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**The article title:**

**Chronic toluene exposure and the hippocampal structure in adolescent and adult brains**

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**Chronic toluene exposure and the structure of the hippocampus**

**Abstract:**

Toluene and toluene containing volatile substances are the most commonly abused solvents with a demonstrative addictive potential for humans. The misuse of these substances is associated with the activation of brain areas involved in both addiction and learning. As potential substrates for both processes, the regions of the mesocorticolimbic system have been identified. A number of clinical and experimental studies indicate that the hippocampus is involved in toluene addiction. In particular, toluene affects learning and memory and provokes various molecular, biochemical and structural alterations in the hippocampus of adolescents and adults. The character of these alterations depends upon the age the organism tested. In the present chapter we review immediate and persistent effects of chronic toluene exposure on the structure of the hippocampus in organisms of different ages.

**Keywords:Toluene. Chronic exposure. Hippocampal structure. Organisms of different ages.**

**Introduction**

 Toluene (also known as methylbenzene, phenylmethane, or toluol) and toluene containing volatile substances are the most commonly abused solvents with a demonstrative addictive potential in humans. Experimentation with these substances is especially common during adolescence: toluene is widely used, easily available and inexpensive (Lubman, Yucel, & Lawrence, 2009; Tormoehlen, Tekulve, & Nanagas, 2014).Moreover, because toluene is broadly used as an industrial solvent in the manufacturing of automotive fuels, chemical pharmaceuticals and multiple household and commercial products, such as paints, inks, glues, rubber cements or other adhesives, it has a high addictive potential for adult employees in direct contact with the substance also. For the same reasons millions of people may potentially be exposed to occupational levels of toluene in their own home (Mistretta & Charlier, 2013; Proctor, Heaton, Smith, Rodrigues, & McClean, 2014).Therefore, toluene abuse has a huge social impact that cannot be denied.

It is now obvious that toluene shares common cellular mechanisms and has similar actions with other drugs of abuse, namely, it activates the major substrate of addiction –the mesolimbic dopaminergic system and related structures (Apawu, Mathews, & Bowen, 2015; Beckley, Evins, Fedarovich, Gilstrap, & Woodward, 2013; Riegel, Zapata, Shippenberg & French, 2007; Tomaszycki, Aulerich, & Bowen, 2013).A number of modifications arise at different levels of the organism**.** However, compared with other substances, the effect of chronic toluene exposure is elucidated in relatively few studies.

**Chronic effect of toluene exposure depends on the age of organism tested**

 Numerous clinical and experimental data indicate that long-term exposure to toluene vapor leads to diverse outcomes at levels ranging from the cell to the whole organism. The central nervous system is one of the main targets. A number of long-lasting neurological and behavioral impairments, as well as various biochemical, molecular and diffuse changes in the white and grey matter of adults and adolescents, were described (Filley, Halliday, & Kleinschmirt-DeMaster, 2004; Bowen, Charlesworth, Tokarz, Wright, & Wiley, J.L., 2007; Paez-Martinez et al., 2013).However, some of these same impairments are not well elucidated. In particular, the effect of toluene on the brain structure is described only in a few studies. Furthermore, most studies concerning neurobiological, neurobehavioral and other alterations provoked by toluene have been performed on adult animals, while the data obtained on younger animals are rare (Huerta-Rivas et al., 2012; O’Leary-Moore et al., 2009; Win-Shwe, Yoshida, Kunugita, Tsukahara, & Fujimaki, 2010).Especially, limited research was conducted investigating the impact of toluene abuse during adolescence,despite the fact that mainly adolescents represent the most numerous group of toluene abusers and the majority of adults who use this substance started as teenager*s* (Filley et al., 2004).

Numerous data indicate that due to the specificity in physiology, adolescents and adults react differently to various drugs, such as amphetamine, cocaine, methylphenidate or alcohol and nicotine (Batis, Hannigan, & Bowen, 2010; Hollis, Gaval-Cruz, Carrier, Dietz, & Kabbaj, 2012; Nikura, Ho, Kreek, & Zhang, 2013). In some studies the age-dependent effect of toluene misuse was described. Chronic inhalational exposure to toluene was found to provoke behavioral, molecular and structural alterations in the organisms of different ages; the character of such alterations depends upon the postnatal age of toluene testing. Specifically, it was revealed that chronic inhalation of high concentrations of toluene affects recognition locomotor behavior in rats. This effect depends on the age of the animal and the pattern of toluene exposure (Batis et al., 2010). Furthermore, adolescents were characterized by having less sensitization to and attenuation of neurochemical responses than adults (Bowen et al., 2007; O’Leary-Moore et al., 2009).

In our recent studies we also described (to our knowledge, for the first time) the age-dependent effect of toluene on learning processes in the multi-branch maze **(**Bikashvili et al., 2012), in recognition memory (Zhvania et al., 2013) and in the structure of the hippocampus (Zhvania, Chilachava, Japaridze, Gelazonia, & Lordkipanidze, 2012).

 Several factors may play a role in this difference. Specifically, at least partly, it could be affected by the age-related difference in toluene pharmacokinetics. A number of data indicate that the levels of hepatic enzymes responsible for toluene metabolism to benzyl alcohol and *o ~* and *p ~* cresol vary across age in drug-naïve rats. As a result, younger rats might metabolize toluene more quickly than adults (Nakajima & Wang, 1994; Sanchez-Serrano, Cruz, & Lamas, 2011; Waxman, Dannan, & Guengerich, 1985). (However, it is noteworthy that adolescent and adult rats differ in sensitivity to the acute effect of some other addictive drugs and CNS depressants, which have other pharmacokinetic mechanisms than toluene).

 Another key factor could be the unique developmental profile of the adolescent brain. The special peculiarities of such a brain are the high level of neuroplasticity, the set of neurobehavioral alterations, the biochemical rearrangement, the neuronal reduction and specific changes in receptors and neurotransmitter systems, including systems which play an essential role in addiction. Specifically, considerable developmental transformations arise in limbic and mesocorticolimbic dopamine systems of different species. These modifications may be crucial for attributing salience to drugs and other stimuli (Spear, 2000).

 While there remain many unanswered questions, studies utilizing experimental animals of different ages might provide important insights into the mechanisms of toluene addiction and the ways of correcting the corresponding pathology in adolescents and adults.

