Sleep disorders and the memory processing at ethanol administration

M.Gogichadze[1], M.Nemsadze[2], N.Lortkipanidze[3], N.Oniani [4]

Research Center "T.Oniani Laboratory of Study Sleep-Wakefulness", Ilia State University, Tbilisi, Georgia
[1]PhD, Doctor of Biological Sciences, Main researcher, [2]PhD, Doctor of Biological Sciences, Professor- Emeritus, [3]PhD, researcher , [4]PhD, Associative Professor, Head of the research Center

Summary

The sleep wakefulness cycle (SWC) generally is sensitive to a variety of pharmacological and non-pharmacological impact, therefore, can be considered as valid model to study the effects of various substances, including ethanol (ET). ET, in various doses, may change excitability of nervous circles, which are involved in regulation of sleep-wakefulness cycle and learning and memory trace consolidation. The aim of the present study was to investigate effects of the various doses of 25% ET solution on the acquisition of the active avoidance reaction, memory trace processing and the sleep wakefulness cycle (SWC) in rats. Experiments conducted on inbred adult rats (weight 180-250 gr. N=50). The following methods used: 1. Stereotaxic, for implantation of electrodes in the brain, oculomotor and cervical muscles; 2. Polygraph registration of the SWC; 3. Passive avoidance test used for study of possibility of memory consolidation; 4. Active avoidance test for study of possibility learning processes (daily 20 trials during 20 days until achievement the learning criterion).5. Animals were injected intraperitonealy of 25% ET (1 ml/kg, 2 ml/kg, 4.5 ml/kg) solution during 20 days. 6. The obtained date treated statistically and significance of the observed changes evaluated according to the Student's *t*-criterion. Low doses of ET (0.5-1 ml/kg), facilitated elaboration of the avoidance reaction on the light compared with control intact group ($p<0.03$). Middle doses (2-2.5 ml/kg) inhibited of the acquisition of the avoidance. High doses (3-4.5 ml/kg) of ET completely blocked implementation of the elaborated reaction of avoidance ($p<0.03$). Consolidation of memory trace was not disturbed at administration of the low doses of ET. High doses (3-4.5 ml/kg) of ET blocked normal course of the phases and stages of SWC. The EEG wave's amplitude noticeably depressed. Recovery of the SWC structure observed after several days.

Abbreviations: Sleep wakefulness cycle – SWC, slow wave sleep - SWS, paradoxical sleep – PS, raped eye movement – REM, ethanol – ET, Electroencephalogram EEG, Electromyogram – EMG, mesencephalic reticular formation - MRF

Key words: sleep wakefulness EEG active avoidance memory, rats

Introduction

At the present time alcohol abuse and dependence are among the most common health problems worldwide. Excessive alcohol-drinking behavior induces development of addiction and in the end there is a development of the alcoholism syndrome that is a multigenic disorder. This problem is very important for those regions also, where wine production and consumption (and not of the high grade of drinks) is a part of the culture. The knowledge obtained during the researches of the factors, which causes alcoholism development, problems associated with the induced disorders becomes deeper. Sleep problems, which can have significant clinical and economic consequences, are more common among alcoholics than among nonalcoholics (Brower, 2009). Alcohol problem severity may be predictive of sleep disturbances (Hartwell et. al., 2015).

Despite the intensity of the study many issues regarding the development of alcoholism remain unclear and require further study, the creation of new models as it would be expedient from the point of view, study of other neurological and psychiatric diseases (Kalueff and Tuohimaa, 2004).

Sleep is a highly evolved global behavioral state in homeothermic vertebrates. Sleep in mammals can be defined by (Hobson, Pace-Schott. 2002) characteristic changes in EEG and posture (Datta, Patterson (2003).

On the basis of long-term investigation of the SWC neurobiological mechanisms and survey of scientific literature a view has been formulated according to the SWC as a valid physiological model. In the pointed cycle the ratio of the stages, their homeostatic nature, reciprocal interdependence, a delicate, regular and consecutive pattern of the phases alteration, make it possible to apply this model as a natural test for studying the pharmacological and non-pharmacological impacts (Oniani et al., 2001; Gogichadze et al., 2000, 2002; Maisuradze et al., 2003; Gvilia et. al ., 2006;Campbell et al., 2007). At the same time a number of psycho-nervous diseases including alcoholism are characterizing by disturbances of the SWC structure (Gillin et al., 1989; Brower 2000; Schreckenberger et al., 2004; Soderlund et al., 2007). The same effects have been observed in the researches connected to the animals (Gogichadze et al., 1988; Ehlers, Slaweski, 2000; Gogichadze et al., 2000, 2002, 2004; Kubota et al., 2002). Among them the problem of correlation of sleep disorders and cognitive deficit in the alcoholics is very interesting, as to a greater extent addiction is followed by cognitive deficit (Liapas et al., 2007; Nowakowska et al., 2008).

