Sleep and Breathing (2019) 23:297–302
https://doi.org/10.1007/s11325-018-1705-8

NEUROLOGY • ORIGINAL ARTICLE

Lack of correlation between CSF glutamate levels and PSQI scores in heavy smokers

Jinzhong Xu[1,2] · Hui Li[3,4] · Fan Wang[3,5] · Zeping Xu[2] · Guohua Li[3] · Cheng Ding[6] · Junnan Wu[2] · Yimin Kang[4] · Hong Li[7] · Heng Xu[3,8] · Yanlong Liu[2]

Received: 16 January 2018 / Revised: 21 July 2018 / Accepted: 31 July 2018 / Published online: 7 August 2018

Abstract

Objective Glutamate is an excitatory neurotransmitter in the central nervous system that participates in initiation and maintenance of sleep and wakefulness. The mechanisms involved occur in the brainstem, lateral hypothalamus, and basal forebrain. Our previous study suggested that higher levels of glutamate in cerebrospinal fluid (CSF) contributed to poorer sleep quality. Smoking has been shown to be harmful to sleep quality. In the present study, we recruited non-smokers and heavy smokers and measured the concentration of CSF glutamate in order to investigate the associations among smoking status, sleep quality, and CSF glutamate levels.

Methods We recruited 147 men ($n = 68$ non-smokers, 30.31 ± 9.10 years; $n = 79$ heavy smokers, 34.54 ± 10.71 years). Glutamate concentrations in CSF were measured by spectrophotometry, and subjective sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI).

Results PSQI total scores were significantly higher in heavy smokers than that in non-smokers ($p < 0.001$). Glutamate concentrations in CSF were lower in heavy smokers than that in non-smokers ($p < 0.001$). CSF glutamate levels positively correlated with PSQI total scores in the non-smokers group ($r = 0.313$, $p = 0.011$, effect size = 0.324). No correlation was found between CSF glutamate levels and PSQI total scores in the heavy smokers group ($p > 0.05$). Multivariable linear regression analysis showed that years of smoking was contributed to the PSQI total scores ($p = 0.008$), and cigarettes smoked per day contributed to the decreased CSF glutamate levels in heavy smokers ($p = 0.001$).

Conclusion Poorer subjective sleep quality and lower CSF glutamate concentrations were observed in the heavy smokers group than in the non-smokers group. In addition, lack of correlation was observed between CSF glutamate levels and PSQI scores in the heavy smokers.

Keywords Cerebrospinal fluid · Glutamate · Sleep · Heavy smoking

Jinzhong Xu, Hui Li and Fan Wang contributed equally to this work.

✉ Heng Xu
changshengx@126.com

✉ Yanlong Liu
benjaminlyl@wmu.edu.cn

[1] The Affiliated Wenling Hospital of Wenzhou Medical University, Wenling 317500, China

[2] College of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou 325035, China

[3] Second Affiliated Hospital of Xinjiang Medical University, Urumqi 830028, China

[4] Psychosomatic Medicine Research Division, Inner Mongolia Medical University, Huhhot 010110, China

[5] Beijing Hui-Long-Guan Hospital, Peking University, Beijing 100096, China

[6] Department of Respiratory Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325035, China

[7] Department of General Surgery, Ningbo Medical Treatment Center, Li Huili Hospital, Ningbo 315000, China

[8] The Second Xiangya Hospital, Central South University, Changsha 410011, China

Introduction

Glutamate is the most common excitatory neurotransmitter; it is broadly distributed in the central nervous system [1], where it plays an important role in initiating and maintaining sleep and wakefulness [2]. Glutamate in the brain stem regulates brain activity, maintains muscle tone during wakefulness, and mediates adjustment of the electroencephalograph in rapid eye movement phase. It is also associated with muscle weakness. Glutamate in the lateral hypothalamus participates in the lateral hypothalamic arousal system by activating orexin neurons. Basal forebrain glutamatergic neurons take part in electroencephalograph synchronization and cause decreases in sleep [2]. Several animal studies suggested that glutamate levels increased after sleep deprivation [3]. Our previous study showed that CSF glutamate levels positively correlated with PSQI total and Component 2 scores, suggesting that higher levels of CSF glutamate contributed to poorer sleep quality, demonstrating a direct link between CSF glutamate levels and sleep quality [4].

