Long-term effects of alcohol use on adolescent brain development

Richard M.A. Houtman

Front page
Alcohol can cause damage to the developing adolescent brain.
[Image adapted from the 2010 campaign of MADD New Mexico (USA) at http://brokenteens.org/]

Title	Long-term effects of alcohol use on adolescent brain development
Name	Richard Mattheus Alouisius Houtman, BSc
Email	R.M.A.Houtman@students.uu.nl
Student number	0215392
Master's program	Neuroscience and Cognition, Utrecht University
Supervisor	Dr Heidi M.B. Lesscher
Second reviewer	Prof Dr Louk J.M.J. Vanderschuren
Place	Rudolf Magnus Institute of Neuroscience (RMI), Department of Neuroscience and Pharmacology, University Medical Centre Utrecht (UMCU)
Period	May – August 2010

Abstract

Alcohol use entails high medical, social and economic costs for our society. Despite laws restricting the age of alcohol users, alcohol use in young Dutch adolescents is quite common. Alcohol consumption among adolescents is characterized by frequent drinking and drinking in high quantities. At the same time during adolescence, the brain undergoes many developmental changes, particularly in the prefrontal cortex, hippocampus, and mesocorticolimbic dopamine system. Human and rodent studies revealed that adolescent alcohol use can cause brain damage and long-term detrimental neurocognitive effects, for example for executive functioning and memory and learning abilities. Moreover, evidence suggests that early onset and high-risk alcohol use among adolescents increases the risk for later alcohol abuse and addiction. Suggestions are given for future research to further elucidate the effect of alcohol use on adolescent brain development and to minimize the negative consequences of this alcohol use. Finally, recommendations for the prevention of underage drinking are discussed.

Table of contents

Abstract……... 2

1. Introduction: Alcohol use and alcoholism……...4-7
1.1 Alcoholism: some definitions……4
1.2 Alcoholism: incidence and consequences……..5
1.3 Thesis outline……7

2. Consequences of alcohol use in adolescents……... 8-26
2.1 Brain structure and functioning……8
Alcohol-induced neural damage……..8
Possible explanations for alcohol-induced neural damage……9
2.2 Neurocognition……..... 10
Attention and decision making……11
Memory and learning……...12
Cue reactivity……...20
2.3 Alcohol abuse and addiction……..21
Adolescent-specific vulnerability to alcoholism…….. 22
Neurobiology of alcoholism……23
Effects of adolescent alcohol use on the mesocorticolimbic DA system.....25

3. Discussion……26-30

References……...31-38

1. Introduction: Alcohol use and alcoholism

Alcohol is an organic solvent especially known for its use for human consumption. The type of alcohol that is found in alcohol beverages is ethanol. Most common alcohol beverages are beer, wines, and spirits. However, even consumption of non-beverage alcohol solutions such as tinctures, perfumes, and mouthwash has been reported, often by severe alcoholics (Leon *et al.*, 2007; Soo Hoo *et al.*, 2003). Ethanol has been known to mankind for its intoxicating effects since ancient times. Short-term positive effects of moderate alcohol consumption include an overall improvement of mood and increased relaxation, self-confidence, courage, and sociability. However, there are also numerous negative effects related to the use of alcohol. For example, the risk for several disorders, including liver cirrhosis, cardiovascular disease, multiple forms of cancer, and neuropsychiatric conditions, increases as a result of alcohol use. Another negative effect of alcohol is its addictive potential. When comparing the adverse effects of alcohol with those caused by other drugs of abuse, categorized as hard drugs, one could argue that alcohol should be classified as a hard drug. Yet, most government laws – at least those from all western countries – consider alcohol as legal under certain minimum age restrictions (Nutt *et al.*, 2007).

1.1 Alcoholism: some definitions

Drug addiction is a chronic brain function disorder that is characterized by compulsive drug use. Drug addiction may comprise both characteristics of physical dependence and physiological dependence. Table 1 summarizes the DSM-IV criteria that are used in the clinic for the diagnosis of alcohol addiction, which is also termed alcohol dependence, compared with those for alcohol abuse. Tolerance can be one of the symptoms of alcohol

Table 1. Criteria from the Diagnostic and Statistical Manual of Mental Disorders IV text revision (DSM-IV-TR) for alcohol abuse and alcohol dependence. Alcohol dependence is also termed alcohol addiction. Adapted from American Psychiatric Association (2000).

Alcohol abuse	Alcohol dependence
A maladaptive pattern of use that leads to clinically significant impairment or distress, as manifested by 1 or more of the following within a 12-month period: ● recurrent alcohol use that results in a failure to fulfil major role obligations at work, school, or home; ● recurrent alcohol use in situations in which it is physically hazardous; ● recurrent alcohol-related legal problems; ● continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the alcohol; and: ● the symptoms have never met the criteria for alcohol dependence.	A maladaptive pattern of use that leads to clinically significant impairment or distress, as manifested by 3 or more of the following within the same 12-month period: ● tolerance; ● withdrawal; ● alcohol is often taken in larger amounts or over a longer period than was intended; ● there is a persistent desire or unsuccessful efforts to cut down or control use; ● a great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects; ● important social, occupational, or recreational activities are given up or reduced because of use; ● alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol.

dependence and is defined as the situation in which an individual becomes less responsive to alcohol and has to use higher doses to be satisfied. Other symptoms can be withdrawal – which covers the group of negative symptoms after alcohol use is stopped abruptly – and the continuation of alcohol use despite knowledge of having problems due to alcohol. Alcohol use disorders (AUDs) include alcohol abuse, alcohol dependence, and harmful use of alcohol.

1.2 Alcoholism: incidence and consequences

Alcohol addiction may in society often be regarded as one of the least problematic substance addictions, but numbers tell a different story. The World Health Organisation (WHO) has recently estimated that 76.3 million people suffer from alcohol use disorders worldwide against at least 15.3 million people who have other drug use disorders. Alcohol has a major contribution to the total number of years of life lost to death and disability, as recorded in disability adjusted life years (DALYs), in 1990 as well as in 2000 (Murray and Lopez, 1996; Rehm *et al.*, 2004) (Table 2). DALYs are a measure of overall disease burden and one DALY is equal to one year of healthy life that is lost. Causes of alcohol-related DALYs are AUDs, neuropsychiatric conditions, and other alcohol-induced diseases and injuries. Alcohol addiction is a risk for the health of the addict as well as for others, e.g. in cases of traffic accidents and violence. Furthermore, addicts can loose their jobs and/or participate in criminal activities. To summarize, alcohol use entails high medical, social and economic costs.

Table 2. Global burden of disease in 1990 (Murray and Lopez, 1996) and in 2000 (Rehm *et al.*, 2004) due to alcohol, tobacco, and illicit drugs. DALYs, disability adjusted life years.

	Murray and Lopez (1996)				**Rehm *et al.* (2004)**
	Deaths [% of total deaths]	Years of life lost [% of total years of life lost]	Years of life disabled [% of total years of life disabled]	DALYs [% of total DALYs]	DALYs [% of total DALYs]
ALCOHOL	774.000 [1.5]	19.287.000 [2.1]	28.400.000 [6.0]	47.687.000 [3.5]	82.962.000 [5.7]
TOBACCO	3.038.000 [6.0]	26.217.000 [2.9]	9.965.000 [2.1]	36.182.000 [2.6]	88.784.000 [6.1]
ILLICIT DRUGS	100.000 [0.2]	2.634.000 [0.3]	5.834.000 [1.2]	8.467.000 [0.6]	14.555.000 [1.0]

Alcohol is one of the most commonly used drugs among adolescents – young people going through the developmental stage from childhood to adulthood. There is no consensus on the age definition of adolescence. According to the WHO for example, adolescents are between the ages of 10 and 19 years. Research groups and organisations from all over the world use various age spans depending on their program, funding sources and/or for sociocultural reasons. Thus, establishing a fixed age span for a developmental period is difficult and further complicated by individual variation and gender differences, with females maturing

more rapidly than males. For clarity, in this thesis adolescents will be referred to as youth between 12 and 25 years of age, thus from early puberty to young adulthood. During adolescence, prevalence of drinking increases, in particular between 12 and 15 years of age until the age of 25, after which alcohol consumption decreases again (Poelen *et al.*, 2005). Moreover, the pattern of drinking among adolescents is different compared with the pattern of alcohol use by adults: drinking behaviour among adolescents is characterized by much more binge drinking and heavy drinking when compared with alcohol use in adults. Binge drinking is often defined as the use of at least 4 (for females) or at least 5 (for males) alcoholic drinks in a row at one occasion, usually in a time window of approximately 2 hours, and often with the aim of becoming drunk (Stolle *et al.*, 2009). Heavy drinking is defined as the use of these amounts of drinks (binges) on the same occasion for at least 5 days in a month.

Despite strict laws concerning age limits, alcohol use in young adolescents is quite common. In the Netherlands, it is illegal to sell light alcoholic beverages to people younger than 16 years and to sell spirits to people under the age of 18. Nevertheless, Dutch adolescents are among the youngest to start drinking and are among the heaviest drinkers compared to other western countries (Currie *et al.*, 2004; Van Laar *et al.*, 2010). 21% of 13-year-old Dutch adolescents drink weekly, while this is on average 12% in other western countries. For the 15-year-olds, 52% of the Dutch adolescents drink weekly, compared with 29% of adolescents in this age group from other western countries. Furthermore, alcohol use among adolescents has increased substantially over the past two decennia. In the 1990s, the frequency of drinking and the quantity of alcohol consumed among Dutch adolescents between 12 and 25 years increased (Poelen *et al.*, 2005). The percentage of adolescents who consume alcohol weekly increased from 10% to 15% between 1993 and 2000. Also, a 50% increase in this period was found in the number of adolescents consuming more than 5 drinks per week (i.e. from 20% to 30%). During the first decennium of the 21st century, alcohol use slightly decreased for 12- to 14-year-olds but remained stable for 15- to 18-year-olds (Monshouwer *et al.*, 2008; Van Laar *et al.*, 2010). Noteworthy, alcohol-related hospital admissions in the Netherlands have risen in the last 4 years, especially for youth of 16 years or younger between 2006 and 2007 (i.e. 42%) (Van Laar *et al.*, 2010). Furthermore, the increase of clients with a primary alcohol problem in Dutch addiction care centres in the past decennium was relative highest in adolescents of 15 to 19 years of age and people older than 60 years (Van Laar *et al.*, 2010). Between 2002 and 2008, the number of 15- to 19-year-old alcohol clients increased with 85%. The consequences of excessive consumption of alcohol among adolescents are only partly understood but there are strong indications that the consequences in the long-term are pronounced. The risk *for* and *of* alcohol use during adolescence may be particularly high for two reasons.

First, adolescents are highly vulnerable to the actual use of alcohol. One of the functions of adolescence is to establish good social skills and other adaptations to prepare for adulthood. However, the rapid expansion of psychological developmental changes goes with conflicting behaviours. Adolescents display enhanced novelty seeking, sensation seeking, and/or reward seeking behaviour accompanied by poor judgment and lack of impulse control. This explorative and risky behaviour promotes experimentation of alcohol and other drugs (Crews *et al.*, 2007; Dahl, 2004; Spear, 2000). In addition, adolescents are particularly prone to peer pressure, which makes proper decision making regarding to alcohol use even harder. Furthermore, they are less sensitive to the sedative effects of alcohol than adults. As a

consequence, adolescents are more likely to use and abuse alcohol, which in turn makes them highly vulnerable to alcohol-induced neurotoxicity (Monti *et al.*, 2005; Spear, 2000).

Second, during adolescence the brain is not yet fully matured but is rather still in development. Although after adolescence brain plasticity remains, the brain undergoes rapid developmental changes until the age of 25. The main developmental processes in the brain that persist during adolescence are increased axonal myelination and revision of synaptic density in grey matter. Magnetic resonance imaging (MRI) studies have demonstrated a loss of grey matter volume during adolescence after a pre-adolescent increase (Giedd *et al.*, 1999; Gogtay *et al.*, 2004). This fluctuation of grey matter volume reflects synaptogenesis followed by rapid synaptic pruning (Giedd *et al.*, 1999), resulting in greater efficiency of signal transmission. The prefrontal cortex (PFC) is one of the last brain areas to fully mature in that way (Gogtay *et al.*, 2004). This brain area in the frontal lobe is critically involved in processing executive functions, which include decision making, behavioural inhibition, attention, problem solving, and reasoning. The maturation of the PFC is in line with the behavioural changes in executive functions that adolescents undergo in maturing to adults. Increases in white matter during adolescence in the same brain areas reflect enhanced myelination in neuronal networks and is thought to be related to increases in cognitive efficiency in a time window during which the brain is not yet fully matured (Barnea-Goraly *et al.*, 2005; Giedd *et al.*, 1999). Alcohol may disturb the normal process of adolescent brain development.

1.3 Thesis outline

Together, adolescents consume high levels of alcohol, the consequences of which are only partly understood. The objective of this thesis is therefore to study what the consequences of adolescent alcohol use are in the long-term. In particular, alcohol use in adolescents may cause cognitive problems and put them at risk for alcohol addiction.

In chapter 2, human and rodent literature will therefore be reviewed to address the long-term consequences of alcohol use in adolescents in relation to neurocognition and alcoholism. Moreover, the neurobiological basis that underlies this enhanced risk for impaired cognitive function and addiction liability will be determined, evidence for which is mainly provided by preclinical studies. To fully understand the consequences of adolescent alcohol exposure and the neurobiological changes that occur as a result of alcohol exposure in adolescence we can benefit from valid animal models for addiction. For example, rodents can show drug self-administration behaviour like humans. The definition of adolescence in rodents is similar to that of humans and covers the period from 28 days to at least 42 days after birth (Spear, 2000). Animal research can shed light on the phenomenon of addiction, the effect of alcohol use on neurocognition, as well as the underlying genetic and neurobiological mechanisms associated with adolescent alcohol use.

In chapter 3, the objective of this thesis will be achieved by formulating a summary and conclusion of the main results. Furthermore, methodological considerations and ideas for further and future research will be given. Chapter 3 will end with a statement of what should be done to prevent early onset of alcohol use in these vulnerable young people who form the future.

2. Consequences of alcohol use in adolescents

As stated earlier, most adolescents use alcohol for the first time at an early age and approximately 50% of all 15-year-old Dutch adolescents drink weekly. 12- to 16-year-olds who drink weekly show more delinquent and aggressive behaviour than non-weekly drinkers (Van Laar *et al.*, 2010). Furthermore, weekly drinking is associated with somatic, anxiety and depressive complaints in 12- and 13-year-old adolescents (Van Laar *et al.*, 2010). The drinking pattern of adolescents is characterized by frequent drinking (e.g. heavy drinking) and drinking in high quantities (e.g. binge drinking). It is likely that alcohol affects brain development but the consequences of high levels of alcohol use in adolescents are only partly understood. In this chapter, literature will be discussed to determine the consequences of adolescent alcohol use on brain structure and functioning (section 2.1), neurocognition (section 2.2), and the risk for alcohol abuse and addiction (section 2.3).

2.1 Brain structure and functioning

Alcohol use in adolescents may affect the development and structure of their maturing brain. In this section, evidence of alcohol-induced brain damage as well as its possible underlying processes will therefore be discussed.

