European Archives of Psychiatry and Clinical Neuroscience (2019) 269:309–315
https://doi.org/10.1007/s00406-018-0900-8

ORIGINAL PAPER

NMDA receptor subunits change in the prefrontal cortex of pure-opioid and multi-drug abusers: a post-mortem study

Hamidreza Daneshparvar[1] · Mitra-Sadat Sadat-Shirazi[2,3] · Monir Fekri[2] · Solmaz Khalifeh[4] · Ali Ziaie[5] · Nasrin Esfahanizadeh[6] · Nasim Vousooghi[2,3] · Mohammad-Reza Zarrindast[2,7]

Received: 25 September 2017 / Accepted: 24 April 2018 / Published online: 16 May 2018

Abstract

Addiction is a chronic relapsing disorder and is one of the most important issues in the world. Changing the level of neurotransmitters and the activities of their receptors, play a major role in the pathophysiology of substance abuse disorders. It is well-established that *N*-methyl-D-aspartate receptors (NMDARs) play a significant role in the molecular basis of addiction. NMDAR has two obligatory GluN1 and two regionally localized GluN2 subunits. This study investigated changes in the protein level of GluN1, GluN2A, and GluN2B in the prefrontal cortex of drug abusers. The medial prefrontal cortex (mPFC), lateral prefrontal cortex (lPFC), and orbitofrontal cortex (OFC) were dissected from the brain of 101 drug addicts brains and were compared with the brains of non-addicts ($N=13$). Western blotting technique was used to show the alteration in NMDAR subunits level. Data obtained using Western blotting technique showed a significant increase in the level of GluN1 and GluN2B, but not in GluN2A subunits in all the three regions (mPFC, lPFC, and OFC) of men whom suffered from addiction as compared to the appropriate controls. These findings showed a novel role for GluN1, GluN2B subunits, rather than the GluN2A subunit of NMDARs, in the pathophysiology of addiction and suggested their role in the drug-induced plasticity of NMDARs.

Keywords Addiction · Opioid · Post-mortem · *N*-Methyl-D-aspartate (NMDA) receptor · Prefrontal cortex

Introduction

Drug addiction is one the most important issues in the world especially in the Middle East [1]. Drug addiction is a chronic relapsing disorder and is characterized by the compulsion to seek drug, loss of control in managing consumption, and negative emotional states (like anxiety and dysphoria) when the drug is not available [2]. Addiction consists of three stages including (1) binge/intoxication, (2) withdrawal/negative effect, and (3) preoccupation/

Hamidreza Daneshparvar and Mitra-Sadat Sadat-Shirazi equal contribution.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00406-018-0900-8) contains supplementary material, which is available to authorized users.

✉ Mohammad-Reza Zarrindast
zarinmr@ams.ac.ir

1 Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran

2 Iranian National Center for Addiction Studies (INCAS), Tehran University of Medical Sciences, Tehran, P.O.Box: 13145-784, Iran

3 Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

4 Cognitive and Neuroscience research Center (CNRC), Islamic Azad University, Tehran Medical Sciences Branch, Tehran, Iran

5 Legal Medicine Center, Tehran, Iran

6 Department of Periodontics, Tehran Dental Branch, Islamic Azad University, Tehran, Iran

7 Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

anticipation (craving). This is a loop and after the craving stage, the patient comes back to the first stage (binge/intoxication). These three steps are responsible for the framework of studying the neurobiology of addiction [3].

Multi-drug abuse described the tendency to use more than one drug. Multi-drug abuse occurs when a given drug is not available, or tolerance occurred. To relieve the boredom or tension and depression of this, a person experienced other kinds of drugs [4, 5].

There are many neurotransmitters involved in substance use disorder such as dopamine, glutamate, enkephalins, γ-aminobutyric acid, norepinephrine, corticotropin-releasing factor (CRF), dynorphin, neuropeptide Y, and endocannabinoids [2]. Glutamate is the primary excitatory neurotransmitter in the brain and directly or indirectly it modulates the activity of the dopaminergic system in drug addiction [6]. Glutamate has two types of receptors: (1) ionotropic receptors including AMPA, *N*-methyl-D-aspartate (NMDA), and kainate (KA) [7] and (2) metabotropic receptors including mGluR I, mGluR II, and mGluR III [8]. NMDA receptors (NMDAR) are tetramer proteins consisting of two GluN1 subunits which are constant in NMDARs and two GluN2 (A-D) or GluN3 (A-B) subunits [9, 10]. The glutamate binding site is located in GluN2 subunits [11]. In the prefrontal cortex (PFC), drug exposure could regulate GluN2 subunits [12–14]. NMDA receptors are expressed in different parts of the brain including PFC, hippocampus, and striatum [15].