A recent tobacco survey found that the prevalence of smoking among adults was 27.7% (52.1% in men and 2.7% in women) in China [5]. In addition to causing lung cancer, smoking has been shown to be harmful to sleep quality [6]. As previously reported, current smokers have greater difficulty in initiating and maintaining sleep than do non-smokers, and they are generally more dissatisfied with their sleep quality [7]. In addition, several studies investigated the effects of smoking on glutamate levels/glutamate receptors as well as the role of glutamate in nicotine addiction [8–10]. Animal studies and human PET imaging suggested that nicotine and cigarette smoking affected glutamate and related neurochemical markers in the brain, implying that smoking reduced extracellular glutamate [8]. In the present study, we recruited non-smokers and heavy smokers to investigate the associations among smoking status, sleep quality, and CSF glutamate levels.

Material and methods

Only male subjects were recruited because of the relatively few female smokers in China. In the present study, 147 male subjects (68 non-smokers and 79 heavy smokers) were recruited at several Chinese hospitals to extract CSF samples before spinal anesthesia in a group of people who were scheduled to undergo surgery for anterior cruciate ligament injuries without other trauma and without any medication including herbal products. All of the participants were Northern Chinese. Subjects with a family history of psychosis and neurological diseases, determined according to criteria based on the Mini-International Neuropsychiatric Interview (Chinese version), were excluded from the study. Individuals with systemic or CNS diseases were also excluded. According to self-reporting and confirmation by the next of kin and family members, subjects without a history of drug abuse or dependence, including alcohol or nicotine abuse, were included in the non-smokers group. All non-smokers had never smoked during their entire lifetimes. The classification of heavy smokers was based on World Health Organization criteria (≥ 10 cigarettes/day over 1 year). They were without a history of other drug abuse or dependence, including alcohol abuse, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition. The study was approved by the Human Ethics Committee of Inner Mongolia Medical University, and written informed consent was obtained directly from the participants.

Lumbar puncture was performed by a licensed anesthetist using sterile technique with the subject in the lateral decubitus position. Cerebrospinal fluid (CSF) was drawn during spinal anesthesia before surgery. The spinal needle was inserted into the L3/L4 or L4/L5 intervertebral space, and a 5-mL CSF sample was obtained from each subject. Each CSF sample was placed in 0.5-mL fractions in polypropylene tubes and immediately frozen at − 80 °C until analysis. Quantification of glutamate levels was performed using a commercial spectrophotometric measurement kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. A 10% quantity of each CSF sample (0.5 mL) was assayed in duplicate.

Subjective sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI). The PSQI consists of seven components, each targeting a major aspect of sleep: subjective sleep quality, sleep onset latency, sleep duration, sleep efficiency, presence of sleep disturbances, use of hypnotic/sedative medication, and presence of daytime disturbances that serve as an indication of daytime alertness. All of the data were expressed as mean ± standard deviation. Partial correlation between CSF glutamate levels and PSQI total scores was analyzed with age and education year as covariates in each group. Multivariable linear regression was performed to evaluate the association between cigarette abuse and CSF glutamate levels or PSQI total scores in heavy smoking group, respectively. Effect size was calculated using the online platform (http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-R-main.php). The difference of CSF glutamate and PSQI total scores was determined using analysis of covariance with age and education years as covariates. Other continuous variables were analyzed using independent-sample t tests. All of the tests were two-tailed, and the α level was set at $p = 0.05$. All analyses were performed using SPSS 20.0 software (Statistical Package for the Social Sciences for Windows, Chicago, IL, USA). A post hoc power analysis was performed to evaluate the power level (1-β), and the result was 0.9968 (G* Power 3.1.9.2 for Windows).

Results

Glutamate concentrations in CSF were assessed in samples from all subjects. As shown in Table 1, higher PSQI total scores were observed in heavy smokers than in non-smokers, indicating poor sleep quality in the former group. We also observed lower glutamate concentrations in heavy smokers than in non-smokers. The association was tested between CSF glutamate levels and PSQI total scores (Fig. 1) in both groups. Partial correlations were then performed, and these showed that CSF glutamate levels positively correlated with PSQI total scores in the non-smokers group ($r = 0.313$, $p = 0.011$, and effect size = 0.324; Fig. 1a). But no correlation was found between CSF glutamate levels and PSQI total scores in heavy smokers group ($r = 0.087$, $p = 0.467$; Fig. 1b).