Despite the intensity of the study many issues regarding the development of alcoholism remain unclear and require further study, the creation of new models as it would be expedient from the point of view, study of other neurological and psychiatric diseases (Kalueff and Tuohimaa, 2004).

The SWC generally is sensitive to a variety of pharmacological and non-pharmacological impact, therefore, can be considered as valid model to study the effects of various substances, including ET. The animal models are important to study of alcohol effects because they allow researchers to use methods that cannot be used with human subjects. ET, in various doses, may change excitability of nervous circles, which are involved in regulation of SWC (Kubota et.al., 2002), and learning and memory trace consolidation (Söderlund et.al., 2007).

Despite alcohol being extensively studied and widely used, the biological processes underlying its beneficial effects on memory particularly in connection with sleep disorders (Silvers et.al. 2003; Matthews, Silvers, 2004) and consequence memory impairment remain unclear. The aim of the present study was to investigate effects of the various doses of 25% ET solution on the acquisition of the active avoidance reaction, memory trace processing and the SWC in rats and to make correlation between memory processing and alteration of the SWC evoked by administration of ET.

Methods and materials

Investigation of SWC structure

1.1. Animals and experimental environment

Inbred albino adult rats (weighing 280–320 g at the beginning of the experiments) were housed groups in the environmental chambers before the surgery. After the surgery the rats individually placed in the experimental cages in natural day/night conditions. Food and water were available *ad libitum* and the constant room temperature was maintained.

1.2. Surgical procedures and recording

Under Hexenal anesthesia (3-4 mg/kg, i.p.), the rats were surgically implanted with chronic constantan cortical (bilaterally sensorimotor area and hippocampal projection) and dorsal neck bipolar electrodes for assessment of sleep-wakefulness states in antiseptic conditions. The electrodes were implanted using stereotaxic coordinates (Buresh et al., 1991, Paxinos, Watson, 1997). The EEG electrodes screws threaded into holes drilled through locations on the skull; the indifferent (silver) electrode was fastened on the comb of the occipital bone. For registration of the rapid eye movement two electrodes were implanted in the oculomotor muscles. The diameter of the tip of uninsulated wires was 100-200 Mk.

Leads from the electrodes were soldered to a special bin and the complete assembly was anchored to the skull with dental acrylic. After rehabilitation period (5-7 days) after surgery, animals were connected to a recording cable and were adapted to the recording procedure for 12–24 h (beginning at 10:00 A.M.) each day. During experiments, EEG and EMG signals were recorded continuously using a polysomnograph recording devices {"Medicor" (Hungary) and 4 channel soviet EEG}. A baseline recording was conducted during several days and after completely stabilization of SWC alcoholization of the animals was begun.

The procedure of alcoholization:

2.1. Acute administration was conducted by 25% solution of ET intraperitoneal injection before10-20 min the registration of SWC or learning session in different doses (1, 2, 2.5 and 4.25 g/kg). The high (narcotic) dose has been taken for define "long sleeper" (non- alcoholic) and "short sleeper" (alcoholic) for determination alcohol preference in the rats **(see** Буров, Ведерникова, 1985).

Analysis of SWS in the rats

Different stages of sleep-wakefulness cycle of the rats were determined by EEG alteration and visual observations. Wakefulness, together with behavioral parameters – motor activity, grooming, feeding, was defined by the presence of low-amplitude and high-frequency EEG activity with higher neck muscle tone. A high-amplitude slow-wave EEG and decreasing EMG tone relative to wakefulness allowed defining SWS. The PS was identified by the presence of slow amplitude of waves in EEG and high theta activity from the hippocampal projection of the cortex, with decreasing neck EMG tonus, arising of REM in the oculogramm and slight twitching of the limbs and whiskers.

