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SYMPOSIUM REPORT

The development of cortical columns: role of Fragile X mental retardation protein

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Neuronal circuits in the brain are complex and precise. Here, I review aspects of the development of cortical columns in the rodent barrel cortex, focusing on the anatomical and functional data describing the maturation of ascending glutamatergic circuits. Projections from layer 4 to layer 3 develop into cortical columns with little macroscopic refinement. Depriving animals of normal sensory experience induces long-term synaptic depression but does not perturb this pattern of development. Mouse models of mental retardation can help us understand the mechanisms of development of cortical columns. *Fmr1* knock-out (ko) mice, a model for Fragile X syndrome, lack Fragile X mental retardation protein (FMRP), a suppressor of translation present in synapses. Because FMRP expression is stimulated by neuronal activity, *Fmr1*-ko mice provide a model to survey the role of sensory input in brain development. Layer 4 to layer 3 projections are altered in multiple ways in the young mutant mice: connection rate is low and layer 4 cell axons are spatially diffuse. Sensory deprivation rescues the connection rate phenotype. The interaction of FMRP and neuronal activity in the development of cortical circuits is discussed.

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The barrel cortex is characterized by a remarkable columnar organization of neuronal circuits, each column corresponding to a whisker. Columns are readily identified in living tissue thanks to the presence of optically dense aggregates in layer 4, the barrels (Woolsey & Loos, 1970; Finnerty *et al.* 1999). Intracortical connections develop mostly during the first 2 postnatal weeks and the role of sensory experience can be tested by manipulating whiskers *in vivo*. It is an excellent model to study such fundamental questions as: How is the columnar pattern of cortical circuits formed? What is the role of sensory experience? Construction of sensory circuits pertains to different developmental programmes in the brain. It proceeds through an initial diffuse growth followed by pruning in retinocollicular (Brown *et al.* 2000) and retinogeniculate connections (Campbell & Shatz, 1992). It is more precise in intralaminar cortical circuits (Katz, 1991; Callaway & Lieber, 1996).

Studying the development of cortical networks in genetic models for mental retardation presents the dual possibility of finding circuit correlates for cognitive defects, and testing the contribution of a particular gene in development. Children with Fragile X syndrome have learning disabilities, seizures and, for some, autism (O'Donnell & Warren, 2002). This disease is due to the silencing of a single gene, *Fmr1*, located on the X chromosome (Verkerk *et al.* 1991; Jin & Warren, 2000). The phenotype of *Fmr1*-ko mice recapitulates some of the symptoms and includes learning deficits (The Dutch-Belgian Fragile X Consortium, 1994; Paradee *et al.* 1999) and audiogenic seizures (Musumeci *et al.* 2000; Chen & Toth, 2001).

Fragile X mental retardation protein (FMRP) is present in spines (Feng *et al.* 1997*b*; Weiler *et al.* 1997; Antar *et al.* 2004) and axons (Antar *et al.* 2006; Hengst *et al.* 2006). Its expression is regulated by sensory stimulation (Todd & Mack, 2000; Gabel *et al.* 2004; Tessier & Broadie, 2008). FMRP regulates translation through interactions with polyribosomes (Siomi *et al.* 1996; Feng *et al.* 1997*a*; Darnell *et al.* 2005) and mRNAs (Ashley *et al.* 1993; Siomi *et al.* 1993; Brown *et al.* 2001; Darnell *et al.* 2001). FMRP-mediated regulation of translation could

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therefore be part of an early response to synaptic input. The functions served by FMRP mRNA targets such as MAP1-B, PSD-95, α-CamKII and Sema3F (reviewed in Bassell & Warren, 2008) imply that FMRP regulates neuronal development and plasticity. Indeed, the best known cellular features of *Fmr1*-ko mice are an increased density of immature-looking spines, the filopodia (Comery *et al.* 1997; Irwin *et al.* 2001; Nimchinsky *et al.* 2001; McKinney *et al.* 2005; Antar *et al.* 2006; Grossman *et al.* 2006), and an enhanced protein synthesis-dependent long-term depression downstream of the activation of group 1 metabotropic glutamate receptors (mGluRs) (Huber *et al.* 2002; Koekkoek *et al.* 2005). Considering the wide spectrum of actions of FMRP, what is the net output for the construction of cortical circuits? Being in most cases a repressor of translation, FMRP could act as a brake in plasticity mechanisms (Bear *et al.* 2004) and one can hypothesize that the lack of FMRP will lead to their exaggeration. This makes the *Fmr1*-ko mouse an interesting tool to assess the impact of neuronal activity on the development of cortical networks.

