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Neuroepigenetics of Arousal: Gamma Oscillations in the Pedunculopontine Nucleus

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Abstract

Four major discoveries on the function of the pedunculopontine nucleus (PPN) have significantly advanced our understanding of the role of arousal in neurodegenerative disorders. The first was the finding that stimulation of the PPN induced controlled locomotion on a treadmill in decerebrate animals, the second was the revelation of electrical coupling in the PPN and other arousal and sleep-wake control regions, the third was the determination of intrinsic gamma band oscillations in PPN neurons, and the last was the discovery of gene transcription resulting from the manifestation of gamma activity in the PPN. These discoveries have led to novel therapies such as PPN deep brain stimulation (DBS) for Parkinson's disease (PD), identified the mechanism of action of the stimulant modafinil, determined the presence of separate mechanisms underlying gamma activity during waking vs REM sleep, and revealed the presence of gene transcription during the manifestation of gamma band oscillations. These discoveries set the stage for additional major advances in the treatment of a number of disorders.

Keywords

Arousal; deep brain stimulation; electrical coupling; gamma activity; histone deacetylation; locomotion; N-type calcium channels; pedunculopontine nucleus; P/Q-type calcium channels; REM sleep; waking

Introduction

1. Basal ganglia projections

The pedunculopontine nucleus (PPN) achieved prominence when it was found to receive basal ganglia outputs in studies on monkeys using silver degeneration methods developed by Nauta (Nauta and Mehler, 1966). The same year, a group of Russian scientists described the mesencephalic locomotor region (MLR), an area in the same region that could be stimulated electrically in decerebrate animals to control locomotion on a treadmill (Shik et al., 1966). In 1979, we began a series of studies to determine if there was co-localization between these

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regions. Schute and Lewis (Schute and Lewis, 1967) described (histochemically) cholinergic projections ascending from this region, and cholinergic neurons were identified (immunocytochemically) in this area in multiple species (Manaye et al., 1999; Mesulam et al., 1984, 1989). Ultimately, triple in situ hybridization identified three cell types, cholinergic, glutamatergic, and GABAergic, in the PPN (Wang and Morales, 2009). The PPN had long been known to be part of the reticular activating system (RAS) in charge of arousal (Garcia-Rill, 2015).

2. Four major discoveries

a. Control of locomotion—Our early studies consisted of identifying the lowest threshold (usually <100 uA) site in the posterior midbrain that would induce controlled locomotion (increasing current could “control” stepping from a walk to a trot to a gallop) using 40–60 Hz frequency of stimulation. We found internal pallidal and nigral projections directly to locomotion-inducing sites (Garcia-Rill et al., 1981, 1983a, 1983b), chemically-induced locomotion at the same sites (eliminating the possibility that electrical stimulation produced the effects) (Garcia-Rill et al., 1985), single cell activity in relation to locomotion in these sites (Garcia-Rill et al., 1983c), and, significantly, locomotion-inducing sites localized within the cholinergic PPN cell group (Garcia-Rill et al., 1987c). In addition, we described descending PPN projections to pontomedullary reticulospinal projection sites (Garcia-rill et al., 1987a, 1987b, 1990; Homma et al., 2002; Kinjo et al., 1990). These studies established the processes whereby descending projections from the PPN could influence both posture and gait by influencing different populations of reticulospinal neurons (Mamiya et al., 2005). The PPN was only one region that could elicit stepping, but it was the one we could reliably identify anatomically in physiological locomotion-inducing studies. We went on to address issues related to spinal cord injury and the use of epidural spinal cord stimulation (see Garcia-Rill, 2015). In the meantime, clinician scientists implemented the methodology for deep brain stimulation (DBS) of the PPN for the treatment of Parkinson’s disease (PD) based on our early work.

Clinical implications (PPN DBS):- The first studies on PD patients reported relief of gait symptoms in patients with severe axial and locomotor deficits (Mazzone et al., 2005; Plaha and Gill, 2005). Subsequent work used imaging and intracerebral recordings to identify stimulation sites (Mazzone et al., 2008, 2009; Zrinzo et al., 2008). Population activity in the PPN showed responses in relation to voluntary and passive movements (Weinberger et al., 2008), as well as imagined movements (Karachi et al., 2010; Tattersall et al., 2014). One group showed decreased falls and freezing of gait using 15 Hz and 25 Hz stimulation (Ferraye et al., 2010), while another group showed decreases in falls and improved motion using 50 Hz and 70 Hz stimulation (Moro et al., 2010). Some reported mild motor effects but better sleep and cognition using 10 Hz and 25 Hz stimuli (Stefani et al., 2010, 2013). Others found no motor improvement but significantly better sleep and cognitive scores (Alessandro et al., 2010). Stimulation at 20–35 Hz decreased reaction time, improved falls, and decreased freezing of gait, and the effect was optimal using bilateral stimulation (Thevathasan et al., 2010, 2012). MRI-aided research established that the optimal site for aiding freezing of gait using 10–30 Hz PPN DBS was indeed within the PPN (Goetz et al., 2018). In summary, PPN DBS has shown considerable promise for the treatment of postural

and gait disturbances in PD, as well as the normalization of sleep patterns and amelioration of cognitive function.

