Neuropeptide S: A Novel Activating Anxiolytic?

Many different neuropharmacological agents modulate arousal and anxiety, yet to date, few endogenous substances have produced arousal with an anxiolytic effect. In this issue of *Neuron*, Xu et al. describe the localization and characterization of a novel neuropeptide, neuropeptide S (and its cognate receptor), that is unique in its arousing and anxiolytic-like properties.

The article by Xu and colleagues (Xu et al., 2004 [this issue of *Neuron*]) provides evidence of a potential novel brain modulator of arousal and anxiety using animal models. The authors describe the anatomical localization and behavioral characterization of a novel neuropeptide, neuropeptide S (NPS), and its cognate receptor. NPS is localized to brain pontine regions, such as the peri-locus coeruleus and Barrington's nucleus. NPS is also localized to the amygdala, an area important for anxiety function. The NPS receptor is seen in highest abundance in amygdala, anterior olfactory nucleus, endopiriform nuclei, paraventricular thalamic nucleus, subiculum, and several cortical regions. Intracerebroventricular administration of NPS at doses of 0.1 nmol and 1 nmol caused increased motor activity and also promoted wakefulness. At similar doses, NPS also was found to have anxiolytic-like properties, using open field, light-dark box, elevated plus maze, and marble burying paradigms. NPS and its receptor are hypothesized to be involved in arousal and anxiety, based on their brain localization and behavioral results. To our knowledge, this manuscript provides the first description of the localization and behavioral effects of NPS. Thus, NPS may be an important novel neuropeptide mediating arousal and anxiety behaviors.

There are several important experimental and conceptual strengths to this work that make it particularly noteworthy. First, from a conceptual framework, NPS appears to produce behavioral activation and induce wakefulness, but at the same time produces an anxiolytic-like effect. To some extent, this is a paradox—most drugs that are arousing and activating ultimately produce anxiety-like effects, not anxiolytic-like effects (for example, cocaine, amphetamines, γ-aminobutyric acid antagonists, and corticotropin-releasing factor [Sutton et al., 1982; Koob and Heinrichs, 1999; Koob et al., 2004]). An exception to this rule is nicotine, a drug which also increases arousal and wakefulness and produces anxiolytic-like, antistress effects. Long documented, this paradox has been held as an example of how arousal and stress do not always follow a monophasic continuum. Nesbitt (1973) allowed smokers to smoke during a stressful experience (sessions where they received painful shocks), and these smokers showed more arousal (increase in pulse rate) but reported less emotion (more pain endurance, more shocks taken) than smokers that were not allowed to smoke but that simulated smoking. These early results were interpreted to support the Nesbitt paradox that is reported by smokers—that their physiological arousal is increased but they report

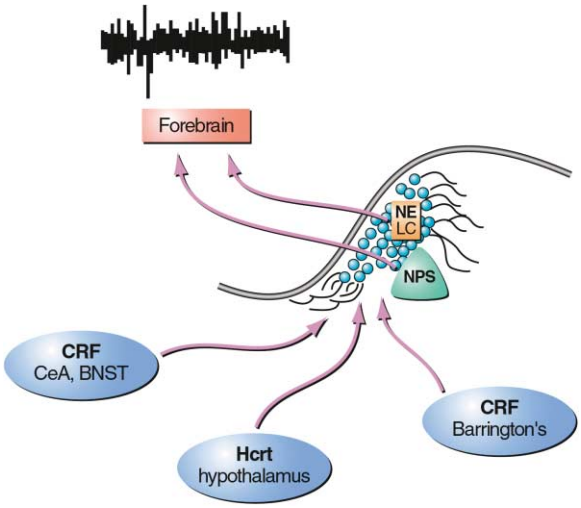

Figure 1. Possible Neuropeptide S Circuit in Arousal

Corticotropin-releasing factor (CRF) neurons in Barrington's nucleus, the central nucleus of the amygdala (CeA), and the bed nucleus of the stria terminalis (BNST) project to the pons, as well as hypocretin/orexinergic neurons (Hcrt) from the hypothalamus. NPS neurons in the peri-locus coeruleus, as well as nearby noradrenergic (NE) neurons in the locus coeruleus (LC), project to the forebrain, possibly as part of an arousal projection (Foote et al., 1980; van Bockstaele et al., 1998; Sutcliffe and de Lecea, 2002).

themselves to be calmer and more relaxed. The comparison of NPS to nicotine leads to the question as to whether an interaction between NPS and nicotine ultimately may be found. NPS and future analogs derived from NPS could be excellent tools for further characterizing arousal and anxiety function in the brain and possibly teasing apart separate systems that may underlie these two behaviors. Interaction with the NPS system could also potentially provide important therapeutic uses for sleep, anxiety disorders, and depression.

Second, the experimental strength of the Xu et al. study lies in the careful attention to the use of validated measures of anxiety to test the hypothesis at hand. Here, NPS produced increases in exploratory behavior in animal models of anxiety where anxiety is manifest as an inhibition in behavior (such as the elevated plus maze) but produced inhibition of behavior in tests where anxiety-like effects are reflected in an active response (marble burying). Such a dual approach to validated animal models lends assurance that nonspecific (locomotor) activation or depression in behavior are not influencing the measures used to hypothesize anxiolytic-like effects and lend more credence to an antianxiety action of NPS. Further statistical analysis within a given animal model of anxiety also confirmed a loading on the anxiolytic measure. Obviously, small molecule compounds will be required to test this hypothesis in humans and to determine if there is any functional role for NPS in the arousal/anxiety domain.

The anatomical localization of NPS also provides some intriguing links to long-hypothesized pontine roles in sleep, waking, and arousal. NPS is not localized within the locus coeruleus (LC). Neuropeptide S-expressing neurons do not produce norepinephrine, and locus coer-

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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Selected Reading

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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,