Alcohol-induced neural damage

Current as well as detoxified alcohol-dependent adults show decreased grey and white matter volumes (Chanraud *et al.*, 2007; Kril *et al.*, 1997; Pfefferbaum *et al.*, 2000; Pfefferbaum *et al.*, 2006). Adolescent alcohol use may be even more harmful because the adolescent brain is still in development (Brown and Tapert, 2004; Chambers *et al.*, 2003; Clark *et al.*, 2008; Crews *et al.*, 2007; Dahl, 2004; Ehlers and Criado, 2010; Kokotailo, 2010; Maldonado-Devincci *et al.*, 2010b; Witt, 2010). Indeed, greater damage of frontal brain regions was found in 35-day-old adolescent rats compared with 80- to 90-day-old adult rats after binge drinking exposure to alcohol (Crews *et al.*, 2000). In that study, 15% alcohol was given intragastrically 4 times per day for 4 days, which means that the rats were exposed to approximately 9-10 g/kg ethanol each day. Moreover, age at first drinking is positively correlated with grey matter volume in frontal brain regions, pons, and cerebellum in alcohol-dependents (Chanraud *et al.*, 2007) and age of AUD onset with hippocampal volume in adolescents with AUDs (De Bellis *et al.*, 2000), which suggests that these regions are highly vulnerable to early-onset alcohol use. In addition, adolescents with AUDs had been reported to have smaller PFC white matter volumes (De Bellis *et al.*, 2005), smaller left hippocampal volumes (De Bellis *et al.*, 2000), and white matter decreases in the splenium of the corpus callosum (Tapert *et al.*, 2003b) compared with controls. On top of that, decreases in white matter in the corpus callosum were significantly related to both longer duration of heavy drinking and larger quantities of recently consumed alcohol (Tapert *et al.*, 2003b). However, the AUD adolescents in former studies had comorbid mental disorders, thus a correlative effect of the comorbid condition cannot be ruled out. Nevertheless, in adolescents with AUD without psychiatric comorbidity, hippocampal volume (Medina *et al.*, 2007; Nagel *et al.*, 2005) and white matter volumes (Medina *et al.*, 2008) showed a similar decrease, suggesting that the reduction in brain volume is related to alcohol use in adolescents and is unlikely to be secondary to comorbid psychiatric conditions.

Little is known about alcohol-induced brain structure changes in adolescents without

AUDs. After binge drinking exposure to alcohol, adolescent rats showed increased neural death in both the hippocampus and the PFC (Pascual *et al.*, 2007). Binge drinking exposure in that study was established by intraperitoneal administration of 25% ethanol once per day for 2 consecutive days at 48 hour intervals over 14 days, resulting in exposure to 3 g/kg ethanol each day per rat. A recent MRI study examined white matter characteristics in 14 binge drinking adolescents without AUDs compared with 14 control adolescents using diffusion tensor imaging (DTI) (Jacobus *et al.*, 2009). DTI measures the restricted diffusion of water molecules in white matter tissue to produce images of localized white matter fiber tracts. An estimate of the directionally dependent movement of water, called the fractional anisotropy (FA), is often used as scalar value in DTI. Higher FA values indicate more white matter. Interestingly, FA was found to be significantly lower in the drinking group than in controls for eight white matter regions, including frontal association fibres such as frontal-occipital and superior longitudinal fasciculi. This implies weakening of connectivity and neural transmission speed within and between the frontal cortex and other brain regions.

Although it is not always clear if the neural changes found in human studies are the cause or consequence of alcohol use, these studies together suggest that the adolescent brain is particularly vulnerable to the detrimental effects of alcohol.

Possible explanations for alcohol-induced neural damage

The mechanisms through which alcohol causes brain damage in adolescents are not yet understood. However, several processes involved in brain (re)generation are affected by alcohol and may be causal to the detrimental effects of alcohol in the adolescent brain.

A proper cerebral blood flow (CBF) is necessary to provide the brain with substances and energy. In addition, stages of increased CBF support periods of rapid brain growth, for example during adolescence (Epstein, 1999). CBF has been measured in eight alcohol-dependent adolescent women of 18 to 25 years old and eight controls by using the blood oxygen level dependent (BOLD) signal in functional MRI (fMRI) (Clark *et al.*, 2007). This study showed decreased perfusion in six prefrontal and parietal brain regions of alcohol-dependent adolescents. Knowing that inadequate CBF can damage brain tissue, these data suggest that alcohol exposure during adolescence may cause neural damage by affecting cerebral blood flow.

Another process that is affected by alcohol exposure is neurogenesis – the process by which new neurons are generated. Neurogenic processes in the forebrain subventricular zone (SVZ) and the hippocampal dentate gyrus (DG) continue into senescence, although the highest levels of neurogenesis are reached during adolescence (He and Crews, 2007). Neurogenesis has been shown to be altered upon exposure to alcohol during the adolescent period. Adolescent male rats – 35 to 40 days old – were treated with one of three acute alcohol doses (1.0, 2.5 or 5.0 g/kg) intragastrically (Crews *et al.*, 2006). Those three groups showed reduced levels of neurogenesis in both SVZ and DG as compared with neurogenesis observed in vehicle-treated rats. The dentate gyrus of the hippocampus is critically involved in the acquisition and representation of memories. Therefore, we may speculate that adolescent binge drinking may have long-term inhibiting effects on neurogenesis, which may disrupt learning and other behaviours during brain maturation.

More specifically, adolescent alcohol use affects GABAergic and glutamatergic neurotransmission. Alcohol directly acts on gamma-aminobutyric acid (GABA) receptors and *N*-methyl-$_D$-aspartic acid (NMDA) receptors. When GABA, the major inhibitory

neurotransmitter of the central nervous system (CNS), binds to the $GABA_A$ receptor, this chloride channel opens and the resulting influx of chloride ions inhibits neuronal activity. Low doses of alcohol enhance the influx of chloride into the cell, resulting in further inhibition of neuronal activity. When high doses of alcohol are consumed repeatedly, $GABA_A$ receptors compensate for the continuous alcohol-induced inhibition by reducing the number of subunits of this receptor (Mihic and Harris, 1997). A subunit of the $GABA_A$ receptor is at its highest level in the frontal cortex of rats in mid-adolescence (Yu *et al.*, 2006), thus repeated alcohol use during adolescence could seriously affect the normal remodelling of GABAergic synapses in this critical brain area. In addition, glutamate, the major excitatory neurotransmitter of the CNS, can bind to NMDA receptors under certain conditions, causing calcium channels to open thus resulting in an increase in neuronal activity. In the presence of alcohol the excitatory NMDA-receptors are inhibited and as a consequence neuronal activity is inhibited. After chronic alcohol use, however, these receptors are upregulated to compensate for the alcohol-induced inhibition of excitation (Tsai *et al.*, 1995). Binding of glutamate to NMDA-receptors in the forebrain cortex is highest in early adolescence (Insel *et al.*, 1990), making the glutamatergic system potentially more vulnerable during adolescence when ethanol may cause NMDA supersensitivity. In short, the direct neurotoxic effect of alcohol on GABAergic and glutamatergic neurotransmission, i.e. inhibition of neurons that produces sedation, may evolve after repeated alcohol use into long-term indirect excitotoxic effects leading to withdrawal. Both these direct and indirect effects may contribute to smaller PFC white matter volumes in alcohol consuming adolescents (De Bellis *et al.*, 2005).

Besides inadequate CBF, neurogenesis, and neurotoxicity, neuroinflammation has been proposed as another mechanism for alcohol-induced brain damage. For example, chronic alcohol intake activates inflammatory mediators in the brain by triggering glial cells and intracellular signalling pathways to generate cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), the proinflammatory cytokines IL-1b and TNF-a, and neural cell death (Blanco *et al.*, 2005; Valles *et al.*, 2004). More important, binge drinking exposure in adolescent rats resulted in an upregulation of COX-2 and iNOS levels and increased neural cell death in the neocortex, hippocampus, and cerebellum (Pascual *et al.*, 2007). Interestingly, the increase in brain inflammatory mediators after adolescent alcohol use was associated with long-term cognitive impairments, as reflected by deficits in both conditional discrimination and object recognition tasks (Pascual *et al.*, 2007).

Taken together, there is profound evidence for alcohol-induced neural damage during adolescence, in particular in brain structures involved in learning and cognition.

2.2 Neurocognition

Given the alcohol-induced brain damage during adolescent brain development, it is likely that adolescent alcohol use causes cognitive dysfunction which may persist into adulthood. Indeed, when compared with control subjects, neuropsychological tests among AUD adolescents revealed deficits in cognitive functions, including attention difficulties, poorer verbal and non-verbal retention, poorer visuospatial functioning, lower verbal and full-scale IQ scores, and lower achievement scores in reading recognition, total reading, and spelling (Brown *et al.*, 2000; Moss *et al.*, 1994; Sher *et al.*, 1997; Tapert *et al.*, 2002). In addition, other human and rodent studies demonstrate the vulnerability of adolescent neurocognitive abilities after alcohol use as well, including long-term alcohol-induced effects on executive

functions, memory, and learning, and neurocognition of cue reactivity, which will be discussed in more detail in this section.

Attention and decision making

Executive functions, e.g. attention and decision making, are critical for successful adaptations and performance during lifetime. Alcohol-induced damage in the PFC of adolescents, may have long-lasting consequences for their executive functioning.

Notably, adult alcohol-dependent subjects showed impaired performance on several executive function tasks, including the Trail Making Test Part-B (TMT-B) for measuring attention ability that was correlated with decreases in the frontal cortex among other brain structures (Chanraud *et al.*, 2007). In addition, a longitudinal study over 8 years reported differences in neuropsychological performance between AUD adolescents and healthy adolescents from the same community (Tapert *et al.*, 2002). For example, attention ability was measured at project intake (mean age: 16 years) and 8 years later by TMT-A, TMT-B, WISC–R0WAIS–R (Arithmetic, Digits Forward, and Digits Backward), PASAT, SDMT, and Stroop tasks. No significant group differences were found for these tests at project intake. However, greater cumulative alcohol use in the following 8 years predicted poorer attention functioning in all tests (significance: $0.014 \leq p \leq 0.042$), which pinpoint the detrimental effects of chronic alcohol use on attention during adolescence.

Furthermore, a rodent study investigated the effect of voluntary alcohol consumption during adolescence on decision making in adulthood (Nasrallah *et al.*, 2009). Adolescent rats with a minimum age of 30 days had access to 10% ethanol or control gelatine presented in a gel matrix for 20 days, thus into early adulthood. Alcohol intake levels in the alcohol group were on average 11.4 g/kg/day (range: 6.4-17.9 g/kg/day). Then, alcohol gel delivery was stopped and a probability-discounting instrumental response task was started to test the influence of uncertainty on the choice behaviour of both the alcohol and control group. Rats were presented two levers: one which certainly delivers two sucrose pellets, the other one which probably delivers four of these pellets (with a probability of 25%, 50%, or 75%). Separated groups of rats (both with alcohol-exposed and control rats) were tested 3 weeks and 3 months after alcohol delivery stopped and task training started. In both experiments the rats with a history of adolescent alcohol use preferred the larger but uncertain and risky

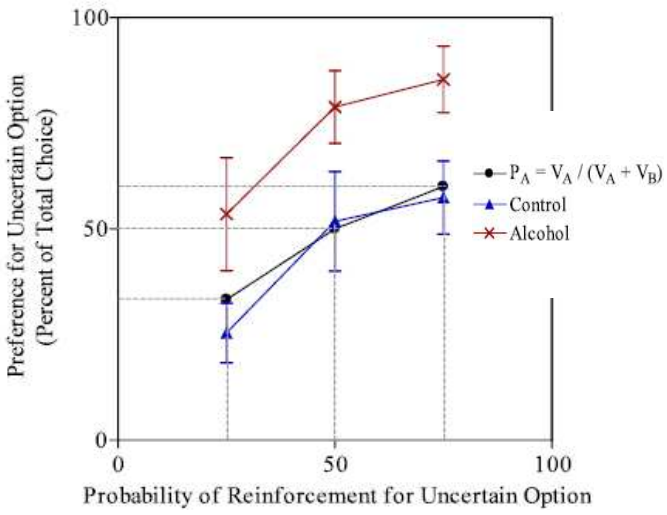

Figure 1. Results from the probability-discounting instrumental response task 3 months after alcohol delivery stopped and task training started for adolescent rats with 20 days of voluntary alcohol consumption (in red) and control rats (in blue). The alcohol group shows a preference for the larger but uncertain option, compared with the control group. Results for the 3 weeks training groups were strikingly similar (not shown). Dashed lines represent risk-neutral choice predictions for 25%, 50%, and 75% probability conditions following the equation: $P_A = V_A / (V_A + V_B)$, where $V_X = \text{Magnitude}_X * \text{Probability}_X$. Error bars show the means ± SEM after ANOVA. Adapted from Nasrallah *et al.* (2009).

option in adulthood (Fig. 1, page 11), which demonstrates impulsivity as a consequence of adolescent alcohol use.

In summary, these studies demonstrate that executive functions could be impaired after pronounced alcohol use during adolescence, thereby eliciting brain and behavioural changes that last into adulthood.

Memory and learning

Memory and learning are of great importance during development, especially in adolescence that covers a time-window with educational challenges and job opportunities. The hippocampus plays pivotal roles in learning, long-term memory, and spatial navigation. Moreover, the hippocampus progressively develops and matures during adolescence. Therefore, the adolescent hippocampus is highly vulnerable and alcohol-induced damage in the adolescent hippocampus may likely cause long-term deficits in above-mentioned neurocognitive skills.

An *in vitro* study revealed that hippocampal slices from 30-day-old adolescent rats were much more sensitive to ethanol-induced disruption of hippocampal NMDA receptor-mediated LTP than slices from 90-day-old adult rats (Pyapali *et al.*, 1999). Since LTP is considered to be the major cellular mechanism underlying – or at least reflecting – learning and memory processes, the finding of this *in vitro* study would suggest greater learning and memory impairments in adolescents than in adults after *in vivo* alcohol use. Indeed, in the Morris water maze task – a widely used task that requires subjects to learn the spatial location of a hidden platform located in a pool of water – acquisition of spatial memory was impaired by alcohol in adolescent rats but not in adult rats (Markwiese *et al.*, 1998). Furthermore, after acute alcohol exposure in a group of light drinking people, late adolescents (aged 21-24 years) showed more alcohol-induced impairments in memory acquisition on both semantic and figural memory tasks than early adults (aged 25-29 years) (Acheson *et al.*, 1998). In addition, 30-day-old adolescent and 60-day-old adult male rats were trained in a Morris water maze for 5 days (Sircar and Sircar, 2005). Each test day, they were intraperitoneally injected with 2 g/kg ethanol solution or saline 30 minutes before testing. Compared with saline-treated control rats, ethanol-treated rats required more time and swam greater distances to reach the hidden platform. In adult rats, however, ethanol negatively affected swim speed and performance on a visual cued task, which would probably explain the longer latencies and swim distances. Indeed, a probe trial verified that spatial learning in the ethanol-treated adult rats was intact, thus excluding the involvement of alcohol-induced spatial memory deficits in performing the Morris water maze task. In contrast, swim speeds and visual task performance of ethanol-treated adolescent rats did not differ from saline-treated rats, indicating that the poorer performance of ethanol-treated adolescent rats on the Morris water maze was not due to motoric or visual impairments but to alcohol-induced spatial memory deficits. In short, this study again suggests that adolescents are more vulnerable to memory deficits after acute alcohol exposure compared with adults.

More importantly, however, is that besides higher sensitivity to acute alcohol-induced deficits, adolescents are more vulnerable to the long-lasting effects of alcohol use. Sircar and Sircar (2005), in the previously mentioned study, investigated long-term effects of alcohol use in both adolescent and adult rats after the last acute alcohol exposure. Rats did not get alcohol or saline injections anymore but were retested on test day 9, 12, and 30 to determine how soon they recovered from their cognitive dysfunction. Thus, the adolescent rats were retested when they were 39, 42, and 65 (so meanwhile adult) days of age and the adult rats

Table 3. Mean time (± SEM), expressed in seconds, required for adolescent and adult rats to find the hidden platform of the Morris water maze task after alcohol-free periods of 4, 7, and 25 days (tested on test day 9, 12, and 30, respectively). On all test days, ethanol-treated adolescent rats required more time to find the hidden platform compared with same-day saline-treated adolescent control rats. In adult rats, ethanol-treated and saline-treated rats showed similar latencies. ***$p < 0.001$, **$p < 0.005$, *$p < 0.05$ compared with saline-treated rats. Adapted from Sircar and Sircar (2005).