Cognitive studies revealed that addiction encompasses neuroplasticity mechanisms like learning and memory [16]. These mechanisms underlie the role of the medial prefrontal cortex (mPFC) [17–19]. Human imaging and rodent studies showed that mPFC had an essential role in the drug relapse model [20–25]. Furthermore, cell bodies in the ventral tegmental area (VTA) and axons in nucleus accumbens (NAc) received glutamatergic inputs from PFC, amygdala, and hippocampus [26, 27], and these regions are involved in drug addiction [28, 29]. Chronic morphine treatment leads to excessive activation of NMDAR via stimulation of μ-opioid receptors [30]. Other investigations revealed that chronic morphine exposure alters the expression of some NMDA subunits with a region-specific pattern in the brain of rats [31].

Post-mortem studies are valuable because they provide a chance to achieve accurate details about illnesses that are not possible with living humans. Finding changes in the brain of post-mortem addicted human, help to develop new medication against addiction. This study was conducted to clarify the changes in NMDAR subunits (GluN1, GluN2A, and GluN2B) in the prefrontal cortex (medial prefrontal cortex (mPFC), lateral prefrontal cortex (lPFC), and orbitofrontal cortex (OFC)) of pure-opioid and multi-drug abusers.

Subjects and methods

Post-mortem brain tissue collection and subject characterization

Post-mortem brains (101 male addicts and 13 male controls) were obtained from Iranian legal medicine organization, Kahrizak, Tehran, Iran after getting informs consent from relatives. A forensic pathologist determined the cause and manner of death using medical records, police reports, autopsy results, and toxicological data. All the cases were tested for common drugs of abuse using rapid test kit (Baharafshan, Iran).

At least one family member and/or friend provided data about the history of substance abuse. The demographic data of subjects is shown in Suppl. Table 1. All samples with a history of neurological or psychiatric illness, post-mortem interval more than 48 h, evidence of neuropathology (e.g., encephalitis), or chronic illness (e.g., cirrhosis, cancer, HIV, and prolonged hospitalization) were omitted. Each abuser subject was matched to the control based on age and post-mortem interval.

All procedures were approved by the ethics review board of the Iranian legal medicine organization. The examined brain regions including mPFC (Brodmann's area 9, 10, 12), lPFC (Brodmann's area 10, 46) and OFC (Brodmann's area 11,12) were dissected (Suppl. Figure.1) from the whole fresh brain (according to Paxinos atlas [32]) and kept at − 80 °C.

Protein extraction and quantification

The autopsy tissues were immediately transferred to liquid nitrogen to avoid protein degradation. Each sample was homogenized in Radio Immuno Precipitation Assay (RIPA) buffer containing protease inhibitor cocktail. After centrifugation at 18,000*g* for 5 min, total protein was collected by stacking up the supernatant. Protein concentration was defined at 230 nm wavelength using a spectrophotometer (Picodrop, Hinxton, UK). Each sample (60 μg) was combined with loading buffer [50 mM Tris–HCl (pH 6.8), 1.5% SDS, 10% glycerol, 2.5% mercaptoethanol and 0.1% bromophenol blue]. Then, the samples were kept at 95 °C for 5 min and prepared to downstream gel electrophoresis.

Western blotting

The GluN1, GluN2A, and GluN2B NMDA receptor subunits were quantified using immunoblot analysis as described previously [33]. Briefly, samples were loaded in 8% polyacrylamide gel, then electrophoresis was conducted at 120 V for 120 min, and blotted onto polyvinylidene fluoride (PVDF)

membranes (Chemicon Millipore Co. Temecula, USA). The membranes were incubated for 90 min in skimmed milk 5% to block non-specific protein binding sites. Subsequently, blots were incubated with a primary antibody (Abcam, 1:1000 diluted in skimmed milk) overnight at 4 °C.

