uleus norepinephrine neurons do not contain NPS receptor mRNA. Indeed, there appears to be a cluster of NPS-expressing neurons in the pons that do not produce either norepinephrine or corticotropin-releasing factor but that are located in-between the locus coeruleus (norepinephrine) and Barrington's nucleus (corticotropin-releasing factor). NPS cells located in the pons, where they are most abundant, could be involved in an arousal projection from the pons to rostral areas of the brain, including the cortex, amygdala, and thalamus, where its cognate receptor is localized (see Figure 1). Thus, the peri-locus coeruleus NPS neuronal system could be a novel and important component of a cortical arousal system that has long been hypothesized to be part of the reticular activating system (Moruzzi and Magoun, 1949).

In summary, the novel neuropeptide NPS has been localized in the brain in areas that are relevant for arousal and wakefulness and at the same time presents with a profile of an anxiolytic in animal models of anxiety. This contrasts with its pontine neighbors norepinephrine (which increases arousal but can have stress-like effects), hypocretin/orexin A (which increases arousal and has little stress-like effects but may have aversive effects), and corticotropin-releasing factor (which increases arousal and has stress-like and aversive effects). Such a symphony of arousal-activating neuropeptides located in the pons may provide insight into the regulation of arousal and wakefulness, and NPS appears to be a new key player.

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RNA Transport (Partly) Revealed!

Specific mRNAs are transported to dendrites where their translation may modify synaptic plasticity. In this issue of *Neuron*, Kanai et al. use affinity chromatography and mass spectrometry to identify a large number of new factors that associate with kinesin, a molecular motor, and employ siRNA technology to demonstrate their importance for RNA transport in neurons.

Often times, cells concentrate certain proteins in particular regions so that the metabolic events that they catalyze occur only locally. How they accomplish this task depends on the cell and the protein, but consider three general possibilities: make the protein everywhere but destroy it where it is not needed, distribute mRNA encoding the protein everywhere but translate it only locally, or transport the mRNA in a silent form to the place where it is to be translated. The transport of silent mRNA would seem to be especially complicated, as it would require three sets of machinery: that needed for moving the cargo, for keeping the RNA silent while it is being moved, and for activating the mRNA once it arrives at its destination. In neurons, a variety of mRNAs are transported into dendrites, quite possibly in a quiescent state; the products of these mRNAs, which are thought to be synthesized at or near activated synapses, may then modify synaptic plasticity (Martin et al., 2000). These working hypotheses are based mostly on reporter RNA assays and/or the application of protein synthesis inhibitors to brain slices or neurons in culture, which have been very useful in identifying *cis* elements that direct mRNAs to dendrites as well as for demonstrating the importance of *some* newly synthesized protein(s) in plasticity. However, without loss-of-function type experiments (i.e., gene knockouts or RNAi knockdowns, e.g., Eom et al., 2003; Huang et al., 2004), the information they yield regarding the factors responsible for the transportation process, the mechanism(s) responsible for activity-dependent translation, or the identity and function of newly synthesized protein in synaptic plasticity is limited. One example where combined experimental approaches have been particularly fruitful is CaMKIIα mRNA, which is transported to dendrites, translated in the synaptodendritic compartment in an activity-dependent manner, and whose protein product modifies synaptic plasticity (Ouyang et al., 1999; Mayford et al., 1996; Miller et al., 2002; Silva et al., 1992). But even in the case of CamKIIα mRNA, very few factors that direct its transport to dendrites are known; our knowledge seems to be even more rudimentary when considering that translational repression and activation are part of the larger picture of mRNA regulation in dendrites (but see Huang et al., 2004). In an experimental tour de force published in this issue of *Neuron*, Kanai et al. (2004) now take a very large step not only in identifying the panoply of factors that *could* mediate mRNA transport in neurons, but in *demonstrating* their functional importance for this process. Their data, in conjunction with those of others, also have wider implications for translational control in neurons.