	Test day 9		**Test day 12**		**Test day 30**	
	Adolescent	Adult	Adolescent	Adult	Adolescent	Adult
SALINE-TREATED	14.17 ± 0.64	23.72 ± 2.85	14.00 ± 1.20	17.52 ± 2.83	10.98 ± 1.73	13.20 ± 1.52
ETHANOL-TREATED	29.28 ± 1.49***	24.83 ± 2.93	21.83 ± 1.73**	13.66 ± 1.48	17.67 ± 1.62*	17.12 ± 1.88

were retested at the age of 69, 72, and 90 days. On all retest days, ethanol-treated adolescent rats required more time (Table 3) and swam greater distances (Fig. 2) to reach the hidden platform than saline-treated adolescent rats, but this time no differences were found anymore between ethanol-treated and saline-treated adults for these variables. Although ethanol-treated adolescent rats showed a significant decline in both latencies and travelling distance between test day 9 and 12, indicating the ability to learn the task, the differences with adolescent control rats that were still present at test day 30 (whilst rats have become adult) for both variables demonstrate long-term spatial memory deficits as a consequence of adolescent alcohol use. In contrast, chronic intermittent alcohol exposure during adolescence did not affect Morris water maze performance in adult rats (Silvers *et al.*, 2003; Silvers *et al.*, 2006), indicating the sensitivity of different alcohol administration paradigms. However, several other studies did report long-lasting memory effects of alcohol use during

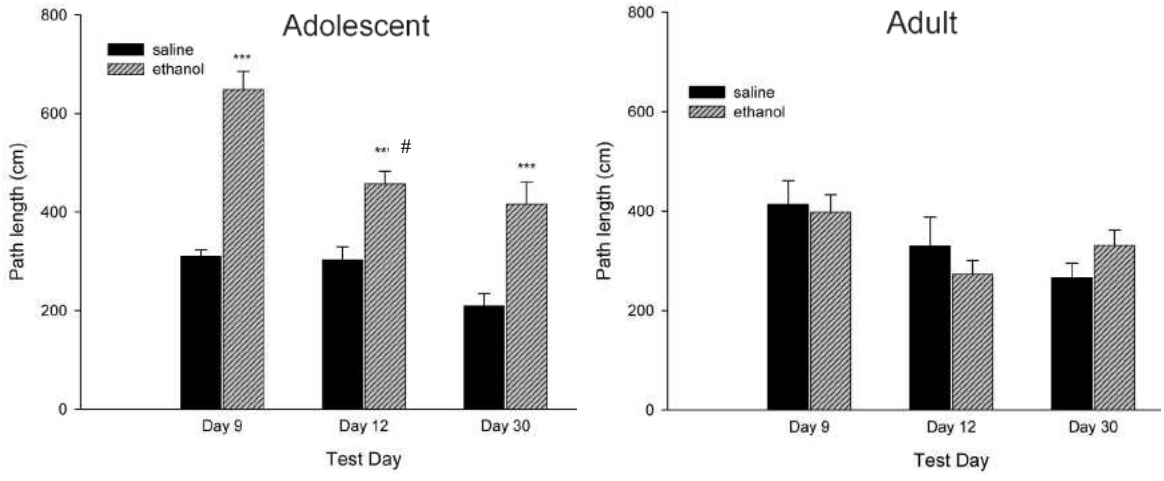

Figure 2. Mean (± SEM) distance travelled to find the hidden platform in the Morris water maze by adolescent (left) and adult (right) rats after alcohol-free periods of 4, 7, and 25 days (tested on test day 9, 12, and 30, respectively). On all test days, ethanol-treated adolescent rats travelled greater distances to find the hidden platform compared with same-day saline-treated adolescent control rats. In adult rats, ethanol-treated and saline-treated rats travelled similar distances to find the platform. ***$p < 0.001$ compared with same-day saline-treated rats; $^{\#}p < 0.005$ compared with day 9 ethanol-treated rats. Adapted from Sircar and Sircar (2005).

adolescence. For example, acute alcohol exposure before a spatial task in adult rats who had been exposed to alcohol binges during adolescence caused a larger decline in spatial working memory performance than in adult rats without a history of adolescent binge drinking exposure to alcohol (White *et al.*, 2000). Furthermore, alcohol use has also been linked to impairments in trace conditioning (Yttri *et al.*, 2004), a form of associative learning. Results from hippocampal-lesioned rats and human fMRI studies indicate that trace conditioning is, in contrast to another form of associative learning called delay conditioning, dependent on the hippocampus (Knight *et al.*, 2004; McEchron *et al.*, 1998). Male and female adolescent rats were administered 0, 1.5, 2.5, or 4.5 g/kg ethanol by acute intragastric gavage at day 28, 30, 32, and 34 after birth (Yttri *et al.*, 2004). Six days after the last alcohol exposure, i.e. at day 40, half of the group was given a delayed condition. In this procedure rats were given 5 pairings of a flashing light of 10 seconds in duration (CS; conditioned stimulus) immediately followed by a 0.5 mA foot shock of 1 second in duration (US; unconditioned stimulus). The other half of the group was also given 5 CS-US pairings, but the US was presented 10 seconds after CS offset – the trace conditioning procedure. 24 hours after the rats were given the conditioning sessions, the subjects were tested for CS-induced freezing behaviour. Freezing is defined as no observable movements except those necessary for respiration. Alcohol-exposed adolescents showed reduced freezing to the CS in trace conditioning, particularly for doses of 2.5 and 4.5 g/kg ethanol, but not in delay conditioning (Fig. 3),

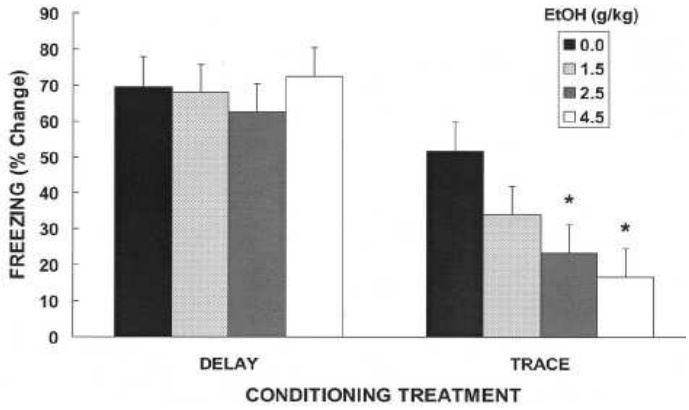

Figure 3. Mean (± SEM) CS-induced freezing behaviour of 41-day-old adolescent rats, recorded 24 hours after delay (left) or trace (right) conditioning. Rats were previously administered 0.0, 1.5, 2.5 or 4.5 g/kg ethanol when they were 28, 30, 32 and 34 days old. The amount of CS-induced freezing remains stable following delay conditioning but is lower following trace conditioning. *$p < 0.05$ compared with 0.0 g/kg ethanol-treated rats. Adapted from Yttri *et al.* (2004).

indicating that CS and US can be easily detected and intermittent alcohol exposure during adolescence results in impaired hippocampal-dependent trace conditioning that may persist. Another type of associative learning was studied in rats performing a conditional discrimination learning task (Pascual *et al.*, 2007). First, rats were intraperitoneally injected with 3 g/kg ethanol at days 25, 26, 29, 30, 33, 34, 37 and 38 after birth to simulate a binge-like alcohol exposure. Control rats were injected with saline in the same way. Then, conditional discrimination learning was tested at 39 days or 60 days of age in a Y-maze with three equally sized arms by 10 trials per day during six sessions. One arm was considered the arm where the rat started a trial, the other two were considered the choice arms. Both choice arms were completely covered with white or black inserts. Rats were rewarded for choosing the left arm when inserts were black or choosing the right arm when inserts were white. Each choice arm had a food cup located at the end: a correct response was rewarded with four food pellets in the food cup of the correct arm, while the incorrect arm contained an empty food

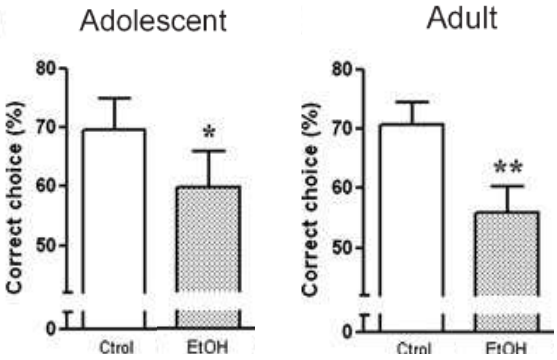

Figure 4. Effects of binge-like alcohol exposure during adolescence on a conditional discrimination learning task, showed as mean (± SEM) percentage of the correct choice in control saline (Ctrol) and 3 g/kg ethanol (EtOH) injected rats. 39-day-old adolescent rats (left) were tested 24 hours after the last injection; 60-day-old adult rats (right) were tested 3 weeks after the last injection. Compared to saline-treated rats, both ethanol-treated age groups show a significant reduction in the percentage of correct choices. $*p < 0.05$, $**p < 0.01$ compared with saline-treated (Ctrol) rats. Adapted from Pascual *et al.* (2007).

cup. Binge drinking exposure elicited significant decrease in the percentage of correct choices in adolescent rats (Fig. 4). Moreover, this effect was also present in adulthood (Fig. 4), suggesting that alcohol causes long-term deficits in the ability to learn conditional discrimination. In addition, these 39-day-old and 60-day-old rats performed an object recognition test. Rats were allowed to explore an empty testing box for two minutes. The next day rats were place in the middle of the same box that then contained two square yellow plastic blocks. Following 3 minutes training (T1) and a 1-minute interval, rats were reintroduced to the box for 3 minutes testing (T2) with objects being replaced by one square yellow plastic block (familiar object) and a round pink block (non-familiar object). Object exploration was defined as the animal orienting its snout towards the object. The difference in exploration time between the two objects in T2 was considered as an index measure of discrimination (d1) between both the new and familiar objects. No significant differences were found between ethanol-treated rats and saline-treated rats in total exploration time of both objects during T1 and T2 (Table 4). However, both adolescent and adult ethanol-treated rats showed a reduction in the discrimination index d1, indicating impaired discrimination between novel and familiar objects, and suggesting that alcohol exposure during adolescence causes long-lasting deficits in object recognition. Interestingly, both conditional discrimination learning and object recognition are sensitive to hippocampal lesions (Ennaceur and Aggleton, 1997; Murray and Ridley, 1999), suggesting a link between

Table 4. Effects of alcohol exposure on a object recognition task. Results are shown as mean (± SEM) time in seconds. Exploration time e1 and e2 is defined as the total exploration time of both objects during T1 and T2, respectively. Discrimination index d1, an index measure of discrimination between both the new and familiar objects, is measured as the difference in exploration time between the two objects in T2. e1 and e2 in ethanol-treated rats are not significantly different from saline-treated rats in both the 39-day- and 60-day-old group. In both these age groups, d1 is reduced in ethanol-treated rats compared with saline-treated rats. $*p < 0.05$, $**p < 0.01$ compared with saline-treated rats. Adapted from Pascual *et al.* (2007).

	39-day-old adolescents			**60-day-old adults**		
	Exploration time e1	Exploration time e2	Discrimination index d1	Exploration time e1	Exploration time e2	Discrimination index d1
SALINE-TREATED	23.75 ± 4.47	19.25 ± 6.98	8.00 ± 2.51	22.25 ± 3.35	18.06 ± 3.04	10.19 ± 1.43
ETHANOL-TREATED	15.11 ± 1.44	7.89 ± 2.00	1.22 ± 0.79*	12.44 ± 2.27	9.28 ± 2.49	2.06 ± 1.89**

impaired performance of these tasks and alcohol-induced hippocampal damage. Taken together, the studies from Sircar and Sircar (2005), White *et al.* (2000), Yttri *et al.* (2004), and Pascual *et al.* (2007) all reported long-term memory and learning deficits that could be due to underlying alcohol-induced hippocampal damage. In support of this idea, binge-like exposure to ethanol in adolescent rats, by exposure to ethanol vapour for 10 days (i.e. from day 30 to day 40 after birth) for 12 hours per day, significantly altered hippocampal event-related potentials (ERPs) in the long-term (Slawecki *et al.*, 2001). ERPs were measured after an alcohol-free period of 49 days, so when rats were 89 days old. Decreases in P2 and P3 hippocampal ERPs in alcohol-exposed rats compared with controls (Fig. 5) may indicate long-lasting alcohol-induced learning and memory deficits (Begleiter *et al.*, 1983; Beracochea *et al.*, 1992; Slawecki *et al.*, 2001).

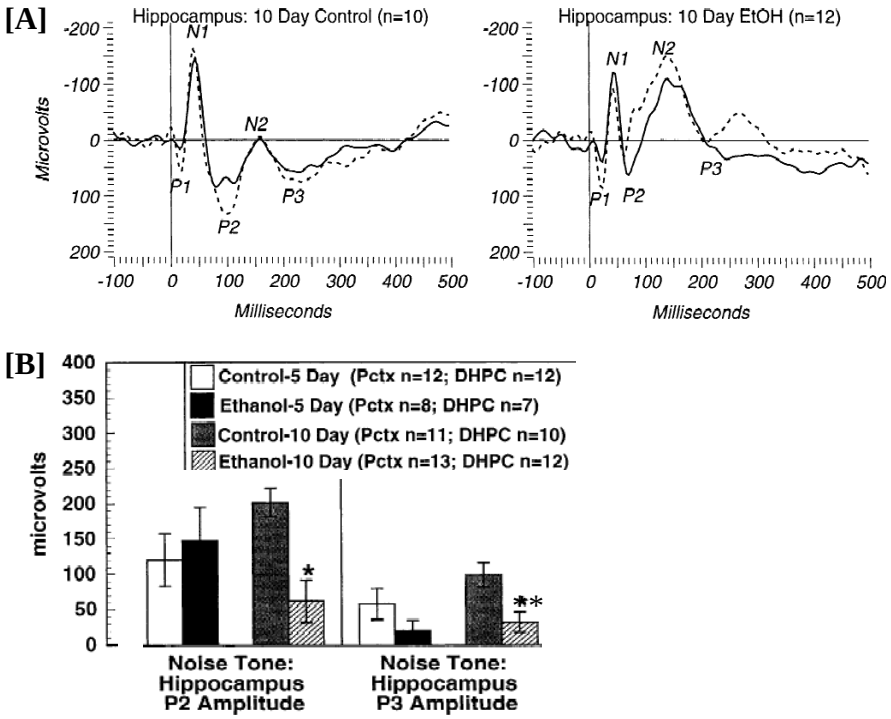

Figure 5. ERP results following auditory stimuli, 49 days after the last alcohol exposure. **[A]** ERP grand averages from the hippocampus of air-exposed control rats (left) and ethanol vapour-exposed rats for 10 days (right). Dashed lines represent the response to a noise tone, solid lines represent the response to a rare tone. **[B]** Bars represent mean (± SEM) hippocampal P2 and P3 amplitudes in rats exposed to ethanol vapour or air (control) for 5 or 10 days. **p* = 0.001, ***p* = 0.005 compared with controls. Adapted from Slawecki *et al.* (2001).