Investigation of learning and memory processing:

4.1. Active avoidance test used for study possibility of learning and reproduction (acquisition) of the skill in different experimental paradigms including ET administration.

The special arrangement - the chamber with front transparent wall for watching the animals (50X20X30sm) was used for studying possibility of learning and acquisition of active avoidance skills. The grill floor of the chamber was electrified and the animal for avoid of the electrical stroke had to jump of the shelf mounted on the left wall of the chamber. The light stimulus (10 sec) preceded the electrical stroke (20 MA, duration 20 sec).The learning sessions passed during 20 days daily 20 trials until achievement the learning criterion – 9 (from 10 signals) consequence correct answers (jumping on the shelf to avoid painful stimulus) on the light.

In the first series of the experiments (n=12) the effects of low dose (1 g.kg) of ethanol was studied. In the second series of the experiments (n=10) the effects of middle dose (2 g.kg) of ethanol was studied. Injections of ethanol in these experiments were made pre-session. In the third series (n=8) effects of high post-session dozes (3.4 and 4.25 g/kg) of ethanol were studied on the acquisition of the avoidance task on the following day.

4.2. Passive avoidance test used for study of possibility of memory consolidation in different experimental paradigms including ET administration. Testing conducted in two chamber cage (Buresh et al., 1991). At the beginning of the test the animals were placed in the well lighted (4 incandescent lamps – 60Vt). Plexiglas transparent cube (20x20x20 Sm).On the one wall was small hole and the animal could enter the dark compartment to avoid the light environment according to natural fear of light. The grill floor of the dark compartment was electrified. After entering the roof of the compartment was closed and the animal received electrical stroke (20 MA) during 20 sec. Retention of the passive avoidance reaction was considered as fulfilled, if the animal, after 24h after stroke did not enter the dark compartment during 10 min. Duration of latency of entering the dark compartment also counted up **(**Oniani, Nemsadze, 1985**).**

5. Statistical analysis. The obtained date treated statistically and significance of the observed changes evaluated according to the Student's *t*-criterion.

At all times, animals will be treated in accordance with guidelines for animal care established by the National Institutes of Health (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996), using protocols approved by Binghamton University Institutional Animal Care and Use Committee.

Results:

1. ***Influence of different doses of Ethanol on the learning and memory processing***

According of the date in which influence of different doses of ET on the memory was discussed, the effects of this substance associated to disturbances in the brain structures, even injures, at using of low doses of alcohol. In the following part of the paper the effects of different doses of ET on the possibility of learning and memory trace consolidation are shown.

1.1. Influence of different doses of ET on the elaboration and acquisition of the active avoidance skills

The animals in two groups were alcoholized by intraperitoneal injections of ET during 20 days while active avoidance testing was performed.

The first experimental group received ET injection 10 min before learning session in dose 1g/kg.

After first injection (the first day of testing) the animals were characterized by facilitate of motor activity what was expressed in spontaneous jumping on the shelf of the experimental chamber and exploration of the experimental environment (intersignal activity). During following testing days inter signal activity in all group fluctuated.

As concerns of second experimental group where the animals were injected by ET in the dose of 2g/kg amount of spontaneous jumping on the shelf was less and exploration activity was low – they did not characterized by high motor activity and inter signal activity expressed in the decline of that parameters (Fig. 1).

Fig 1. Dynamics of spontaneous intersignal activity of rats in the active avoidance test
On the abscise – the experimental days
On the ordinates – frequency of jumping on the shelf

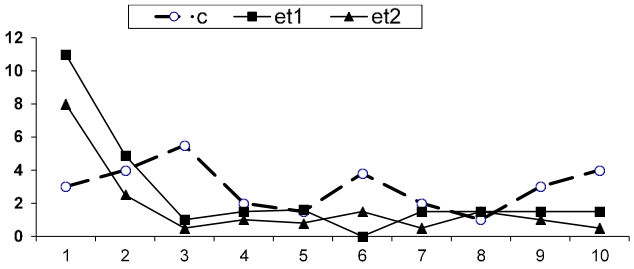
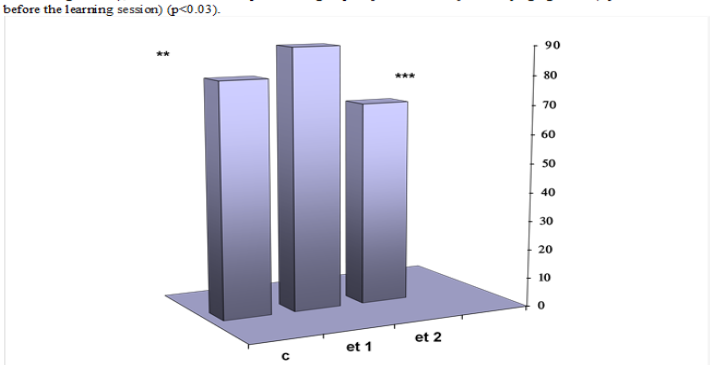


