

Research Article

Different Levels in Orexin Concentrations and Risk Factors Associated with Higher Orexin Levels: Comparison between Detoxified Opiate and Methamphetamine Addicts in 5 Chinese Cities

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Received 2 April 2013; Accepted 1 August 2013

Academic Editor: Alfredo Conti

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This study sought to explore the degree of orexin levels in Chinese opiate and methamphetamine addicts and the differences between them. The cross-sectional study was conducted among detoxified drug addicts from Mandatory Detoxification Center (MDC) in five Chinese cities. Orexin levels were assayed with radioimmunoassay (RIA). Mann-Whitney *U* test and Kruskal-Wallis test were used to detect differences across groups, and logistic regression was used to explore the association between orexin levels and characteristics of demographic and drug abuse. Between November 2009 and January 2011, 285 opiates addicts, 112 methamphetamine addicts, and 79 healthy controls were enrolled. At drug withdrawal period, both opiate and methamphetamine addicts had lower median orexin levels than controls, and median orexin levels in opiate addicts were higher than those in methamphetamine addicts (all above $P < 0.05$). Adjusted odds of the above median concentration of orexin were higher for injection than “chasing the dragon” (AOR = 3.1, 95% CI = 1.2–7.9). No significant factors associated with orexin levels of methamphetamine addicts were found. Development of intervention method on orexin system by different administration routes especially for injected opiate addicts at detoxification phase may be significant and was welcome.

1. Introduction

Orexins (also named hypocretins) are neuropeptides initially discovered almost simultaneously by de Lecea [1] and Sakurai [2] and are identified as neurotransmitters in lateral hypothalamus (LH) neurons. Since its discovery, studies on orexin have been growing rapidly and made a series of achievements. Many studies have confirmed that the function of orexin neurons was mainly reflected through the regulation of arousal and sleep [3–9], stimulating feeding [10–14], regulating drinking behavior [15], and activating the function of adrenal [16] and cardiovascular system [17]. Later investigations on the orexin system have suggested a possible role of orexins in addictive behaviors [18]. In support of this proposition, a number of research groups began to evaluate the role of orexin on cocaine [19–21], nicotine [22–25], ethanol [26–31], and morphine [11, 32]. For instance, Mori et al. [33] reported

that orexin mutant mice have attenuated physical withdrawal from morphine. Li et al. [11] found that after 5 days of treatment (4 mg/kg) twice a day with chronic amphetamine, cAMP-CRMB in orexin neurons was activated. These findings all support the important role of orexin neuropeptides in addiction [28, 31, 34–36].

However, most literatures mainly dealt with that problem based on animal models. Intense research has focused on describing the pathways and mechanisms by which orexins exert their diverse array of functions. To our best knowledge, there were few studies on orexin among illicit drug addicts, especially in China. The aim of this study was to describe and compare orexin levels among Chinese opiates addicts, methamphetamine addicts, and healthy controls and to test whether orexin concentrations were associated with history of drug use by different abused drugs under present data setting.

2. Methods

2.1. Ethical Statement. The study was approved by the ethical committee of Peking University Health Center (Grant number IRB00001052-10026). Before the interviews and blood sampling were conducted, subjects were informed about the purpose of this study and confidentiality of all information they would provide. Respondents' consent was gathered. The survey was conducted in an isolated room, and the survey procedures were designed to protect privacy of subjects by allowing for anonymous and voluntary participation. Participants in the study could stop at any time if they desired.

2.2. Site and Population. A multicenter survey was conducted from the Mandatory Detoxification Center (MDC) in 5 cities, including Beijing, Guangzhou (Guangdong Province), Shenzhen (Guangdong Province), Taiyuan (Shanxi Province), and Xi'an (Shanxi Province). The research locations were chosen for practical reasons. Beijing, Guangzhou, and Shenzhen are metropolitan and most densely populated in China. Taiyuan and Xi'an city were capital cities and smaller in size. Each city represents different geographic regions including northland, midland, southland and westland urban regions of China. Moreover, MDC is administered by the Ministry of Public Security, and for repeat drug offenders, the Ministry of Public Security does not provide drug replacement therapy to addicts [37], which helped facilitate organization and recruitment of participants.