In addition to alcohol-induced hippocampal-dependent learning deficits, amygdala-dependent learning deficits in adulthood after adolescent alcohol exposure are reported as well. 28-day-old adolescent and 80-day-old adult rats had restricted access to 10% ethanol or only water (control group) for 18 days before they performed an auditory fear conditioning task – a delay conditioning paradigm (Bergstrom *et al.*, 2006). In contrast to the delay conditioning paradigm of Yttri *et al.* (2004), Bergstrom and colleagues inserted a long abstinence period of 30 days after the last alcohol exposure to minimize possible withdrawal effects. After the abstinence period, rats had a short conditioning training with three presentations of a 80 dB tone of 20 seconds in duration (CS) immediately followed by a 0.5 mA scrambled foot shock of 2 seconds in duration (US). Two days later, the 77-day-old adult rats of the adolescent group and the 129-day-old adult rats of the adult group were tested for retention of auditory fear conditioning by measuring freezing behaviour after three presentations of the CS alone. The alcohol-exposed rats of the adolescent group showed reduced freezing behaviour

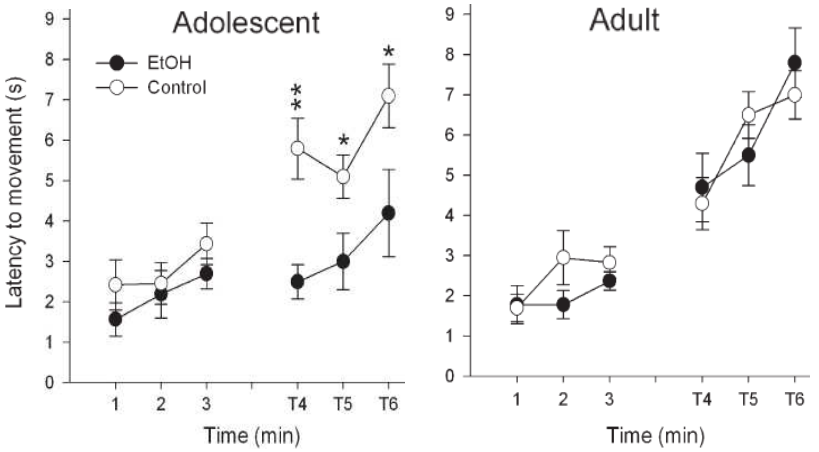

Figure 6. Mean (± SEM) freezing time of adult male rats when exposed to the test chamber (minute 1, 2 and 3) and after tone trials (minute T4, T5 and T6). Prior exposure to alcohol when adolescent (left) decreased freezing to tone compared to controls. Adult groups (right) did not show differences in freezing. $^{**}p < 0.05$, $^{*}p < 0.01$ compared with alcohol-exposed rats. Adapted from Bergstrom *et al.* (2006).

compared with adolescent controls and compared with all rats of the adult group, who did not show any alcohol-dependent differences (Fig. 6), indicating impaired fear learning in adulthood after adolescent alcohol exposure. This may be caused by alcohol-induced damage of the amygdala during adolescence considering that lesions in the amygdala impair acquisition and consolidation of auditory fear conditioning (Blair *et al.*, 2001).

Besides behavioural rat studies, some human imaging studies are also used in investigating the effects of alcohol on the adolescent brain in learning and memory tasks. Longitudinal imaging studies are not yet reported, but a few cross-sectional studies are

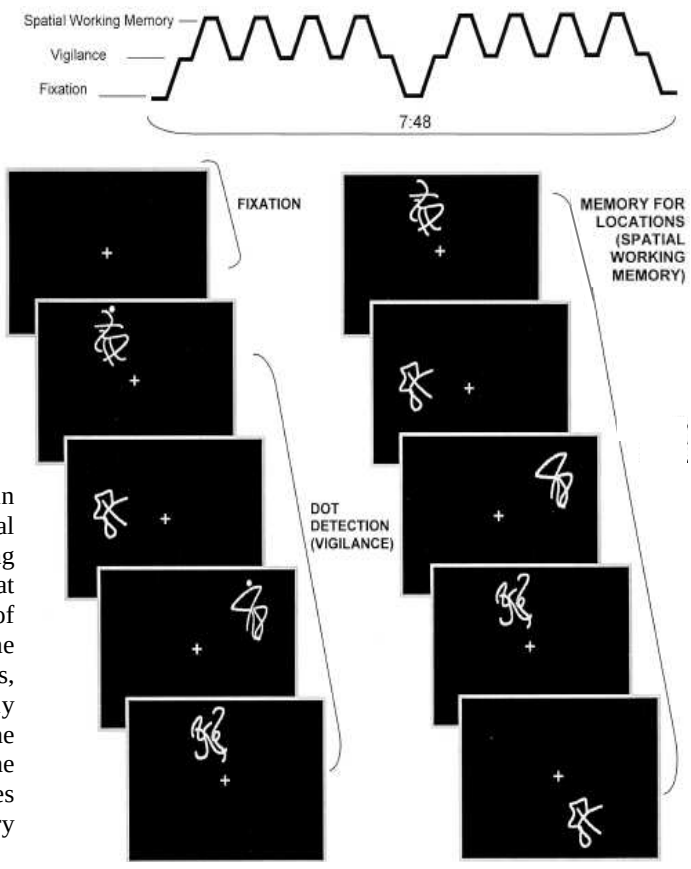

Figure 7. This spatial working memory task consisted of 18 blocks of 20 seconds in duration that altered between experimental (spatial working memory) and baseline (vigilance) conditions for a total time of 7 minutes and 48 seconds. In the beginning, middle, and end of the task a resting block (fixation) was situated, during which a fixation cross appeared on the centre of a screen that was situated at the foot of the MRI machine. In the spatial working memory condition, figures were presented one at a time for 1000 milliseconds in one of eight locations. Subjects were asked to press a button when a figure appeared on a location where another figure had previously appeared within that block. The implementation of verbal labelling strategies was minimized by using abstract line drawings (Kimura figures) that were not presented at the four cardinal points of a compass. In the vigilance condition, the same figures were presented in the same locations, but subjects were asked to press the button only if they saw a figure with a dot. In this way, the vigilance condition provided a control for the simple motor and visual attention processes involved in the spatial working memory condition. Adapted from Tapert *et al.* (2001).

indicative for long-term effects of alcohol use during adolescence and provide insight in where differences between alcohol (ab)using adolescents and light or non-drinking controls are located in the brain. Tapert and colleagues performed two such cross-sectional studies by measuring the BOLD response during a spatial working memory task in an MRI machine (Tapert *et al.*, 2001; Tapert *et al.*, 2004b) (see Fig. 7 on page 17 for task description). In one of their studies, functional brain activity in 15- to 17-year-old adolescents who met AUD criteria for one to two years differed from that of light drinking age- and gender-matched controls (Tapert *et al.*, 2004b). The adolescents with AUDs showed decreased activation in the left precentral gyrus, bilateral occipital and cerebellar regions, but increased activation in bilateral parietal regions, compared with light drinkers (Fig. 8A). In addition, those with greater alcohol consumption showed greater abnormalities. Despite the functional brain differences, the behavioural performance of the spatial working memory task was similar in the AUD adolescents and light drinking adolescents, suggesting that the more active brain regions of the AUD adolescents compensate for the lesser functioning brain regions during the spatial working memory task. However, in another study, adolescent women of 18 to 25 years old with alcohol dependence for 4 to 5 years for which they had received treatment performed the same spatial working memory task worse than light drinking age- and gender-matched controls (Tapert *et al.*, 2001). Moreover, their functional brain activity differed from that of the 15- to 17-year-old AUD adolescents: the 18- to 25-year-old alcohol-dependent adolescents only showed decreased activation in parietal and frontal regions, particularly in the right hemisphere, compared with light drinkers (Fig. 8B). The mean age of onset of AUD or alcohol dependence in both studies was 15 years of age, ruling out this factor to be related to the differences of functional brain activity between the chronic heavy drinking adolescent

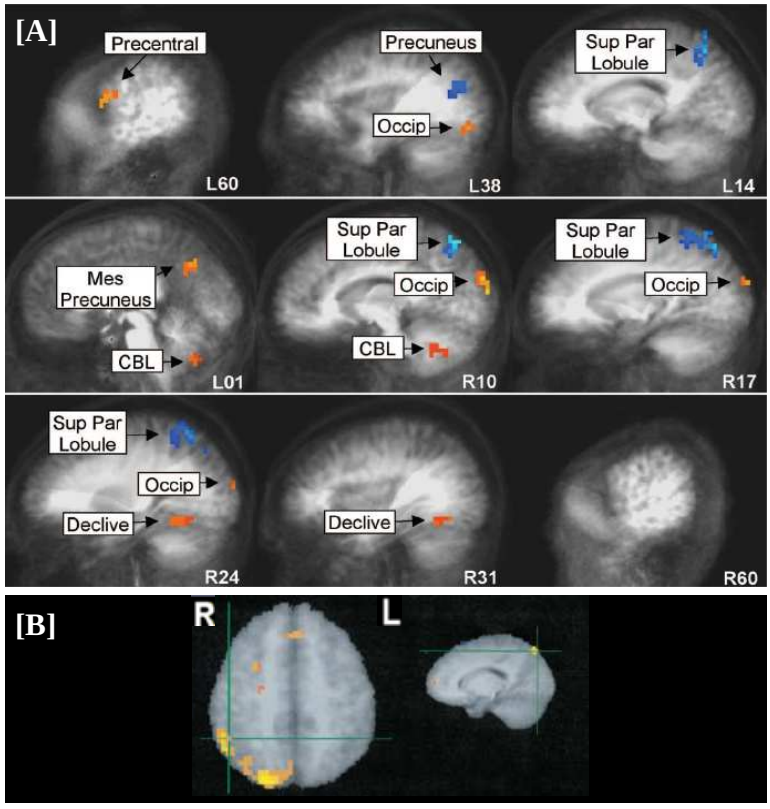

Figure 8. Between-group BOLD response differences during a spatial working memory task in human adolescents. Yellow and orange clusters represent brain regions where adolescents with AUD showed less response than light drinking controls; blue indicates where adolescents with AUD showed more response than light drinking controls ($p < 0.05$). **[A]** Results from AUD adolescents and light drinking controls of 15-17 years old. Numbers refer to sagittal slice positions. Occip, occipital; Sup Par, superior parietal; Mes, mesial; CBL, cerebellum. Adapted from Tapert *et al.* (2004b). **[B]** Results from AUD women and light drinking controls of 18-25 years old. Green lines to assess locations of the horizontal and saggittal slice. Side of the brain: R, right; L, left. Adapted from Tapert *et al.* (2001).

groups of both studies. However, clear differences between the two studies were found in gender ratio (Tapert *et al.*, 2004b: 67% men and 33% women; Tapert *et al.*, 2001: only women), mean age of subjects (in 2004: 16.77 years; in 2001: 19.6 years), and the mean duration of their AUD or alcohol dependence (in 2004: 1-2 years; in 2001: 4-5 years). When the cause of the differences of functional brain activity between the chronic heavy drinking adolescent groups of both studies lies in the mean age of subjects or the mean duration of the adolescent AUD or alcohol dependence (Brown and Tapert, 2004; Tapert and Schweinsburg, 2005), this could be explained as follows. Young heavy drinking adolescents or adolescents in the early stages of AUD may show alcohol-induced brain reorganisations that include compensating brain changes without interfering spatial working memory performance. Adolescents who initiate heavy drinking in a later stage of their brain maturation or adolescents in later stages of AUD regardless of their age may be less able to compensate for alcohol-induced neural damage which then will impair behavioural functioning. Further research, preferable longitudinal studies, should elucidate which of the three mentioned factors (gender ratio, mean age, and mean duration of heavy drinking) together or alone could explain the findings of the studies of Tapert and colleagues, and if subtle brain changes are already visible in alcohol using adolescents without AUDs. Up to now, one imaging study did investigate possible functional brain changes between alcohol using adolescents without AUDs and non-drinking controls in a novel word pairing encoding task (Schweinsburg *et al.*, 2010). Alcohol using subjects were 16-18 years old, had been drinking for an average of 3 years, and had been abstinent for an average of 33 days at the time of scanning. These subjects were characterized as binge drinkers, but did not met criteria for heavy drinking. Control subjects were demographically similar non-drinkers. First, all subjects were trained on a verbal learning task. They had to memorize 16 highly associated pairs of monosyllabic nouns that were presented for 5 second each. Training stopped when they had learned to recall 10 of the 16 pairs by verbalizing the second member of a pair when the first member of that pair was presented. Only 1 of 12 non-drinkers, but 5 of 12 binge drinkers did not adequately recall 10 of the 16 pairs on the first training session yet, indicating a trend that binge drinkers have poorer verbal learning performance. After training was performed on criterion, fMRI scanning was started and subjects were presented with 32 pairs of associated words for 5 seconds each. These 32 pairs of associated words included word pairs of their training session (repeated word pair encoding condition) and novel word pairs (novel word pair encoding condition). For both the repeated and novel word pair encoding condition, subjects were asked to learn the word pairs. During scanning, binge drinkers recalled 7% fewer word pairs in the repeated word pair encoding condition

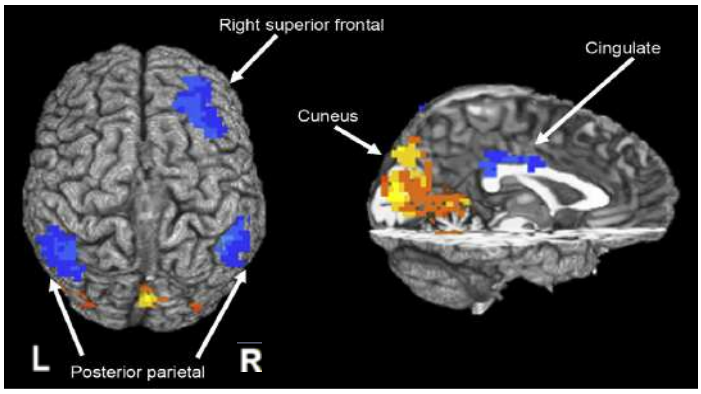

Figure 9. Differences in BOLD response between human adolescent binge drinkers and non-drinkers of both 16-18 years old during a novel word pair encoding condition of a verbal learning task. Orange and yellow indicate where binge drinkers showed less response than non-drinkers; blue clusters represent regions where binge drinkers showed more response than non-drinkers ($p < 0.05$). Side of the brain: L, left; R, right. Adapted from Schweinsburg *et al.* (2010).

correctly, compared with non-drinkers. Again, although not significant, this result may be indicative of a poorer verbal learning performance in binge drinkers. In addition to the subtle differences in verbal learning performance, significant differences in BOLD response were found between binge drinkers and controls during the novel word pair encoding condition. While encoding new word pairs, binge drinkers demonstrated significantly less brain activation than controls in a large posterior cluster that included bilateral cuneus, lingual gyrus and parahippocampal gyrus, as well as the right medial precuneus (Fig. 9, page 19). A similar pattern was observed in the adolescents with AUD during a spatial working memory task (Tapert *et al.*, 2004b) (Fig. 8A, page 18), indicating at least less involvement of visual processing when learning verbal and spatial information. Furthermore, binge drinkers exhibited significantly more brain activation than controls in the right superior frontal gyrus, left superior and inferior parietal lobule, right inferior parietal lobule, and bilateral cingulate gyrus (Fig. 9, page 19). A trend of reduced activity in the right hippocampus, together with the significant patterns of decreased parahippocampal gyrus and increased right frontoparietal response among binge drinkers during novel word encoding suggests an alcohol-induced compensating shift in brain processing to more frontal working memory use and less hippocampal memory consolidation. Although the study had a low power due to a small sample size, the preliminary results nevertheless demonstrate that a relatively short and problem-free period of adolescent binge drinking could alter brain functioning during verbal encoding and may impair verbal learning in the long-term.

In summary, behavioural rat studies show that alcohol use could cause hippocampal-dependent and amygdala-dependent learning and memory deficits that are long-lasting. In addition, cross-sectional human imaging studies demonstrate that binge drinking as well as chronic heavy drinking during adolescence causes significant differences in brain functioning during learning and memory tasks as compared with light drinking or non-drinking adolescents, which at an early age or in an early drinking stage may include compensating brain processes but ultimately may lead to impaired memory and learning abilities after years of severe alcohol use.