Fig.2 Influence of different doses of ethanol 25% solution on the criterion of learning in the active avoidance test

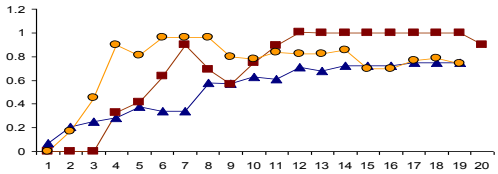
c – control group, **et1**- the first experimental group at pre- session injection by 1g/kg of ET (injected 10 min. before the learning session); **et2** – the second experimental group at pre- session injection by 2g/kg of ET (injected 10 min. before the learning session) ($p<0.03$).



Elaboration and acquisition of the active avoidance reaction at alcoholization of rats by different doses of 25% solution of ET alleviated at low dose. The rats of the first experimental group (et.1) easily got trained in the active avoidance test (Fig.2).

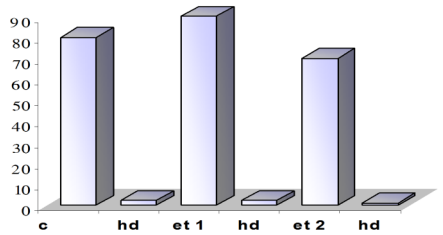
On the Fig.3 the dynamics of elaboration of the active avoidance in the three groups of the animals – control, exposure by low and middle doses of ET is shown. Low dose of ET facilitated the process of learning and consolidation of memory in the active avoidance test.

Fig.3.The dynamics of acquisition of active avoidance reaction at 20 consequence days in 20 trials session. ▲ – Control group; ■ - at injection of ET in doses 1g/kg; ● - at injection of ET in doses 2g/kg. The correct answers on the light is showing on the ordinate, on the abscise – the days of elaboration



In the special experiment influence of high (4.25 g/kg of 25% solution, evoking "anesthetic" sleep, and see below in next division) on the elaborated skill of active avoidance was studied. This dose of ET was administrated after learning session were rats demonstrated high level of the response. The high dose of ET that strongly disturbed the structure of SWC (see Fig.) elicited blackout and the animals in the all three groups (Fig.4).

Fig. 4. *Influence of high doses (hd) of ET 3.4-4.25 g/kg at post-session injection on the performance of active avoidance reaction after 24 h after injection*
c –control group, hd'–after post-session injection; et1 – the first experimental group, hd'' – after post-session injection; et2 – the second experimental group, hd''' – after post-session injection. ($p<0.03$ for all groups).



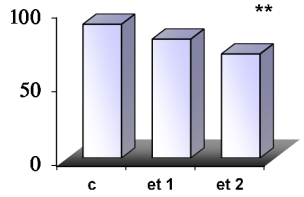
1.2. Influence of different doses of ET on the reaction of passive avoidance

For studying influence of different doses of ET on possibility of consolidation of memory trace the passive avoidance test was used. At pre-session administration of ET low dose possibility of memory trace was not prevented and animals did not enter the dark compartment of the experimental device after 24h of electroshock. Slight impairment of memory was observed in the second experimental group, which administrated by 2 g/kg of ET (Fig.5).

Fig. 5 The possibility of consolidation of memory traces at ethanol pre – session administration in a passive avoidance test

On the ordinate the percentage of the criterion of passive avoidance reaction is shown

***c** – The control group; **et1** – the first experimental group with low dose (1g/kg) of ET injection; **et2** – the second experimental group with middle dose (2g/kg) of ET injection; p<0,01*



is possible to conclude that low dose of ET improved memory processing, middle dose elicited slight impairment of this process, and administration of high dose provoked blackout, and animals of the all groups are not able to implement a previously trained skill.

