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# Arousal and drug abuse

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ABSTRACT

The reticular activating system (RAS) is not an amorphous region but distinct nuclei with specific membrane properties that dictate their firing during waking and sleep. The locus coeruleus and raphe nucleus fire during waking and slow wave sleep, with the pedunculopontine nucleus (PPN) firing during both waking and REM sleep, the states manifesting arousal-related EEG activity. Two important discoveries in the PPN in the last 10 years are, 1) that some PPN cells are electrically coupled, and 2) every PPN cell manifests high threshold calcium channels that allow them to oscillate at beta/gamma band frequencies. The role of arousal in drug abuse is considered here in terms of the effects of drugs of abuse on these two mechanisms. Drug abuse and the perception of withdrawal/relapse are mediated by neurobiological processes that occur only when we are awake, not when we are asleep. These relationships focus on the potential role of arousal, more specifically of RAS electrical coupling and gamma band activity, in the addictive process as well as the relapse to drug use.

## 1. The control of arousal

### 1.1. Waking and drug abuse

Drug abuse only occurs during waking. We experience withdrawal or relapse to drug abuse only when awake. Higher motor activation while awake has been associated with greater addiction liability. The ventral tegmental area (VTA), a key neural substrate for the modulation of drug abuse, is modulated by reticular activating system (RAS) output, particularly from the pedunculopontine nucleus (PPN). The RAS modulates oscillating rhythms between the thalamus, hypothalamus, basal forebrain, and cortex that are characterized in the EEG during wake-sleep states [1]. The RAS is not an amorphous, unspecific region but rather a group of distinct nuclei with specific intrinsic membrane properties that dictate their firing frequencies during waking and sleep. All three main nuclei in the RAS, the PPN that is partly cholinergic, the locus coeruleus (LC), which is mainly noradrenergic, and the raphe nucleus (RN), which is mainly serotonergic, have been described as affecting neural substrates related to addiction like the VTA. These VTA inputs are critical because the basal activity of VTA neurons can be functionally associated to vulnerability to drug abuse [2].

The PPN contains cholinergic, glutamatergic, and GABAergic neurons [3]. The cholinergic output has a net excitatory effect on LC and RN neurons [4,5]. Importantly, one of the targets of PPN non-cholinergic neurons is the VTA [6]. Cholinergic efferents from the PPN to the VTA form a loop that includes the medial prefrontal cortex (mPFC). This loop is composed of mPFC glutamatergic efferents to dopaminergic (DA) and GABAergic neurons in the VTA and to the nucleus accumbens (NAcc) through a polysynaptic circuit that includes the PPN. In addition, VTA sends dopaminergic and GABAergic efferents to the NAcc. Activation of the PPN thus increases VTA dopaminergic output, and increases extracellular DA levels in the NAcc and mPFC [7], which suggests that the PPN in part regulates the reward and motivational functions of the VTA [8]. In turn, increased glutamatergic efferent activation from the mPFC would in turn reduce VTA dopaminergic output through its direct activation of local GABAergic interneurons in the VTA. Recent optogenetic experiments confirmed that PPN-VTA pathway stimulation can induce psychostimulant-like behavior in the absence of drug administration [9].

The noradrenergic input from the LC to the PPN is inhibitory, presumably via alpha 2 adrenergic receptors [10]. Furthermore, the RN sends inhibitory serotonergic projections to both PPN and the LC [11]. Other inhibitory inputs to the PPN come from GABAergic neurons in the substantia nigra (SN), an area also involved in the locomotor activation produced by psychostimulant administration. Since midbrain dopaminergic neurons originating in the VTA and SN *pars compacta* (SNc) have been previously described as the neural substrates underlying individual vulnerability to drug addiction [12–14], understanding the functional modulation of the VTA and SNc by the RAS is key to

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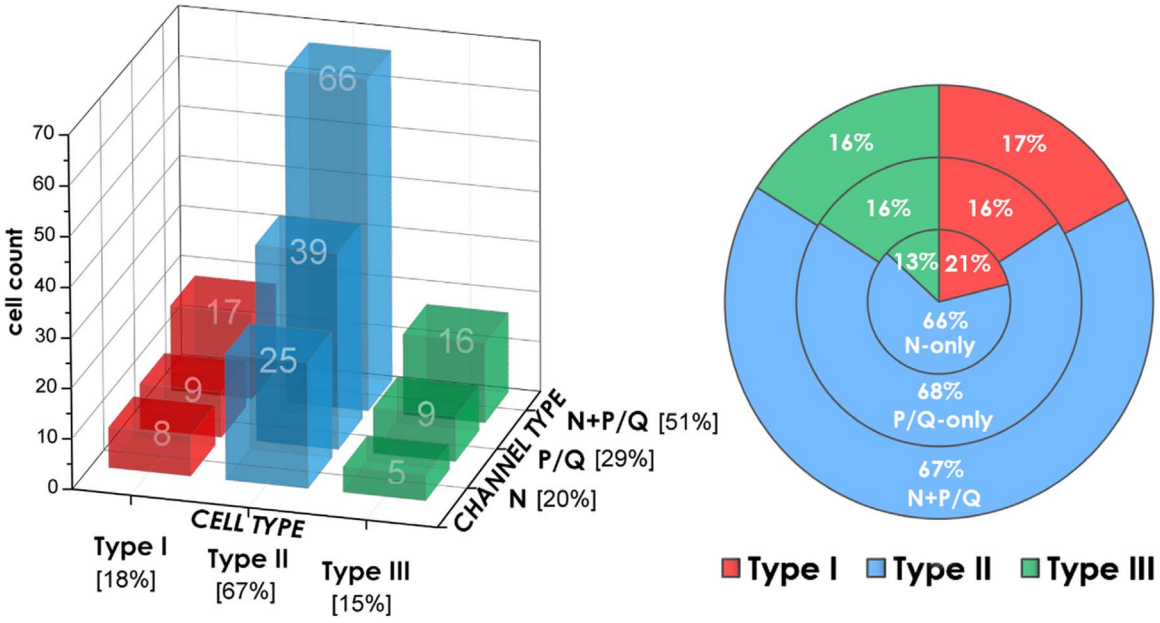