Wiring diagrams can be depicted from the anatomy of neuronal projections. But, even if the overlap of an axon and a dendrite is a necessary step for forming a synapse, it is not necessarily sufficient. In addition, synaptic properties change throughout development and they are modified by sensory experience. Therefore, only the combination of anatomical and functional methods can provide a comprehensive view of the construction of networks. This short review synthesizes the current view on the development of projections from layer 4 barrels to the layer 3 pyramidal cells, a main ascending excitatory circuit in barrel cortex. It brings together anatomical and physiological data from *in vivo* and *in vitro* preparations to describe their normal and pathological development and the role of sensory experience. The projections are altered both functionally and morphologically in Fragile X mice suggesting an important role for FMRP in this process.

The development of cortical columns

Due to a series of technical developments in optics and photochemistry, the spatial organization of circuits can now be studied in brain slices using functional methods. Patterns of connections are mapped in a few minutes in brain slices with the technique of laser-scanning photo-stimulation (LSPS) (Callaway & Katz, 1993; Shepherd & Svoboda, 2005). This technique combines glutamate uncaging to stimulate neurons and patch recording to monitor synaptic activity: connectivity is surveyed through the detection of synaptic events. The functional mapping of excitatory connections in rat barrel cortex at different developmental stages revealed that ascending connections from layer 4 to layer 3 were organized in columns even at the earliest stage, when they were still growing (Bureau *et al.* 2004). Consistent with this observation, whole-cell patch clamp recordings in neonates *in vivo* showed that cells in layer 3 had sharp receptive fields (Bureau *et al.* 2004) even at a time when whisker-evoked responses were still weak and could rarely drive neurons to spike (Armstrong-James, 1975). The development of the anatomical connections from layer 4 to layer 3 is more in debate as the reconstruction of layer 4 cell axons filled with biocytin showed either an increase of correct targeting during the second postnatal week (Bender *et al.* 2003) or a mature pattern of innervations at all ages (Bureau *et al.* 2004). In all cases, pruning was never encountered.

Sensory experience plays a role in the development of intracortical connections because whisker clipping induced a long-term depression of synapses between layer 4 and layer 3 neurons (Allen *et al.* 2003; Bender *et al.* 2006). However, deprivation did not impair the formation of cortical columns because the growth of layer 4 cell axons (Bender *et al.* 2003) and the columnar pattern of functional connections (Shepherd *et al.* 2003) were intact. Together, these observations indicate that this intracortical circuit grows precisely into columns without going through a phase of large-scale refinement instructed by sensory inputs. What are the guiding mechanisms then?

The development of cortical circuits in Fragile X mice

Mapping intracortical connections with LSPS in *Fmr1*-ko mice revealed that pyramidal cells in layer 3 received weaker glutamatergic input from layer 4 (Bureau *et al.* 2008). This difference was attributed to a lower connection rate because the synaptic strength of individual connections was normal. The lack of FMRP reduced the connection rate within layer 4 microcircuits too, affecting the excitatory projections onto inhibitory neurons (Gibson *et al.* 2008). The development of cortical circuits is characterized by intense rearrangements during which spines form and dissolve rapidly (Lendvai *et al.* 2000). These lower connection rates in the mutant suggested that FMRP promoted the stabilization of synaptic contacts. A reduced connectivity rate was also described in cultured hippocampus (Braun & Segal, 2000; Antar *et al.* 2006; Hanson & Madison, 2007). In cerebellum, the lack of FMRP decreased the early multi-innervations of Purkinje cells by climbing fibres (Koekkoek *et al.* 2005). The clever use of *Fmr1* mosaic mutant, lacking FMRP in subpopulations of neurons only, demonstrated that the reduced connection rate originated from the mutation in the presynaptic neurons in hippocampus (Hanson & Madison, 2007). Interestingly, the rescue of FMRP expression in mutant hippocampal neurons also decreased the connectivity by a mechanism sitting in the

postsynaptic neurons (Pfeiffer & Huber, 2007). It is still an open question whether, in the barrel cortex, connectivity between layer 4 and layer 3 is controlled from the presynaptic or the postsynaptic side.