**Chronic toluene** **exposure and long-term outcomes**

 One more approach which is important for determining the neural mechanisms of toluene addiction is the elucidation of long-term outcomes for toluene abusers of different ages. It should be noted that the biggest part of existing clinical and experimental studies has been focused on toluene’s acute effects with limited work clarifying delayed outcomes in toluene abusers who abstain from toluene abuse (persistent or long-term effects of toluene misuse). As a result, there are insufficient data concerning the reversibility/irreversibility of toluene-induced pathologies once the toxic effect of toluene no longer persists. Nevertheless, several clinical and autopsy data demonstrate that chronic toluene exposure provokes relatively specific damage to myelin accompanied with axonal sparing (Filley et al., 2004; Rosenberg et al., 1988). Based on such data, it was proposed that the brain damage induced by chronic exposure to toluene may be considered as potentially reversible with abstinence or even compliant to future treatments designed to restore myelin using the existing scaffolding of preserved axons (Filley et al., 2004). Indeed, clinical experience suggests that toluene abusers who abstain from toluene misuse may show partial recovery (Al-Hajri & Del Bigio, 2010; Filley et al., 2004; Kornfeld et al., 1994). However, long-term effects of chronic toluene misuse were mostly described inadult patients and adult experimental animals only**.** Furthermore, such data are not sufficient**.** To fully understand the nature of toluene addiction, immediate and persistent effects of chronic toluene exposure in the organisms of different ages should be elucidated and compared.

**Addiction and the hippocampus**

It is well-established that the presence of addictive drugs to abusers provokes the activation of brain regions that are involved in both addictive and learning processes (Belujon & Grace, 2011; Fowler et al., 2014; Van den Oever et al,. 2013). Based on such studies, it was suggested that addiction, learning and memory share the nervous substrates. As possible substrates, the regions of the mesocorticolimbic system and related structures have been identified. The hippocampus is one such region. Several data indicate that the hippocampus, the neural substrate for learning and memory, significantly contributes to the pathophysiology of addiction. In particular, it is well-documented that the hippocampus (along with amygdala) is critical for cue-elicited drug-seeking consumption (Arguello et al., 2009; See, 2002; Zhao et al., 2006). Hippocampal pathways are highly implicated in drug-seeking that is achieved by contextual stimuli (Belujon & Grace 2011; Garcia-Fuster, Clinton, Watson, & Ajil, 2009; Gould, 2006). The hippocampus participates in strengthening connections in the areas which are involved in addiction. Based on such data it was suggested that drug-induced changes in the hippocampus should produce long-lasting functional alterations (Belujon and Grace 2011; Garcia-Fuster, Clinton, Watson, & Ajil, 2009). Furthermore, it was shown that the operation of hippocampal cells alters dopamine levels and firing rates of dopaminergic cells in the areas of the mesolimbic system – neural substrate, which significantly contributes to the pathophysiology of drug abuse (Brandon, Marinelli, & White, F.J., 2003; Eisch & Harturg, 2006; Riegel, Ali, Torinese, & French, 2004**).** Therefore, the hippocampus is considered as an “exciting” part of brain in regard to addiction. The ability of addictive drugs to alter hippocampus and other neural substrates involved in learning and memory may explain the capacity of these substances to produce long-term alterations in behavior and cell physiology (Bergstrom, McDonald, French, & Smith, 2008; Campbell, Wood, & Spear, 2000; Fuchs, et al., 2005; Evans, & Ledford, 2005).

There are many reports that chronic misuse of nicotine, alcohol, cocaine, methamphetamine or other abusive drugs, provokes structural modifications in the mesocorticolimbic areas and related regions of the brain (Garcia-Fuster, Clinton, Watson, & Ajil, 2009; McDonald et al., 2005). In particular, such mistreatments produce significant alterations in the dendritic branching, and the density and number of spines in specific neurons of the nucleus accumbens, the tegmental area, the prefrontal cortex and the hippocampus (Fowler et al., 2014; McDonald et al., 2005). However, in comparing with other mesocorticolimbic areas, structural pathologies of the hippocampus from the addicted brain were described only in a few studies. Even so, such pathologies associated with the modifications in hippocampal learning-related cell signaling present strong documentation of the significance of learning and memory in addiction.

**Toluene addiction and the hippocampus**

Several clinical and experimental data demonstrate that the hippocampus is involved in toluene addiction. Thus, it was shown that toluene affects learning and memory in organisms of different ages (Bowen et al., 2007; Filley et al., 2004; Gerasimov et al., 2003; Lin, Ou, Chung, Pang, & Chen, 2010) and provokes various biochemical, molecular and structural modifications in the hippocampus - brain region which plays one of the key roles in learning and memory (Ahmed et al., 2007; Bale, Tu, Carpenter-Hyland, Chandler, & Woodward, 2005; Huerta-Rivas et al., 2012; Win-Shwe et al., 2010). However, while behavioral, biochemical and molecular alterations have been studied extensively, the effect of toluene abuse on the hippocampal structure is described only in a few studies (Gelazonia, Japaridze, & Svanidze, 2006; Korbo, 1999; Ladefoged et al., 2004; Terashi, Nagata, Satoh, Hirata, & Hatazawa, 1997; Zhvania et al., 2012).We still need to clarify the age-dependent effect of chronic toluene exposure on the structure of the hippocampus and to reveal if the hippocampus of adults and adolescents are equally sensitive to such treatment.

**Chronic toluene misuse and the structure of the hippocampus**

Several experimental data indicate that chronic exposure to toluene provokes hippocampal cell death. For example, it was shown that the exposure of adult rats to 1500 ppm of toluene over six weeks is associated with the decreased number of cells in the inferior regions (CA3 and CA2) of the hippocampus (Korbo, 1999). Such pathology has been revealed even after a four-month period without exposure. The authors of this test consider this fact as the structural basis of chronic toxic encephalopathy, the major symptoms of which is a memory deficit. Furthermore, high vulnerability of pyramidal cells of the hippocampus to toluene abuse was detected in young rats (Gelazonia et al., 2006).

In our comparative study we investigated immediate and persistent effects of chronic toluene exposure on hippocampal cell loss and the structure of neurons in adolescent and adult Wistar rats. The period of adolescence varies across the species. In rats it is a two-week period: P28-P42 (Spear & Varlinskaya, 2005). In our experiments we used animals from two age groups: 28-30 days as adolescents and 90-100 days as adults. The animals inhaled 2 000 ppm of toluene over 40 days. The immediate effect of toluene exposure was studied directly after the end of chronic inhalation, and the persistent effect after 90 days. So, these animals had a three-month period without exposure. We have been focused on two main hippocampal areas: CA1 and CA3. Both areas have specific structures, molecular peculiarities and neuronal networks. In these regions we evaluated the effect of toluene misuse on interneuron and pyramidal cell loss and dendritic arbor of pyramidal neurons. In parallel to a morphological analysis, we studied the behavior of abused rats in a multi-branch maze, their exploratory activity in open field and their locomotor activity.