**Fig. 1.** Distribution of PPN neurons according to electrophysiological type (I, II, or III) and high threshold calcium channel type (N + P/Q, N only, P/Q only). Left side. Graph of the distribution of cells by cell type (Type I red, Type II blue, Type III green columns) and by channel type (N only, P/Q only, N + P/Q). Note that the numbers in each column are cell counts of recorded cells, and the percentage of cell type or channel type are in brackets in the axis legends. Right side. Pie chart of the percentage of cells by cell type and channel type. Note that the numbers inside the chart represent percent, not cell counts. Basically, the sample of almost 200 PPN cells shows that all cell types manifest all three types of channel expression. Since Type I cells are non-cholinergic, Type II cells are 2/3 cholinergic, and Type III cells are 1/3 cholinergic, it is highly likely that all three transmitter types (cholinergic, glutamatergic, and GABAergic) manifest all three channel types. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

understanding how reinforcing, drug craving, and the effects of drugs of abuse are modulated by a wake-promoting nucleus such as the PPN.

### 1.2. State-dependent activity

The LC and RN fire during waking and also during slow wave sleep, with the pedunculopontine nucleus (PPN) being the only RAS cell group firing during both waking and rapid eye movement (REM) sleep, states of high frequency beta/gamma band EEG activity [15]. Single cell recordings in PPN *in vivo* identified several categories of thalamic projecting PPN cells distinguished by their firing properties relative to ponto-geniculo-occipital wave generation [16]. Some PPN neurons fired at low rates (< 10 Hz), but most fired in the beta/gamma range (20–80 Hz). PPN neurons exhibit beta/gamma frequencies *in vivo* during active waking and REM sleep, but not during slow wave sleep [16–21]. Similarly, the presence of gamma band activity has been confirmed in the PPN in relation to the cortical EEG of the cat *in vivo* when the animal is active [16,22]; and in the region of the PPN in humans during stepping, but not at rest [23]. A recent study showed that PPN neurons exhibited low firing frequencies ~10 Hz at rest, but the same neurons fired at gamma band frequencies when the animal woke up, or when the animal began walking on a treadmill [24]. That is, the same cells were involved in both arousal and motor control. Thus, there is ample evidence for gamma band activity during active waking and movement in the PPN *in vitro*, *in vivo*, and across species, including man.

PPN neurons showed increased firing rates during REM sleep, named "REM-on" cells, or both waking and REM sleep, named "Wake/REM-on" cells, and some cells fired only during waking, called "Wake-on" cells [16,17,19], suggestive of increased excitation only during activated states. Stimulation of the PPN potentiated the appearance of beta/gamma oscillations in the cortical EEG, outlasting stimulation by 10–20 s [25]. These findings emphasize the fact that PPN neurons are mainly active during states of arousal marked by high frequency EEG activity such as waking and REM sleep.

### 1.3. Major Breakthroughs

#### 1.3.1. Electrical coupling in the RAS-

We demonstrated the presence of cells in the PPN, as well as in its ascending target the Parafascicular nucleus (Pf), and in its descending target, the Subcoeruleus nucleus dorsalis (SunCD), a proportion (7–10%) of which were electrically coupled [26]. This was the first finding suggesting a role for electrical coupling in wake-sleep control. Electrical coupling through (Cx36) 36 gap junctions has been described in the reticular nucleus of the thalamus, the site of slow wave generation [27–29], so that its presence in wake-sleep regions is well known. In fact, gap junction blockers such as halothane and propofol are among the most rapidly acting anesthetics [30,31]. The role of electrical coupling is to allow neurons to fire together, regardless of frequency. Modafinil, a stimulant used for the treatment of narcolepsy and excessive daytime sleepiness, was found to increases gap junctions, driving coherence at high frequencies to promote arousal [32]. We also found that input resistance in PPN and SubCD, mainly GABAergic, neurons was reduced by modafinil [33]. Thus, electrical coupling in the RAS serves to synchronize activity across populations of cells, blockade of electrical coupling induces anesthesia, and such coupling is increased by the stimulant modafinil to induce arousal.

#### 1.3.2. Gamma band intrinsic membrane oscillations

We also discovered that all PPN cells fired maximally at gamma band frequency when depolarized using current steps [34]. We identified the mechanism responsible for this ceiling in firing as high threshold, voltage-dependent calcium channels [35]. Basically, ramp-induced membrane depolarization resulted in beta/gamma frequency intrinsic membrane oscillations at −30 mV to −10 mV, suggesting that the location of these channels was away from the cell body [35]. This was confirmed using fast calcium imaging demonstrating that the channels are located all along the dendrites of these cells [36]. Pharmacological studies established that the calcium channels involved were N- and P/Q-type [35,37,38].

The most impressive element in these findings was that all PPN neurons manifested these channels, regardless of transmitter type,