2. Changes in electrical activity of the cortical areas during administration of high doses of Ethanol

Administration of low and middle doses of ETG did not elicit significant alteration on the duration, architecture and the ratio of SWC in the rats (Fig.6).

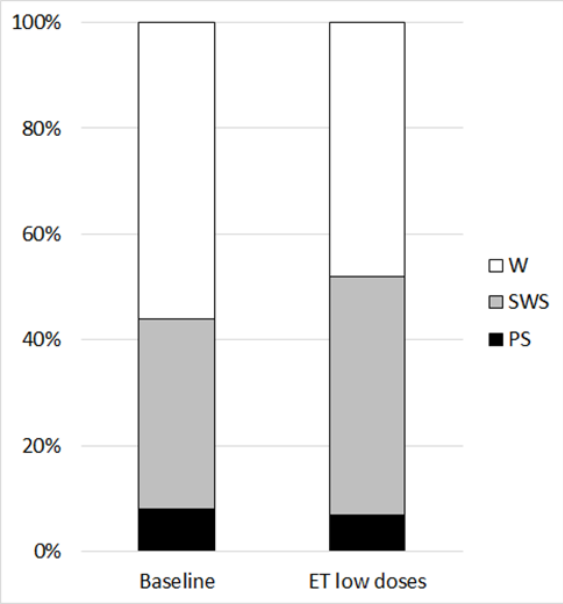


Fig.6. Influence of low doses of ET on the volume ratio of the SWC

2.1 Influence of ET high (anesthetic) dose on the EEG and SWC structure in the rats

Selection of the inbreed rats according to predisposition for alcohol consumption, beside of free choose method (McBrideae et.al., 2014) performed by injection of ET in high narcotic dose – 4,25 g/kg of 25 % solution (Буров и др., 1983).

Duration of "sleep" testified that "short sleepers" (20-80 min) as these animals do prefer alcohol in the freely choosing paradigm. The "long sleepers" (duration of narcotic sleep – more than 120 min) considered as "non- alcoholics"– they do not prefer alcohol. On the base of comparison of different experimental data in our experiments we have given preference to the injection method for selection of animals. More over data considered to the characters of the narcotic sleep evoked by ET narcotic dose are negligibly little.

On the fig.7 influence of high doses of ET on rats' EEG is illustrated. Sharp alteration of the electrical activity of the sensor motor area and hippocampal projection of the cortex was observed after 15 min from injection. Depression of the electrical activity accompanied by complete immobilization of the animals and corresponded with cataplexy. After 2h after injection specific EEG activity - low amplitude synchronous activity generated. It is distinguished that hippocamal activity is more is sensitive to high dose of ET.

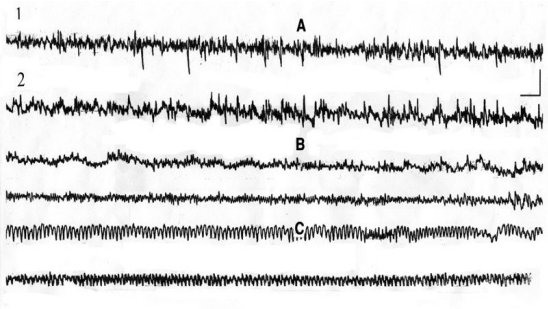


Fig.7. Influence of high doses of ET on rats' EEG.

*A – baseline, B – after 15 min from injection, C - after 2 h. **1-** sensor motor area, 2 – hippocampal projection of the cortex*

High doses (3-4.5 ml/kg) of ET blocked normal course of the phases and stages of SWC. During depression of EEG parameters the phases of SWC were not generated. The disturbances of the structure of SWC in the whole continuum were approximately 35-40 %. After completion of EEG depression amount of W was greater than the baseline and respectively duration of SWS and PS – decreased (Fig8). Recovery of the SWC structure observed after several days.