Numerous clinical and experimental data indicate that alterations provoked by chronic misuse of toluene are dose-dependent. The dose used in our experiments is 2 000 ppm. This dose of toluene is considered as dangerous for health and life (Occupational Safety and Health Administration). Clinically, this dose is comparable to the inhaled exposure which produces euphoria in humans (euphoria usually appears at levels near 800-1 500 ppm, but individuals may be more or less sensitive to the effect of inhaled toluene). Because euphoria is the desired effect, the individuals abusing toluene purposely expose themselves to toluene levels of at least 800-1 500 ppm or higher. For instance, the person who has used toluene by sniffing glue, may reach levels of exposure estimated at 500-1 200 ppm. On the other hand, chronic addicts usually use 5 000-12 000 ppm of toluene (Filley et al., 2004). Therefore, the 2000 ppm level of toluene may be considered a comparatively low doze. However, even this dose affects the level of brain neurotransmitters, gene expression and some types of behavior. In particular, repeated exposure to toluene provokes alterations in the dynamics of dopamine, acetycholine and GABA (Apawu, Mathews, & Bowen, 2005; Bale et al., 2007). In other studies the activation of catecholaminergic cells (Tomaszycky et al., 2013) and reduced avoidant behavior were described (Huerta-Rivas et al., 2012; Benignus, Boyes, Kenyon, & Bushnell, 2007).

 In our experiments we revealed that chronic exposure (40 days) to 2000 ppm of toluene produces modifications in the hippocampal structure and different types of memory in adolescent and adult rats. Specifically, in both main areas of the hippocampus (CA1 and CA3) we detected significant cell loss in the pyramidal cell layer, those with a high concentration of pyramidal cells) and the radial and oriental layers (where interneurons were mostly located) (Figs. 1 and 2). In both age groups, the loss of pyramidal cells was much more substantial than the loss of interneurons. In addition to the loss of cells, the structure of some pyramidal cells was modified. In particular, we detected a decrease in the total length of the dendrite tree, its secondary branches and in the total number of spines. Such alterations should be associated with the reorganization of synaptic networks in the hippocampus and the corresponding behavioral outcome.

 Some literature data indicate that the other addictive drugs may also produce modifications in the dendritic tree of specific types of neurons. In particular, it was shown that chronic nicotine exposure provokes significant alterations in the dendritic arborization of pyramidal cells (McDonald et al., 2005). In another study, the effect of repeated treatment with amphetamine and cocaine on the morphology of dendrites and dendritic spines was described (Robinson, Gorny, Mitton, & Kolb, 2001). Unlike our data, these results indicate the increase of dendritic branching and the density of spines in specific neurons: pyramidal cells in layer V of the prefrontal cortex and medium-spiny neurons in the shell of the nucleus accumbens. Persistent neurobehavioral outcomes should be related to the ability of these drugs to modify synaptic connectivity in the above-mentioned brain regions. The analysis of such data indicates that the character of modifications provoked by drug abuse depends on the chemical nature of these drugs, their dosage, duration and type of treatment. The age, species and some other factors should also be taken into consideration.

What peculiarities of hippocampal pyramidal cells provoke high susceptibility to toluene is unknown. Immunohistochemical approaches could help to classify the neurochemistry of vulnerable neurons and to identify possible upregulation/downregulation of various peptides, neurotransmitters and their receptors in these cells**.** Moreover, novel activity-related markers could be used to determine a direct link between alterations in some types of neurons and the reorganization of corresponding neuronal networks. Such an approach could provide important information about pathological processes provoked by the abuse of toluene.

**Different vulnerability of hippocampal areas to chronic toluene exposure**

Another finding observed in our study was the greater vulnerability of the hippocampal CA1 area to chronic toluene exposure in comparison with the CA3 area (Tables 1, 2 and 3). Such a feature was observed in both age groups. In particular, the cell loss - predominantly of pyramidal neurons - was more prominent in the CA1 areas of adolescents and adult rats (Table 1; Fig. 3, 4).

Among the hippocampal regions, the CA1 area is known as one of the most susceptible to various experimental treatments, hormone actions and disorders (Counts, Alldred, Che, Ginsberg & Mufson, 2014; Hatanaka et al., 2009; Kerchner et al., 2012). Moreover, some data indicate the different roles of the CA1 and CA3 areas in memory processes. For example, it was shown that both hippocampal areas contribute to the acquisition of context-dependent extinction, but only area CA1 is required for contextual memory retrieval (Ji & Maren, 2008). Other scientists describe specific modifications to the apical dendrites of pyramidal cells in stratum-radiatum/stratum lacunosum-moleculare of CA1 areas. Authors suggest that such modifications represent the earliest signs of Alzheimer’s disease. On the other hand, the changes in the width of the dentate girus/CA1 area do not correlate significantly with different aspects of memory performance (Kerchner et al., 2012). Furthermore, it was proposed that there is some correlation between the CA1 area’s spine properties and spatial acquisition (which was not observed in the CA3 area) (McLaughlin et al., 2010). Some other data also indicate that various addictive drugs provoke significant structural modifications to the CA1 region (Stramiello & Wagner, 2010; Swant, Chirwa, Stanwood, & Khoshbouerm, 2010). Finally; in a few studies the special effect of toluene on the hippocampal CA1 area was described. In particular, the increased number of active caspase-3 immunoreactive cells (sign of apoptosis) was observed in mice of PND21 (post-natal day 21) even after low occupational levels (50 ppm) of toluene exposure (Win-Shwe et al., 2010). Furthermore, it was shown that chronic toluene exposure alters the pyramidal cells of the CA1 area only in some mouse strains (Whin-Shwe et al.).

 At this stage it is difficult to differentiate which peculiarities of the CA1 area determine such a high vulnerability of this part of the hippocampus to toluene. Some structural and molecular specificities of this region are well-known. In particular, in comparison with the CA3 area, the CA1 region is characterized by a comparatively large number of electrical synapses along with chemical forms; another peculiarity of this area is a large assortment of Ca+2 binding proteins**.** In addition, such susceptibility could, at least partly, be related to the dopamine system which plays an important role in both drug addiction and novelty acquisition. In the CA1 area this system acts via D1/D5 receptors which are well presented in pyramidal cells in this area. Recently, a strong functional difference between pyramidal neurons from different sub-layers of the CA1 areas was described. Nevertheless, all pyramidal neurons possess DA1/DA5 receptors and address their targets jointly or separately depending on brain state (Mizuseki, Diba, Pastalkova, & Buzsaki, 2011). It has been proposed that the activation of CA1 pyramidal neurons through dopamine afferents triggers *de novo* protein synthesis in these neurons. These new proteins may then be sequestered to synaptic tags resulting in a focused facilitation or degradation in transmission (Mizuseki et al., 2011).

