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Supplementary information S4: HSFs and brain function

HSFs play essential roles in brain function and development through the modulation of neuronal migration, the formation and maintenance of neuronal synapses and providing resistance to proteotoxic stress. HSF1 is needed to maintain a normal neuronal differentiation program, brain structure, and to establish and maintain synaptic fidelity for memory consolidation [1,2]. In the hippocampus the absence of HSF1 causes a decrease in the dendrite length of the dentate gyrus granule neurons and decreased dendritic spine density, resulting in reduced synapse formation. *Hsf1* $^{-/-}$ mice also show reduced dendrite length in pyramidal neurons of the *cornu ammonis* supporting the essential role of HSF1 in maintaining the structural integrity of hippocampal neurons [3]. In addition, the absence of HSF1 reduced neuronal progenitor proliferation, enhanced premature neuronal differentiation and caused increased anxiety and depression [3-5].

The loss of HSF1 also results in ataxia and other motor deficits, attributed to hippocampus, basal ganglia, cerebellar and hindlimb dysfunction as a consequence of astrogliosis and demyelination [6]. More recently, the motor deficits associated with an early stage of cerebellar ataxia have been linked to a role for HSF1 in regulating Ca^{2+} homeostasis through its activation of calbindin expression in cerebellar Purkinje cells [7]. HSF1 also plays a fundamental role in lipid raft formation [1,3,8,] which ensures proper post-synaptic consolidation that ultimately leads to the activation of memory receptors and long-term memory retention. HSF1 also activates expression of brain-derived neurotrophic factor (BDNF) [1], which contributes to the survival of peripheral and CNS

neurons and is an important regulator of synaptogenesis and synaptic plasticity mechanisms underlying learning and memory [9].

HSFs have important protective roles in response to stresses during brain development, prenatal exposure to stressful conditions and in neuropsychiatric disorders [10]. Although HSF1 activation is very robust in the embryonic brain, it is compromised in mature neurons in the adult brain [11]. Prenatal exposure to stressful conditions such as alcohol, methylmercury or maternal epileptic seizure induces HSF1 nuclear localization and binding to the HSP70 promoter in cortical cells, activating HSP70 expression [10]. Prenatal exposure to alcohol activates HSF1 in a manner that differs from that classically described for proteotoxic stress. While HSF1 hyper-phosphorylation is not detected in ethanol exposed embryos, reduced acetylation and SUMOylation are observed (see also Supplementary information S3 (box) for details on post-translational modifications of HSF1) [12]. When *Hsf1*$^{-/-}$ mice are exposed to similar prenatal stressful conditions, structural abnormalities appear in the cerebral cortex, associated with increased incidence of lepto-meningeal heterotopia in the frontal cortex. These alterations increase susceptibility to epilepsy and correlate with some features of schizophrenia [10].

Maternal alcohol consumption during pregnancy leads to Fetal Alcohol Syndrome (FAS) in a mechanism that is, in part, controlled by HSF2 [12]. Both HSF1 and HSF2 are activated in the brain cortex by chronic prenatal alcohol exposure, forming ethanol-induced HSF1-HSF2 heterotrimers that activate microtubule regulators controlling radial neuronal migration and neuronal positioning [10,12], [13]. *Hsf2*$^{-/-}$ mice exposed to prenatal alcohol

consumption revealed decreased neural abnormalities in the cortex and showed decreased HSF1 binding to its target genes, demonstrating the role of HSF2 in mediating defects that are characteristic of FAS [12].

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