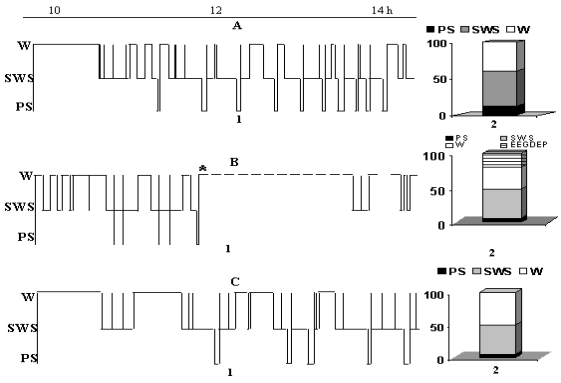


*Fig.8 .Influence of high doses of Ethanol on the SWC structure in the rats. A-baseline B- during ET administration, C- recovery day. **1** – cyclogram of SWC during 4h of registration, **2-** percentage of the amount the phases of the SWC in the same period.*

Discussion:

Obtained results are discussed in the following view points:

1. Low doses of ET (1-1.5 mg/kg) in all probability activate arousal system of MRF, posterior hypothalamus, according of elevate eiphoria, that is common during consumption of these doses of alcohol, stimulate awarding system in the mesolimbic and limbic as well opioid systems, contribute EEG desinchronisation, facilitate motor activity and promote learning and memory processing (Ониани, 1980; Kitamura, et al., 2017).
2. Middle doses of ET (2-2.5 mg/kg) induce partial inactivation of arousal system of MRF, posterior hypothalamus, induce slow down of motor activity does not contribute learning and memory processing.
3. Acute administration of high doses of ET (3-4.5 mg/kg) via blocking of MRF, inactivates of arousal system, inhibits spinal reflexes, and induces EEG depression, complete instability SWC and total disruption of memory processing.

Animal models, including genetic models of alcoholism that may be relevant to some forms of alcoholism, and sophisticated genetic research strategies have been directed at this specific question. Moreover, in contrast to most other drugs of abuse, the actions of which are confined to a more limited number of neurochemical or receptor systems, the pharmacological effects of ethanol that support alcohol reward and alcohol seeking behavior involve actions at multiple receptors and neurochemical systems occurring at widespread neuroanatomical sites throughout the brain (Weiss, Porrino 2002; Spanagel et. al., 2005).

Alcohol has many effects on brain functions and hence on human behavior (as well on animal behavior), ranging from anxiolytic and mild disinhibitory effects, sedation and motor incoordination, amnesia, emesis, hypnosis and eventually unconsciousness (Harrison et al., 2017).

It has dose dependent effect and probably impact on neurotransmission in the mostly all of the brain structures and may modify as modification of CNS inhibitory and excitatory synaptic transmission occurs in response to acute and chronic alcohol (reviewed by Roberto and Varodayan, 2017).

Impact of alcohol involving limbic domains, such as the nucleus accumbence (NAc) and the dorsal striatum, which mediate motivational and emotional alterations (Graybiel, 2008).

It was demonstrated that both dopamine neurotransmission and κ –opioid receptors sensitivity are dysregulated in the NAc core and precommisural dorsolateral caudate of male cynomolgus macaques following 6 months of voluntary ethanol self-administration (Siciliano et.al., 2015).

Under alcohol, decreased brain responses in right frontotemporal areas might slow down the attentional capture of infrequent stop-signals and subsequent updating of action plans which leads to impaired inhibitory control. In turn, pronounced alcohol-induced impairment of inhibitory control may enhance alcohol consumption in young adults which might promote future alcohol problems including cognitive impairment (Angarita et. al., 2016).

Sleep problems, including insomnia observed and well documented among adults with alcohol dependence (Stein and Friedmann, 2006; Brower, 2009; Brower and Perron, 2010; Angarita, et. al., 2016).

The relationship between sleep and memory is widely discussed in the current neuroscientic researches (see Rasch and Born, review 2013), despite significant contradictions which were discussed and refuted in Oniani's earlier works (see Oniani, 1970, 1977, 1980, 1984).

In the present article destruction, even completely blocking of previously elaborated avoidance reaction and sleep phases elimination after application of the narcotic dose of ET is established. It is estimated that SWC disturbances correlate with memory trace recall. Although it is possible the main reason is severe intoxication produced by applied high dose of ET that with its own line disables the influence of activating reticular (arousal) system.

Conclusion:

Obtained results allow suggesting that the main reason of the deficit learning and memory during alcoholization with middle and high doses of ethanol solution depends on disruption of SWC structure and mainly on deactivation of the brain arousal system.