Special studies are needed to fully evaluate the unique role of pyramidal cells in the hippocampal CA1 area in toluene addiction.

**Immediate and persistent effects of chronic toluene exposure in adolescents and adults**

Several data indicate that the character of structural and behavioral alterations provoked by toluene addiction strongly depends upon the post-natal age of the animals tested (Huerta-Rivas et al., 2012; O’Leary-Moore et al., 2009; Win-Shwe et al., 2010). Our morphological studies also point to the age-dependent difference in toluene effects. In particular, the immediate and persistent effects in both hippocampal areas of adolescent rats were almost the same. Therefore, in animals of this age group, the alterations do not progress over 90 days of toluene withdrawal. On the contrary, the most prominent alteration in adult rats was the persistent effect. So, structural pathology in the hippocampal CA1 area in these animals progresses during abstinence periods (Fig. 4a). Such age-dependence might be associated with several factors. Some possible factors were considered above (Division: The effect of toluene depends on the age of the organism). One more reason could be related to the character of toluene-induced cell loss – apoptosis, necrosis, aponecrosis – which often depends on the brain region, the cell type, the developmental stage of animals and the duration, dosage and/or type of toluene exposure (Dalgaard, Hossaini, Hougaard, Hass, & Ladefoged, 2003; Ladefoged et al., 2004).Moreover, it was shown that the expression of several peptides that participate in apoptosis and necrosis differs in adult and adolescent brain. Some of these peptides, for example, the p75NTR - a member of the TNF receptor superfamily - are shown to contribute to toluene-induced cell death (Pascual et al., 2004). It is very likely that the unique character of hippocampal cell death in adolescents and adults as a result of toluene misuse is, at least partly, related to the difference in expression of p75NTR and other peptides participating in toluene-provoked apoptosis and necrosis. Comparative studies of toluene misuse, such as the identification of cell death pathways, the detection of factors responsible for cell death or the determination of the level of their expression in organisms of different ages are considered as future directions for our research.

Of particular interest is the coincidence of some morphological and behavioral data in our research. Using the same experimental design, we clarified the effect of chronic toluene exposure on behavior in multi-branch maze, on object and spatial recognition memory and on locomotor activity in adult and adolescent rats (Bikashvili et al., 2012; Zhvania et al., 2013). Like in our morphological studies in adolescent rats - and in behavioral experiments also - immediate and persistent effects were almost the same (alterations did not progress over 90 days of abstinence), while in adult rats persistent effects predominated (alterations progress over 90 days of toluene withdrawal).

As was mentioned above, (Division: chronic toluene exposure and long-term outcomes), the brain damage induced by chronic toluene exposure could be considered as potentially reversible with abstinence or even amenable to future treatments designed to restore myelin using a scaffolding of preserved axons (Filley et al., 2004). In particular, clinical data indicate that toluene abusers who abstain from toluene may show partial recovery once the toxic effect no longer persists. In our research we showed that in adolescent rats (but not in adult rats) the alterations provoked by chronic exposure to toluene in the learning process, in several types of memory and in hippocampal structures do not progress significantly over a period of abstinence: the long-term outcome was almost the same as the outcome observed immediately after the end of toluene misuse. Moreover, some parameters were even normalized. At least partly, this phenomenon may be related to high neuroplasticity of the adolescent brain. Therefore, it is very likely that in adolescents long-term abstinence is associated with the reversibility of toluene-induced pathologies.

Our data present additional evidence that the hippocampus - the neural substrate for learning and memory - may significantly contribute to the pathophysiology of toluene addiction in organisms of different ages. In particular, the principal (pyramidal) neurons of the hippocampus, first and foremost of the CA1 area, may play a substantial role in this pathology. Future clarification of this phenomenon is of special importance.

Independently from the outcome of additional investigations, further studies of toluene-induced pathologies in the brain structure of adolescents and adults could facilitate the progress in our understanding of toluene-induced age differences which will be important in developing effective therapeutic treatment.

**Applications to Other Addictions and Substance Misuse**

 In the present chapter, the literatures and our own data, concerning the effect of toluene indicates that the misuse of addictive drugs is associated with the activation of brain regions that are involved in addiction and learning processes. As potential substrate for these processes, the areas of mesocorticolimbic system, including the hippocampus, has been proposed. Recently, the involvement of the hippocampus in toluene addiction was demonstrated. In particular, toluene affects learning and memory in organisms of different ages and produces various alterations in the hippocampus. Therefore, the hippocampus **-** the neural substrate of learning and memory - may significantly contribute to the pathophysiology of toluene abuse. There is relatively little data concerning the effects of toluene on the hippocampal structure. However, this data strongly indicate that chronic exposure to toluene provokes some disorder in the structure of the hippocampus and the level of this disorder depends on the age of the animal.

Some literature data indicate that chronic exposure to other abusive drugs such as cocaine, methamphetamine, morphine, or nicotine and alcohol also provoke structural alterations in the hippocampus of experimental animals of different age groups. Specifically, such mistreatments produce neuronal cell death and modifications in dendritic arbor of special types of neurons in the hippocampus of adults and adolescents. Moreover, since the specific physiology of different ageing groups, adolescents and adults react differently to these drugs. It was proposed that persistent neurobehavioral outcomes to such treatments are related to the ability of these drugs to modify synaptic connectivity in the hippocampus and other regions of the mesocorticolimbic system. The character of such modifications may depend on the chemical nature of addictive drugs, the dosage of the drug, and the duration and type of treatment**.**

While there remain many unanswered questions, studies utilizing experimental animals of different ages might provide important insights into the mechanisms of addiction and the ways of correcting corresponding pathology in adolescents and adults.

**Mini-Dictionary of Terms:**

TOLUENE – is the most commonly abused solvent with an addictive potential for humans. Experimentation with toluene is especially common during adolescence: toluene is easily accessible and inexpensive. Moreover, because toluene is largely used as an industrial solvent in the manufacturing of automotive fuels, pharmaceuticals and numerous household and commercial products, it has the highest addictive potential for adults also.

ADDICTION – the presence of addictive drugs for abusers is associated with the activation of brain regions that are involved in addictive and learning processes. It is suggested that addiction and learning and memory share the same nervous substrate. As a potential substrate for these processes, areas of the mesolimbic system - including the hippocampus - have been identified.

THE MESOLIMBIC SYSTEM – is the part of the brain which plays a central role in motivated behavior, different types of reward and cognitive processes. Its participation in goal-directed behavior, addiction and processes of learning and memory is well-documented.