Cue reactivity

Cue reactivity in alcohol using people comprises physiological, cognitive and neural responses to alcohol-related stimuli that differ from those of non-drinkers. Cue reactivity is highly pronounced in alcohol-dependents but has also been reported for light drinkers. The first study on cue reactivity in adolescents also showed neurocognitive responses to alcoholic beverage pictures among 14- to 17-year-old adolescents who met AUD criteria for 1 to 2 years (Tapert *et al.*, 2003a). During fMRI scanning, subjects were presented 20 alcoholic and 20 non-alcoholic beverage pictures from advertisements, matched by colour, visual complexity, and presence of people. Each subject was presented a personalized set of images, based on his or her preferences and experiences with the beverages, to ensure familiarity with the stimuli. AUD adolescents showed greater brain activation (as measured by BOLD response) to alcoholic beverage pictures than demographically similar light drinkers in several regions throughout the brain, including the prefrontal area, nucleus accumbens, hypothalamus, posterior cingulate, and left temporal lobe (Fig. 10A, page 21). In addition, adolescents of 18 to 25 years old also exhibit cue reactivity to alcohol-related words, for example by increased activation in the nucleus accumbens (Tapert *et al.*, 2004a). Moreover, alcoholic picture-induced activation in the right posterior cingulate and precuneus limbic

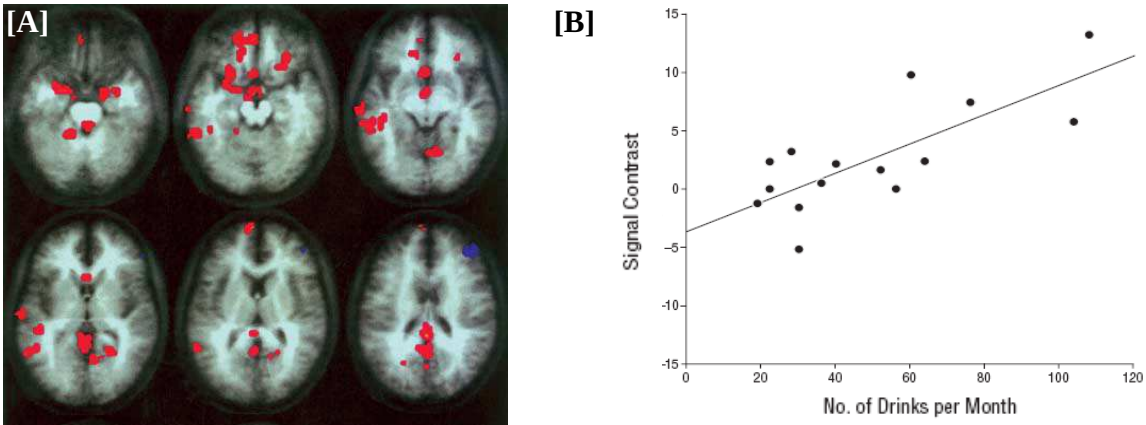

Figure 10. FMRI results of 14- to 17-year-old adolescents during exposure to alcoholic beverage pictures relative to non-alcoholic beverage pictures. **[A]** Red and yellow clusters represent brain regions where AUD adolescents showed more response than light drinkers; blue indicate the right middle and inferior frontal gyrus where AUD adolescents showed less response than light drinkers ($p < 0.05$). Side of the brain: L, left; R, right. **[B]** BOLD response signal contrast in the right posterior cingulate and precuneus limbic region as a function of drinks consumed per month for AUD adolescents ($p < 0.001$). Adapted from Tapert *et al.* (2003a).

region was correlated with more frequent drinking (Tapert *et al.*, 2003a) (Fig. 10B).

The reported high cue reactivity in reward, desire, positive emotion, and episodic recall brain areas of drinking adolescents suggests that alcohol-related stimuli have been conditioned with drinking experiences. More important, liking and remembering alcoholic beverage pictures and advertisements are associated with more frequent drinking among adolescents (Connolly *et al.*, 1994; Pulido *et al.*, 2009). Together, these findings suggest that alcohol-related stimuli trigger several brain areas that influence the pattern of adolescent alcohol use in a negative way. The long-term effects of actual adolescent alcohol consumption will be discussed in the next section that mentions aspects of alcohol abuse and dependence during the vulnerable time window of adolescent brain development.

2.3 Alcohol abuse and addiction

Long-lasting alcohol-induced neurocognitive changes could arise during adolescence, but neurobiological changes in brain addiction systems may occur as well. For instance, adolescents with AUDs and patients with Korsakov's Syndrome – of which a growing number of relative young Dutch adult cases are reported the past years – can suffer from neurocognitive deficits as a consequence of alcohol abuse or addiction. This section first discusses the effects of adolescent alcohol use on the risk for later alcohol abuse and addiction. Then, an overview of the key neurobiological brain system involved in alcohol addiction – the mesocorticolimbic dopamine system – is given. This section will end with a review on long-lasting behavioural and neurobiological alterations as a consequence of alcohol-induced changes in the mesocorticolimbic dopamine system during adolescence.

Adolescent-specific vulnerability to alcoholism

During the past decennium, on average 60% of young Dutch adolescents already had consumed their first alcoholic drink at 12 years of age, an increase compared to 1992 in which less than 40% of 12-year-olds had this experience (Van Laar *et al.*, 2010). Furthermore, the number of actual drinkers among 12- to 18-year-old adolescents is high with more than half of this age range consuming alcohol, often frequent (e.g. heavy drinking) and in high quantities (e.g. binge drinking). Thus, the initiation and continuation of alcohol use among Dutch adolescents is at an early age. In addition, as stated earlier, the number of 15- to 19-year-old adolescents with a primary alcohol problem in Dutch addiction care centres increased with 85% between 2002 and 2008. Does the early initiation and specific continuation of alcohol use during adolescence make adolescents highly vulnerable to the development of alcoholism?

Indeed, the above-mentioned facts are particularly alarming, since many prospective and retrospective human studies have reported an association of early onset of alcohol use with a higher prevalence of developing problem drinking in adolescence (Ellickson *et al.*, 2003; Fergusson *et al.*, 1994; Friedman and Humphrey, 1985; Gruber *et al.*, 1996; Hawkins *et al.*, 1997; Pedersen and Skrondal, 1998) as well as later alcohol abuse or alcohol dependence (Chou and Pickering, 1992; DeWit *et al.*, 2000; Deykin *et al.*, 1987; Grant *et al.*, 2001; Grant and Dawson, 1997; Pitkanen *et al.*, 2005; Robins and Przybeck, 1985; Warner and White, 2003; York *et al.*, 2004). For example, for those aged 18 years or younger at first use in the USA, the prevalence of lifetime alcohol *abuse* was about 8%, which is nearly double the risk for alcohol abuse for individuals that initiated alcohol use at the age of 21 years (4.8%) (Grant and Dawson, 1997). Moreover, the prevalence of lifetime alcohol *dependence* for those who initiated alcohol use at 18 years was about 17%, while individuals who initiated alcohol use at the age of 12 years or younger had an almost 2.5 times higher risk for alcohol dependence (40.6%). Although minimum age restrictions for alcohol consumption are more conservative in the USA (i.e. alcohol use under the age of 21 is not allowed) compared to Europe, the incidence and morbidity associated with alcohol use disorders in adolescents are similarly high for these two areas (Chambers *et al.*, 2003). At least one study did not find an association between age at first alcohol use and alcohol abuse or dependence at either 20 or 30 years of age (Labouvie *et al.*, 1997). However, the reliability of the retrospectively recalled ages of onset of use in this study is questionable, especially for the older aged participants. Vulnerability to alcoholism among human adolescents was further studied by an antisaccadic eye test performed during an fMRI scan (McNamee *et al.*, 2008). In this way, response inhibition in 25 adolescents without AUDs could be measured. A high neurobehavioral disinhibition score, which is a measure for risk for AUD and other substance use disorders (SUDs), was associated with less activation in frontal regions in response to the antisaccadic eye test. This suggests that adolescents with impaired executive processes, e.g. as a consequence of alcohol use (see paragraph 'Attention and decision making' of section 2.2), have a high vulnerability for risk for AUD (or other SUDs).

Early onset of alcohol use in humans may rather be serving as a marker, than being a causal precursor for later alcohol problems. Rodent studies may solve this apparent chicken-and-egg problem. Rodents show a similar drinking pattern during adolescence as humans, with adolescents consuming more alcohol relative to adult animals (Truxell *et al.*, 2007). However, adult drinking patterns after different onsets of adolescent alcohol exposure are not yet studied in animals. Nevertheless, rodent studies did examine the question *if* alcohol

exposure during adolescence has an effect on the extent of alcohol intake in adulthood. Concerning the vulnerability of adolescence brain development and the former results from human studies, one should expect that rodents show an increase in adult alcohol use after adolescent alcohol exposure. However, the results of rodent studies are not conclusive. Three rat studies did not find an increase in adult alcohol consumption after alcohol exposure during adolescence. In these studies, rats were presented alcohol via forced exposure in an alcohol vapour paradigm (Slawecki and Betancourt, 2002) or via constant voluntary access in a two-bottle (one with water, one with ethanol) choice paradigm (Tolliver and Samson, 1991; Vetter *et al.*, 2007). In contrast, two mouse studies did find increased alcohol intake in adulthood after constant voluntary access in a two-bottle paradigm during adolescence (Ho *et al.*, 1989; Yashimoto, 1988). In addition, rats who underwent a binge-like alcohol exposure during adolescence did also show increases in adult alcohol preferences and intake (Maldonado-Devincci *et al.*, 2010a; Pascual *et al.*, 2009), indicating that alcohol exposure itself is capable of causing a long-term addictive-like effect. Species differences in alcohol sensitivity in the studies with the two-bottle choice paradigm may explain the different outcomes of these studies. Further research is needed to elucidate the value of this paradigm for human relevance. Given the results of the binge-like exposure paradigm in rats, we may at least speculate that binge-like alcohol exposure in human adolescents may be a risk for later alcohol abuse or addiction.

In summary, human and rodent studies indicate that early onset of alcohol use and binge-like continuation of alcohol use may be risk factors for later alcohol abuse or addiction. The underlying neurobiological system that may be disturbed by alcohol during adolescence is the mesocorticolimbic dopamine system. Before studies concerning adolescent alcohol use effects on this brain system are discussed, the system's maturation and its contribution to alcohol abuse and addiction will first be elucidated in the next paragraph.

Neurobiology of alcoholism

The mesocorticolimbic dopamine system (further: mesocorticolimbic DA system) is widely accepted as the brain reward system. The mesocorticolimbic DA system is important for the rewarding and reinforcing effects of alcohol and other drugs of abuse and undergoes important remodelling during adolescence, thereby playing a critical role in the risk for alcohol abuse and addiction. The mesocorticolimbic DA system originates in the ventral tegmental area (VTA) which projects to the prefrontal cortex (PFC) as well as to the nucleus accumbens (NAcc) and other structures of the limbic system (Fig. 11). Ethanol is thought to increase the activity of dopaminergic neurons of the VTA by decreasing GABAergic transmission in the VTA (Pierce and Kumaresan, 2006). The subsequent increased release of dopamine (DA) in the NAcc is a key factor in the reward and reinforcing effects in alcohol

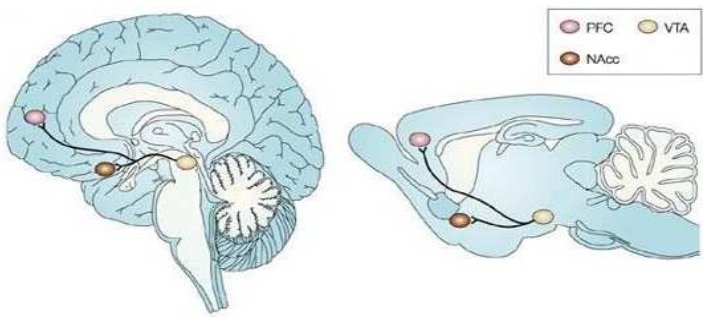

Figure 11. The mesocorticolimbic dopamine system in human (left) and rat (right) brains, showing the projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) and prefrontal cortex (PFC). Adapted from Laviolette and van der Kooy (2004).

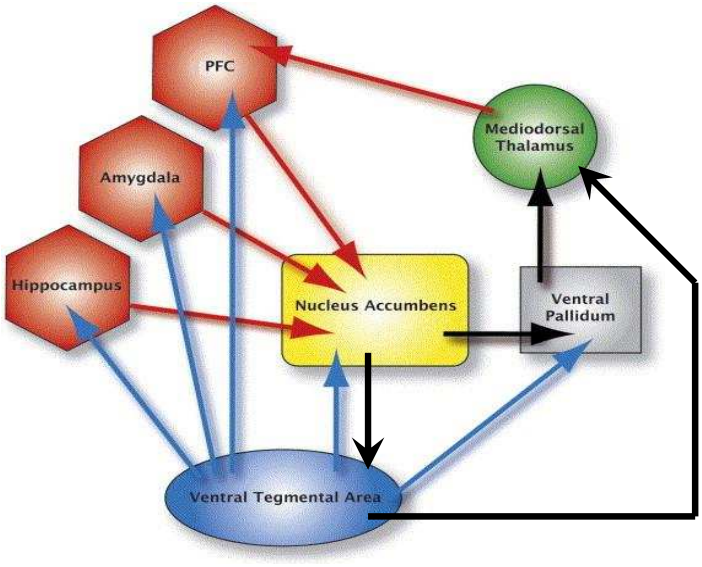

Figure 12. Schematic diagram showing major neuronal inter-connections of the mesocorticolimbic dopamine system. For example, the nucleus accumbens (NAcc) receives input from both the dopamine neurons of the ventral tegmental area and the glutamatergic neurons of the prefrontal cortex (PFC), amygdala and hippocampus. Main outputs of the NAcc include GABAergic projections to the ventral pallidum and ventral tegmental area. Blue arrows indicate dopaminergic pathways; red arrows indicate glutamatergic pathways; black arrows indicate GABAergic pathways. Adapted from Pierce and Kumaresan (2006).

addiction. Repeated alcohol use sensitizes the mesocorticolimbic DA system, so that alcohol-related or alcohol-associated stimuli also begin to release DA resulting in a further increase of alcohol use (see paragraph 'Cue reactivity' of section 2.2) that can lead to alcohol abuse or dependence.

The nucleus accumbens (NAcc) integrates sensory, affective, and contextual memory information by receiving input from both the DA neurons of the VTA and the glutamatergic neurons of the PFC, amygdala and hippocampus (Chambers *et al.*, 2003) (Fig. 12). It is thought that such actions of the NAcc are controlled by the PFC (Spear, 2000). Furthermore, the level of DA activity in the PFC is generally inversely related to DA release in the NAcc among other subcortical regions (Spear, 2000). In fact, a similar pattern in DA synthesis takes place in adolescence, during which the mesocorticolimbic DA system exhibits considerable reorganization. Levels of basal DA synthesis in rat PFC highly increase until early adolescence (i.e. 30 days after birth) before levels decline to lower levels in late adolescence (i.e. 40 days after birth) (Andersen *et al.*, 1997). In addition, young adolescent rats display a higher basal DA turnover in PFC relative to adults. Conversely, basal DA synthesis and turnover in rat NAcc is lower in early adolescence than in later stages (Andersen *et al.*, 1997; Maldonado-Devincci *et al.*, 2010b). DA D1, D2, and D4 receptors in rat frontal cortex, hippocampus, and entorhinal cortex, highly increase until 35 days after birth and then stabilize to adulthood, whereas overproduced DA receptors in rat NAcc undergo subsequent pruning during adolescence, suggesting maturational remodelling of the mesocorticolimbic DA system (Tarazi and Baldessarini, 2000). Furthermore, adolescent-associated increases in DA concentrations, DA transporters, and fibre densities are found in the PFC of rats and non-human primates (Spear, 2000). Finally, connectivity between PFC and NAcc, VTA, and amygdala continues to develop between adolescence and young adulthood as evidenced by increased densities of dopaminergic and glutamatergic projections (Witt, 2010). Thus, these data provide evidence for the continued development of the mesocorticolimbic DA system through adolescence.