Reference:

1. Angarita, G. A. Emadi N, Hodges S. Morgan P.T. 2016. Sleep abnormalities associated with alcohol, cannabis, cocaine, and opiate use: a comprehensive review Addict Sci Clin Pract 11:9 DOI 10.1186/s13722-016-0056-7
2. Brower, K. J. 2009. Alcohol's Effects on Sleep in Alcoholics. Alcohol Res Health.PMC PMC2778757, NIHMSID: NIHMS4273.
3. Brower KJ, Perron BE. (2010) Prevalence and correlates of withdrawal related
4. insomnia among adults with alcohol dependence: results from a national survey. Am J Addiction 19:238–44.
5. Brower KJ, Aldrich MS, Robinson EA, et al. (2001) Insomnia, self-medication,
6. and relapse to alcoholism. Am J Psychiatry 158:399–404.
7. Datta S, Patterson E.H. 2003Activation of phasic pontine wave (P-wave): a mechanism of learning and memory processing. In: Maquet P, Smith C,Stickgold R, editors. Sleep and brain plasticity. New York: Oxford University Press; p. 379.
8. Froehlich J.C. 1997. Opioid Peptides. Alcohol, Health and Research World, 21, 2, 132-136.
9. Gan G., Guevara A., Marxen M., Neumann M., Jünger E, Kobiella A., Mennigen E., Pilhatsch M., Schwarz D., Zimmermann U. S. Smolka M.N. 2014. Alcohol-induced impairment of inhibitory control is linked to attenuated brain

responses in right fronto-temporal cortex *Biol Psychiatry*. November 1; 76(9): 698–707. doi:10.1016/ j.biopsych.2013.12.017.