Multivariable linear regression using PSQI total scores as dependent variable showed that years of smoking contributed to the PSQI total scores ($p = 0.008$) after adjusting education year, BMI, pain level, and CSF glutamate levels (Table 2). In addition, multivariable linear regression analysis using CSF glutamate as dependent variable showed cigarettes smoked per day contributed to the decreased CSF glutamate levels in heavy smokers ($p = 0.001$) (Table 3).

Discussion

We observed poorer subjective sleep quality and lower CSF glutamate concentrations in heavy smokers than in non-smokers. In addition, we also observed positive correlation between CSF glutamate levels and PSQI total scores in non-smokers and lack of correlation between CSF glutamate levels and PSQI scores in the heavy smokers.

Smoking has been shown to be harmful to sleep quality. As previously reported, current smokers have greater difficulty in initiating and maintaining sleep than do non-smokers, and they are generally more dissatisfied with their sleep quality [6, 11]. Several studies reported a negative association between cigarette smoking and sleep quality in adults and adolescents in both population-based and laboratory and clinical studies using polysomnography [12]. Heavier smoking was associated with poor sleep quality among young adult smokers. Higher cigarette consumption was associated with poor sleep quality [13]. As in these studies, we also found poor subjective sleep quality in the present cohort of Northern Chinese heavy smokers.

Substance dependence is associated with varying glutamate concentrations in the dorsal anterior cingulate cortex (dACC) [14]. Studies have identified glutamate imbalances in smokers. There were higher baseline glutamate concentrations in the dorsal anterior cingulate cortex in smokers than in non-smokers [14]. However, in the present study, we found lower glutamate concentrations in heavy smokers than in non-smokers. The differences between these two studies may have resulted from measurement and location differences. The former study measured glutamate concentrations in the dorsal anterior cingulate cortex using proton magnetic resonance spectroscopy, which was not optimized to differentiate between glutamate, glutamine, and glutathione [14]. In the present study, CSF glutamate levels were measured using a commercial spectrophotometric measurement kit.

Glutamate is known to play a critical role in sleep–wake homeostasis [13]. Several animal studies have shown that glutamate levels increased after sleep deprivation [2]. Glutamate levels rose upon sleep deprivation in animal models [15]. Our previous study showed that CSF glutamate levels positively correlated with PSQI total and Component 2 scores, suggesting that higher levels of CSF glutamate contributed to poorer sleep quality, demonstrating a direct link between CSF glutamate levels and sleep quality [16]. Following up on this previous study, in the present study we found that CSF glutamate levels positively correlated with PSQI total scores in the non-

Table 1 Inter-group differences in variables

Variables	Non-smokers ($n = 68$) Mean± SD	Heavy smokers ($n = 79$) Mean ± SD	p
Age (years)	30.31 ± 9.10	34.54 ± 10.71	0.011*
Education (years)	13.21 ± 2.30	11.76 ± 3.12	0.002*
BMI (Kg/m^2)	24.70 ± 3.75	25.92 ± 3.72	0.05
Pain level	1.60 ± 0.56	1.77 ± 1.17	0.258
CSF glutamate (U/mol)	97.87 ± 8.53	57.05 ± 56.21	< 0.001*
PSQI total	2.28 ± 2.27	4.16 ± 2.45	< 0.001*
Age at smoking onset	/	19.80 ± 3.66	/
Cigarettes smoked per day	/	14.77 ± 7.53	/
Years of smoking	/	14.51 ± 9.55	/

Note: The data are expressed as mean ± standard deviation. The differences of CSF glutamate and PSQI total scores were determined using analysis of covariance with age and education years as covariates. Other continuous variables were analyzed using independent-sample t test, *$p < 0.05$