Participants were recruited using cluster sampling. The eligibility criteria were (1) meet the (Diagnostic and Statistical Manual of Mental Disorders, four edition (DSM-IV); American Psychiatric Association, 1994b) criteria for opiate dependence and methamphetamine dependence; (2) men aged 18 or over; (3) for opiate addicts, predominate drugs of choice were only opiates; and for methamphetamine addicts, predominate drugs of choice were only methamphetamine in the baseline survey; (4) received mandatory detoxification treatment for less than 12 months; (5) willing to sign an informed consent; and (6) adhered sufficiently to the study protocol. Meanwhile, the exclusionary criteria were made: (1) unwilling to participate or could not provide written informed consent; (2) could not discriminate the primary drug of abuse; (3) severe psychotic disorders; (4) severe body diseases (e.g., prostrate disease of liver or kidney); (5) be apt to drop out; and (6) deficiency in or lack of language and comprehensive problems. For better understanding, men from community were selected as healthy controls. The criteria were (1) aged 18 and above; (2) without a history of drug abuse before the study and no evidence of drug abuse during study; and (3) provided informed consent.

Based on registration information of MDC, samples of drug addicts were stratified into opiate and methamphetamine addicts. It is necessary to explain that opiates are a category of drugs including heroin and morphine. Within this study, heroin was the main opiate used by respondents. In addition, participants did not simultaneously use both opiates and methamphetamine. Polydrug users were excluded from this study. Overall, a total of 476 eligible subjects were recruited between November 2009 and January

2011. The sample of analysis included 285 opiates addicts, 112 methamphetamine addicts, and 79 healthy men.

2.3. Characterization of Demographics and History of Drug Use. The baseline survey by self-administered questionnaire included age of subjects, ethnicity, marital status, regular dosage by gram each time, months of drug abuse, and routes of drug abuse. The survey period was set from initiation phase to enter MDC. Responses for opiates and methamphetamine use on administration routes were chasing the dragon (which refers to inhaling the vapor from heated morphine, heroin, or opium that has been placed on a piece of foil), intravenous injection, intramuscular injection, smoking in a cigarette, snorting, and other routes. Due to sparseness of data and lack of meaningful differences in some routes of administration, intravenous injection and intramuscular injection were grouped into category of injection. Smoking a cigarette, snorting, and other routes were classified as others. Therefore, the response options for administration routes were defined as chasing the dragon, injection, and others.

2.4. Measurement of Orexin Concentration. In view of circadian rhythm of orexin hormone secretion and in order to avoid the influence of food, therefore, the collection of blood specimen was fixed before breakfast. All drug abusers were requested to collect blood specimen with a limosis status. Five milliliter (mL) blood samples were obtained by venipuncture and immediately cooled on ice. Plasma was separated by centrifugation with 4000 rpm for 8 minutes and then stored at below 20 centigrade. The assays were performed in one batch as soon as the study was completed. To measure orexin, radioimmunoassay (RIA) with reagent box (produced by Xinwan Biology and Science Inc., Tianjin, China) was used. And intra-assay coefficients of variation were all less than 10.0% and interassay coefficients of variation were all less than 15.0%.

2.5. Study Design and Procedures. The cross-sectional survey was employed in this study. Each investigation included an administered questionnaire and a specimen. In order to eliminate and prevent possible bias, a pilot trial was carried out. At the beginning of formal survey, the experienced research staff explained the purpose of this study. Investigation was conducted in private and undisturbed rooms. All finished questionnaires were reviewed by researchers for completeness and consistency. Following the survey, respondents were asked to provide blood specimen for orexin testing. The process would take about 30 min for each subject.

2.6. Data Analyses. As a result of orexin concentrations were not normally distributed; the median with interquartile range was used to present the skewed distributions of this variable. The differences of orexin concentrations in numerical variables were compared using Kruskal-Wallis test. Where necessary, Mann-Whitney *U* test was carried out. The chi-square test was used in qualitative variables. To understand factors related to orexin levels, the outcome variables were recoded into two categories (if higher than the median level

TABLE 1: Demographic characteristics and history of drug use among three different groups.

Variables	Opiates (N = 285)	MA* (N = 112)	Healthy [‡] (N = 79)
Demographic characteristics			
Age (year) (valid total [†])	36.4 ± 8.2 (285)	31.3 ± 7.6 (106)	32.8 ± 9.8 (79)
Han ethnicity (%) (valid total [†])	76.8 (285)	51.8 (112)	—
Married (%) (valid total [†])	54.7 (285)	18.8 (112)	—
History of drug use			
Median months of drug abuse (valid total [†])	7.7 (280)	12.0 (71)	—
Regular median dosage (g/each time) (valid total [†])	0.4 (277)	0.2 (72)	—
Chasing the dragon versus injection (%) (valid total [†])	63.5 (181) versus 27.7 (79)	51.8 (58) versus 0	—

[†]Total number may not be equal to the number of cases due to excluding missing data.

[‡]Data of healthy group were collected only for age of cases.

