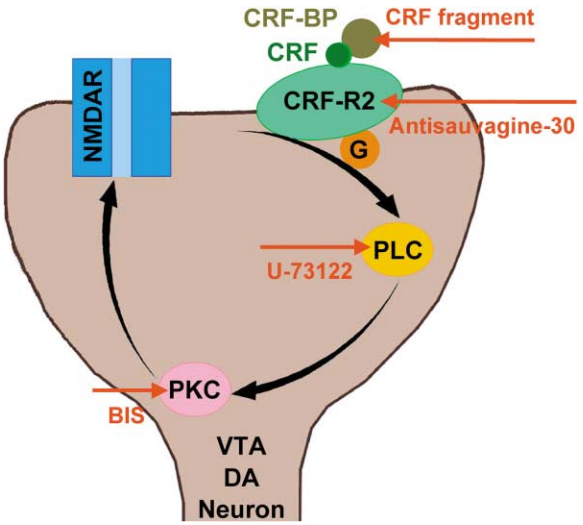

Figure 1. A Didactic Representation of the Action Initiated by Corticotropin-Releasing Factor at a Glutamatergic Postsynaptic Bouton on a VTA DA Neuron

Corticotropin-releasing factor (CRF) interacts with CRF binding protein (CRF-BP) to activate the G protein-linked CRF receptor-2 (CRF-R2). Subsequently, phospholipase C (PLC), and in turn, protein kinase C (PKC), are activated. This activity ultimately increases the current mediated by the NMDAR component of glutamatergic synaptic transmission. Inhibition of the various components is shown by labeled red arrows, with BIS representing Bisindolyeamide 1.

nent in a midbrain slice preparation. It is not obvious how the two finds can be reconciled when considering the in vivo consequences of stress. Until further research is completed, we cannot be certain that CRF potentiation of the NMDAR-mediated EPSCs has a biological role. There is, however, a speculative explanation that unifies the findings.

During the stress response, CRF is released early along the hypothalamic-pituitary-adrenal axis, and it is also released from extrahypothalamic neurons that could innervate the midbrain. In that way, the early arrival of CRF could transiently increase the NMDAR component of excitatory transmission as described by Ungless et al. (2003). This temporary increase could take place in a matter of seconds to minutes via nongenomic processes. Because LTP at glutamatergic synapses onto VTA DA neurons requires a sufficient calcium signal mediated by NMDARs, the elevated NMDAR component caused by CRF makes these synapses more amenable to LTP induction. Thus, when glucocorticoids arrive (within the stress response), they can more easily contribute to LTP and cause the insertion of AMPARs. Under this theory, stress boosts both components of the glutamatergic excitation of DA neurons, but the increased NMDAR component is temporary. For society, possibly the most important issue is stress-induced drug cravings that can cause relapse even after years of abstinence. The final answers must await further research, but this interesting study suggests pathways of investigation in the link between stress and addictive drug use.

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Selected Reading

Carrasco, G.A., and Van de Kar, L.D. (2003). Eur. J. Pharmacol. *463*, 235–272.

Doherty, M.D., and Gratton, A. (1997). Synapse *26*, 225–234.

Goeders, N.E. (2002). J. Pharmacol. Exp. Ther. *301*, 785–789.

Lewis, K., Li, C., Perrin, M.H., Blount, A., Kunitake, K., Donaldson, C., Vaughan, J., Reyes, T.M., Gulyas, J., Fischer, W., et al. (2001). Proc. Natl. Acad. Sci. USA *98*, 7570–7575.

Linton, E.A., Behan, D.P., Saphier, P.W., and Lowry, P.J. (1990). J. Clin. Endocrinol. Metab. *70*, 1574–1580.

Malinow, R., and Malenka, R.C. (2002). Annu. Rev. Neurosci. *25*, 103–126.

Saal, D., Dong, Y., Bonci, A., and Malenka, R.C. (2003). Neuron *37*, 577–582.

Thierry, A.M., Tassin, J.P., Blanc, G., and Glowinski, J. (1976). Nature *263*, 242–244.

Tsigos, C., and Chrousos, G.P. (2002). J. Psychosom. Res. *53*, 865–871.

Ungless, M.A., Whistler, J.L., Malenka, R.C., and Bonci, A. (2001). Nature *411*, 583–587.

Ungless, M.A., Singh, V., Crowder, T.L., Yaka, R., Ron, D., and Bonci, A. (2003). Neuron *39*, this issue, 401–407.