After washing three times with Tris-buffered saline (TBS) and Tween 20 (TBST), blots were incubated with secondary antibody (Horseradish peroxidase-linked goat anti-rabbit IgG, Abcam, 1:5000) for 1 h. Enhanced chemiluminescence (ECL; Amersham, UK) Western blot detection system was used to detect bounds. It was visualized by exposure to autoradiographic films for 1–10 min.

Hair analysis for drugs

Hair samples were collected for drug test to confirm chronic substance abuse. About 50 mg of the hair sample was washed three times with deionized water. Then, the hair was drenched in petroleum benzene five times; each lasted for 5 min. Finally, the samples were kept in dichloromethane and vortex. The hair sample was dried and cut into small fragments (1 mm). Thin layer chromatography (TLC) was performed for drugs (including morphine, codeine, tramadol, methadone, diphenoxylate, Δ9-Tetrahydrocannabinol (THC), methamphetamine/amphetamine, benzodiazepine (BDZ), phenothiazine, acetaminophen, Tricyclic antidepressant (TCA), and pseudoephedrine), using TLC kit (Baharafshan, Tehran, Iran) according to the manufacturer's protocol.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM), and was processed using SPSS version 21. Quantification of the Western blotting result was performed by densitometric scan of films using the Image J software in comparison with beta-actin as a housekeeping protein (endogenous control). One-way analysis of variance (ANOVA) and post hoc test (Bonferroni) were performed to detect the differences between groups. P value less than 0.05 ($P < 0.05$) was considered to be statistically significant ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$).

Results

Demographic data

As shown in Table 1, the mean age of abusers was 37.139 ± 0.91 SEM as compared to the control group with 36.308 ± 2.57 SEM which is not statistically different. The average year for the duration of substance consumption was 7.71 ± 0.482 SEM. The total number of subjects and the causes of death are shown in Table 2.

The level of GluN1 and GluN2B NMDA receptor subunits' protein level increased in the mPFC of drug abusers

Figure 1 shows that protein level of GluN1 subunits increased among substance abusers when compared with the control group. As shown in Fig. 1a, GluN1 increased in both pure-opioid and multi-drug abusers in comparison to the control group (2.17 fold, $P < 0.01$ and 2.03 fold, $P < 0.05$, respectively). In the case of GluN2A (Fig. 1b), there were no significant differences in GluN2A protein level in mPFC between the groups. However, the GluN2B protein level was elevated in pure-opioid and multi-drug abusers (5.25 and 5.95 fold, respectively, $P < 0.001$). In every three subunits, there was no significant difference between pure-opioid and multi-drug abusers. Also, the manner of death and PMI did not correlate with the level of proteins of NMDA receptor subunits in the mPFC. The details

Table 1 The mean of age and duration of drug consumption in abusers presented in mean ± SEM

	Control	POA	MDA	Total addict
Number	13	56	45	101
Age (year)	36.308 ± 2.57	38.39 ± 1.14	35.572 ± 1.44	37.139 ± 0.91
Duration of drug consumption (year)	0	8.6 ± 0.71	6.93 ± 0.56	7.83 ± 0.47

There was no significant difference in age between groups

Table 2 The manner of death among substance abusers which defined by legal medicine center

	Pure-opioid abusers			Multi-drug abusers		
Cause of death	Overdose	Non-overdose	Unknown	Overdose	Non-overdose	Unknown
Number of objects	11	41	4	18	27	1

The cause of death in four subjects of pure-opioid abusers and one subject of multi-drug abusers could not be found