Neurons contain several isoforms of kinesin (KIFs), the molecular motor that directs cargos to the plus ends of microtubules. Because the microtubules are arrayed with their plus ends extending into dendrites, the kinesins have long been thought to direct RNA-containing cargoes, which appear granular or particulate in nature,

into these processes. Kanai et al. constructed affinity columns composed of various regions of one kinesin isoform, KIF5, fused to GST and then applied extracts derived from the mouse brain to them. One particular region of KIF5, a 59 amino acid "minimal binding sequence" in the tail (as opposed to the motor domain) interacted with up to 42 proteins, which were identified by mass spectrometry or immunoblotting. These proteins include several RNA helicases (e.g., DDX1, vasa), hnRNP proteins (e.g., hnRNP U, hnRNP A1), factors involved in general protein synthesis (e.g., EF-1α, eIF2α, eIF2β), factors perhaps involved in specific mRNA translation (e.g., FMR1, FXR1), and not surprisingly, one factor already thought to be involved in RNA transport (staufen) (Kiebler et al., 1999; Tang et al., 2001). The authors show that the proteins reside in huge (up to $\sim$1000 S) complexes, together with the mRNAs encoding CamKIIα and ARC (activity regulated cytoskeleton-associated protein). At least based on size and their inclusion of staufen, eIF2α, and CaMKIIα mRNA, these complexes may be analogous to those identified by Krichevsky and Kosik (2001), who isolated huge RNP particles from polysome sucrose gradients of rat brain extract. Ultrastructural analysis of the Krichevsky and Kosik particles showed that they contained ribosomes, but because the initiation factors eIF4E and eIF4G were not detected, they assumed that the particles were not engaged in mRNA translation. With the exception of one ribosomal protein (L3), Kanai et al. surprisingly did not detect any other component of the ribosome. Perhaps a possible association of ribosomes with KIF5 is too indirect or weak to be retained on an affinity column.

Kanai et al. next obtained or generated antibody against many of the proteins and performed an extensive series of coimmunoprecipitation experiments and found that RNA was necessary for most of the proteins to reside in the complex. Thus, many of the interactions are probably indirect and occur through RNA intermediates. More interesting, immunocytochemical analysis of cultured mouse hippocampal neurons revealed that many of the proteins are in dendrites. Furthermore, a double in situ hybridization for CaMKIIα mRNA and indirect immunofluorescence for one of the proteins in the complex (Pur α, an RNA binding protein) showed them to be colocalized. While additional experiments demonstrated that particle movement was reduced by a dominant-negative KIF5, the pièce de résistance of the paper clearly is found in the final figure; here the authors use siRNA to knock down six of the factors and find that in four of the cases (Pur α, hnRNP U, PSF, a polypyrimidine tract binding protein-associated factor, and staufen), the transport of an CaMKIIα 3′ UTR in dendrites is substantially reduced. It will be of great interest to determine whether the transport of other mRNAs to dendrites is reduced when these or other factors are knocked down by siRNA, and whether such knockdowns lead to premature translation.

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A Rhythmic Ror

The circadian clock mechanism in mammals involves two interlocking transcriptional feedback loops. Rev-erb α, through its role as a transcriptional repressor, was thought to be the primary determinant of the feedback loop that regulates *Bmal1* transcription. Results reported by Sato et al. in this issue of *Neuron* now show that the transactivator Rora acts coordinately with Rev-erb α and that their competing activities on the same promoter element drive the rhythm in *Bmal1* transcription. This finding defines the second feedback loop in mammals.

Organisms time their physiology and behavior to cope with the predictable daily alterations in the environment that result from the Earth's rotation. Such biological timing is controlled by genetically determined timers called circadian clocks. These self-sustained clocks can oscillate for months in constant conditions, while maintaining a remarkably stable period length of about 24 hr. A key question in circadian biology is how a stable molecular oscillator with such a long cycle length is generated. In this issue of *Neuron*, Sato et al. (2004) take us a step closer to answering this question by identifying a new element important for adding stability to the mammalian clock mechanism.

The circadian clocks of animals were initially envisioned to be comprised of a single intracellular negative transcriptional feedback loop (Figure 1, core loops). In *Drosophila* (the organism in which the loop was first described), the model posited that the heterodimeric transcription factor complex of dCLOCK/dCYCLE (dCLK/dCYC) positively regulates the expression of *period (per)* and *timeless (tim)* by binding to E box elements in their