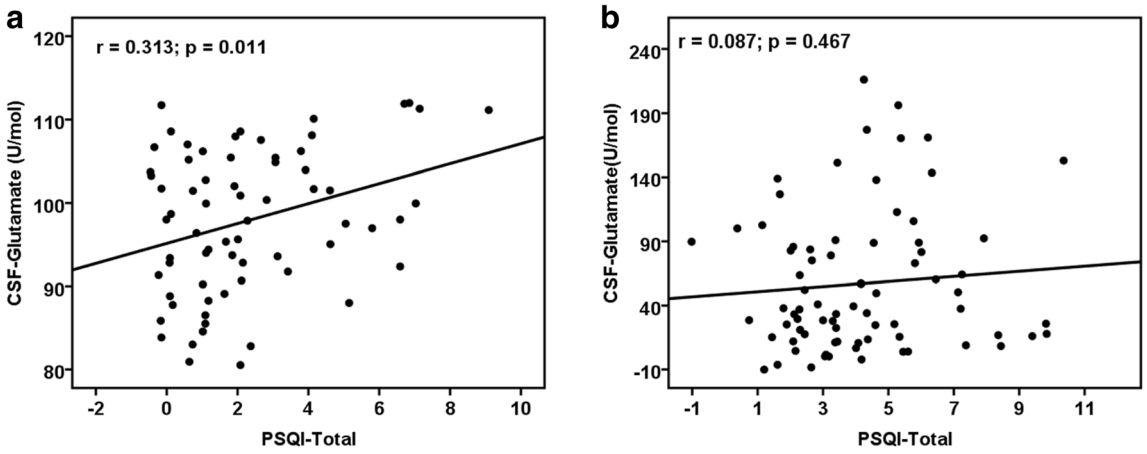

Fig. 1 **a** Positive correlation between CSF glutamate levels and PSQI total scores in non-smokers group. **b** No correlation between CSF glutamate levels and PSQI total scores in heavy smokers group. Partial correlation between CSF glutamate levels and PSQI total scores was performed with age and education years as covariates

smokers group, but not in the heavy smokers group, suggesting that heavy smoking disturbed the positive correlation between CSF glutamate levels and PSQI total scores. Glutamate does not penetrate the blood brain barrier under normal physiological conditions [17]; however, nicotine could change the penetration of the blood brain barrier and induce diminution of passive diffusion of some compounds across the blood–brain barrier, particularly under conditions of chronic exposure [18, 19]. This could be one reason for decreased CSF glutamate levels in heavy smokers. Thus, the positive correlation between CSF glutamate levels and PSQI total scores in healthy people was no longer maintained after chronic nicotine exposure. This could further explain the lack of correlation between CSF glutamate levels and sleep quality in heavy smokers, although we found that smoking decreased CSF glutamate levels and sleep quality.

In addition, in the present study, multivariable linear regression analysis showed that years of smoking was contributed to the PSQI total scores, and cigarettes smoked per day contributed to the decreased CSF glutamate levels in heavy smokers. These distinct correlations may indicate possible mechanisms involved in the lack of correlation between CSF levels and PSQI scores. Negative correlation between CSF glutamate levels and cigarettes smoked per day, but not between years of smoking and CSF glutamate levels, indicated that recent cigarette use has a stronger impact on CSF levels compared to long history of tobacco use or abuse.

Correlation between the results of sleep investigations and neuroendocrine analyses to detect changes in sleep-regulating mechanisms is a compelling topic. Relevant neurotransmitters such as acetylcholine, dopamine, serotonin, norepinephrine, and glutamate, as well as hypothalamic-pituitary axis (HPA) functions are not only known to be influenced by nicotine, but they also play a crucial role in sleep–wake regulation [20]. All these neurotransmitters and HPA changes may be responsible for smoking-induced sleep impairment. In addition, disturbed sleep may be one link in the two-way relationship between smoking and depression. Thus, smoking-induced sleep impairment is induced by a complex neurological network.