b. Electrical coupling—Our PPN recording studies revealed the presence of spikelets in some PPN neurons, a result that led to the description of pairs of PPN cells that were electrically and dye-coupled (Garcia-Rill et al., 2007). We also found electrically coupled neurons, mainly GABAergic cells, in an ascending (Parafascicular-Pf intralaminar thalamus) and a descending (Subcoeruleus dorsalis-SubCD nucleus) target of the PPN (Garcia-Rill et al., 2007, 2008; Heister et al., 2007). The presence of 5–10% of cells in these nuclei with such coupling helped develop a novel theory of sleep-wake control based on electrical coupling. The idea is that such electrical coupling promotes coherence between groups of neurons, allowing firing in coordination to create a signal that is mirrored accurately by other groups of neurons. Some of the fastest acting anesthetics such as propofol and halothane are gap junction blockers (Evans and Boitano, 2001; He and Burt, 2000). Agents that block gap junctions such as carbenoxolone and mefloquine also induce soporific effects (Rozental et al., 2001). On the other hand, agents that increase electrical coupling should have stimulatory or wake-promoting effects. The clinical implications of sleep-wake control modulated by electrical coupling driving coherence are considerable.

Clinical implications (modafinil)—: The mechanism of action of modafinil, an atypical stimulant, was described as increasing electrical coupling in neurons of the cortex, reticular nucleus of the thalamus, and cerebellum (Urbano et al., 2007). Soon after, we described similar results for the PPN, Pf, and SubCD (Garcia-Rill et al., 2007). The idea is that, by increasing electrical coupling in small GABAergic neurons, their usual high input resistance is decreased, promoting a decrease in GABA release and thus disinhibition of target sites. This disinhibits a host of systems, leading to overall higher coherence in the RAS, thalamocortical systems, and elsewhere. Therefore, this promotes arousal. On the other hand, blocking gap junctions leads to rapid loss of consciousness, such as when using the fast-acting anesthetics. Modafinil is the only agent that appears to have the property of increasing electrical coupling, a process that increases arousal by disinhibiting many systems and pathways. We carried out a number of additional studies on this agent for its effects on arousal in vivo, in vitro, in animals and humans, as well as on modulating spasticity (see Garcia-Rill, 2015). The mechanism of electrical coupling remains an untapped resource in the control of sleep-wake states, although the use of modafinil for narcolepsy and excessive daytime sleepiness is without equal. Its use off-label for a number of disorders of arousal is increasing.

c. Gamma oscillations—Neurons in the PPN have long been known to fire at beta/gamma frequencies during waking and REM sleep (Boucetta et al., 2014; Datta and Siwek, 2002; Kayama et al., 1992; Sakai, 1980; Steriade, 1999, see review in Garcia-Rill, 2015). In the feline, PPN neurons fire at gamma frequencies when the animal is active or awakens (Steriade et al., 1990), while in the monkey the same PPN neurons fired at low frequencies (~10 Hz) at rest but increased to gamma frequency when they awakened or walked on a treadmill (Goetz et al., 2016), and in the human, activity in the region of the PPN was shown to be at gamma frequencies during stepping but not at rest (Fraix et al., 2013). Therefore, in

vitro, in vivo, in the rodent, feline, primate, and human, PPN neurons are known to fire at gamma frequencies during waking and motor activity, the same frequencies that elicit locomotion when the PPN is stimulated. The reason for these results is that all PPN neurons manifest intrinsic gamma oscillations.

We discovered that all PPN neuronal transmitter types fired at beta/gamma frequencies (Simon et al., 2010), that the firing was mediated by intrinsic oscillations generated by P/Q- and N-type calcium channels (Kezunovic et al., 2011), and that ~25% cells manifested only N-type channels and were probably “REM-on” cells, ~25% had only P/Q-type channels and were probably “Wake-on” cells, while 50% had both channels and were probably “Wake-REM on” cells (Luster et al., 2015, 2016). Further studies showed that CaMKII modulates P/Q-type channels and cAMP/PKA modulates N-type channels (Garcia-Rill, 2015, 2017; Garcia-Rill et al., 2014a,b; Urbano et al., 2012). Moreover, previous work had shown that injections of NMDA into the PPN preferentially drove waking, while injections of kainic acid (KA) preferentially induced REM sleep (Datta et al., 2001; Datta, 2002). Thus, Figure 1 summarizes the organization of the intracellular pathways and mechanisms that control the generation of gamma band activity during waking, compared to the generation of gamma band activity during REM sleep.