HIPPOCAMPUS – is a part of the mesolimbic system. The hippocampus plays an essential role in different types of memory. The hippocampus is composed of structurally and functionally different sub-regions. Alterations in learning and memory are associated with changes in hippocampal areas. Drug abuse provokes alterations in learning and memory and various modifications of the hippocampus.

ADOLESCENCE – is a developmental period, characterized by high levels of neuroplasticity, specific neurobehavioral changes and a set of biochemical, molecular and structural rearrangements in different regions of the central nervous system. Considerable developmental transformations are known to develop across a variety of species. In rats the unique developmental profile of the adolescent brain is a two weeks period: P28-P42.

CELL LOSS – is the reduction in the number of cells. Among other reasons, it may be caused by various pathologies. Cell loss indicates alterations in corresponding neuronal networks. Various pathological conditions, including alterations in learning and memory, provoke cell loss in different regions of the hippocampus.

**Key Facts of Toluene Effects on the Hippocampal Structure**

* It is suggested that addiction and learning and memory can share the same nervous substrates.
* As potential bases for both processes, the regions of the brain’s mesocorticolimbic system, including the hippocampus, is identified.
* The hippocampus plays an essential role in learning and memory. It contains several structurally and functionally different sub-regions. The CA1, CA3 and dentate girus are among these sub-regions.
* Toluene is an addictive substance. Toluene initiates changes in learning and memory and provokes several molecular and biochemical modifications in different sub-regions of the hippocampus.
* In addition to these modifications, chronic toluene exposure provokes hippocampal cell death and modifications in the structure of special types of hippocampal neurons.
* The character of cell death differs in diverse regions of the hippocampus.
* Hippocampal cell death and the reorganization of the neuronal structure indicate the pathologies in the functioning of the hippocampal network.
* The character of structural alterations provoked by chronic exposure to toluene in the hippocampus depends upon the post-natal age of tested animals.

**Summary Points:**

* This Chapter focuses on immediate and persistent effects of chronic exposure to toluene on the structure of the hippocampus in adolescents and adults.
* Toluene is the most commonly abused solvent there is with demonstrative addictive potential for humans.
* Long-term exposure to toluene vapor leads to diverse outcomes at different levels of the organism; the character of these outcomes could differ in organisms of different ages.
* Chronic exposure to toluene alters the structure of the hippocampus in adolescents and adults provoking both immediate and persistent effects.
* The character of structural alterations provoked by chronic exposure to toluene on the hippocampus differs in adolescents and adults.
* Hippocampal regions are vulnerable to chronic toluene exposure in different ways.
* Chronic exposure to toluene alters the structure of hippocampal pyramidal cells and interneurons. Alterations are more presented in pyramidal cells.
* The hippocampus - the neural substrate for learning and memory - significantly contributes to the pathophysiology of toluene addiction.
* While there remain many unanswered questions, studies utilizing experimental animals of different ages might provide important insights into the mechanisms of toluene addiction and the ways of correcting corresponding pathology in adolescents and adults.

**Titles to all figures and tables:**

**Figure 1. Persistent effects of chronic exposure to toluene (90 days after withdrawal) on pyramidal cells in the hippocampal CA3 area of adolescent rats.**

**Figure 2. Persistent effects of chronic exposure to toluene (90 days after withdrawal) on pyramidal cells in the hippocampal CA1 area of adult rats.**

**Figure. 3. The number of pyramidal cells in the CA1 and CA3 areas of adolescent rats after chronic exposure to toluene: immediate and persistent effects.**

**Figure 4. The number of pyramidal cells in the CA1 and CA3 areas of adult rats after chronic exposure to toluene: immediate and persistent effects.**

**Table 1. Immediate and persistent effects of chronic exposure to toluene on the number of pyramidal cells in the hippocampal CA1 and CA3 areas in adolescent and adult rats**. **Summary of one-way ANOVA results.**

**Table 2. Immediate and persistent effects of chronic exposure to tolueneon the number of pyramidal cells in the hippocampal CA1 and CA3 areas of adolescent rats. Summary of two sample*t*-test.**

**Table 3. Immediate and persistent effects of chronic exposure to toluene on the number of pyramidal cells in the hippocampal CA1 and CA3 areas of adult rats. Summary of two sample*t*-test.**

**Legends to all figures and tables:**

**Legend to Figure 1.** Micrographs demonstrate depletion of pyramidal cell layer in the hippocampal CA3 area of adolescent experimental animals (b) in comparison to control group (a). Magnification 200x; scale = 25 μm.

**Legend to Figure 2.** Micrographs demonstrate depletion of pyramidal cell layer in the hippocampal CA1 area of adult experimental animals (b) in comparison to control group (a). Magnification 200x; scale = 25 μm..

**Legend to Figure 3.** Data are given as a percentage of mean ± percentage of SEM. \**p*≤ 0.05; \*\**p*≤ 0.01.

**Legend to Figure 4.** Data are given as a percentage of mean ± percentage of SEM. \**p*≤ 0.05; \*\**p*≤ 0.01.

**Legend to Table 1.** *F*- variance ratio from one-way ANOVA, *P* - probability.

**Legend to Table 2.** Data are given as mean ± SE; † - indicates vs. control group, ‡ - indicates vs. immediate effect group, ¥ - indicates vs. control of immediate effect group. The 4 sets of measurements per each group were used.

**Legend to Table 3.** Data are given as mean ± SE; † - indicates vs. control group, ‡ - indicates vs. immediate effect group, ¥ - indicates vs. control of immediate effect group.

The 4 sets of measurements per each group were used.

**Reference List**

1. Ahmed, S., Win-Shwe, T.T., Yamamoto, S., Tsukahara, S., Kunugita, N., Arashidani, K., & Fujimaki, H. (2007). Increased hippocampal mRNA expression of neuronal synaptic plasticity related genes in mice chronically exposed to toluene at a low-level human occupational-exposure. *Neurotoxicology, 28*,168-174.

2. Al-Hajri, Z., & Del Bigio, M.R. (2010). Brain damage in a large cohort of solvent abusers. *Acta Neuropathologica, 119*, 435-445**.**

3. Apawu, A.K., Mathews, T.A., & Bowen, S.E. (2015). Striatal dopamine dynamics in mice following acute and repeated toluene exposure. *Psychopharmacology (Berl), 232,* 173-184.

4. Arguello, A.A., Fischer, S.J., Schonborn, J.R., Markus, R.W., Brekken, R.A., & Eisch, A.J. (2009). Effect of chronic morphine on the dentate gyrus neurogenic microenvironment. *Neuroscience, 159*, 1003–1010.