The important remodelling of the mesocorticolimbic DA system during adolescence may contribute to the adolescent-specific vulnerability to alcohol addiction. Indeed, developmental and alcohol-induced changes of the mesocorticolimbic DA system have been

reported to play a major role in the risk *for* and *of* alcohol use. First, immaturity of the mesocorticolimbic DA system may contribute to the early initiation of alcohol use. The novelty seeking, sensation seeking, and/or reward seeking behaviour of adolescents that go with experimentation with alcohol use may be the consequence of either an overactive mesocorticolimbic DA system in which they experience enhanced reward from using alcohol (Chambers *et al.*, 2003; Cohen *et al.*, 2010) or an underactive mesocorticolimbic DA system in which they require alcohol to compensate for reward deficiency (Bjork *et al.*, 2004b; Spear, 2000). Second, the mesocorticolimbic DA system may be particularly vulnerable to disruptions by alcohol during adolescent brain development. The remodelling of DA neurotransmission throughout adolescence may play a critical role in stabilizing behaviours that are established during adolescence (Crews *et al.*, 2007). This process of brain maturation may be altered by alcohol use. Indeed, alcohol-induced changes in the mesocorticolimbic DA system during adolescence have been reported with long-lasting behavioural consequences. This will be discussed in next paragraph.

Effects of adolescent alcohol use on the mesocorticolimbic DA system

As previously mentioned, increased adult alcohol preferences and intake have been reported after binge-like alcohol exposure during rat adolescence (Maldonado-Devincci *et al.*, 2010a; Pascual *et al.*, 2009). In one of these studies, those long-term behavioural effects were associated with changes in the mesocorticolimbic DA system (Pascual *et al.*, 2009). For instance, reduced protein levels of the DA receptor subunit (DRD2) and phosphorylated glutamate receptor subunit (NMDAR2B) were found in alcohol-treated adolescents compared with alcohol-treated adults, indicating alterations in dopaminergic and glutamatergic neurotransmission. Furthermore, changes in acetylation of histones H3 and H4 were found in NAcc and PFC of alcohol-treated adolescents compared with alcohol-treated adults, suggesting chromatin remodelling changes in the mesocorticolimbic DA system. Moreover, ethanol elicited a similar prolonged DA response in alcohol-treated adolescents and alcohol-treated adults, but basal DA levels were higher (160%) in these adolescents than in the adults (Pascual *et al.*, 2009). Increased extracellular DA levels in alcohol-treated adolescents may persist into adulthood (Badanich *et al.*, 2007), which, together with the previous findings, suggests that alcohol-induced increases in extracellular DA levels during adolescence may mediate alcohol abuse or dependence on the long-term.

In addition to neurobiological studies, behavioural rat studies also show evidence of adolescent alcohol use-induced long-term consequences for alcohol abuse and dependence. For example, adolescent rats chronically exposed to alcohol during adolescence showed increased novelty seeking behaviour by exhibiting greater novel object exploration than saline-treated adolescent rats (Stansfield and Kirstein, 2007). Given that novelty seeking has been associated with increased drug intake (Bevins *et al.*, 1997), adolescents consuming high amounts of alcohol throughout adolescence may be at higher risk for greater alcohol intake in adulthood. Furthermore, a conditioned place preference (CPP) paradigm was used to evaluate alcohol-seeking behaviour (Maldonado, Badanich and Kirstein, unpublished data, in Maldonado-Devincci *et al.*, 2010b). First, rats were pretreated with chronic exposure to alcohol or saline during adolescence (i.e. from 30 to 50 days after birth). Then, after an abstinence period of 14 days, rats were conditioned with alcohol or saline in a two-chambered apparatus in young adulthood (i.e. from 65 to 70 days after birth). Adolescents that were pretreated with alcohol and conditioned with alcohol in young adulthood showed a

significantly greater amount of time spent in the alcohol-paired chamber than adolescents pretreated with saline and conditioned with alcohol in young adulthood. Thus, alcohol-seeking behaviour in adulthood is higher when rats were already exposed to alcohol during adolescence, indicating long-term behavioural consequences of adolescent alcohol use related to alcohol abuse and dependence.

In summary, these studies together indicate that the adolescent mesocorticolimbic DA system is sensitive to alcohol-induced changes resulting in an increased risk of seeking for and actual consumption of alcohol in adulthood which may even lead to alcohol abuse or dependence.

3. Discussion

The objective of this thesis was to study the literature on long-term consequences of alcohol use on adolescent brain development. Studies in the entire spectrum of neuroscience have shed light on this social and economic relevant topic.

First, structural changes in grey and white matter volumes have been observed in rats and humans as a consequence of alcohol use during adolescence. Grey and white matter reductions have been found throughout the brain, particularly in hippocampus and frontal brain regions (e.g. the PFC). This volume loss may be directly or indirectly caused by alcohol-induced reduced cerebral blood flow, reduced neurogenesis, neurotoxicity, and/or neuroinflammation. Second, functional changes have been reported in humans after adolescent alcohol use compared to light or non-drinking adolescents, as evidenced by differences in fMRI BOLD-responses during learning and memory tasks and on a cue reactivity task. Furthermore, altered hippocampal ERPs have been found in adult rats after binge-like alcohol exposure during adolescence. These structural and functional changes may underlie impairments in neurocognitive performance. Indeed, long-lasting deficits in attention, decision making, and especially learning and memory have been reported in adolescents and adults after alcohol use during adolescence. This neurocognitive evidence is mainly provided by studies in which rats underwent a binge-like alcohol exposure. In addition, early alcohol use in humans is likely to be a risk factor for later alcohol abuse and alcohol addiction. Moreover, binge-like alcohol exposure in adolescent rats increases the extent of alcohol intake in adulthood. Alcohol-induced changes in the mesocorticolimbic DA system contribute to the increased risk of seeking for and actual consumption of alcohol in adulthood. In summary, human and rat studies have demonstrated many harmful effects of alcohol on adolescent brain development resulting in long-term deficits in neurocognition and increased risk for later alcohol abuse and addiction.

However, some important methodological considerations should be mentioned. For instance, sample sizes in most studies were rather small. Therefore, these studies should be replicated with larger sample sizes, which increases power, to ultimately confirm the findings of previous studies. Furthermore, in most rat studies describing the detrimental effects of alcohol, rats underwent a binge-like alcohol exposure. However, chronic alcohol exposure, alcohol vapour exposure and the two-bottle choice paradigm showed conflicting results. Further research should further elucidate the value of each ethanol administration paradigm in animals for human relevance. Moreover, to unravel whether early onset of alcohol use in humans may rather be serving as a marker than being a causal precursor for later alcohol problems, adult drinking patterns after different onsets of adolescent alcohol exposure should

be studied in rats. In addition, most human studies on the effect of adolescent alcohol use included adolescents who met criteria for AUD. In some of these studies adolescents with AUD had comorbid psychiatric conditions, including other SUDs, which may influence results. Ideally, prospective studies should include healthy adolescents that are followed in their drinking pattern and cognitive performance during adolescence into adulthood. On top of that, longitudinal human imaging studies are needed to investigate long-term temporal changes in brain functioning (e.g. in a learning task performance) as a consequence of adolescent alcohol use. In contrast, retrospectively collected data of alcohol consumption are not favourable considering the insufficient reliability of the assessment, for example when adolescents are asked how old they were when they initiated their alcohol use (Engels *et al.*, 1997). Besides, human studies should take into account age-related differences in the effects of alcohol use. For example, as previously stated, binge drinking is often defined as the use of at least 4 (for females) or at least 5 (for males) alcoholic drinks in a row at one occasion. However, given that this definition is originally made for adults and that adolescents conceive alcohol in another way, it has been proposed that the definition of binge drinking should be adjusted for adolescents (Donovan *et al.*, 2009). For example, for 12- and 13-year-old adolescents and 14- to 17-year-old girls the definition can be adjusted to 3 or more drinks, while it should be defined in 14-15 year old boys as 4 or more drinks on a row at one occasion (Donovan *et al.*, 2009). Next, beyond the scope of this thesis were gender differences and the role of stress, hormones, and serotonin in investigating the long-term effects of alcohol use on adolescent brain development. 12- to 15-year-old adolescents show no gender differences in alcohol use (Poelen *et al.*, 2005; Young *et al.*, 2002). However, after the age of 15 years gender differences in alcohol use could arise as a consequence of the biological difference in sensitivity to alcohol, with females being generally more vulnerable to adverse alcohol effects than males (Guerri and Pascual 2010). Alcohol-induced structural differences between male and female adolescents include smaller PFC volumes in females with AUD (Medina *et al.*, 2008) and less evident D1 and D2 receptor overproduction and subsequent pruning in female rats (Andersen *et al.*, 1999). Moreover, alcohol-induced functional differences between human adolescent males and females with AUD include less frontal brain activation in females during a spatial working memory task as measured by BOLD response (Caldwell *et al.*, 2005). Thus, it would be favourable to separate female and male subjects in further research concerning the effects of alcohol use during adolescence. In addition, stressors during adolescence may contribute to DA level changes in the mesocorticolimbic DA system by selectively activate the mesocorticolimbic DA projections (Spear 2002). Hormones may affect the developing mesocorticolimbic DA system in the adolescent brain as well (Chambers *et al.*, 2003). Furthermore, few studies suggest that serotonin may also contribute to adolescent drinking (McBride *et al.*, 2005). Thus, further research should elucidate the precise role of stressors, hormones, serotonin and other neurotransmitters in the risk for alcohol abuse and addiction.

Now, some new ideas for future research. For instance, it would be interesting to investigate the possibility of automatic recovery of neurocognitive abilities following an abstinence period after alcohol use during adolescence. If the human adolescent brain is plastic enough, it remains to be seen what abstinence time (e.g. a couple of years?) will be necessary to bring neurocognitive performance at a predrinking level. Longitudinal fMRI studies initiating at the onset of drinking can be useful in solving this question. Another interesting topic for future research is genetic variation for risk of alcohol abuse and

addiction. Non-drinking adolescents with a family history of alcoholism showed impaired behavioural inhibition on several neurocognitive tasks similar to adult alcohol-dependents (Bjork *et al.*, 2004a; Lawrence *et al.*, 2009; Schweinsburg *et al.*, 2004), indicating an inherited addiction vulnerability in the non-drinking adolescents. Moreover, a strong correlation has also been found between family history of alcoholism and the risk of initiating drinking under the age of 15 (Dawson, 2000). However, variation of initiation and frequency of drinking in adolescent twins differed between studies and could not clearly be explained by either heritability or common environmental factors alone (for an overview, see Poelen *et al.*, 2008), which may suggest gene-environment interactions in both initiation and frequency of alcohol use during adolescence. Gene-environment interactions are thought to be mediated by epigenetic changes in the genome (Liu *et al.*, 2008). For example, epigenetic mechanisms have been involved in changes in gene expression in the mesocorticolimbic DA system that are thought to contribute to the pathogenesis and persistence of drug addiction (Guerri and Pascual 2010). Together, this raises the question if we should protect genetically high risk individuals from alcohol (ab)use in adolescence. Indeed we should, considering that the alcohol abuse or dependence phenotype may only emerge when early alcohol use and genetic risk factors occur together. Prevention of early alcohol use could be one of the protective actions which will further be discussed in the last subparagraph of this section. In addition, the prevention of relapse – falling back into the pattern of problem drinking – is one of the most desired treatments for alcohol abuse and addiction. fMRI results in methamphetamine-dependent adults revealed that a combination of right middle frontal gyrus, middle temporal gyrus, and posterior cingulate activation best predicted the time to relapse (Paulus *et al.*, 2005). Less activation in these areas may likely indicate poor-decision making that precedes relapse. Future research should determine if similar patterns in brain activity can predict relapse in AUD adolescents. Also, it should be possible to predict relapse with biomarkers (Bearer *et al.*, 2010). However, how could we suppress or even prevent the negative effects of alcohol? Adolescent rats which were administered the nonsteroidal anti-inflammatory drug indomethacin before exposure to alcohol did not show alcohol-induced behavioural deficits. Indomethacin provides this protective effect by inhibiting COX-2 activity, iNOS expressing and neuronal cell death. However, indomethacin has many serious side effects thus can not be used in everyday clinic. Other results from clinical trials in alcohol-dependent patients suggest that the opioid antagonist naltrexone (NTX) may decrease the chance of relapse for 36% and lower the risk of treatment withdrawal in alcohol-dependent patients for 28% (Srisurapanont and Jarusuraisin, 2005). Future research in rodents should elucidate if other opioid antagonist or a combination of opioid antagonists may decrease the risk for these negative alcohol effects. Recently, David Nutt and his colleagues have developed a pill which serves as a substitute for alcohol (see, e.g., http://tvblik.nl/nieuwslicht/8-april-2010). It induces the positive effects of alcohol without getting drunk. Another pill will be developed to immediately undo the effects of the alcohol pill. Off course, future research should clarify long-term positive and negative effects of these pills but the first results are promising.

However, as in many other situations: prevention is better than cure. Therefore, it is important to make a statement on preventing early onset and high-risk alcohol use in adolescents. First, a short overview of alcohol prevention developments in the Netherlands in the past 15 years is given, before some recommendations for discouraging underage drinking will be formulated. In 1996, the NIGZ (*Nationaal Instituut voor Gezondheidsbevordering en*

Ziektepreventie; Health Promotion Institute) started the campaign *DRANK maakt meer kapot dan je lief is* ('ALCOHOL causes more damage than you care for') by order of the Dutch government. Objective of this campaign is to make people aware of and give them knowledge about the detrimental effects of alcohol, in adolescents for example by giving the information on camping sites and high schools. In 2005, the Netherlands Institute of Mental Health and Addiction (*Trimbos-instituut*) did scientific research by order of the Dutch government in order to (1) review the harmful effects of and (2) trends in alcohol use in adolescents under the age of 16 and (3) the proposed effect of a new campaign preventing underage alcohol use aimed at both young adolescents and their parents (Verdurmen *et al.*, 2006). From that moment on, the NIGZ campaign used a slogan in their campaign aimed at parents, that remind parents to prevent alcohol-induced damage during development of their child (Fig. 13). In addition, STIVA (*Stichting Verantwoord Alcoholgebruik*; existing of

Figure 13. Flyers from the Dutch campaign *DRANK maakt meer kapot dan je lief is* ('ALCOHOL causes more damage than you care for') with the slogan *Voorkom alcoholschade bij uw opgroeiende kind* ('Prevent alcohol-induced damage in your developing child') (NIGZ, 2006-present).

manufacturers and importers of the Dutch alcohol beverage branch) started the slogan *Alcohol onder de 16, nog even niet* ('Alcohol under the age of 16, not yet') in 2006, that two years later changed into the slogan *Alcohol onder de 16, natuurlijk niet* ('Alcohol under the age of 16, of course not'). These slogans were presented in alcohol beverage commercials broadcasted on television and cinemas. In late 2009 STIVA started a cooperation with the Dutch government and again changed its slogan, which is still: *Geen 16? Geen druppel* ('Aren't you 16 years old? No drop of alcohol'). The impact of these slogans remains to be seen. Nevertheless, adolescents have a high risk for alcohol use because of their enhanced novelty seeking, sensation seeking, and/or reward seeking behaviour that promotes experimentation with alcohol. Increasing the age restrictions for alcohol use may be preferred but, when comparing Europe and the USA, this is apparently not a remedy for alcohol-related problems and therefore should not be focussed on. More important, for example, is to maintain the minimum legal drinking age and to provide information about alcohol effects by education in order to discourage underage drinking. Mandatory education at elementary schools and high schools, as well as public education, are essential in reducing early onset and high-risk alcohol use in adolescents (Kokotailo *et al.*, 2010). In addition, approaching

parents, besides adolescents, is a new trend that is not less important in preventing underage drinking. Dutch adolescents may start drinking regularly at a relatively young age, because of permissive attitudes of parents towards drinking (Van Der Vorst *et al.*, 2005). Notably, several studies revealed that when parents make strict rules prohibiting alcohol use this can postpone the initiation of alcohol drinking and lower the chance to engage in problem drinking (Van Laar *et al.*, 2010). However, only 25% of Dutch parents make such rules with their child (Verdurmen *et al.*, 2008). Furthermore, parental drinking patterns and insufficient knowledge of adolescent alcohol effects among parents degrade the value of these rules and negatively influence the consumption of alcohol in their child (Verdurmen *et al.*, 2008). Therefore, improved education for parents, for example via commercials and flyers, is recommended. In addition, it is known that higher exposure to alcohol advertisements is associated with higher amounts of alcohol consumption that can last after adolescence (Snyder *et al.*, 2006). Moreover, knowing that adolescents visit supermarkets on a regular basis, it is alarming that exposure to in-store beer displays in supermarkets could predict the initiation of alcohol use in 13-year-olds two years later (Ellickson *et al.*, 2005). Taken these two studies together, it is needed to reduce the exposure to alcohol advertisements and other alcohol-related stimuli among adolescents to prevent early onset and high-risk alcohol use. Another problem seen in the Netherlands is the observance of age restrictions of 16 years for light alcoholic beverages and 18 years for spirits. Despite the strict age restriction criteria, it is not difficult for underage adolescents to purchase alcoholic beverages. For example, spirits could be easily purchased in liquor stores by 73% of 14- and 15-year-olds and by 85% of 16- and 17-year-olds (Bieleman *et al.*, 2002). Given the long-lasting harmful effects of alcohol in adolescents it is necessary to reduce exposure to alcohol-related stimuli among adolescents and strengthen the observance of age restrictions from the Dutch law. Although it takes time to fully accomplish, it is expected that prohibiting sale of alcoholic beverages in supermarkets and extending liquor stores under the restriction of stricter age restriction observance may reduce early onset and high-risk alcohol use in adolescents as well. Accomplishing this, together with the continuation of providing improved education for adolescents and their parents on the long-lasting detrimental effects of adolescent alcohol use, would be a good signal from Dutch government to improve the future abilities of our youth.