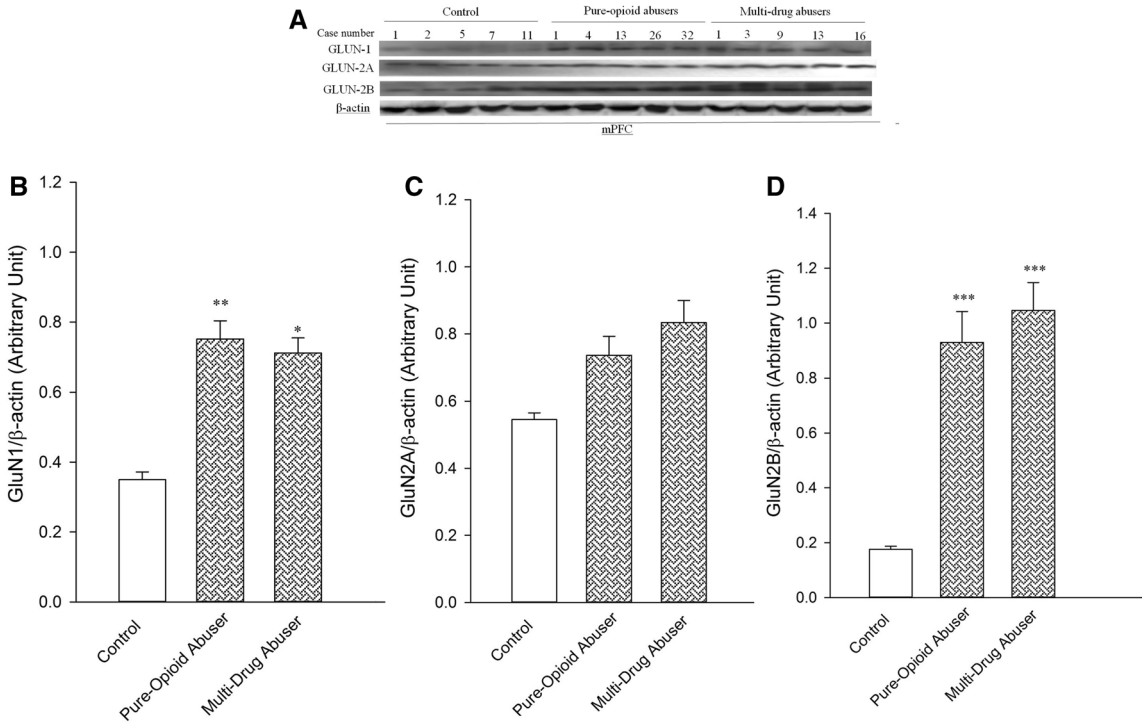

Fig. 1 The protein level of GluN1 and GluN2B subunits increased among substance abusers. Evaluation of protein level in the mPFC of pure-opioid and multi-drug abusers performed by western blotting (**a**). Sixty micrograms of proteins were separated on SDS-PAGE, Western blotted, probed with anti-GluN1 (**b**), GluN2A(**c**), and GluN2B (**d**) antibodies, and reprobed with anti-β-actin antibody (one representative Western blot was shown). The densities of the corresponding bands were measured, and the ratio of β-actin bands was calculated. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to the control group

of densitometric data for all three brain regions are represented in Suppl. Table 2.

GluN1 and GluN2B NMDA receptor subunits' protein levels increased in lPFC of substance abusers

Figure 2 shows that substance abusers (both pure-opioid and multi-drug abusers) had an elevated level of GluN1 (Fig. 2a) and GluN2B (Fig. 2c) proteins when compared with the control group. Figure 2-A shows that the GluN1 protein level was elevated in both pure-opioid ($P<0.01$) and multi-drug ($P<0.05$) abusers. As shown in Fig. 2c, the level of GluN2B subunit was significantly higher in both pure-opioid and multi-drug abusers ($P<0.001$), when compared with the control group. There was no significant difference in the level of GluN2A in lPFC among the groups (Fig. 2-B).

In addition, the route of death and PMI did not change the expression level between groups.

The protein level of GluN1 and GluN2A and GluN2B NMDA receptor subunits increased in OFC of substance abusers

Figure 3 shows that all three subunits of NMDA receptor increased in the OFC of subject who are drug addicts (both pure-opioid and multi-drug abusers). GluN1 ($P<0.001$), GluN2A ($P<0.05$), and GluN2B ($P<0.01$) were upregulated in both multi-drug and pure-opioid abusers when compared with the control group. As shown in Fig. 3a, GluN1 was increased to 2.87 fold in pure-opioid and 2.89 fold in multi-drug abusers as compared to the control group ($P<0.001$). Figure 3b shows that an increase in the level of GluN2A occurred in the pure-opioid (1.85 fold) and multi-drug abusers (2.06 fold) when compared with the control group. In that order, the GluN2B protein level increased (6.15 fold) in pure-opioid abusers ($P<0.01$) as compared to the control group. This subunit was upregulated 7.75 fold