5. Bale, A.S., Jackson, M.D., Krantz, Q.T., Benignus,V.A., Bushnell, B.J., Shafer, T.J., & Boyes, W.K. (2007). Evaluating the NMDA-glutamate receptor as a site of action for toluene, in vivo. *Toxicol Sci. 98,* 159–166.

6. Bale, A.S., Tu, Y., Carpenter-Hyland, E.P., Chandler, L.J., & Woodward, J.J. (2005). Alterations in glutamatergic and gabaergic ion channel activity in hippocampal neurons following exposure to the abused inhalant toluene. *Neuroscience, 130*, 197–206.

7.Batis, J.C., Hannigan, J.H., & Bowen, S.E. (2010). Differential effects of inhaled toluene on locomotor activity in adolescent and adult rats. *Pharmacol Biochem Behav. 96,* 438-448.

8. Beckley, J.T., Evins, C.E., Fedarovich, H., Gilstrap, M.J., & Woodward, J.J. (2013). Medial prefrontal cortex inversely regulates toluene-induced changes in markers of synaptic plasticity of mesolimbic dopamine neurons. *J Neurosci. 33,* 804-813.

9 Belujon, P., & Grace, A.A. (2011). Hippocampus, amygdala, and stress: interacting systems that affect susceptibility to addiction. *Ann N Y Acad Sci. 1216,*  114–121.

10. Benignus, V.A., Boyes, W.K., Kenyon, E.M., & Bushnell, P.J. (2007). Quantitative comparisons of the acute neurotoxicity of toluene in rats and humans, *Toxicol Sci. 100,* 146–155.

11. Bergstrom, H.C., McDonald, C.G., French, H.T., & Smith, R.F. (2008). Continuous nicotine administration produces selective, age-dependent structural alteration of pyramidal neurons from prelimbic cortex. *Synapse, 62,* 31-39.

12. Bikashvili T.Z., Chilachava, L.R., Gelazonia, L.K., Japaridze, N.J., Zhvania, M.G., Lordkipandize, T.G. & Okuneva, V.G. (2012). Effect of chronic inhalation of toluene on behavior of rats of various age groups in multi-branched maze. *Bull Exp Biol Med.152,* 587-589.

13. Bowen, S.E., Charlesworth, J.D., Tokarz, M.E., Wright, M.J.Jr., &nWiley, J.L. (2007). Decreased sensitivity in adolescent vs. adult rats to the locomotor activating effects of toluene. *Neurotoxicol Teratol. 29,* 599-606.

14. Brandon, C.L., Marinelli, M., & White, F.J. (2003). Adolescent exposure to methylphenidate alters the activity of rat midbraim dopamine neurons. *Biol Psychiatry, 54,* 1338-1344.

15. Campbell, J.O., Wood, R.D., & L.P. Spear, L.P. (2000). Cocaine and morphine-induced place conditioning in adolescent and adult rats. *Physiol Behav. 68,* 487–493.

16. Counts, S.E., Alldred, M.J., Che, S., Ginsberg, S.D., & Mufson, E.J. (2014) Synaptic gene dysregulation within hippocampal CA1 pyramidal neurons in mild cognitive impairment. *Neuropharmacology, 79,* 172-179.

17. Dalgaard, M., Hossaini, A., Hougaard, K.S., Hass, U., & Ladefoged, O. (2003). Developmental toxicity of toluene in male rats: effects on semen quality, testis morphology, and apoptotic neurodegeneration. *Arch Toxicol.75*, 103–109.

18. Eisch, A.J., & Harburg, G.C. (2006). Opiates, psychostimulants, and adult hippocampal neurogenesis: insights for addiction and stem cell biology. *Hippocampus, 16,* 271-86. Review.

19. Filley, C.M., Halliday, W., & Kleinschmidt-DeMaster, B.K. (2004). The effects of toluene on the central nervous system. *J. Neuropathol Exp Neurol. 63,* 1-12.

20. [Fowler, A.K](https://www.ncbi.nlm.nih.gov/pubmed?term=Fowler%20AK%5BAuthor%5D&cauthor=true&cauthor_uid=25188266)., [Thompson, J](https://www.ncbi.nlm.nih.gov/pubmed?term=Thompson%20J%5BAuthor%5D&cauthor=true&cauthor_uid=25188266)., [Chen, L.,](https://www.ncbi.nlm.nih.gov/pubmed?term=Chen%20L%5BAuthor%5D&cauthor=true&cauthor_uid=25188266)[Dagda, M](https://www.ncbi.nlm.nih.gov/pubmed?term=Dagda%20M%5BAuthor%5D&cauthor=true&cauthor_uid=25188266)., [Dertien, J](https://www.ncbi.nlm.nih.gov/pubmed?term=Dertien%20J%5BAuthor%5D&cauthor=true&cauthor_uid=25188266)., [Dossou, K.S](https://www.ncbi.nlm.nih.gov/pubmed?term=Dossou%20KS%5BAuthor%5D&cauthor=true&cauthor_uid=25188266)., [Moaddel, R](https://www.ncbi.nlm.nih.gov/pubmed?term=Moaddel%20R%5BAuthor%5D&cauthor=true&cauthor_uid=25188266)., [Bergeson, S.E., &](https://www.ncbi.nlm.nih.gov/pubmed?term=Bergeson%20SE%5BAuthor%5D&cauthor=true&cauthor_uid=25188266) [Kruman, I.I](https://www.ncbi.nlm.nih.gov/pubmed?term=Kruman%20II%5BAuthor%5D&cauthor=true&cauthor_uid=25188266). (2014). Differential sensitvity of prefrontal cortex and hippocampus to alcohol-induced toxicity. *PLoS One 9,* Sep 9 (9):e106945. doi: 10.1371/journal.pone.0106945.

21. Fuchs, R.A., Evans, K.A.,Ledford, C.C., Parker, M.P., Case, J.M., Mehta, R.H., & See, R.E. (2005) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology, 30,* 296–300.

22. Garcia-Fuster, M-J., Clinton, S. M., Watson, S.J., & Akil, H. (2009). Effect of cocaine on Fas-associated protein with death domain in the rat brain: individual differentces in a model of differential vulnerability to drug abuse. *Neuropsychopharmacology 34,* 1123-1134**.**

23. Gelazonia, L., Japaridze, N., Svanidze, I. (2006). Pyramidal cell loss in hippocampus of young rats exposed to toluene. *Georgian Med News, 135*, 126–128.

24. Gerasimov, M.R., Collier, L., Ferrieri, A., Alexoff, D., Lee, D., Gifford, A.N., & Balster R.L. (2003). Toluene inhalation produces a conditioned place preference in rats. *Eur J Pharmacol. 477*, 45-52.