Thalamocortical Topography Reloaded: It's Not Where You Go, but How You Get There

When looking for the origin of topography within the thalamocortical system, attention has largely focused either on the target (cortex) or on the projecting neurons (thalamus). Two papers in this issue of *Neuron* (Dufour et al., 2003; Seibt et al., 2003) point to an intermediate target, the ventral telencephalon, as the key region where thalamocortical topographic mapping is established, and to the ephrin-A/EphA signaling system as the molecular mediator of this process.

The orderly growth of axons to form topographic connections between distant structures is one of the most fascinating features of the vertebrate nervous system. The retinotectal system is perhaps the best-studied model for dissecting the mechanisms controlling the early development of topographic specificity. In this system, retinal axons from the most nasal part of the retina project to the most posterior region of the tectum, whereas axons from progressively more temporal locations in the retina project to progressively more anterior regions of the tectum. Sperry's elegant experiments strongly suggested that axon-target detection in the retinotectal system requires the recognition of specific chemical labels in the tectum (Sperry, 1963). In other words, the establishment of topographic connections

in the retinotectal system relies on information located in the projecting and targeted structures.

The identification of the Eph receptor tyrosine kinases and their ligands, the ephrins, as Sperry's "recognition molecules" has provided further insights into the molecular mechanisms underlying the formation of topographic projections in the retinotectal system (Cheng et al., 1995; Drescher et al., 1995). Briefly, in the retinotectal system, EphA receptors and their ephrin-A ligands are distributed in complementary gradients in the retina and tectum, and binding of ephrins to their receptors inhibits axonal growth. Consequently, axons expressing high levels of EphA receptors map to the region of the tectum with lower ephrin-A protein levels, while axons with low EphA expression project to the region of the tectum with the higher concentration of ephrin-A ligands (Wilkinson, 2001). The experiments performed in the retinotectal system support Sperry's initial suggestion that topographic mapping requires a molecular dialog between the projecting and targeted structure. Now, is this essential for the establishment of all topographic maps in the brain?

The development of topographic connections between the dorsal thalamus and the largest region of the mammalian cortex, the six-layered isocortex (i.e., neocortex), has been also extensively studied because of their prominent functional role in processing sensory and motor information. Thalamocortical projections display two levels of topographic organization. First, specific dorsal thalamic nuclei, which relay distinct modalities of sensory (e.g., visual, somatosensory, auditory) or motor information, project to specific neocortical regions (inter-areal topography). For example, the visual thalamus (dorsal lateral geniculate nucleus, dLGN) projects to the occipital cortex (visual cortical region), whereas the motor thalamus (ventromedial nucleus, VM) projects to the frontal cortex (motor cortical region). In rodents, these thalamic nuclei are roughly arranged following a caudolateral to rostromedial gradient within the dorsal thalamus, whereas their corresponding targets are found in caudorostral progression in the cortex (Figure 1). Such inter-areal topography arises early during embryonic development and appears to be largely independent of functional activity within the thalamocortical radiation. The second level of topographic organization corresponds to the projections that each thalamic nucleus sends to a specific cortical region. For example, projections from the somatosensory thalamus (ventrobasal complex, VB) create a topographic representation of the somatosensory information derived from different parts of the body within the somatosensory cortex (i.e., a somatotopic map). This second level of topographic mapping emerges later in development than inter-areal topography, and it is refined during the first postnatal weeks, partly through activity-dependent mechanisms (López-Bendito and Molnar, 2003).

Influenced by the findings of the retinotectal system, the prevailing model for the establishment of topographic connections in the thalamocortical system proposes that the expression of localized cues within the cortex controls the targeting of thalamic axons. According to this model, projection neurons from each thalamic nucleus (i.e., visual, somatosensory, auditory) would specifically recognize information from their corresponding cortical region to establish orderly thalamocortical connections. The specific cues that provide this targeting information would be located in the cortex itself or, alternatively, in the reciprocal corticothalamic axons that grow toward the thalamus (Molnár and Blakemore, 1995). An alternative hypothesis to this model was proposed recently, when Garel et al. (2002) described that, unexpectedly, the establishment of topographic thalamocortical projections is not strictly determined by cues located in the cortex, but instead is influenced by the relative position of thalamic axons inside the ventral telencephalon. In short, the topographic organization that thalamic axons adopt in the cortex is already set up upon their entry in the telencephalon, and any alteration of their topography in this intermediate target results in a parallel perturbation of their topography in the cortex. In other words, cues located in an intermediate target of the thalamic projections, the ventral telencephalon, are largely responsible for imposing the topographic organization on thalamocortical projections.