References

Acheson, S. K., Stein, R. M., and Swartzwelder, H. S. (1998) Impairment of semantic and figural memory by acute ethanol: age-dependent effects. *Alcohol Clin. Exp. Res.* **22,** 1437-1442.

Andersen, S. L., Dumont, N. L., and Teicher, M. H. (1997) Developmental differences in dopamine synthesis inhibition by (+/-)-7-OH-DPAT. *Naunyn Schmiedebergs Arch. Pharmacol.* **356,** 173-181.

Andersen, S. L., Rutstein, M., Benzo, J. M., Hostetter, J. C., and Teicher, M. H. (1997) Sex differences in dopamine receptor overproduction and elimination. *Neuroreport* **8,** 1495-1498.

Badanich, K. A., Maldonado, A. M., and Kirstein, C. L. (2007) Chronic ethanol exposure during adolescence increases basal dopamine in the nucleus accumbens septi during adulthood. *Alcohol Clin. Exp. Res.* **31,** 895-900.

Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., Dant, C. C., and Reiss, A. L. (2005) White matter development during childhood and adolescence: a cross-sectional diffusion tensor imaging study. *Cereb. Cortex* **15,** 1848-1854.

Bearer, C. F., Bailey, S. M., and Hoek, J. B. (2010) Advancing alcohol biomarkers research. *Alcohol Clin. Exp. Res.* **34,** 941-945.

Begleiter, H., Porjesz, B., Chou, C. L., and Aunon, J. I. (1983) P3 and stimulus incentive value. *Psychophysiology* **20,** 95-101.

Beracochea, D., Micheau, J., and Jaffard, R. (1992) Memory deficits following chronic alcohol consumption in mice: relationships with hippocampal and cortical cholinergic activities. *Pharmacol. Biochem. Behav.* **42,** 749-753.

Berger, M., Gray, J. A., and Roth, B. L. (2009) The expanded biology of serotonin. *Annu. Rev. Med.* **60,** 355-366.

Bergstrom, H. C., McDonald, C. G., and Smith, R. F. (2006) Alcohol exposure during adolescence impairs auditory fear conditioning in adult Long-Evans rats. *Physiol Behav.* **88,** 466-472.

Bevins, R. A., Klebaur, J. E., and Bardo, M. T. (1997) Individual differences in response to novelty, amphetamine-induced activity and drug discrimination in rats. *Behav. Pharmacol.* **8,** 113-123.

Bieleman, B., Jetzes, M., Kruize, F. (2002) *Alcoholverstrekking aan Jongeren: Naleving Leeftijdsgrenzen 16 en 18 Jaar uit de Drank- en Horecawet: Metingen 1999 en 2001*. Intraval, Groningen/Rotterdam.

Bjork, J. M., Hommer, D. W., Grant, S. J., and Danube, C. (2004a) Impulsivity in abstinent alcohol-dependent patients: relation to control subjects and type 1-/type 2-like traits. *Alcohol* **34,** 133-150.

Bjork, J. M., Knutson, B., Fong, G. W., Caggiano, D. M., Bennett, S. M., and Hommer, D. W. (2004b) Incentive-elicited brain activation in adolescents: similarities and differences from young adults. *J. Neurosci.* **24,** 1793-1802.

Blair, H. T., Schafe, G. E., Bauer, E. P., Rodrigues, S. M., and LeDoux, J. E. (2001) Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn. Mem.* **8,** 229-242.

Blanco, A. M., Valles, S. L., Pascual, M., and Guerri, C. (2005) Involvement of TLR4/type I IL-1 receptor signaling in the induction of inflammatory mediators and cell death induced by ethanol in cultured astrocytes. *J. Immunol.* **175,** 6893-6899.

Brown, S. A. and Tapert, S. F. (2004) Adolescence and the trajectory of alcohol use: basic to clinical studies. *Ann. N. Y. Acad. Sci.* **1021,** 234-244.

Brown, S. A., Tapert, S. F., Granholm, E., and Delis, D. C. (2000) Neurocognitive functioning of adolescents: effects of protracted alcohol use. *Alcohol Clin. Exp. Res.* **24,** 164-171.

Caldwell, L. C., Schweinsburg, A. D., Nagel, B. J., Barlett, V. C., Brown, S. A., and Tapert, S. F. (2005) Gender and adolescent alcohol use disorders on BOLD (blood oxygen level dependent) response to spatial working memory. *Alcohol Alcohol* **40,** 194-200.

Chambers, R. A., Taylor, J. R., and Potenza, M. N. (2003) Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *Am. J. Psychiatry* **160,** 1041-1052.

Chanraud, S., Martelli, C., Delain, F., Kostogianni, N., Douaud, G., Aubin, H. J., Reynaud, M., and Martinot, J. L. (2007) Brain morphometry and cognitive performance in detoxified alcohol-dependents with preserved psychosocial functioning. *Neuropsychopharmacology* **32,** 429-438.

Chou, S. P. and Pickering, R. P. (1992) Early onset of drinking as a risk factor for lifetime alcohol-related problems. *Br. J. Addict.* **87,** 1199-1204.

Clark, C. P., Brown, G. G., Eyler, L. T., Drummond, S. P., Braun, D. R., and Tapert, S. F. (2007) Decreased perfusion in young alcohol-dependent women as compared with age-matched controls. *Am. J. Drug Alcohol Abuse* **33,** 13-19.

Clark, D. B., Thatcher, D. L., and Tapert, S. F. (2008) Alcohol, psychological dysregulation, and adolescent brain development. *Alcohol Clin. Exp. Res.* **32,** 375-385.

Cohen, J. R., Asarnow, R. F., Sabb, F. W., Bilder, R. M., Bookheimer, S. Y., Knowlton, B. J., and Poldrack, R. A. (2010) A unique adolescent response to reward prediction errors. *Nat. Neurosci.* **13,** 669-671.

Connolly, G. M., Casswell, S., Zhang, J. F., and Silva, P. A. (1994) Alcohol in the mass media and drinking by adolescents: a longitudinal study. *Addiction* **89,** 1255-1263.

Crews, F., He, J., and Hodge, C. (2007) Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol. Biochem. Behav.* **86,** 189-199.

Crews, F. T., Braun, C. J., Hoplight, B., Switzer, R. C., III, and Knapp, D. J. (2000) Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. *Alcohol Clin. Exp. Res.* **24,** 1712-1723.

Crews, F. T., Mdzinarishvili, A., Kim, D., He, J., and Nixon, K. (2006) Neurogenesis in adolescent brain is potently inhibited by ethanol. *Neuroscience* **137,** 437-445.

Currie, C., Roberts, C., Morgan, A., Smith, R., Settertobulte, W., Samdal, O., Barnekow Rasmussen, V. (2004) *Young People's Health in Context. Health Behavior in School-Aged Children (HBSC) Study: International Report From the 2001/2002 Survey*. WHO, Copenhagen.

Dahl, R. E. (2004) Adolescent brain development: a period of vulnerabilities and opportunities. Keynote address. *Ann. N. Y. Acad. Sci.* **1021,** 1-22.

Dawson, D. A. (2000) The link between family history and early onset alcoholism: earlier initiation of drinking or more rapid development of dependence? *J. Stud. Alcohol* **61,** 637-646.

De Bellis, M. D., Clark, D. B., Beers, S. R., Soloff, P. H., Boring, A. M., Hall, J., Kersh, A., and Keshavan, M. S. (2000) Hippocampal volume in adolescent-onset alcohol use disorders. *Am. J. Psychiatry* **157,** 737-744.

De Bellis, M. D., Narasimhan, A., Thatcher, D. L., Keshavan, M. S., Soloff, P., and Clark, D. B. (2005) Prefrontal cortex, thalamus, and cerebellar volumes in adolescents and young adults with adolescent-onset alcohol use disorders and comorbid mental disorders. *Alcohol Clin. Exp. Res.* **29,** 1590-1600.

DeWit, D. J., Adlaf, E. M., Offord, D. R., and Ogborne, A. C. (2000) Age at first alcohol use: a risk factor for the development of alcohol disorders. *Am. J. Psychiatry* **157,** 745-750.

Deykin, E. Y., Levy, J. C., and Wells, V. (1987) Adolescent depression, alcohol and drug abuse. *Am. J. Public Health* **77,** 178-182.

Donovan, J. E. (2009) Estimated blood alcohol concentrations for child and adolescent drinking and their implications for screening instruments. *Pediatrics* **123,** e975-e981.

Ehlers, C. L. and Criado, J. R. (2010) Adolescent ethanol exposure: does it produce long-lasting electrophysiological effects? *Alcohol* **44,** 27-37.

Ellickson, P. L., Collins, R. L., Hambarsoomians, K., and McCaffrey, D. F. (2005) Does alcohol advertising promote adolescent drinking? Results from a longitudinal assessment. *Addiction* **100,** 235-246.

Ellickson, P. L., Tucker, J. S., and Klein, D. J. (2003) Ten-year prospective study of public health problems associated with early drinking. *Pediatrics* **111,** 949-955.

Engels, R. C., Knibbe, R. A., and Drop, M. J. (1997) Inconsistencies in adolescents' self-reports of initiation of alcohol and tobacco use. *Addict. Behav.* **22,** 613-623.

Ennaceur, A. and Aggleton, J. P. (1997) The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behav. Brain Res.* **88,** 181-193.

Epstein, H. T. (1999) Stages of increased cerebral blood flow accompany stages of rapid brain growth. *Brain Dev.* **21,** 535-539.

Fergusson, D. M., Lynskey, M. T., and Horwood, L. J. (1994) Childhood exposure to alcohol and adolescent drinking patterns. *Addiction* **89,** 1007-1016.

Friedman, J. and Humphrey, J. A. (1985) Antecedents of collegiate drinking. *Journal of Youth and Adolescence* **14,** 11-21.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., Paus, T., Evans, A. C., and Rapoport, J. L. (1999) Brain development during childhood and adolescence: a longitudinal MRI study. *Nat. Neurosci.* **2,** 861-863.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., Nugent, T. F., III, Herman, D. H., Clasen, L. S., Toga, A. W., Rapoport, J. L., and Thompson, P. M. (2004) Dynamic mapping of human cortical development during childhood through early adulthood. *Proc. Natl. Acad. Sci. U. S. A* **101,** 8174-8179.

Grant, B. F. and Dawson, D. A. (1997) Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J. Subst. Abuse* **9,** 103-110.

Grant, B. F., Stinson, F. S., and Harford, T. C. (2001) Age at onset of alcohol use and DSM-IV alcohol abuse and dependence: a 12-year follow-up. *J. Subst. Abuse* **13,** 493-504.

Gruber, E., DiClemente, R. J., Anderson, M. M., and Lodico, M. (1996) Early drinking onset and its association with alcohol use and problem behavior in late adolescence. *Prev. Med.* **25,** 293-300.

Guerri, C. and Pascual, M. (2010) Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. *Alcohol* **44,** 15-26.

Hawkins, J. D., Graham, J. W., Maguin, E., Abbott, R., Hill, K. G., and Catalano, R. F. (1997) Exploring the effects of age of alcohol use initiation and psychosocial risk factors on subsequent alcohol misuse. *J. Stud. Alcohol* **58,** 280-290.

He, J. and Crews, F. T. (2007) Neurogenesis decreases during brain maturation from adolescence to adulthood. *Pharmacol. Biochem. Behav.* **86,** 327-333.

Heitzeg, M. M., Nigg, J. T., Yau, W. Y., Zubieta, J. K., and Zucker, R. A. (2008) Affective circuitry and risk for alcoholism in late adolescence: differences in frontostriatal responses between vulnerable and resilient children of alcoholic parents. *Alcohol Clin. Exp. Res.* **32,** 414-426.

Ho, A., Chin, A. J., and Dole, V. P. (1989) Early experience and the consumption of alcohol by adult C57BL/6J mice. *Alcohol* **6**, 511-515.

Insel, T. R., Miller, L. P., and Gelhard, R. E. (1990) The ontogeny of excitatory amino acid receptors in rat forebrain--I. N-methyl-D-aspartate and quisqualate receptors. *Neuroscience* **35,** 31-43.

Jacobus, J., McQueeny, T., Bava, S., Schweinsburg, B. C., Frank, L. R., Yang, T. T., and Tapert, S. F. (2009) White matter integrity in adolescents with histories of marijuana use and binge drinking. *Neurotoxicol. Teratol.* **31,** 349-355.

Knight, D. C., Cheng, D. T., Smith, C. N., Stein, E. A., and Helmstetter, F. J. (2004) Neural substrates mediating human delay and trace fear conditioning. *J. Neurosci.* **24,** 218-228.

Kokotailo, P. K. (2010) Alcohol use by youth and adolescents: a pediatric concern. *Pediatrics* **125,** 1078-1087.

Kril, J. J., Halliday, G. M., Svoboda, M. D., and Cartwright, H. (1997) The cerebral cortex is damaged in chronic alcoholics. *Neuroscience* **79,** 983-998.

Labouvie, E., Bates, M. E., and Pandina, R. J. (1997) Age of first use: its reliability and predictive utility. *J. Stud. Alcohol* **58,** 638-643.

Laviolette, S. R. and van der Kooy, D. (2004) The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. *Nat. Rev. Neurosci.* **5,** 55-65.

Lawrence, A. J., Luty, J., Bogdan, N. A., Sahakian, B. J., and Clark, L. (2009) Impulsivity and response inhibition in alcohol dependence and problem gambling. *Psychopharmacology (Berl)* **207,** 163-172.

Leon, D. A., Saburova, L., Tomkins, S., Andreev, E., Kiryanov, N., McKee, M., and Shkolnikov, V. M. (2007) Hazardous alcohol drinking and premature mortality in Russia: a population based case-control study. *Lancet* **369,** 2001-2009.

Liu, L., Li, Y., and Tollefsbol, T. O. (2008) Gene-environment interactions and epigenetic basis of human diseases. *Curr. Issues Mol. Biol.* **10,** 25-36.

Maldonado-Devincci, A. M., Alipour, K. K., Michael, L. A., and Kirstein, C. L. (2010a) Repeated binge ethanol administration during adolescence enhances voluntary sweetened ethanol intake in young adulthood in male and female rats. *Pharmacol. Biochem. Behav.*

Maldonado-Devincci, A. M., Badanich, K. A., and Kirstein, C. L. (2010b) Alcohol during adolescence selectively alters immediate and long-term behavior and neurochemistry. *Alcohol* **44,** 57-66.