25. Gould, T.J. (2006). Nicotine and hippocampus-depending learning. *Mol Neurobiol. 34*, 93–107.

26. Hatanaka, Y., Mukai, H., Mitsuhashi, K., Hojo, Y., Murakami, G., Komatsuzaki, Y., Sato, R., & Kawato, S. (2009) Androgen rapidly increases dendritic thorns of CA3 neurons in male rat hippocampus. *Biochem Biophys Res Commun. 381*,728-732.

27. Hollis, F., Gaval-Cruz, M., Carrier, N., Dietz, D.M., & Kabbaj, M. (2012). Juvenile and adult rats differ in cocaine reward and expression of zif268 in the forebrain. *Neuroscience, 3,* 91-98.

28. Huerta-Rivas, A., Lopez-Rubalcava, C., Sanchez-Serrano, S.L., Valdez-Tapia, M., Lamas, M., & Cruz, S.L. (2012). Toluene impairs learning and memory, has antinociceptive effects, and modifies histone acetylation in the dentate gyrus of adolescent and adult rats. *Pharmacol Biochem Behav. 102,* 48-57.

29. Ji, J., & Maren, S. (2008). Differential roles for hippocampal areas CA1 and CA3 in the contextual encoding and retrieval of extinghushed fear. *Learn Mem. 15*,244-251.

30. Kerchner, G.A., Deutsch, G.K., Zeineh, M., Dougherty, R.F., Saranathan, M., & Rutt, B.K. (2012). Hippocampal CA1 apical neuropil atrophy and memory performance in Alzheimer disease. *Neuroimage, 63*,194-202.

31. Korbo, L. (1999). Glial cell loss in the hippocampus of alcoholics. *Alcohol Clin Exp Res. 23*, 164-168.

32. Kornfeld, M., Moser, A.B., Moser, H.W., Kleinschmidt-DeMasters, B, Nolte, K., & Phelps, A. (1994). Solvent vapor abuse leukoencephalopathy. Comparison to adrenoleukodystrophy. *J Neuropathol Exp Neurol. 53*, 389-398.

33. Ladefoged, O., Hougaard, K.S., Hass, U., Sorensen, I.K., Lund, S.P., Svendsen, G.W., & Lam, H.R. (2004). Effects of combined prenatal stress and toluene exposure on apoptotic neurodegeneration in cerebellum and hippocampus of rats. *Basic Clin Pharmacol Toxicol. 94,* 169–176.

34. Lin, B.F., Ou, M.C., Chung, S.S., Pang, C.Y., & Chen, H.H. (2010). Adolescent toluene exposure produces enduring social and cognitive deficits in mice: an animal model of solvent-induced psychosis. *World J Biol Psychiatry, 11,* 792-802.

35. Lubman D.I., Yucel, M., & Lawrence, A.J. (2008). Inhalant abuse among adolescents: neurological considerations. *Br J Pharmacol. 154*,316-326.

36. McDonald, C.G., Dailey, V.K., Bergstrom, H.C., Wheeler, T.L., Eppolito, A.K., Smith, L.N., & Smith, R.F. (2005). Periadolescent nicotine administration produces enduring changes in dendritic morphology of medium spiny neurons from nucleus accumbens. *Neurosci Lett. 385*, 163–167.

37. McLaughlin, K.J., Wilson, J.O., Harnman, J., Wright, R.L., Wieczorek, L. Gomez, J., Korol, D.L., & Conrad, C.D. (2010). Chronic 17β-estradiol or cholesterol prevets stress-induced hippocampal CA3 dendritic retraction in ovariectomized female rats: Possible correspondence between CA1 spine properties and spatial acquisition. *Hippocampus, 20,* 768-786.

38. Mistretta, V., & Charlier, C. (2013). Biomonitoring of exposure to chemical products in environmental and workplace toxicology. *Ann Biol Clin (Paris). 71,* 257-267.

39. Mizuseki, K., Diba, K., Pastalkova, E., & Buzsaki, G. (2011). Hippocampal CA1 pyramidal cells form functionally distinct sublayers. *Nature Neuroscience, 14*, 1174-1181.

40. Nakajima, T., & Wang, R.S. (1994). Induction of cytochrome P450 by toluene. *Int J Biochem. 26,* 1333–1340.

41. NiIkura, K., Ho. A., Kreek, M.J., & Zhang, Y. (2013). [Oxycodone-induced conditioned place preference and sensitization of locomotor activity in adolescent and adult mice.](https://www.ncbi.nlm.nih.gov/pubmed/23827650) *Pharmacol Biochem Behav. 110*, 112-116.

42. O’Leary-Moore, S.K., Galoway, M.P., McMechan, A.P., Irtenkauf, S., Hannigan, J.H., & Bowen, S.E. (2009) Neurochemical changes after acute binge toluene inhalation in adolescent and adult rats: a high-resolution magnetic resonance spectroscopy study. *Neurotoxicol Teratol. 31*, 382–389.

43. Paez-Martinez, N., Aldrete-Audiffred, J., Gallardo-Tenorio, A., Castro-Garcia, M., Estrada-Camarena, E., & Lopez-Rubalcava, C. (2013). [Participation of GABAA, GABA(B) receptors and neurosteroids in toluene-induced hypothermia: evidence of concentration-dependent differences in the mechanism of action.](https://www.ncbi.nlm.nih.gov/pubmed/23085024) *Eur J Pharmacol. 698*, 178-185.

44. Pascual, J., Moron, L., Zarate, J., Gutierrez, A., Churruca, I., & Echevarria, E. (2004). Toluene alters p75NTR expression in the rat brainstem. *Ind Health, 42,* 75–78.

45. Proctor, S., Heaton, K., Smith, K., Rodrigues, E., & McClean, M. (2014). 0019 The occupational JP8 exposure neuroepidemiology study; evaluation of neuropsychological effects. *Occup Environ Med. Suppl 1:A1-2.* doi: 10.1136/oemed-2014-102362.3.

46. Riegel, A.C., Ali, S.F., Torinese, S.G., & French, E.D. (2004). Repeated exposure to the abused inhalant toluene alters levels of neurotransmitters and generates peroxynitrite in nigrostriatal and mesolimbic nuclei in rat. *Ann N Y Acad Sci.* 1025, 543–551.

47. Riegel, A.C., Zapata, A., Shippenberg,T.S., & French, E.D. (2007). The abused inhalant toluene increases dopamine release in the nucleus accumbens by directly stimulating ventral tegmental area neurons. *Neuropsychopharmacology, 32*, 1558–1569.