One limitation of the present study is that we did not recruit female subjects because of the small number of female smokers in China. The use of subjective, instead of objective, measures of sleep in the present study presented certain limitations. In addition, we did not record daytime or nighttime smoking, possibly explaining the lack of correlation between CSF glutamate levels and PSQI scores in the heavy smoker

Table 2 Multivariable linear regression analysis of associations between PSQI total scores and cigarette abuse in heavy smokers

Variables	Model 1[a]		Model 2[b]		Model 3[c]	
	B (95%CI)	*p*	B (95%CI)	*p*	B (95%CI)	*p*
Age at smoking onset	− 0.09 (− 0.25; 0.067)	0.26	− 0.11 (− 0.28; 0.049)	0.17	− 0.092 (− 0.27; 0.085)	0.30
Cigarettes smoked per day	− 0.018 (− 0.094; 0.058)	0.64	− 0.004 (− 0.079; 0.07)	0.90	0.011 (− 0.07; 0.09)	0.78
Smoking years	0.067 (0.004; 0.13)	0.037	0.086 (0.022; 0.15)	0.009	0.088 (0.024; 0.16)	0.008

[a] Unadjusted

[b] Adjusted by education year and BMI

[c] Adjusted by education year, BMI, pain level, and CSF glutamate levels

Table 3 Multivariable linear regression analysis of association between CSF glutamate levels and cigarette abuse in heavy smokers

Variables	Model 1[a]		Model 2[b]		Model 3[c]	
	B (95%CI)	*p*	B (95%CI)	*p*	B (95%CI)	*p*
Age at smoking onset	− 2.51 (− 6.06; 1.04)	0.16	− 1.17 (− 4.82; 2.48)	0.53	− 3.01 (− 6.53; 0.51)	0.09
Cigarettes smoked per day	− 2.23 (− 3.94; − 0.51)	0.012	− 2.36 (− 4.03; − 0.69)	0.006	− 2.55 (− 4.07; − 1.03)	0.001
Smoking years	0.45 (− 0.98; 1.88)	0.53	− 0.058 (− 1.48; 1.37)	0.94	− 0.55 (− 1.91; 0.82)	0.43

[a] Unadjusted

[b] Adjusted by education year and BMI

[c] Adjusted by education year, BMI, pain level, and PSQI total scores

group. Because nicotine is a CNS stimulant, smoking later in the day could be associated with poorer sleep quality in heavy smokers. Moreover, more subjects are needed to validate our results.

Conclusion

Poorer subjective sleep quality and lower CSF glutamate concentrations were observed in the heavy smokers group than in the non-smokers group. In addition, lack of correlation was observed between CSF glutamate levels and PSQI scores in the heavy smokers.