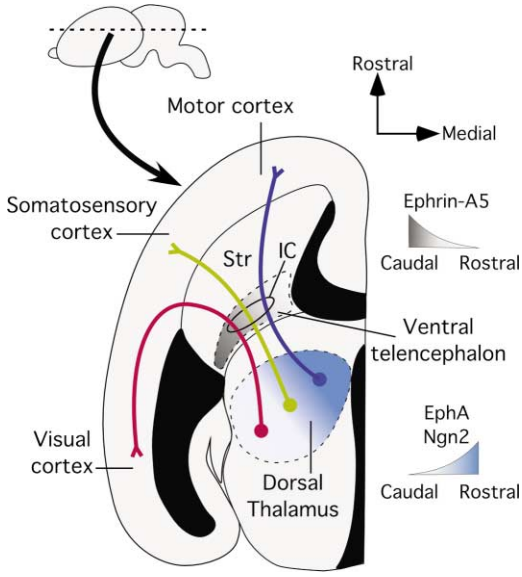

Figure 1. A Schematic Representation of the Organization of Thalamocortical Projections and the Role of *Ngn2* and EphA/ephrin-A Protein Interactions in This Process

The topographic organization of thalamocortical projections is depicted in a schema of a horizontal hemi-section of the embryonic forebrain. Dorsal thalamic projections following a developmental caudolateral to rostromedial gradient target the cortex in a caudorostral direction. The topographic organization of these projections takes place at the entrance of the ventral telencephalon, where a gradient of ephrin-A ligands matches the distribution of EphA receptors in dorsal thalamic axons. *Ngn2* is also expressed in the dorsal thalamus in a similar gradient to the EphA receptors.
IC, internal capsule; Str, striatum.

In this issue of *Neuron*, two studies corroborate the hypothesis that the development of inter-areal topography within the thalamocortical system occurs through the sorting of thalamocortical axons in the ventral telencephalon (Dufour et al., 2003; Seibt et al., 2003). In addition, these studies elegantly shed light on the transcriptional machinery behind the selection of specific targets by distinct thalamic nuclei and identify candidate molecules controlling the topographic organization of thala-

mocortical axons within the ventral telencephalon. These molecules turn out to be the usual suspects in topographic mapping—the ephrins.

Transcriptional Control of Topographic Thalamocortical Projections

Since each dorsal thalamic nucleus projects to a specific region of the isocortex, dorsal thalamic neurons are able to distinguish cues provided by the target structure in a cell-autonomous manner. To get insight into the mechanisms controlling this process, in this issue of *Neuron*, Seibt et al. (2003) analyzed the role of the bHLH transcription factor *Neurogenin2* (*Ngn2*) in specifying the targeting of thalamic projections to specific regions of the cortex. *Ngn2* is regionally expressed in the developing dorsal thalamus in a rostral-high to caudal-low gradient, both in progenitor cells and in postmitotic neurons. Using heterozygous mice from a transgenic line where IRES-EGFP has been knocked in the endogenous *Ngn2* gene (*Ngn2*$^{KIGFP/+}$), the authors determined that GFP (*Ngn2*) is preferentially expressed in thalamic neurons projecting to rostral cortical areas. These neurons belong to several rostromedial thalamic groups, such as the anterior group, the ventrolateral nucleus (VL), and the VM. Analysis of *Ngn2* mutants at late embryonic stages showed that *Ngn2* is required to specify the projection of rostral thalamic neurons to the rostral cortex. Specifically, the projection of the rostral region of the dorsal thalamus to the cortex is caudally shifted in the absence of *Ngn2*. Remarkably, this caudal shift is already present in the ventral telencephalon, reinforcing the notion that the organization of thalamocortical projections in this intermediate target dictates their final mapping in the cortex (see also Garel et al., 2002; Dufour et al., 2003). Thus, *Ngn2* controls the projection of rostral thalamic neurons to rostral territories of the ventral telencephalon and, consequently, to the rostral cortex.