Ample evidence shows that neurons possess more filopodia in the mutant but there is less indication that the lack of FMRP affects the morphology of axons (Antar *et al.* 2006). In the *Fmr1* mutant *Drosophila*, axons of the mushroom body failed to stop at the midline (Michel *et al.* 2004; Pan *et al.* 2004). In the barrel cortex, the axonal arbors of layer 4 cells grew more spatially diffuse (Bureau *et al.* 2008). This finding naturally prompted the hypothesis that it was a developmental delay. But the overall length of layer 4 cell axons was normal indicating that this phenotype was not one of arrested maturation but instead indicated a defect in mechanisms for guiding or stabilizing axons. Identifying the mRNA targets of FMRP in axons could help our understanding of the mechanisms controlling the growth of cortical circuits into columns.

Can we reconcile the morphology and physiology of cortical neurons in the mutant?

The comparison of the anatomical and functional data in *Fmr1*-ko mouse barrel cortex raises some questions. First, are the connectivity and axon phenotypes merely concurrent or causally related? Even if the geometry of the layer 4 cell axons was changed it was not to the point of outreaching the span of dendrites of their target cells: layer 4 and layer 3 cells still overlapped (Supplemental materials in Bureau *et al.* 2008). This argues for two independent phenotypes. Rescue experiments may be able to resolve this more definitively. The strategy could include the elegant approach carried out by Bear and collaborators who genetically diminished the expression of mGluR5 in *Fmr1*-ko mice and rescued an impressive number of phenotypes ranging from the level of filopodia to ocular dominance plasticity and behaviour (Dölen *et al.* 2007). Today, whether the morphology of cortical axons is regulated by protein synthesis downstream of mGluRs is unknown. However, mGluR5 stimulates connectivity in 2-week-old neocortex (Le Bé & Markram, 2006).

Second, layer 4 cell axons in the mutant crossed the barrel boundaries to innervate neighbouring columns. But there was no evidence that these incorrect innervations made functional synaptic contacts. Were they silent synapses? It is striking to note that higher NMDA/AMPA ratios, commonly taken as signs of synaptic immaturity, were not detected in structures where an overabundance of filopodia was described (Huber *et al.* 2002; Desai *et al.* 2006; Pfeiffer & Huber, 2007; Hu *et al.* 2008). Again, this challenges the hypothesis that the *Fmr1*-ko phenotype is the expression of a developmental delay. The functional characterization of filopodia in the mutant could shed some light on this paradox.

FMRP participates in activity-dependent developmental programmes

FMRP expression and targeting to synapses are stimulated by the activation of mGluRs and NMDA receptors (Todd *et al.* 2003; Antar *et al.* 2004; Gabel *et al.* 2004; Ferrari *et al.* 2007). In barrel cortex, the expression of FMRP is driven up by whisker stimulation (Todd & Mack, 2000). One can therefore speculate that the lack of FMRP will affect cortical circuits under specific states of sensory input. In barrel cortex, the low connection rate was specific to the ascending projections in the barrel columns in mutants. Outside in septum-related columns, which are points of convergence of uncorrelated inputs from different whiskers, the same projections appeared normal. The impact of neuronal activity on *Fmr1*-ko phenotype was more directly tested with sensory deprivation and enrichment. The connection rate in the mutant barrel columns was restored to wild-type level after whisker clipping (Bureau *et al.* 2008). This restoration coincided with a long-term depression of synaptic transmission at these synapses. The disappearance of the connectivity phenotype could reflect the activity-dependency of FMRP function. Alternatively, the rescue could result from homeostasis, balancing connection rate with synaptic strength. Other examples of interference of neuronal activity and *Fmr1*-ko phenotypes were described. Impairing synaptic plasticity through the genetic down-regulation of mGluR5 rescued the accumulation of filopodia in visual cortex and restored normal ocular dominance plasticity (Dölen *et al.* 2007). In *Drosophila*, axon pruning controlled by FMRP required sensory stimulation and neuronal activity (Tessier & Broadie, 2008). It is interesting to note that environmental stimulation can also alleviate *Fmr1*-ko phenotypes. Indeed, enrichment rescued the spine morphology in *Fmr1*-ko mouse visual cortex and suggested the induction of an FMRP-independent form of structural plasticity (Restivo *et al.* 2005).

Conclusion

Investigating the neuronal defects in developmental diseases such as Fragile X and describing their molecular mechanisms will help us understand the rules for the construction of cortical circuitry. An important area for the future is to try to link the circuit phenotypes with behavioural phenotypes to gain more insight into how circuits subserve specific functions, and to explore the importance of accuracy in circuit development.

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