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Windows of opportunity: timing in neurodevelopmental disorders

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Developmental processes disrupted in neurodevelopmental disorders occur rapidly and with temporal precision. During development, individual gene activity can dynamically engage different signaling networks; thus genetic mutations can lead to different functional changes at different times. Interpretation of phenotypes can be further complicated if initial functional changes trigger compensatory mechanisms. Examining genetic mouse models of neurodevelopmental disorders reveals cellular phenotypes that change over the course of development and exist long before behavioral deficits are assessed. Correspondingly, earlier genetic interventions in these disorder models have often been more effective at improving behavioral deficits than late interventions. The restricted period of effective intervention demonstrates that identifying a target window is an essential component of treatment.

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Introduction

Neurodevelopmental disorders such as autism arise from the disruption of developmental processes, which are under precise temporal control. To develop treatments for neurodevelopmental disorders, it is critical to consider the circuit-specificity and timing of these developmental processes. For example, neuronal proliferation and migration, axon guidance, synaptogenesis, and activity-dependent refinement each occur at distinct times in different circuits during development. Moreover initial functional changes can be closely followed by homeostatic mechanisms which may confound analysis [1]. Thus expression of initial defects may only be obvious during specific time-periods in different circuits. Recent work with monogenic mouse models has supported the importance of timing in neurodevelopmental disorders [2,3]. Although our understanding remains incomplete, new models enabling temporally and spatially specific manipulations of autism-associated genes, including of Mecp2, Syngap1, Ube3a, FMRP, and Shank3, have allowed investigation of disease progression and reversibility. Careful mapping of the emergence of disease-related phenotypes in these models has revealed early and sometimes transient phenotypes, such as altered timing of synaptic maturation. Critically, only early interventions are able to effect later changes in behavior in many genetic models, suggesting treatment timing may need to be closely linked to the timing of deficit emergence and the underlying affected developmental process.

Neurodevelopmental disorder progression highlights sensitive windows in development

Examining developmental trajectories in mouse models of neurodevelopmental disorders shows that functional changes emerge during distinct periods of development. Phenotypes can include a shift in developmental timing or change over time, for instance from synaptic hypoactivity to hyperactivity. This speaks to changing gene activity and function at different stages as well as involvement of homeostatic or compensatory mechanisms, highlighting the importance of carefully cataloging deficits across development. For instance, several mouse models of loss-of-function genes found in autism show changing deficits that appear during the narrow developmental window of active synapse formation and refinement. Expression of Syngap1, a synaptic RasGAP (SynGAP) largely found in dendritic spines, peaks at postnatal day 14 (P14) in the mouse hippocampus and at this time point heterozygous loss of Syngap1 results in prematurely slowed dendritic spine dynamics and increased mEPSC amplitude [4••]. However, earlier in development at P9 no deficits are observed and later in development at P21 normalization to wildtype has occurred [4••]. A similar accelerated development following Syngap1 loss is seen in layer 5 of barrel cortex at the level of spine formation and pruning [5]. A phenotype that changes across development is also present in the striatum following homozygous loss of Shank3, a scaffolding protein of the postsynaptic density. Initial characterization of adult *Shank3B* mutants reported decreased cortical–striatal drive [6]. However, during the period when cortico-striatal activity emerges and stabilizes, at P14, Shank3 loss leads to

increased cortical–striatal drive [7••]. As the cortico-striatal circuit continues to mature, cortico-striatal drive in mutants plateaus so that by P30, control and mutant animals have similar mEPSC frequency. By P60, wildtype drive overtakes mutant [7••] congruent with original observations of *decreased* mEPSC frequency in adult Shank3b mutants [6]. Similarly, deletion of FMRP1, a translational regulator of many synapse-associated mRNAs, leads to multiple transitory deficits [8] including a different trajectory of synaptic potentiation between P4 and P10 at the thalamocortical synapse that normalizes by P14 [9] and short-term synaptic deficits at the cortical layer 4 to 3 synapse [10]. Although some of these early changes can seem impermanent, they nonetheless have important consequences for activity-dependent formation and refinement of neural circuits [5] and thus can have long-lasting impact, presenting as later behavioral phenotypes.

