of striatal neurons to complex II deficiency, the most accepted theory is that due to their high levels of dopamine, striatal neurons are particularly vulnerable to energy impairment and oxidative stress, and that secondary excitotoxicity due to cortical projections contributes to neurodegeneration (Tünez et al., 2010). Contrary to this hypothesis, our findings support the notion that instead, it is the crosstalk between genetic variants and striatal-specific mechanisms of mRNA processing leading to cell cycle re-entry that underlies striatal vulnerability.

Although our research focused on mechanisms of striatal resistance to 3-NP using a sub-acute regimen, our findings may also have implications for the pathology of other diseases, and in particular of Huntington’s disease (HD). HD is an autosomal dominant neurodegenerative disorder caused by expansion of a polyglutamine tract in the amino-terminal portion of the protein huntingtin, which is characterized by extensive striatal degeneration of medium spiny neurons, which comprise about 95% of striatal neurons (McCollan and Tabrizi, 2018). It is important to note that from the several genetic and chemical models of the disease that have been generated over the past decades, only 3-NP models closely recapitulate HD striatal loss and phenotypic manifestations, and therefore have been extensively used to investigate the mechanisms underlying HD (Tünez et al., 2010). The use of sub-acute 3-NP administration, as in our analyses (Dietrich et al., 2022), causes extensive loss of striatal neurons and interneurons in B6, while chronic administration of 3-NP at low doses for a period of 3 to 6 weeks results in a more specific loss of medium spiny neurons in HD (Tünez et al., 2010). 3-NP exposure however elicits similar cellular events and responses in both treatments (Tünez et al., 2010), suggesting that cell death is triggered by the same mechanisms in both 3-NP models. Significantly, complex II activity is severely reduced in HD brains and evidence of cell cycle re-entry is observed in HD patients’ striata (Tünez et al., 2010). Although the possible direct role of cyclin D1 and cell cycle re-entry in HD striatal neuronal cell death has not been investigated so far, increased expression of cell cycle-related genes has been shown to be positively associated with the severity of HD in mice (Langfelder et al., 2016). Since genetic variants are known to modulate the age-of-onset and the progression of striatal neuronal cell death in HD (McCollan and Tabrizi, 2018), it is possible that genetic polymorphisms in CCND1 and/or other genes involved in the cell cycle re-entry pathway might modulate HD neuropathology.

In conclusion, the use of BXD recombinant inbred strains allowed us to unravel the molecular bases of the selective 3-NP-induced neurotoxicity in mice. Further analyses of resistant and susceptible BXD strains using in vivo and in vitro approaches will allow us to address the open questions related to the fate of neurons in different scenarios. Since genetic variants involved in DNA repair and cell cycle re-entry also exist in the human population, further understanding of the mechanisms underlying neuronal survival in conditions of cellular stress will likely open new avenues for novel therapeutic strategies for different neurodegenerative disorders.

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