Short communication

Subcutaneous administration of nicotine changes dorsal raphe serotonergic neurons discharge rate during REM sleep

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Accepted 10 October 2000

Abstract

In the present study nicotine (0.1 mg/kg, s.c.) increased discharge rate of putative dorsal raphe (DRN) serotonergic neurons of behaving rats during REM sleep (362.61%), without any significant change during waking and non-REM sleep. Since serotonergic DRN neurons gate PGO onset, these results suggest that nicotine-induced suppression of PGO spikes during REM sleep previously reported is achieved through stimulation of dorsal raphe serotonergic cells.

In previous studies we have shown that nicotine suppresses the ponto-geniculo-occipital spikes (PGOs) of rapid eye movement (REM) sleep [21] and improves mood during REM sleep [21]. It is possible that these effects are serotonin-mediated since pharmacological [8] and electrophysiological [9] studies indicated that serotonergic neurons from the dorsal raphe nucleus (DRN) play a permissive role in PGO spikes generation and most of treatments currently employed in depression increase brain serotonin (5-HT) traffic [2]. In vitro experiments have shown that nicotine increases discharge rate of serotonergic DRN neurons and 5-HT release in a dose-dependent manner [12,15]. Systemic administration of nicotine in rats facilitates 5-HT release as measured by microdialysis in frontal cortex [19]. However less is known regarding nicotine actions on serotonergic DRN neurons in behaving rats.

In this study we hypothesize that nicotine increases discharge rate of putative serotonergic neurons (PSNs) during REM sleep. This may account for PGO spikes suppression [21] and mood improvement previously reported [17]. This hypothesis is supported by the presence of nicotinic receptors in the DRN of rats [18] and by reports showing anatomical projections [5] from PSNs to the PGO wave generator [4].

Experiments were conducted in six Sprague–Dawley rats (300–350 g). Under deep anesthesia (ketamine [80 mg/kg] + xylazine [10 mg/kg], i.p.) and aseptic conditions, rats were surgically prepared for chronic recording of DRN unit activity along with electroencephalogram (EEG) and electromyogram (EMG) across the sleep–wake cycle, as previously described [7].

In order to record DRN unit activity, stainless steel
microelectrodes (20 μm) inserted into a microdrive were stereotaxically aimed at the DRN (AP −7.8, L 0.0, H 4.5). The microdrive was advanced in steps of 25 μm until stable single-units (signal:noise, 3:1) were found. For each cell, recordings were performed across 2 to 3 sleep–waking complete cycles for identification of the REM-off pattern.

After baseline recording, nicotine (bitartrate Sigma 0.1 mg/kg, dissolved in sodium chloride 0.9%) was administered s.c. in a volume less than 1 ml [10]. After nicotine administration recordings continued for 2 h. Action potential waveform of individual neurons was continuously monitored during the experiments to ensure that the same cell was recorded.

Mean discharge rate per second (spikes/s) was calculated from 30 s epochs both during baseline and after nicotine injection. Cells were classified as wake-related, sleep-related and as state-indifferent according to its NREM/Wake ratio as previously established [1].

Since previous analysis of the DRN PSNs stated that these cells cease discharge before the onset of PGO spikes during the NREM–REM transition, we carried out an analysis of this transition [7]. In order to achieve this goal we selected periods of NREM sleep (60–75 s in duration) which preceded a REM sleep period (defined by the presence of desynchronization and atonia longer than 1 min), being divided into thirds (20–25 s each one). The mean discharge rate was calculated in each third as well as the initial 20–25 s of REM sleep.

The final position of the microwires was histologically identified in Nissl-stained sections.

A total of 36 cells were recorded from the DRN. Sixteen of these cells were classified as PSNs according to the criteria previously established [7]. The mean duration of the action potential was 2.74±0.11 ms. Firing rates declined progressively across the sleep–wake cycle (1.28±0.19 spikes/s during waking, 0.45±0.06 during NREM sleep and 0.10±0.01 in REM sleep). The mean percentage of reduction in discharge rate from waking to REM sleep was −90.39±1.61%. In addition in six of these presumed serotonergic neurons systemic administration of (±)8-OH-DPAT displayed a marked suppression of unit activity (−94.55±3.31%, P<0.01, paired t-test).

The remaining 20 neurons did not meet the criteria for PSNs and were classified as sleep-related (n=3), state-indifferent (n=5) and wake-related neurons (n=12).