Markwiese, B. J., Acheson, S. K., Levin, E. D., Wilson, W. A., and Swartzwelder, H. S. (1998) Differential effects of ethanol on memory in adolescent and adult rats. *Alcohol Clin. Exp. Res.* **22,** 416-421.

McBride, W. J., Bell, R. L., Rodd, Z. A., Strother, W. N., and Murphy, J. M. (2005) Adolescent alcohol drinking and its long-range consequences. Studies with animal models. *Recent Dev. Alcohol* **17,** 123-142.

McEchron, M. D., Bouwmeester, H., Tseng, W., Weiss, C., and Disterhoft, J. F. (1998) Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. *Hippocampus* **8,** 638-646.

McNamee, R. L., Dunfee, K. L., Luna, B., Clark, D. B., Eddy, W. F., and Tarter, R. E. (2008) Brain activation, response inhibition, and increased risk for substance use disorder. *Alcohol Clin. Exp. Res.* **32,** 405-413.

Medina, K. L., McQueeny, T., Nagel, B. J., Hanson, K. L., Schweinsburg, A. D., and Tapert, S. F. (2008) Prefrontal cortex volumes in adolescents with alcohol use disorders: unique gender effects. *Alcohol Clin. Exp. Res.* **32,** 386-394.

Medina, K. L., Schweinsburg, A. D., Cohen-Zion, M., Nagel, B. J., and Tapert, S. F. (2007) Effects of alcohol and combined marijuana and alcohol use during adolescence on hippocampal volume and asymmetry. *Neurotoxicol. Teratol.* **29,** 141-152.

Mihic, S. J. and Harris, R. A. (1997) GABA and the GABAA receptor. *Alcohol Health Res. World* **21,** 127-131.

Monshouwer, K., Verdurmen, J., Van Dorsselaer, S., Smit, E., Gorter, A., Vollebergh, W. (2008) *Jeugd en riskant gedrag 2007: kerngegevens uit het Peilstationsonderzoek scholieren: roken, drinken, drugsgebruik en gokken onder scholieren vanaf tien jaar*. Utrecht: Trimbos-instituut.

Monti, P. M., Miranda, R., Jr., Nixon, K., Sher, K. J., Swartzwelder, H. S., Tapert, S. F., White, A., and Crews, F. T. (2005) Adolescence: booze, brains, and behavior. *Alcohol Clin. Exp. Res.* **29,** 207-220.

Moss, H. B., Kirisci, L., Gordon, H. W., and Tarter, R. E. (1994) A neuropsychologic profile of adolescent alcoholics. *Alcohol Clin. Exp. Res.* **18,** 159-163.

Murray, C. J. L., Lopez, A. (1996) *The Global Burden of Disease: A Comprehensive Assessment of Mortality and Disability from Diseases, Injuries and Risk Factors in 1990 and Projected to 2020.* Boston, Harvard School of Public Health on behalf of the World Health Organization and the World Bank.

Murray, T. K. and Ridley, R. M. (1999) The effect of excitotoxic hippocampal lesions on simple and conditional discrimination learning in the rat. *Behav. Brain Res.* **99,** 103-113.

Nagel, B. J., Schweinsburg, A. D., Phan, V., and Tapert, S. F. (2005) Reduced hippocampal volume among adolescents with alcohol use disorders without psychiatric comorbidity. *Psychiatry Res.* **139,** 181-190.

Nasrallah, N. A., Yang, T. W., and Bernstein, I. L. (2009) Long-term risk preference and suboptimal decision making following adolescent alcohol use. *Proc. Natl. Acad. Sci. U. S. A* **106,** 17600-17604.

Nutt, D., King, L. A., Saulsbury, W., and Blakemore, C. (2007) Development of a rational scale to assess the harm of drugs of potential misuse. *Lancet* **369,** 1047-1053.

Pascual, M., Blanco, A. M., Cauli, O., Minarro, J., and Guerri, C. (2007) Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural alterations in adolescent rats. *Eur. J. Neurosci.* **25,** 541-550.

Pascual, M., Boix, J., Felipo, V., and Guerri, C. (2009) Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. *J. Neurochem.* **108,** 920-931.

Paulus, M. P., Tapert, S. F., and Schuckit, M. A. (2005) Neural activation patterns of methamphetamine-dependent subjects during decision making predict relapse. *Arch. Gen. Psychiatry* **62,** 761-768.

Pedersen, W. and Skrondal, A. (1998) Alcohol consumption debut: predictors and consequences. *J. Stud. Alcohol* **59,** 32-42.

Pfefferbaum, A., Adalsteinsson, E., and Sullivan, E. V. (2006) Supratentorial profile of white matter microstructural integrity in recovering alcoholic men and women. *Biol. Psychiatry* **59,** 364-372.

Pfefferbaum, A., Sullivan, E. V., Hedehus, M., Adalsteinsson, E., Lim, K. O., and Moseley, M. (2000) In vivo detection and functional correlates of white matter microstructural disruption in chronic alcoholism. *Alcohol Clin. Exp. Res.* **24,** 1214-1221.

Pierce, R. C. and Kumaresan, V. (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci. Biobehav. Rev.* **30,** 215-238.

Pitkanen, T., Lyyra, A. L., and Pulkkinen, L. (2005) Age of onset of drinking and the use of alcohol in adulthood: a follow-up study from age 8-42 for females and males. *Addiction* **100,** 652-661.

Poelen, E. A., Derks, E. M., Engels, R. C., van Leeuwe, J. F., Scholte, R. H., Willemsen, G., and Boomsma, D. I. (2008) The relative contribution of genes and environment to alcohol use in early adolescents: are similar factors related to initiation of alcohol use and frequency of drinking? *Alcohol Clin. Exp. Res.* **32,** 975-982.

Poelen, E. A., Scholte, R. H., Engels, R. C., Boomsma, D. I., and Willemsen, G. (2005) Prevalence and trends of alcohol use and misuse among adolescents and young adults in the Netherlands from 1993 to 2000. *Drug Alcohol Depend.* **79,** 413-421.

Pulido, C., Mok, A., Brown, S. A., and Tapert, S. F. (2009) Heavy drinking relates to positive valence ratings of alcohol cues. *Addict. Biol.* **14,** 65-72.

Pyapali, G. K., Turner, D. A., Wilson, W. A., and Swartzwelder, H. S. (1999) Age and dose-dependent effects of ethanol on the induction of hippocampal long-term potentiation. *Alcohol* **19,** 107-111.

Rehm, J., Room, R., Monteiro, M., Ghmel, G., Graham, K., Rehn, N., Sempos, C. T., Frick, U., and Jernigan, D. (2004) Alcohol use. In: Ezzati, M., Lopez, A., Rodgers, A., Murray, C. J. L., eds. *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Due to Selected Major Risk Factors*. Geneva, World Health Organisation.

Robins, L. N. and Przybeck, T. R. (1985) Age of onset of drug use as a factor in drug and other disorders. *NIDA Res. Monogr* **56,** 178-192.

Schweinsburg, A. D., McQueeny, T., Nagel, B. J., Eyler, L. T., and Tapert, S. F. (2010) A preliminary study of functional magnetic resonance imaging response during verbal encoding among adolescent binge drinkers. *Alcohol* **44,** 111-117.

Schweinsburg, A. D., Paulus, M. P., Barlett, V. C., Killeen, L. A., Caldwell, L. C., Pulido, C., Brown, S. A., and Tapert, S. F. (2004) An FMRI study of response inhibition in youths with a family history of alcoholism. *Ann. N. Y. Acad. Sci.* **1021,** 391-394.

Sher, K. J., Martin, E. D., Wood, P. K., and Rutledge, P. C. (1997) Alcohol use disorders and neuropsychological functioning in first-year undergraduates. *Exp. Clin. Psychopharmacol.* **5,** 304-315.

Silvers, J. M., Tokunaga, S., Mittleman, G., and Matthews, D. B. (2003) Chronic intermittent injections of high-dose ethanol during adolescence produce metabolic, hypnotic, and cognitive tolerance in rats. *Alcohol Clin. Exp. Res.* **27,** 1606-1612.

Silvers, J. M., Tokunaga, S., Mittleman, G., O'Buckley, T., Morrow, A. L., and Matthews, D. B. (2006) Chronic intermittent ethanol exposure during adolescence reduces the effect of ethanol challenge on hippocampal allopregnanolone levels and Morris water maze task performance. *Alcohol* **39,** 151-158.

Sircar, R. and Sircar, D. (2005) Adolescent rats exposed to repeated ethanol treatment show lingering behavioral impairments. *Alcohol Clin. Exp. Res.* **29,** 1402-1410.

Slawecki, C. J. and Betancourt, M. (2002) Effects of adolescent ethanol exposure on ethanol consumption in adult rats. *Alcohol* **26,** 23-30.

Slawecki, C. J., Betancourt, M., Cole, M., and Ehlers, C. L. (2001) Periadolescent alcohol exposure has lasting effects on adult neurophysiological function in rats. *Brain Res. Dev. Brain Res.* **128,** 63-72.

Snyder, L. B., Milici, F. F., Slater, M., Sun, H., and Strizhakova, Y. (2006) Effects of alcohol advertising exposure on drinking among youth. *Arch. Pediatr. Adolesc. Med.* **160,** 18-24.

Soo Hoo, G. W., Hinds, R. L., Dinovo, E., and Renner, S. W. (2003) Fatal large-volume mouthwash ingestion in an adult: a review and the possible role of phenolic compound toxicity. *J. Intensive Care Med.* **18,** 150-155.

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

Spear, L. P. (2002) Alcohol's effects on adolescents. *Alcohol Res. Health* **26,** 287-291.

Srisurapanont, M. and Jarusuraisin, N. (2005) Opioid antagonists for alcohol dependence. *Cochrane. Database. Syst. Rev.* CD001867.

Stansfield, K. H. and Kirstein, C. L. (2007) Chronic cocaine or ethanol exposure during adolescence alters novelty-related behaviors in adulthood. *Pharmacol. Biochem. Behav.* **86,** 637-642.

Stolle, M., Sack, P. M., and Thomasius, R. (2009) Binge drinking in childhood and adolescence: epidemiology, consequences, and interventions. *Dtsch. Arztebl. Int.* **106,** 323-328.

Tapert, S. F., Brown, G. G., Baratta, M. V., and Brown, S. A. (2004a) fMRI BOLD response to alcohol stimuli in alcohol dependent young women. *Addict. Behav.* **29,** 33-50.

Tapert, S. F., Brown, G. G., Kindermann, S. S., Cheung, E. H., Frank, L. R., and Brown, S. A. (2001) fMRI measurement of brain dysfunction in alcohol-dependent young women. *Alcohol Clin. Exp. Res.* **25,** 236-245.

Tapert, S. F., Cheung, E. H., Brown, G. G., Frank, L. R., Paulus, M. P., Schweinsburg, A. D., Meloy, M. J., and Brown, S. A. (2003a) Neural response to alcohol stimuli in adolescents with alcohol use disorder. *Arch. Gen. Psychiatry* **60,** 727-735.

Tapert, S. F., Granholm, E., Leedy, N. G., and Brown, S. A. (2002) Substance use and withdrawal: neuropsychological functioning over 8 years in youth. *J. Int. Neuropsychol. Soc.* **8,** 873-883.

Tapert, S. F. and Schweinsburg, A. D. (2005) The human adolescent brain and alcohol use disorders. *Recent Dev. Alcohol* **17,** 177-197.

Tapert, S. F., Schweinsburg, A. D., Barlett, V. C., Brown, S. A., Frank, L. R., Brown, G. G., and Meloy, M. J. (2004b) Blood oxygen level dependent response and spatial working memory in adolescents with alcohol use disorders. *Alcohol Clin. Exp. Res.* **28,** 1577-1586.

Tapert, S. F., Theilmann, R. J., Schweinsburg, A. D. (2003b) Reduced fractional anisotropy in the splenium of adolescents with alcohol use disorder. *Magn Reson Med.* **11,** 8217.

Tarazi, F. I. and Baldessarini, R. J. (2000) Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. *Int. J. Dev. Neurosci.* **18,** 29-37.

Tolliver, G. A. and Samson, H. H. (1991) The influence of early postweaning ethanol exposure on oral self-administration behavior in the rat. *Pharmacol. Biochem. Behav.* **38,** 575-580.

Truxell, E. M., Molina, J. C., and Spear, N. E. (2007) Ethanol intake in the juvenile, adolescent, and adult rat: effects of age and prior exposure to ethanol. *Alcohol Clin. Exp. Res.* **31,** 755-765.

Tsai, G., Gastfriend, D. R., and Coyle, J. T. (1995) The glutamatergic basis of human alcoholism. *Am. J. Psychiatry* **152,** 332-340.

Valles, S. L., Blanco, A. M., Pascual, M., and Guerri, C. (2004) Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. *Brain Pathol.* **14,** 365-371.

Van Der Vorst, H., Engels, R. C. M. E., Meeus, W., Dekovic, M., Van Leeuwe, J. (2005) The role of alcohol-specific socialization in adolescents' drinking behaviour. *Addiction* **100,** 1464–1476.

Van Laar, M. W., Cruts, A. A. N., Van Ooyen-Houben, M. M. J., Meijer, R. F., Brunt, T., eds. (2010) *Nationale drug monitor. Jaarbericht 2009*. Utrecht: Trimbos-instituut.

Verdurmen, J., Abraham, M., Planije, M., Monshouwer, K., Van Dorsselaer, S., Schulten, I., Bevers, J., Vollebergh, W. (2006) *Alcoholgebruik en jongeren onder de 16 jaar: schadelijke effecten en effectiviteit van alcoholinterventies*. Utrecht: Trimbos-instituut.

Verdurmen, J., Smit, E., Van Dorsselaer, S., Monshouwer, K., Schulten, I. (2008) *Ouders over alcohol-, roken en drugspecifieke opvoeding 2007: kerngegevens uit het Peilstationsonderzoek Ouders*. Utrecht: Trimbos-instituut.

Vetter, C. S., Doremus-Fitzwater, T. L., and Spear, L. P. (2007) Time course of elevated ethanol intake in adolescent relative to adult rats under continuous, voluntary-access conditions. *Alcohol Clin. Exp. Res.* **31,** 1159-1168.

Warner, L. A. and White, H. R. (2003) Longitudinal effects of age at onset and first drinking situations on problem drinking. *Subst. Use. Misuse.* **38,** 1983-2016.

White, A. M., Ghia, A. J., Levin, E. D., and Swartzwelder, H. S. (2000) Binge pattern ethanol exposure in adolescent and adult rats: differential impact on subsequent responsiveness to ethanol. *Alcohol Clin. Exp. Res.* **24,** 1251-1256.

Witt, E. D. (2010) Research on alcohol and adolescent brain development: opportunities and future directions. *Alcohol* **44,** 119-124.

Yashimoto, K. (1988) The influence of age upon alcohol drinking behavior. *Med Sci Res* **16**, 755-756.

York, J. L., Welte, J., Hirsch, J., Hoffman, J. H., and Barnes, G. (2004) Association of age at first drink with current alcohol drinking variables in a national general population sample. *Alcohol Clin. Exp. Res.* **28,** 1379-1387.

Young, S. E., Corley, R. P., Stallings, M. C., Rhee, S. H., Crowley, T. J., and Hewitt, J. K. (2002) Substance use, abuse and dependence in adolescence: prevalence, symptom profiles and correlates. *Drug Alcohol Depend.* **68,** 309-322.

Yttri, E. A., Burk, J. A., and Hunt, P. S. (2004) Intermittent ethanol exposure in adolescent rats: dose-dependent impairments in trace conditioning. *Alcohol Clin. Exp. Res.* **28,** 1433-1436.

Yu, Z. Y., Wang, W., Fritschy, J. M., Witte, O. W., and Redecker, C. (2006) Changes in neocortical and hippocampal GABAA receptor subunit distribution during brain maturation and aging. *Brain Res.* **1099,** 73-81.