Clinical implications (arousal, two channels two states)-: The manifestation of intrinsic gamma oscillations in PPN neurons helps explain how electrical stimulation of this nucleus at its preferred frequency leads to ascending arousal as well as descending driving of locomotor pattern generators (Garcia-Rill, 2015). In addition, PPN DBS at gamma frequencies may result in promotion of gamma activity throughout the neuraxis, perhaps accounting for its ameliorative effects on sleep patterns and cognitive function. Moreover, the presence of different calcium channels controlling waking vs REM sleep now allows for more specific modulation of each state. This may be possible through modulation of calcium channel types or of their respective intracellular pathways. In addition, we advanced the idea that, in at least some cases, insomnia may represent not too little sleep, but too much waking, perhaps through over expression of P/Q-type calcium channels (Garcia-Rill et al., 2015). This could be demonstrated with P/Q-type channel knock-in technology. Finally, these findings suggest that the cortical EEG manifestation of gamma band activity during waking has fundamentally different origins during waking vs REM sleep, although this difference is indistinguishable in the EEG record.

d. Neuroepigenetics—Recently, we demonstrated that *in vitro* exposure to the histone deacetylation inhibitor trichostatin A (TSA) eliminated the manifestation of PPN intrinsic membrane oscillations in the gamma band range, but not lower frequency oscillations (Urbano et al., 2018). We also found that long-term incubation with TSA also decreased PPN gamma band oscillations and significantly reduced calcium currents (Urbano et al., 2018). This discovery signifies a specific effect on gamma oscillations when histone deacetylation is blocked, but more importantly suggesting that, without such blockade, arousal-related gamma oscillations modulate gene transcription. Preliminary results showed that the proteins modulated by TSA included proteins in charge of intracellular calcium regulation, such as neuronal calcium sensor protein-1 (NCS-1), calcineurin, etc. These

findings point to the potential role of gene transcription resulting from the manifestation of gamma oscillations as a daily remodeling of PPN neurons and of the updating of sensory experience (Urbano et al., 2018). We will soon report that similar effects are induced when the animal is exposed in vivo to histone deacetylation inhibitors. We concluded that, ultimately, that such transcription upon waking represents daily formulation of the self, given that during sleep there is no sense of self, but it is recalled immediately upon waking (Garcia-Rill, 2019).

Clinical implications (formation of the self)-: Blocking histone deacetylation with TSA may have deleterious effects on intrinsic gamma oscillations in the PPN, presumably inhibiting arousal, which may account for some of the soporific side effects of this medication. More endemic to clinical application, we know that gene regulation can be mediated by voltage-dependent calcium channels (Barbado et al., 2009), and that a number of human disorders result when P/Q-type channels mutate or become dysfunctional (Schorge and Rajakulendran, 2012). Therefore, our findings point to a number of potentially fruitful areas of clinical research that needs to be pursued. The role of arousal in a number of neurodegenerative disorders, including PD, Alzheimer's disease, REM Behavior Disorder, and Narcolepsy, is discussed in an upcoming book (Garcia-Rill, 2019).

Conclusions

The four major advances described were generated over 40 years of research, which is perhaps a good indication of the time it takes to advance neuroscience in a significant manner. Without long-term funding and institutional support, it is unlikely that the S-curve of funding, that needs at least 10 years to reach a plateau of accomplishment, would be marked by significant breakthroughs.

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Significance Statement

Four major discoveries on the function of the PPN have significantly advanced our understanding of the role of arousal in neurodegenerative disorders. These discoveries have led to novel therapies such as PPN DBS for PD, identified the mechanism of action of the stimulant modafinil, determined the presence of separate mechanisms underlying gamma activity during waking vs REM sleep, and revealed the presence of gene transcription during the manifestation of gamma band oscillations. These discoveries set the stage for additional major advances in the treatment of these disorders.

Figure 1. Modulation of waking vs REM sleep by two different transmitters through two different calcium channel types.

P/Q-type calcium channels are modulated by CaMKII and may be involved in generating gamma oscillations during waking, while N-type calcium channels are modulated by cAMP/PKA and may be involved in generating gamma oscillations during REM sleep. In the PPN, activation of NMDA receptors drives waking, while activation of kainic acid receptors drives REM sleep. Interestingly, the gamma activity in the cortical EEG in relation to waking manifests coherence between distant sites during waking, but cortical EEG gamma activity during REM sleep shows a lack of coherence across distant areas of cortex (for review see Garcia-Rill, 2015).

Figure 2. Neuroepigenetic regulation of P/Q-type calcium channel-dependent gamma oscillations.

TSA was found to block P/Q-type calcium channel-dependent gamma oscillations through its inhibition of HDACs, both I and II. However, the HDAC I blocker MS275 did not affect the generation of gamma oscillations, while the HDAC IIa blocker MS1568 inhibited the manifestation of gamma oscillations. This suggests that, normally, gamma oscillations lead to gene transcription that is blocked by TSA or MS1568 when present (Urbano et al., 2018).