The effect of systemic administration of nicotine was examined in all 16 PSNs. Nicotine administration increased discharge rate during REM sleep by 362.61±145.29% (P<0.05, repeated measures ANOVA followed by Bonferroni test). No statistical differences were observed in the mean discharge rate during waking and NREM sleep (Fig. 1A). Fig. 2A and B illustrates polysomnographic recordings both during baseline and
after nicotine administration of PSNs recorded across the sleep–wake cycle.

There was an increase in discharge rate after nicotine administration during the NREM–REM transition (Fig. 1B). This increase was significant in the last third of the NREM sleep (20–25 s prior to REM sleep onset, \( P<0.05 \), unpaired \( t \)-test) and the 20–25 initial seconds of the REM sleep stage (\( P<0.05 \) unpaired \( t \)-test).

The other cell populations (state-indifferent, sleep-related and wake-related groups) exhibited a decrease in discharge rate across the sleep–wake cycle after systemic nicotine administration but this trend was not statistically
significant. For those cells classified as wake-related the percentage of reduction during REM sleep after nicotine administration was 63.95 ± 17.33, 77.22 ± 45.03 in sleep-related neurons and 49.45 ± 10.91 in state-indifferent neurons.

Additionally we measured the mean duration of each REM sleep episode. Nicotine induced an increase in duration of REM sleep episodes from 118.09 ± 3.62 to 138.01 ± 3.51 s (P < 0.05, unpaired t-test) without significantly affecting the frequency of events (2.24 ± 0.16 during baseline vs. 2.38 ± 0.23 after nicotine administration).

The principal finding of this report is that nicotine (0.1 mg/kg) increased the discharge rate of PSNs during REM sleep. In addition there was a significant increase in firing rate during the NREM–REM sleep transition. Taken together these data support the view that PGO spikes suppression [21] and mood improvement by nicotine [17] is mediated by its stimulatory effect on DRN PSNs during REM sleep.

This interpretation is consistent with the observation of Jacobs et al. [9], that electrical stimulation of the DRN during REM sleep suppresses the presence of the PGO spikes.

Aside from the increase in discharge rate during REM sleep, as mentioned above, it was observed that nicotine increased discharge rate in the DRN PSNs in the 20–25 s prior to REM sleep. It has been shown that in cats PGO spikes appear 30–60 s before REM sleep onset [3], coincidentally with the lowest discharge level of 5-HT DRN cells [13,14]. Therefore it is possible that the increase in discharge rate of DRN PSNs induced by nicotine during the NREM–REM sleep transition accounts for the inhibition of PGO spikes. This is consistent with the report that sertraline (an 5-HT reuptake inhibitor) decreases the presence of PGO spikes in the NREM–REM sleep transition [16].

Our study also is in agreement with previous reports in vitro, showing that nicotine increases PSNs discharge rate as well as serotonin release [15].

As concerns sleep-related, state indifferent and wake-related neurons, the consistent finding in this study was a reduction in the discharge rate along the sleep–wake cycle which, however, was not statistically significant. It is evident that these neurons represent different neuronal populations, which agrees with reports showing that DRN neuronal composition is not homogeneous and serotoninergic neurons represent only one third of the neurons in this nucleus [11].

As concerns the influence of nicotine on sleep architecture, the mean duration of each REM sleep period was increased without affecting the frequency of episodes. No significant changes were observed during waking and NREM sleep. Similar findings were reported previously [10] in studies that used similar doses and routes of administration. This increase in REM sleep duration along with an increase in discharge rate of 5-HT neurons seems paradoxical, since DRN serotonergic neurons inhibit [20] the pedunculopontine (PPT) and laterodorsal tegmental (LDT) neurons, generators of REM sleep [6]. In our experimental conditions however it may be assumed that nicotine increased REM sleep by directly stimulating PPT and LDT neurons. The previously reported PGO spike suppression by nicotine may be explained by serotoninergic inhibition of PGO wave generator located in rats in the subcoeruleus nuclei [4].

In sum, our study shows that nicotine increases discharge rate selectively during REM sleep in PSNs. This effect could be responsible at least in part for mood improvement in patients with major depression having nicotine patches [17] and suppression of PGO spikes [21] in cats after nicotine administration.

Acknowledgements

This work was supported by V.A. Medical Research Service. RGM is recipient of a scholarship from the Consejo Nacional de Ciencia y Tecnología (CONACYT) and DGEP-UNAM. Support from the UC-Mexus Program to RD-C is also acknowledged. We wish to thank Janice King, Darrell Thomson and Lindsay Chiu for their excellent technical support, and Mrs Ma. Teresa Torres-Peralta for typing the manuscript.

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