When taking a developmental approach to neurodevelopmental disorders, it is also necessary to investigate in a circuit — specific way. In different neural circuits, the same mutation can lead to emergence of neurodevelopment deficits at different times. The timing of deficits may reflect the different timing of developmental events, such as activity dependent maturation, or temporal regulation of gene expression in individual circuits. For instance, refinement of thalamic inputs in the barrel cortex occurs between P0 and P4, while cortical layer 2/3 development occurs between P13 and P16 [11]. Correspondingly, mEPSC synaptic deficits in layer 4 barrel cortex are observed between P4 and P7 in *Syngap1* mutant mice [12]. By contrast in layer 2/3 medial prefrontal cortex at P14, synaptic deficits are absent despite being present in adult *Syngap1* mutants [13•] while in layer 2/3 barrel cortex, enlarged spines are already observed [5]. Depending on the circuit, cortical region, and layer, phenotypes appear at different times in the same *Syngap1* mutant.

Neurodevelopmental disorders arising from different genetic mutations follow different developmental progressions; not all exhibit peak changes during circuit formation as described above. A different developmental progression is seen following loss of Mecp2. Mecp2 is an X-linked gene and is thought to act broadly throughout the genome regulating transcription of many genes, especially neuronal ones [14]. Loss of Mecp2 in girls leads to Rett syndrome, which shares features with autism such as stereotyped hand movements, and milder Mecp2 mutations have been associated with multiple psychiatric disorders [15]. At neonatal ages, Mecp2 is expressed at low levels only reaching maximum expression around 5 weeks of age in mice [16]. Correspondingly, in mouse models development proceeds relatively normally for about the first three weeks followed by regression with difficulties developing in multiple domains including motor dysfunction as measured by rotarod test, walking on a grid and nest building, as well as impaired fear conditioning, learning and memory and other deficits by 5 weeks of age [15,17,18]. The progressive behavioral deterioration seen in this disorder is also seen at the biochemical level; as the disorder progresses, transcriptional misregulation increases in severity [14]. Human baby girls show a similar trajectory with relatively normal early development, including learning to walk, until 6–18 months of age, when both mental and motor regression begins [15].

Experiments that induce mutations during specific time windows also support the existence of sensitive but limited time frames that are particularly affected by gene loss. The periods sensitive to gene perturbation reflect the trajectories of gene expression and functional importance. For instance, removal of Mecp2 after 4 months of age initiates multiple neurological symptoms with a similar time course to that of the full knockout, including early mortality in males [19–21]. By contrast, adult removal of Syngap1 has little effect at either the synaptic or behavioral level, consistent with its role in early synapse formation and circuit development [13•]. Using these developmental approaches to establish periods when deficits emerge and when gene activity is required can help predict windows of opportunity for effective treatment.

Limited windows for functional rescue revealed through controlled gene expression

Recent technical advances in genetic manipulations have led to some of the clearest demonstrations of the temporal limits for intervening in neurodevelopmental disorders by achieving temporally controlled reintroduction of genes. We focus here on genetic rather than pharmacological strategies due to the difficulties in interpreting the actions of drugs on a mechanistic level, particularly due to the notoriously promiscuous nature of drug activity [22]. Furthermore, ongoing development of safer and more efficient viral vectors means that gene therapy is becoming an increasingly viable therapeutic option [23]. In the meantime, new mouse genetic strategies have enabled restoration of endogenous gene function using Cre-induced removal of floxed stop cassettes [4••,24,25••] or re-orientation of double-floxed inverted exons [26••]. Achieving endogenous patterns and levels of gene expression is advantageous, considering many brain-specific genes show strong dosage effects; many disorders result from both deletions and duplications, as is the case with Mecp2 [15]. Approaches capable of restoring endogenous levels of isoform-specific gene expression in the correct cell types will therefore provide the best opportunity to rescue the phenotypes seen in neurodevelopmental disorders with minimal side effects.

Investigations using such novel genetic approaches are still at an early stage and may yet reveal further