48. Robinson, T.E., Gorny, G., Mitton, E., & Kolb, B. (2001). Cocaine-self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse, 39*,257-266.

49. Rosenberg, N.L., Kleinschmidt-DeMasters, B.K., & Davis, K.A. Dreisbach, J.N., Hormes, J.T., & Filley, C.M. (1988) Toluene abuse causes diffuse central nervous system white matter changes. *Ann Neurol. 23,* 611-614.

50. Sanchez-Serrano, S.L, Cruz, S.L., & Lamas, M. (2011). Repeated toluene exposure modifies the acetylation pattern of histones H3 and H4 in the rat brain. Neurosci Lett. 489, 142–147.

51. See, R.E. (2002). Neural substrates of conditioned-cued relapse to drug-seeking behavior. *Pharmacol Biochem Behav*. *71,* 517–529.

52. Spear, L.P. (2000). The adolescent brain and age-related behavioral manifestations. Neurosci *Biobehav Rev. 24*, 417–463.

53. Spear, L.P., & E. Varlinskaya, E.I. (2005). Adolescence. Alcohol sensitivity, tolerance, and intake. *Recent Dev Alcohol. 17*, 143–159.

54. Stramiello, M., & Wagner, J.J., (2010). Cocaine enhancement of long-term potentiation in the CA1 region of rat hippocampus: lamina-specific mechanisms of action. *Synapse, 64,*  644-648.

55 Swant, J., Chirwa, S., Stanwood, G., & Khoshbouei, H. (2010). Methamphetamine reduces LTP and increases baseline synaptic transmission in the CA1 region of mouse hippocampus. *PLoS One, 5, e11382*. doi: [10.1371/journal.pone. 0011382](http://dx.doi.org/10.1371/journal.pone.0011382%22%20%5Ct%20%22pmc_ext).

56. Terashi, H., Nagata, K., Satoh, Y., Hirata, Y., & Hatazawa, J. (1997). Hippocampal hypoperfusion undelying dementia due to chronic toluene intoxication. *Rinsho Shinkeigaku 37*, 1010-1013.

57. Tomaszycki, M.L., Aulerich, K.E., & Bowen, S.E. (2013). Repeated toluene exposure increases c-Fos in catecholaminergic cells of the nucleus accumbens shell. *Neurotoxicol Teratol. 40*, 28-34.

58.Tormoehlen, L.M., Tekulve, K.J., & Nanagas, K.A. (2014) Hydrocarbon toxicity: A review. *Clin Toxicol. (Phila), 52*, 479-489.

59. Van den Oever, M.C., Rotaru, D.C., Heinsbroek, J.A., Gouwenberg, Y., Deisseroth, K., Stuber, G.D., Mansvelder, H.D., & Smit, A.B. (2013). Ventromedial prefrontal cortex pyramidal cells have a temporal dynamic role in recall and extinction of cocaine-associated memory. *J Neurosci. 33,* 18225-18233.

60. Win-Shwe, T.T., Yoshida,Y., Kunugita, N., Tsukahara, S., & H. Fujimaki, H. (2010). Does early life toluene exposure alter the expression of NMDA receptor subunits and signal transduction pathway in infant mouse hippocampus? *Neurotoxicology, 31*, 647–653.

61. Waxman, D.J., Dannan, G.A., & Guengerich, F.P. (1985). Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry, 24*, 4409–4417.

62. Zhao, Y., Dayas, C.V., Aujla, H., Baptista, M.A., Martin-Fardon, R., & Weiss, F. (2006). Activation of group II metabotropic gluatamate receptors attenuates both stress and cue-induced ethanol-seeking and modulates c-fos expression in the hippocampus and amygdala. *J Neurosci. 27,* 9967-9974.

63. Zhvania, M.G., Chilachava, L. R., Japaridze, N.J., Gelazonia, L.K., & Lordkipanidze, T.G. (2012). Immediate and persisting effect of toluene chronic exposure on hippocampal cell loss in adoelscent and adult rats. *Brain Res Bull. 87,* 187-182.

64. Zhvania, M.G., Japaridze, N.J., Chilachava, L.R., Dashniani, M.G., Bikashvili, T.Z., Gelazonia, L.K., & Burjanadze, M.A. (2013). Immediate and persisting effect of chronic toluene exposure on hippocampal cell loss, learning and memory in adolescent and adult rats. Marco C. Palminteri (Ed.), *Toluene. Chemical Properties, Applications and Toxicology (pp. 53-92). New York: Nova Publishers Inc.*

**Tables:**

**Table 1.**

|  |  |  |
| --- | --- | --- |
| **Age** | **Adolescent** | **Adult** |
|  | ***F*(3, 12)** | ***p*** | ***F*(3, 12)** | ***p*** |
| **CA1 Area****Pyramidal layer** | **17.97** | **0.000** | **27.03** | **0.000** |
| **CA3 Area****Pyramidal layer** | **5.39** | **0.014** | **3.74** | **0.042** |

**Table 2.**

|  |
| --- |
| **The Adolescent Rats** |
| **Age** | **Hippocampus, CA1 Area** | **Hippocampus, CA3 Area** |
| **Effect** | **Immediate** | **Persisting** | **Immediate** | **Persisting** |
| **Groups** | **Control** | **Exp** | **Control** | **Exp** | **Control** | **Exp** | **Control** | **Exp** |
| **Mean** | **51.73±3.3** | **36.92+2.0** | **50.03±2.5** | **30.90+1.2** | **32.75±2.3**  | **25.07±1.4** | **34.92±2.5** | **23.30+0.68** |
| ***p*-value** |  | **0.019†** | **0.699 ¥** | **0.002†****0.064‡** |  |  **0.037†** | **0.640¥** | **0.023†****0.327‡** |

**Table 3.**

|  |
| --- |
| **The Adult Rats** |
| **Age** | **Hippocampus, CA1 Area** | **Hippocampus, CA3 Area** |
| **Effect** | **Immediate** | **Persisting** | **Immediate** | **Persisting** |
| **Groups** | **Control** | **Exp** | **Control** | **Exp** | **Control** | **Exp** | **Control** | **Exp** |
| **Mean** | **76.08±2.3** | **60.45±0.87** | **34.92±2.5** | **23.30+0.68** | **45.22+3.3** | **41.11+2.2** | **44.93±3.3** | **33.82±1.8** |
| **P-value** |  | **0.008†** | **0.640¥** | **0.023†****0.327‡** |  | **0.350†** | **0.951¥** | **0.043 †****0.053‡** |

**Figures:**

**Figure 1.**

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**Figure 2.**

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**Figure 3.**

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**Figure 4.**