One caveat for this conclusion is that *Ngn2* is not only expressed in the rostral thalamus, but also in the ventral telencephalon and in the cortex. Given this, it is reasonable to argue that defects in the cortex of *Ngn2* mutants could be the basis of the perturbation of the topographic mapping found in the thalamocortical system. Indeed, a similar weakness is present in the analysis of other mutant mice where defects in thalamocortical topography were previously ascribed to the ventral telencephalon (Garel et al., 2002). To solve this problem, Seibt et al. (2003) set up a sort of "open-book preparation" for the telencephalic vesicle, which they named "telencephalic whole-mount" assay. This elegant assay represents a significant development in the analysis of thalamocortical projections, because it allows a fairly complete two-dimensional visualization of thalamocortical axons pathfinding in vitro when the telencephalic vesicle is cocultured with a dorsal thalamic explant. The beauty of the assay is that depending on the source of the telencephalic preparation and thalamic explant (wild-type or mutant), it can be determined whether the defect observed in thalamocortical pathfinding is cell autonomous or not. In addition, because the telencephalic preparation includes the whole vesicle, all territories normally traversed by thalamic axons, and not just the final cortical targets, are represented in the assay. Using this assay, the authors determined that different rostrocaudal levels of the developing dorsal thalamus respond differentially to cues located in the ventral telencephalon. Moreover, they unequivocally demonstrate that *Ngn2* cell-autonomously specifies the projection of thalamic neurons to frontal cortical areas.

At what level does *Ngn2* control the specificity of rostral thalamic projections to the cortex? The authors suggest that loss of *Ngn2* does not modify the molecular identity of the dorsal thalamus, supporting the view that specification of dorsal thalamic neurons is not altered, and therefore *Ngn2* most likely influences rostral thalamic pathfinding by directly or indirectly controlling the expression of guidance receptors in this set of neurons. While it is possible that the molecular identity of the dorsal thalamus as a whole is maintained in *Ngn2* mutants (note, however, that *Dlx1* seems to be ectopically expressed in the ventricular zone of the dorsal thalamus of *Ngn2* mutants at E15.5; Figure 7L in Seibt et al., 2003), it remains to be tested if the molecular identity of the rostral thalamus is specifically altered in the absence of *Ngn2*.

ephrins Organize the Topography of Thalamocortical Axons in the Ventral Telencephalon

Several lines of evidence support a model in which the early topographic sorting of thalamocortical axons in the ventral telencephalon determines their final topographic mapping in the cortex. What are the molecules that control this process? To address this question, Dufour et al. (2003) (this issue of *Neuron*) analyzed the expression of ephrin-A ligands and their EphA receptors in the embryonic forebrain at the time thalamocortical axons first invade the telencephalon. Remarkably, ephrin-A5 is expressed in a rostral-low to caudal-high gradient within the ventral telencephalon, whereas EphA3, EphA4, and EphA7, three receptors that bind ephrin-A ligands with high affinity, are expressed in a complementary gradient within the dorsal thalamus, rostromedial-high to caudolateral-low. This matching expression pattern is consistent with the hypothesis that rostral thalamic axons navigate through the ventral telencephalon in a relatively rostral position in relation to caudal thalamic axons by a mechanism involving ephrin-A5-mediated repulsion (Figure 1). To test this hypothesis, the authors used the telencephalic whole-mount assay to study the behavior of rostral thalamic axons when a soluble Eph receptor extracellular domain protein (EphA3-Fc) is added to the culture medium to inhibit the function of endogenous ephrin-A ligands. Under these conditions, rostral thalamic neurons lose their preference to navigate rostrally through the ventral telencephalon. Moreover, wild-type rostral thalamic axons are caudally shifted in the ventral telencephalon when an ephrin-A5 mutant telencephalon is used in the coculture experiments. This series of experiments elegantly demonstrates in vitro that interactions between ephrin-A ligands and EphA receptors are required to establish the initial topographic arrangement of thalamocortical connections as the axons enter the ventral telencephalon, at least for the rostral thalamus.

To determine if this mechanism plays a significant role in the topographic mapping of thalamocortical axons in vivo, the authors studied the organization of thalamocortical connections in ephrin-A/EphA mutant mice. Analysis of postnatal ephrin-A5/EphA4 double mutants revealed a caudal shift in the topography of thalamocor-

tical pathfinding. Thus, axons from the VL, which normally project to the rostral cortex (motor cortex), target instead the parietal cortex (somatosensory cortex; caudal to the motor cortex) in the absence of ephrin-A5 and EphA4. This defect is observed with a low frequency in ephrin-A5 (33%), EphA4 (12.5%), and EphA7 (50%) single mutants, supporting the view that several EphA receptors and ephrin-A ligands are involved in vivo in setting up the topographic mapping of thalamocortical axons. Finally, analysis of thalamocortical connectivity in ephrin-A5/EphA4 double mutants at the time when thalamic axons are invading the ventral telencephalon showed that the topographic sorting of thalamocortical axons is already shifted in this intermediate region—additional evidence supporting the role of the ventral telencephalon in this process.