Funding This work was supported by the Technology Support Project of Xinjiang (2017E0267), Opening Project of Zhejiang Provincial Top Key Discipline of Pharmaceutical Sciences funding, Projects of Medical and Health Technology Program in Zhejiang Province (2017KY720) and Basic and public research project of Zhejiang province (LGF18H020001), Advanced key Scientific and Technological Programs of Ningbo (2013C51009), Public Technology Application Research Project of Zhejiang (2017C35002), and Project of Zhejiang Medical and Health Platform Plan (2016DTA009). The sponsor had no role in the design or conduct of this research.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (the Human Ethics Committee of Inner Mongolia Medical University) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Reis HJ, Guatimosim C, Paquet M, Santos M, Ribeiro FM, Kummer A, Schenatto G, Salgado JV, Vieira LB, Teixeira AL, Palotas A (2009) Neuro-transmitters in the central nervous system & their implication in learning and memory processes. Curr Med Chem 16(7):796–840
2. Shi YF, Yu YQ (2013) The roles of glutamate in sleep and wakefulness. J Zhejiang Univ 42(5):583
3. Dash MB, Douglas CL, Vyazovskiy VV, Cirelli C, Tononi G (2009) Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. J Neurosci 29(3):620–629
4. Wang G, Wang F, Li Q, Yang X, Fan H, He J, Sun H, Lu L, Liu Y (2015) Positive correlation between cerebrospinal fluid glutamate levels and Pittsburgh Sleep Quality Index scores in northern Chinese subjects. Sleep Med 23:123–124
5. Zou X, Jia M, Wang X, Zhi X (2017) Changing epidemic of lung cancer & tobacco and situation of tobacco control in China. Zhongguo Fei Ai Za Zhi 20(8):505–510. https://doi.org/10.3779/j.issn.1009-3419.2017.08.01
6. Liu JT, Lee IH, Wang CH, Chen KC, Lee CI, Yang YK (2013) Cigarette smoking might impair memory and sleep quality. J Formos Med Assoc 112(5):287–290
7. McNamara JPH, Wang J, Holiday DB, Warren JY, Paradoa M, Balkhi AM, Fernandez-Baca J, Mccrae CS (2013) Sleep disturbances associated with cigarette smoking. Psychol Health Med 19(4):410–419. https://doi.org/10.1080/13548506.2013.832782
8. O'Neill J, Tobias MC, Hudkins M, Oh EY, Hellemann GS, Nurmi EL, London ED (2014) Thalamic glutamate decreases with cigarette smoking. Psychopharmacology 231(13):2717–2724. https://doi.org/10.1007/s00213-014-3441-5
9. D'Souza MS, Markou A (2013) The "stop" and "go" of nicotine dependence: role of GABA and glutamate. Cold Spring Harb Perspect Med 3(6):a012146. https://doi.org/10.1101/cshperspect.a012146
10. Cross AJ, Anthenelli R, Li X (2018) Metabotropic glutamate receptors 2 and 3 as targets for treating nicotine addiction. Biol Psychiatry 83(11):947–954. https://doi.org/10.1016/j.biopsych.2017.11.021
11. McNamara JP, Wang J, Holiday DB, Warren JY, Paradoa M, Balkhi AM, Fernandez-Baca J, McCrae CS (2014) Sleep disturbances associated with cigarette smoking. Psychol Health Med 19(4):410–419. https://doi.org/10.1080/13548506.2013.832782
12. Dugas EN, Sylvestre MP, O'Loughlin EK, Brunet J, Kakinami L, Constantin E, O'Loughlin J (2017) Nicotine dependence and sleep quality in young adults. Addict Behav 65:154–160. https://doi.org/10.1016/j.addbeh.2016.10.020
13. Nam HW, McIver SR, Hinton DJ, Thakkar MM, Sari Y, Parkinson FE, Haydon PG, Choi DS (2012) Adenosine and glutamate signaling in neuron-glial interactions: implications in alcoholism and

sleep disorders. Alcohol Clin Exp Res 36(7):1117–1125. https://doi.org/10.1111/j.1530-0277.2011.01722.x

14. Schulte M, Goudriaan AE, Kaag AM, Kooi DP, van den Brink W, Wiers RW, Schmaal L (2017) The effect of N-acetylcysteine on brain glutamate and gamma-aminobutyric acid concentrations and on smoking cessation: a randomized, double-blind, placebo-controlled trial. J Psychopharmacol 31(10):1377–1379. https://doi.org/10.1177/0269881117730660
15. Datta K, Kumar D, Mallick HN (2013) Intragastric administration of glutamate increases REM sleep in rats. Physiol Behav 122:178–181. https://doi.org/10.1016/j.physbeh.2013.09.007
16. Wang G, Wang F, Li Q, Yang X, Fan H, He J, Sun H, Lu L, Liu Y (2016) Positive correlation between cerebrospinal fluid glutamate levels and Pittsburgh Sleep Quality Index scores in northern Chinese subjects. Sleep Med 23:123–124. https://doi.org/10.1016/j.sleep.2015.06.023
17. Hawkins RA (2009) The blood-brain barrier and glutamate. Am J Clin Nutr 90(3):867S–874S
18. Lockman PR, Van der Schyf CJ, Abbruscato TJ, Allen DD (2005) Chronic nicotine exposure alters blood-brain barrier permeability and diminishes brain uptake of methyllycaconitine. J Neurochem 94(1):37–44. https://doi.org/10.1111/j.1471-4159.2005.03162.x
19. Manda VK, Mittapalli RK, Bohn KA, Adkins CE, Lockman PR (2010) Nicotine and cotinine increases the brain penetration of saquinavir in rat. J Neurochem 115(6):1495–1507. https://doi.org/10.1111/j.1471-4159.2010.07054.x
20. Jaehne A, Unbehaun T, Feige B, Lutz UC, Batra A, Riemann D (2012) How smoking affects sleep: a polysomnographical analysis. Sleep Med 13(10):1286–1292. https://doi.org/10.1016/j.sleep.2012.06.026