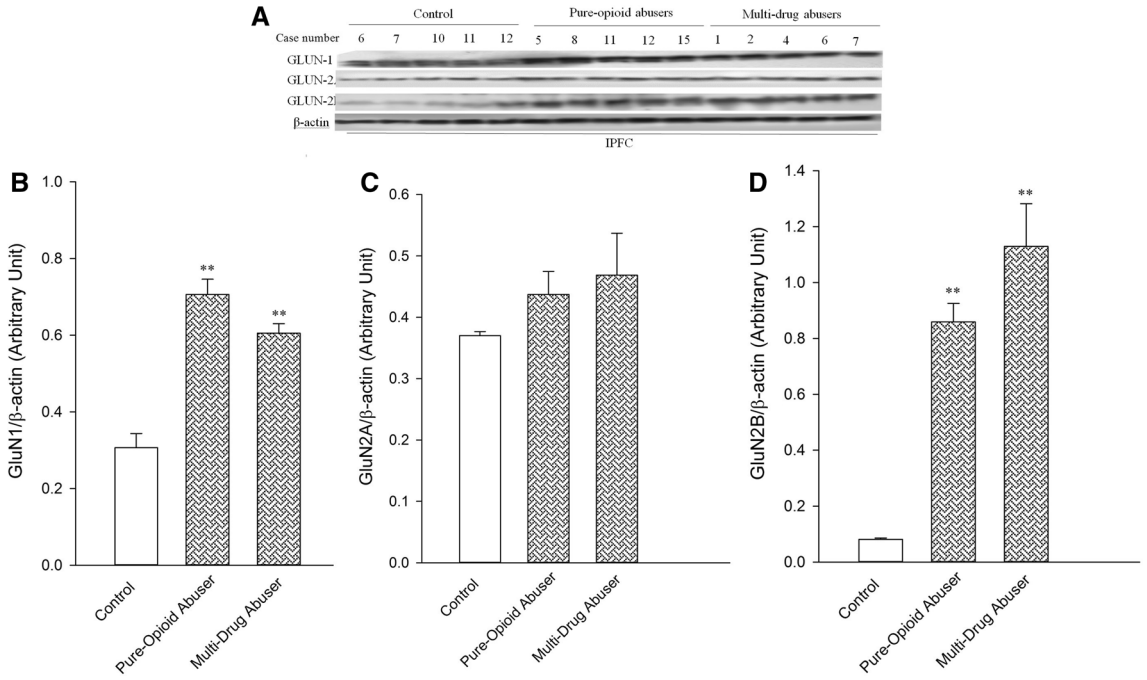

Fig. 2 GluN1 and GluN2B NMDA receptor subunits' protein levels increased in the lPFC of substance abusers. Representative Western blots are shown (**a**). Densitometry analysis of GluN1 (**b**), Densitometry analysis of GluN2A (**c**) and Densitometry analysis of GluN2B (**d**) are shown. β-actin was used as a control. Each point shows the mean ± SEM. $^{**}P < 0.01$ significantly different from the control group