ephrins Contribute to the Formation of the Cortical Somatotopic Map

In addition to their role in establishing the topographic organization of thalamic projections to distinct cortical regions, Dufour et al. (2003) investigated whether ephrin-A/EphA interactions are required for topographic mapping within an individual cortical area. Ephrins have been previously shown to play a role in the formation of other intra-areal topographic maps (Wilkinson, 2001), but their implication in cortical mapping has remained elusive. Projections from the VB to the barrel field of the somatosensory cortex are less precisely organized in ephrinA5/EphA4 double mutants than in control mice, suggesting that the topographic arrangement of somatosensory projections from the dorsal thalamus to the cortex is compromised in the absence of normal ephrin-A/EphA signaling. These results are consistent with the postnatal pattern of expression of ephrin-A5 and EphA4 in the cortex and dorsal thalamus, respectively. Thus, although additional experiments need to be performed to extend these observations to other cortical regions, another important message of this paper is that the same set of mapping labels is used differentially for the generation of topographic maps among different thalamocortical projections (inter-areal topography) and within individual cortical regions (intra-areal topography).

Open Questions

The recent findings by the Polleux and Vanderhaeghen groups have opened new venues for understanding how thalamocortical mapping is established. For example, several questions are raised regarding the role of *Ngn2* in specifying rostral thalamic axon responsiveness to ventral telencephalic cues. What are the downstream effectors of *Ngn2* in the dorsal thalamus? Is *Ngn2* sufficient to direct connections from any dorsal thalamic nucleus toward the rostral cortex? What factor(s) prevent the expression of *Ngn2* in the caudolateral dorsal thalamus, thus enabling this region to project toward the caudal cortex? The identification of ephrin-A ligands as mediators of the topographic mapping of thalamocortical axons, along with the high expression of EphA receptors in the rostral thalamus, would suggest that *Ngn2* directly or indirectly induces the expression of these receptors in the thalamus. Consistent with this hypothesis, Kania and Jessell (2003) have recently shown that the establishment of topographic projections between spinal cord motor neurons and the limb mesenchyme is controlled, in part, through LIM homeodomain protein control of the expression of EphA receptor and ephrin-A ligands in motor neurons and limb mesenchymal cells, respectively. It will be of interest to see whether a bHLH transcription factor can also control the expression of EphA receptors.

Another intriguing issue derived from these studies is the mechanism guiding corticothalamic axons. Is the topography of corticothalamic projections also controlled by the ventral telencephalon or by the reciprocal thalamocortical axons? Additional studies are required to reevaluate previous models on the light of the recent findings. Finally, it is clear that there is much work to be done on the field of intra-areal topography in the cortex; future studies should be directed to increase our knowledge on the role of ephrins in this process as well as to test the potential role of other guidance cues.

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Selected Reading

Cheng, H.J., Nakamoto, M., Bergemann, A.D., and Flanagan, J.G. (1995). Cell *82*, 371–381.

Drescher, U., Kremoser, C., Handwerker, C., Loschinger, J., Noda, M., and Bonhoeffer, F. (1995). Cell *82*, 359–370.

Dufour, A., Seibt, J., Passante, L., Depaepe, V., Ciossek, T., Frisén, J., Kullander, K., Flanagan, J.G., Polleux, F., and Vanderhaeghen, P. (2003). Neuron *39*, this issue, 453–465.

Garel, S., Yun, K., Grosschedl, R., and Rubenstein, J.L. (2002). Development *129*, 5621–5634.

Kania, A., and Jessell, T.M. (2003). Neuron *38*, 581–596.

López-Bendito, G., and Molnar, Z. (2003). Nat. Rev. Neurosci. *4*, 276–289.

Molnár, Z., and Blakemore, C. (1995). Trends Neurosci. *18*, 389–397.

Seibt, J., Schuurmans, C., Gradwhol, G., Dehay, C., Vanderhaeghen, P., Guillemot, F., and Polleux, F. (2003). Neuron *39*, this issue, 439–452.

Sperry, R.W. (1963). Proc. Natl. Acad. Sci. USA *50*, 703–710.

Wilkinson, D.G. (2001). Nat. Rev. Neurosci. *2*, 155–164.

"Resistant" Channels Reluctantly Reveal Their Roles

Presynaptic calcium influx is mediated by a variety of different calcium channel subtypes with distinct pharmacological and biophysical properties. In this issue of *Neuron*, Dietrich et al. show that although $Ca_V2.3$ calcium channels do not contribute to fast transmitter release at hippocampal mossy fiber synapses, they play a specialized role in induction of multiple presynaptic forms of synaptic plasticity.