*Methamphetamine.

of orexin as dependent variables; higher than or equal to the median level = 1; lower than the median level = 0). The age of subjects (≤ 35 years and > 35 years), regular median dosage (≤ 0.5 and > 0.5) (g/each time), and months of drug use (≤ 12 and > 12 month) were categorized into two categories, respectively. The factors associated with orexin levels were analyzed using binary logistic regression models. If possible, multiple logistic regression was used to explore the related factors after adjustment. Data were entered twice with Epi-Data software and then validated until two inputs were completely the same. Statistical analyses were conducted using SPSS. A two-tailed alpha for statistical difference was set at 0.05.

3. Results

3.1. Characteristics of Demographic and History of Drug Use. Demographic characteristics and history of drug use were summarized in Table 1. At baseline, significant difference was observed in respondent's average age ($F = 16.280, P < 0.001$) among three groups. There were significant differences in ethnicity ($\chi^2 = 23.935, P < 0.001$), marital status ($\chi^2 = 42.145, P < 0.001$), median months of drug use (Mann-Whitney $U = 6782.000, P < 0.001$), and administration routes ($\chi^2 = 23.448, P < 0.001$) between opiate and methamphetamine groups, respectively. No statistical difference was found between opiate and methamphetamine groups in regard to median dosage of drug use (Mann-Whitney $U = 8635.500, P = 0.078$).

3.2. Comparison of Orexin Levels among Opiates, Methamphetamine, and Healthy Groups. Figure 1 detailed the median with interquartile range concentrations of orexin in the drug addicts and controls. In total, Kruskal-Wallis test showed a significant difference in median orexin concentrations among opiate, methamphetamine, and healthy groups ($\chi^2 = 32.690, P < 0.001$). For opiate and methamphetamine addicts, both of them had lower median orexin levels than healthy controls. Specifically, the median concentration of orexin in methamphetamine addicts was lower than that in healthy males (Mann-Whitney $U = 2595.000, P < 0.001$). However, Mann-Whitney U test just failed to show a significant difference

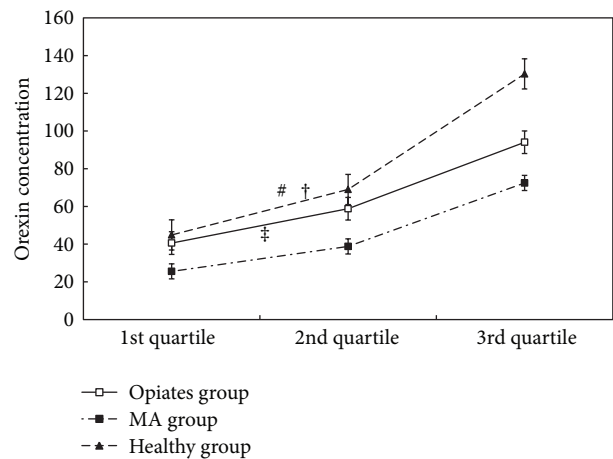


FIGURE 1: Quartiles of orexin median concentrations (pg/mL) in opiate group, MA group, and healthy group. *Notes.* Statistical significance between groups was set at 0.0167 in order for type I error levels to be guaranteed. Significant differences of orexin concentrations for opiates, methamphetamine (MA), and healthy groups were found by Kruskal-Wallis test ($\chi^2 = 32.690, P < 0.001$). [#] $P > 0.0167$ in comparison with the median of healthy group (Mann-Whitney $U = 9472.000, P = 0.031$). [†] $P < 0.0167$ in comparison with the median of MA group (Mann-Whitney $U = 10956.500, P < 0.001$). [‡] $P < 0.0167$ in comparison with the median of healthy group (Mann-Whitney $U = 2595.000, P < 0.001$).

in median orexin levels between opiate and healthy groups (Mann-Whitney $U = 9472.000, P = 0.031$). Otherwise, the median concentration of orexin in opiate addicts was higher than that in methamphetamine addicts (Mann-Whitney $U = 10956.500, P < 0.001$).