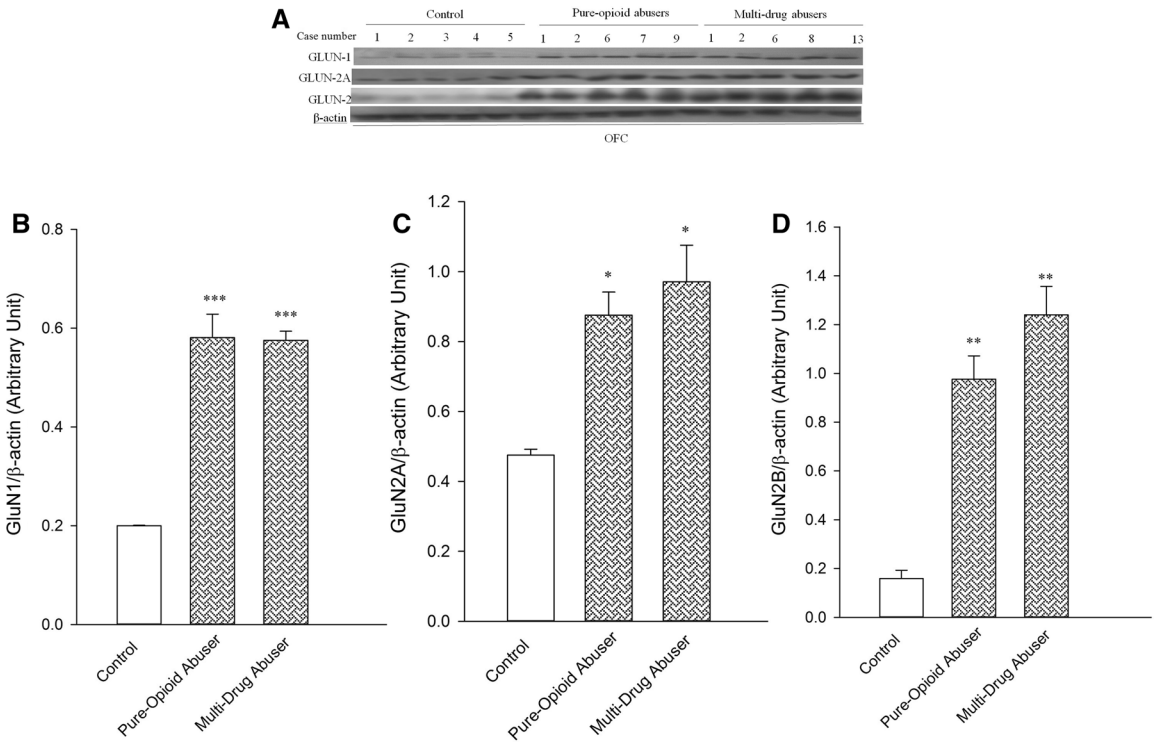

Fig. 3 The protein level of GluN1 and GluN2A and GluN2B NMDA receptor subunits increased in OFC of substance abusers. Western blot technique was used to detect GluN1 and GluN2A and GluN2B protein level in the OFC. The density of bands was measured and their ratio to β-actin was calculated. Each bar is mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the control group

in multi-drug abusers when compared with the control group ($P < 0.001$).

Like mPFC and lPFC, there is no significant correlation between the cause of death and PMI and protein expression level of NMDA subunits among the groups.

Discussion

Drugs of abuse such as morphine could induce deficiency in the cognitive function like goal-directed behavior, executive function, and problem-solving ability [24]. Koob and Volkow showed that drug abuse leads to dysfunction in the normal activity of frontal cortex (OFC, dlPFC, and cingulate gyrus) [34]. Evidence revealed that PFC volume and consequently executive function reduced in crack cocaine abusers even after six weeks of abstinence [35]. The PFC is involved in driven preoccupation/anticipation stage of drug addiction [2] and glutamate plays a major role in neurotransmission activity of the PFC [36]. Activation of glutamatergic system is the most important neuroplastic changes in developing drug addiction. Evidence showed that glutamatergic transmission in mPFC could potentiate the reward saliency of opioids [37].

Ionotropic glutamate receptors (NMDA, AMPA, and KA) are involved in drug-induced neuroplasticity that is essential for incentive salience of drug cues [38]. The present study provides a comparison of NMDA receptor subunits' protein level in the human who abuse opioid and/or stimulant drugs.

The results of the present study showed that chronic opioid or stimulant exposures increase the level of NMDAR subunits. Based on the study, changes in NMDA subunits' protein level were not altered between pure-opioid and multi-drug abusers.

Previously, an electrophysiological study showed that chronic morphine treatment (for fourteen consequence days) increased field potentials that evoke in frontal cortex via NMDA and AMPA receptors [39]. Additionally, chronic morphine treatment enhances mRNA level of the NMDA receptors' GluN1 subunit in some regions of rat brain. This increase in mRNA level of the NMDA receptor has a significant role in tolerance to and dependence on morphine [40]. Bajo et al. [41] reported that chronic morphine treatment in the rat increased the protein level of GluN1 and GluN2B in the NAC. In the present study, NR1 subunit was found to increase in OFC, mPFC, and lPFC of pure-opioid and multi-drug abusers. One animal study in morphine-dependent C57/Bl mice showed that the protein level of GluN1, GluN2A, GluN2B, GluN2C, and GluN2D in the preaquadactal gray matter and ventromedial medulla did not change [42]. However, it was reported that chronic morphine and methamphetamine administration could alter AMPA and mGLU subunits in mPFC of rat [43].