1. 10. Gogichadze M., Omiadze N., Mchedlidze O. 2002. High doses ethanol effects on the structure of the sleep-wakefulness cycle in rats. J. Neurobiol. of Sleep-Wakefulness Cycle, 2, 1, 1-5.
11. Gogichadze M., Oniani N., et.al. 2004. Influence of acute administration of high doses of ethanol on some features of the sleep-wakefulness cycle in rats. Proc. Georgian Acad. Sci., Ser. A, 30, No 3, 329-335.
12. Gogichadze M., et al. 1988. The effect of alcohol intoxication on the structure of sleep-wakefulness cycle. In: "Neurobiology of sleep-wakefulness cycle", ed. by T.Oniani. "Metsniereba", Tbilisi, 433-445.
13. Graybiel A.M. 2008. Habits, rituals, and the evaluative brain. Annu Rev Neurosci 31:359 –387.
14. Gvilia I., Feng Xu, McGinty D, Szymusiak R. 2006. Homeostatic regulation of sleep: A role for preoptic area neurons. J Neurosci. 13;26(37):9426-33.
15. Harrison N.L., Skelly M.J,. Grosserode E. K,. Lowes D. C, Zeric T., Phister S., Salling M. C. 2017. Neuropharmacology http://dx.doi.org/10.1016/j.neuropharm.2017.04.007 0028-3908/© 36-45 www.elsevier.com/locate/neuropharm
16. Hobson JA, Pace-Schott EF. 2002. The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. Nat Rev Neurosci 3:679–93.)
17. Horne J. Why we sleep. 2004, Oxford, N.Y., Tokyo. J Ment Health Policy Econ. 7, 107-125.
18. Jouvet M. 1999. The paradox of sleep: The story of Dreaming. MIT Press.
19. Kalueff A. Tuohimaa P.2004. Experimental modeling of anxiety and depression. Acta Neurobiol Exp 64: 439-448.
20. Kubota T., De A., Brown R.A., Simasko S.M., Krueger J.M. 2002, Diurnal effects of acute and chronic administration of ethanol on sleep in rats. Alcohol. Clin. Exp. Res., 26, 8, 1153-1161.
21. Kitamura, T., Ogawa, S.K., Roy, Dh.S., Okuyama, T.,Morrissey, M. D., Smith L. M., Roger L. R., Tonegawa S. 2017. Engrams and circuits crucial for systems consolidation of a memory. Science. 356, 73–78. pp.2-6.
22. Maisuradze L., Lortkipanidze N., Eliozishvili M., Oniani N., Oniani T. 2003. A qualitative and quantitative assessment of the sleep-wakefulness cycle architecture in the post-paradoxical sleep deprivation period. Sleep Res. Online, 5, 2, 59-66. (www.sro.org).
23. McBridea, .W.J, Rodda Z. A., Bell R. L., Lumengb l.,Ting-Kai. 2014. The alcohol-preferring (P) and high-alcohol-drinking (HAD) rats –Animal Models of Alcoholism *Alcohol*. 48(3): 209–215. doi:10.1016/j.alcohol.2013.09.044.
24. Nowakowska K.. Jabłkowska K, Borkowska A. 2007. Cognitive dysfunctions in patients with alcohol dependence Psychiatr Pol. 41(5):693-702.
25. Oniani T.N. Does paradoxical sleep deprivation disturb memory consolidation? Physiol. Behavior, 1984, 33.
26. Oniani T.N. On neurophysiological mechanisms of short-term memory. Biology of memory. Acad. Kiado, Budapest, 1970.
27. Oniani T.N. On the functional significance of sleep. Acta Neurobiol. Exp., 1977, 37.
28. Oniani T., Mgaloblishvili M., Gogichadze M., Lordkipanidze N., Maisuradze L., Chidjavadze E., Mandjavidze Sh., Oniani N., Nachkebia N., Koridze M., Kavkasidze M. Dynamics of the level of exitability and activity of the brain stem and diencephalic ascending activating structures in the sleep-wakefulness cycle. Proc.Georgian Acad. Sci., Biol.ser., 2001, 27, No 1-3, 205-224.
29. Paxinos G., Watson Ch, 1997.The Rat Brain in Stereotaxic Coordiinates.Elsevier.
30. Rasch B. and Born J. 2013 ABOUT SLEEP'S ROLE IN MEMORY. *Physiol Rev* 93: 681–766, doi:10.1152/ physrev.00032.2012
31. Roberto, M., Varodayan, F., 2017. Synaptic targets: chronic alcohol actions. Neuropharmacology doi: 10.1016/ j.neuropharm.2017.01.013.
32. Siciliano, C.A. Calipari,. Verginia E.S C. Carlson C., Helms C.M., Lovinger D.M., Grant K.A., Jones S.R. 2015. Voluntary Ethanol Intake Predicts _-Opioid Receptor Supersensitivity and Regionally Distinct Dopaminergic Adaptations in Macaques. The Journal of Neuroscience, 35(15):5959 – 5968.
33. Söderlund H., Grady C.L., Easdo C., Tulving E. 2007. Acute effects of alcohol on neural correlates of episodic memory encoding. NeuroImage 35 928–939.
34. Spanagel R, Pendyala G, Abarca C, Zghoul T, Sanchis-Segura C, Magnone MC, Lascorz J, Depner M, Holzberg D, Soyka M, Schreiber S, Matsuda F, Lathrop M, Schumann G, Albrecht U. 2005. The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. Nat Med 11:35– 42.
35. Spanagel R., Zink M., Sommer W.H. 2013.Neurobiology of Alcohol Addiction D.W. Pfaff (ed.), Neuroscience in the 21st Century, DOI 10.1007/978-1-4614-1997-6_LLC .
36. Stein MD, Friedmann PD. 2006. Disturbed sleep and its relationship to alcohol use. Subst Abuse 26:1–13.).
37. Stuke H., Gutwinski, S.,Wiers, C. E., Schmidt T. T., Grupper, S., Parnack, J., Gawron Ch.,Attar C. H., Spengler S., Walter H., Heinz A., Bermpohl F. 2016.To drink or not to drink: Harmful drinking is associated with hyperactivation of reward areas rather than hypoactivation of control areas in men. J Psychiatry Neurosci; 41(3) E24-36.
38. Weiss F., Porrino L.J. 2002. Behavioral Neurobiology of Alcohol Addiction: Recent Advances and Challenges.The Journal of Neuroscience, May 1, *22*(9):3332–3337.
39. Буров Ю.В., Кампов-Полевой А.Б., Каменска Л.Н. 1983. Некоторые особенности эмоционального поведения животных с выраженной алкогольной мотивацией. Ж. ВНД., 33, вып. 5, 941-944.
40. Буров Ю.В., Ведерникова Н.Н. 1985. Нейрохимия и фармакология алкоголизма. М. - "Медицина". с.340
41. Ониани Т.Н.1980. Итегративная фунция лимбической системы.»Мецниереба», Тбилиси.