3.3. Factors Associated with Orexin Levels in Opiate and Methamphetamine Addicts. Kruskal-Wallis test found significant differences between administration routes and the median of orexin concentrations ($\chi^2 = 10.201, P < 0.01$) (Figure 2). The median level of orexin for injection administration (74.29 pg/mL) was approximately 19 pg/mL higher than chasing the dragon (55.61 pg/mL) (Mann-Whitney $U = 5577.000, P < 0.0167$). However, no significant differences were

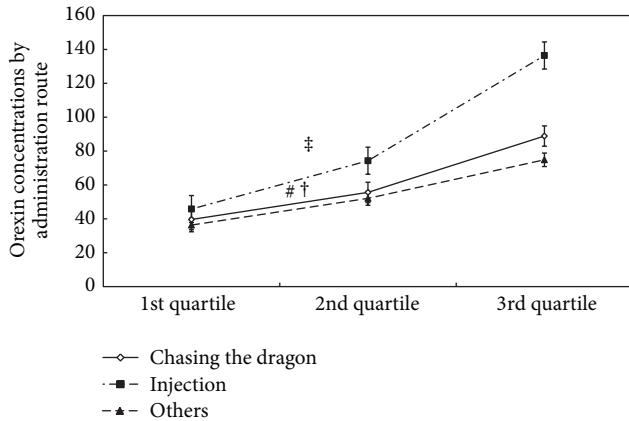


FIGURE 2: Quartiles of orexin median concentrations (pg/mL) by administration routes in opiate addicts. In total, significant differences of orexin concentrations for chasing the dragon, injection, and other routes of drug administration were found by Kruskal-Wallis test ($\chi^2 = 10.201$, $P < 0.01$). As a result of that, $\alpha = 0.0167$ was used when tests were calculated between any two groups. [#]No significant difference in comparison with other routes of drug administration (Mann-Whitney $U = 1991.500$, $P = 0.332$). [†]Significantly different from administration route of injection (Mann-Whitney $U = 5577.000$, $P = 0.005$). [‡]Significantly different from other routes of drug administration (Mann-Whitney $U = 661.000$, $P = 0.013$).

observed between months, regular dosage of drug use, and the median orexin concentrations. The binary logistic regression analysis showed that other routes of administration were more likely to have higher median of orexin concentrations than chasing the dragon (Odds Ratio, OR = 2.904, $P < 0.05$) (Table 2). Although injection drug users tended to report higher median levels than users choosing chasing the dragon method, this effect did not reach statistical significance (OR = 1.609, $P > 0.05$). Using the above median concentration of orexin as the referent, the multiple logistic regression showed that the adjusted odds were higher for injection than chasing the dragon (OR = 3.1, 95% CI = 1.2–7.9) (Table 3). The adjusted variables included age, marital status, months of drug use, and regular administrated dosage.

4. Discussion

The present study showed that both opiate and methamphetamine (MA) addicts had lower orexin levels than healthy controls, and orexin levels in opiate addicts were higher than those in methamphetamine addicts. To our knowledge, this comparison was not found in other studies. For opiate addicts, route of injection was associated with higher levels of orexin. But no association was found between orexin levels and demographic characteristics and drug abuse history for methamphetamine addicts.

In this study, we found that both opiate and methamphetamine addicts had lower median orexin levels than healthy men. Firstly, the served mandatory detoxification may have partly contributed to this effect. Some previous studies have found that stress-induced reinstatement was critically dependent on the drug withdrawal period [38, 39].

Wang et al. [40] found ventral tegmental area (VTA) perfusion of orexin stress reinstated cocaine seeking, which demonstrated that orexin was involved in the process of stress-induced relapse. Withdrawal duration may be an important stress factor for inhibiting orexin. However, this hypothesis needs support from comparison among detoxified addicts in different withdrawal period. Data were collected only for less than 12 months of detoxification in this survey, for those who served more than 12 months, if orexin levels would restore to normal compared to healthy controls needs further study. Secondly, it was reasoned that orexin receptors in the lateral hypothalamus (LH) or the mesolimbic dopamine system were inhibited [34, 41] during the initial detoxification, which expressed itself as the inhibited desire for drug seeking. For example, Narita et al. [42] suggested that the excitation of brain orexin neurons and/or the tonic activation of orexin receptors were specifically required for the rewarding effect of opiates.

Data here also suggest higher median levels of orexin in opiate addicts than in methamphetamine addicts. The underlying reasons may be the following: effect of abused drugs on the orexin system was drug-specific [24], and regional variations within the LH have been reported in response to some drugs. For instance, Fos expression was increased in orexin neurons following acute administration of methamphetamine [37], and acute morphine fails to alter orexin mRNA levels [43]. However, it could not provide more information about the significance of this comparison, because the pharmacological, cytological, and neurobiological data were not obtained in the current investigation. Notwithstanding, it led us to assume that the degree of inhibited orexin system in opiates addicts was slighter than that in methamphetamine addicts, which beget opiate addicts were more liable to rehabilitation.