In the case of methamphetamine abusers, methamphetamine neurotoxicity was shown to induce apoptotic mechanisms [44] via glutamate systems [45]. A recent study using MRS revealed that glutamate concentration in the prefrontal cortex decreased in crack cocaine abusers, which is correlated to the years of usage [46]. Thus, it can be concluded that neurons increased their glutamate receptors in the cell surface to get the same response.

Conclusion

There is scarcity of data about the molecular mechanism of drug abuse. This is a pressing public health concern since the use of different opioids is common among people. The level of GluN1 and GluN2B subunits, rather than the GluN2A subunit of NMDARs, was found to be higher in pure-opioid and multi-drug abusers in mPFC, lPFC, and OFC. Also, the route of death (overdose or non-overdose) and the kind of drugs abused did not affect NMDAR expression level. This study suggests that there are subtle but significant changes in the glutamatergic receptors' subunits of different drug abusers which might be one of the mechanisms underlying drug addiction.

Acknowledgements The authors acknowledge the grant support of the legal medicine research center.

Compliance with ethical standards

Conflict of interest Authors have no conflict of interest to declare.

References

1. Richard Isralowitz PAF (2016) Mental health and addiction care in the middle east. Springer International Publishing, New York
2. Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry 3:760–773
3. Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52–58
4. Kaufman E (1976) The abuse of multiple drugs. I. Definition, classification, and extent of problem. Am J Drug Alcohol Abuse 3:279–292
5. Kaufman E (1977) Polydrug abuse or multidrug misuse: it's here to stay. Br J Addict Alcohol Other Drugs 72:339–347
6. Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 130:1007S–1015S
7. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK et al (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 62:405–496
8. Muto T, Tsuchiya D, Morikawa K, Jingami H (2007) Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. Proc Natl Acad Sci USA 104:3759–3764
9. Zhuo M (2009) Plasticity of NMDA receptor NR2B subunit in memory and chronic pain. Mol Brain 2:4

10. Glasgow NG, Siegler Retchless B, Johnson JW (2015) Molecular bases of NMDA receptor subtype-dependent properties. J Physiol 593:83–95
11. Kendrick SJ, Lynch DR, Pritchett DB (1996) Characterization of glutamate binding sites in receptors assembled from transfected NMDA receptor subunits. J Neurochem 67:608–616
12. Cozzoli DK, Goulding SP, Zhang PW, Xiao B, Hu JH, Ary AW et al (2009) Binge drinking upregulates accumbens mGluR5-Homer2-PI3K signaling: functional implications for alcoholism. J Neurosci 29:8655–8668
13. Cozzoli DK, Courson J, Wroten MG, Greentree DI, Lum EN, Campbell RR et al (2014) Binge alcohol drinking by mice requires intact group 1 metabotropic glutamate receptor signaling within the central nucleus of the amygdala. Neuropsychopharmacology 39:435–444
14. Liddie S, Itzhak Y (2016) Variations in the stimulus salience of cocaine reward influences drug-associated contextual memory. Addict Biol 21:242–254
15. Paoletti P (2011) Molecular basis of NMDA receptor functional diversity. Eur J Neurosci 33:1351–1365
16. Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nat Rev Neurosci 8:844–858
17. Van den Oever MC, Spijker S, Smit AB, De Vries TJ (2010) Prefrontal cortex plasticity mechanisms in drug seeking and relapse. Neurosci Biobehav Rev 35:276–284
18. Tzschentke TM (2000) The medial prefrontal cortex as a part of the brain reward system. Amino acids 19:211–219
19. Robbins TW, Ersche KD, Everitt BJ (2008) Drug addiction and the memory systems of the brain. Ann N Y Acad Sci 1141:1–21
20. LaLumiere RT, Kalivas PW (2008) Glutamate release in the nucleus accumbens core is necessary for heroin seeking. J Neurosci 28:3170–3177
21. Peters J, Kalivas PW, Quirk GJ (2009) Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 16:279–288
22. Peters J, LaLumiere RT, Kalivas PW (2008) Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J Neurosci 28:6046–6053
23. Botelho MF, Relvas JS, Abrantes M, Cunha MJ, Marques TR, Rovira E et al (2006) Brain blood flow SPET imaging in heroin abusers. Ann N Y Acad Sci 1074:466–477
24. Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642–1652
25. Kozlenkov A, Jaffe AE, Timashpolsky A, Apontes P, Rudchenko S, Barbu M et al (2017) DNA methylation profiling of human prefrontal cortex neurons in heroin users shows significant difference between genomic contexts of hyper- and hypomethylation and a younger epigenetic age. Genes 8:152–170
26. Christie MJ, Summers RJ, Stephenson JA, Cook CJ, Beart PM (1987) Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H] aspartate and [3H]GABA. Neuroscience 22:425–439
27. Kelley AE, Domesick VB, Nauta WJ (1982) The amygdalostriatal projection in the rat—an anatomical study by anterograde and retrograde tracing methods. Neuroscience 7:615–630
28. Everitt BJ, Morris KA, O'Brien A, Robbins TW (1991) The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience 42:1–18
29. Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. Ann N Y Acad Sci 877:412–438
30. Mayer DJ, Mao J, Holt J, Price DD (1999) Cellular mechanisms of neuropathic pain, morphine tolerance, and their interactions. Proc Natl Acad Sci USA 96:7731–7736
31. Zhu H, Brodsky M, Gorman AL, Inturrisi CE (2003) Region-specific changes in NMDA receptor mRNA induced by chronic morphine treatment are prevented by the co-administration of the competitive NMDA receptor antagonist LY274614. Brain Res Mol Brain Res 114:154–162
32. Mai JK, Paxinos G (2011) The human nervous system. Elsevier Science, Amsterdam
33. Ashabi G, Sadat-Shirazi MS, Khalifeh S, Elhampour L, Zarrindast MR (2017) NMDA receptor adjusted co-administration of ecstasy and cannabinoid receptor-1 agonist in the amygdala via stimulation of BDNF/Trk-B/CREB pathway in adult male rats. Brain Res Bull 130:221–230
34. Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217–238
35. Fein G, Di Sclafani V, Meyerhoff DJ (2002) Prefrontal cortical volume reduction associated with frontal cortex function deficit in 6-week abstinent crack-cocaine dependent men. Drug Alcohol Depend 68:87–93
36. Peters J, De Vries TJ (2012) Glutamate mechanisms underlying opiate memories. Cold Spring Harbor Perspect Med 2:a012088
37. Bishop SF, Lauzon NM, Bechard M, Gholizadeh S, Laviolette SR (2011) NMDA receptor hypofunction in the prelimbic cortex increases sensitivity to the rewarding properties of opiates via dopaminergic and amygdalar substrates. Cereb Cortex 21:68–80
38. Koob GF, Arends MA, Moal ML (2014) Drugs, addiction. Elsevier Science, Amsterdam
39. Bobula B, Hess G (2009) Effects of morphine and methadone treatments on glutamatergic transmission in rat frontal cortex. Pharmacol Rep 61:1192–1197
40. Zhu H, Jang CG, Ma T, Oh S, Rockhold RW, Ho IK (1999) Region specific expression of NMDA receptor NR1 subunit mRNA in hypothalamus and pons following chronic morphine treatment. Eur J Pharmacol 365:47–54
41. Bajo M, Crawford EF, Roberto M, Madamba SG, Siggins GR (2006) Chronic morphine treatment alters expression of *N*-methyl-D-aspartate receptor subunits in the extended amygdala. J Neurosci Res 83:532–537
42. Kozela E, Popik P (2007) A complete analysis of NMDA receptor subunits in periaqueductal grey and ventromedial medulla of morphine tolerant mice. Drug Alcohol Depend 86:290–293
43. Herrold AA, Persons AL, Napier TC (2013) Cellular distribution of AMPA receptor subunits and mGlu5 following acute and repeated administration of morphine or methamphetamine. J Neurochem 126:503–517
44. Anglin MD, Burke C, Perrochet B, Stamper E, Dawud-Noursi S (2000) History of the methamphetamine problem. J Psychoact drugs 32:137–141
45. Cisneros IE, Ghorpade A (2014) Methamphetamine and HIV-1-induced neurotoxicity: role of trace amine associated receptor 1 cAMP signaling in astrocytes. Neuropharmacology 85:499–507
46. Crocker CE, Purdon SE, Hanstock CC, Lakusta B, Seres P, Tibbo PG (2017) Enduring changes in brain metabolites and executive functioning in abstinent cocaine users. Drug and alcohol dependence 178:435–442