This study found that orexin levels for opiate addicts differed by administration routes ($P = 0.033$) and injected opiate addicts had higher median orexin levels. This may be supported by the finding that injection administration was associated with the above median levels of orexin among opiate addicts (AOR = 3.076, 95% CI: 1.194–7.909). Previous studies have shown that routes of drug administration differed significantly by drugs of abuse [44]. We speculate that administration routes affect the orexin levels of opiate addicts, because injected opiate appeared to activate orexin neurons in this population. Similarly, Harris et al. [45] found that injected morphine could stimulate LH orexin neurons during conditioned place preference (CPP). However, the underlying mechanism between administration routes and orexin levels still needs further examination.

Limitations of this study should be discussed. Although it has been shown that central and peripheral orexin levels increase and decrease in similar proportions, the first limitation of this study may be attributed to the peripheral analysis of orexin levels as a centrally generated metabolite [46]. Second, this study could not neglect the effect of demographic characters, owing to the fact that significant differences between groups were observed. We had tried to match confounding factors such as age and marital status. Thus, demographic characteristics were used as a control in real analysis.

TABLE 2: Unadjusted odds ratios and 95% confidence interval for the relationship between a concentration above the median of the orexin (pg/mL) and demographic characteristics and history of drug use among different groups.

Variables	Opiate group			MA* group				
	N	Median (IQR [†])	P	OR (95% CI)	N	Median (IQR [†])	P	OR (95% CI)
Age (year)								
≤35	119	58.13 (40.89–87.78)	0.787	0.937 (0.585–1.501)	78	42.42 (28.06–77.70)	0.153	1
>35	166	60.24 (39.98–103.14)		1	28	31.90 (23.04–58.78)		1.899 (0.788–4.577)
Regular median dosage (g/each time)								
≤0.5	196	56.92 (39.20–93.73)	0.303	1	63	40.47 (25.14–92.00)	0.342	1
>0.5	89	62.08 (41.55–95.38)		0.768 (0.464–1.270)	49	36.33 (25.96–50.81)		1.439 (0.680–3.046)
Routes of administration at addiction phase								
Chasing the dragon	181	55.61 (39.60–88.87)	0.033	1	58	43.09 (29.56–77.70)	0.450	1
Injection	79	74.29 (45.80–136.40)		1.609 (0.676–3.831)	—	—		—
Others	25	52.07 (36.34–74.86)		2.904 (1.140–7.393)	54	36.33 (24.50–62.00)		0.751 (0.357–1.579)
Months of drug use								
≤12	210	59.87 (40.73–94.51)	0.610	1	36	43.31 (24.66–90.76)	0.419	1
>12	75	57.72 (40.04–92.73)		0.872 (0.514–1.478)	76	37.14 (26.44–62.37)		1.389 (0.626–3.081)

Exposure was defined as the above median concentration of orexin.

[†]IQR; interquartile range.

* Methamphetamine.

TABLE 3: Multiple logistic regression for associations between administration routes of drug use and a concentration above the median of the orexin (pg/mL) among opiate addicts.

Administration routes of drug use	N	Adjusted OR	95% CI	P
Chasing the dragon	181	1		
Injection	79	3.073	1.194–7.909	0.020
Others	25	1.720	0.687–4.304	0.247

Adjusted for age of subjects, marital status, months of drug use, and dosage of drug use.

Moreover, female participants were precluded, but a finding [47] showed that female injected drug users (IDUs) in Tijuana had an even higher prevalence of methamphetamine (MA) use compared to males which warrant exploration in future studies. Third, in spite of many efforts to increase the sample size of blood specimen, the overall number of MA and healthy controls remained little. To some extent, it may not meet for sufficient power to detect significant differences in logistic analysis. Larger scale survey is needed to provide detailed information and extend present findings. Last but not least, comparison of orexin levels before and after detoxification was not executed due to lack of related data, which more or less limited the interpretation of results.

5. Conclusions

Despite these limitations, as far as we know, less was known about the levels and influenced factors of orexin concentrations among different groups in China. From a clinical point of view, the discovery of the linkage between orexin levels and injection administration may lead to the development of a special detoxified means for opiate addicts and to the expectation of the ultimate development of novel drugs for treatment of opiate addicts.

Conflict of Interests

All the authors declare that they have no conflict of interests.

Acknowledgments

The authors would especially like to thank the staff at MMT agencies in Xi'an City of Shanxi Province, Guangzhou and Shenzhen of Guangdong Province, Taiyuan of Shanxi Province, and Beijing. The survey would not have been possible without their hard work. Also they gratefully thank the participants for agreeing to take part in the investigation. This study was granted by the Major State Basic Research Project (973 program, Grant no. 2009CB522007) and by the National "Twelfth Five-Year" Plan for Science and Technology Support Program (Grant no. 2012BAI01B07), China.

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