The role of the amygdala in social behavior and alcoholism

Richelle C. Kosse

Master Neuroscience and Cognition, Graduate School of Life Sciences, Utrecht University, Utrecht, The Netherlands
Contact: r.c.kosse@students.uu.nl

12 December 2013

Abstract

Alcoholism is a major health risk, since 4% of all deaths worldwide are attributed to alcohol. The development of alcoholism is influenced by genetic, psychosocial and environmental factors. The reward feeling, derived of alcohol consumption, can also stimulate the development of an alcohol problem. This reward is mediated by the mesolimbic reward pathway in the brain. Social behavior, especially social play, can also result in a reward feeling and there is a relation between social behavior and alcohol. This relation is most clearly seen in adolescents, where alcohol facilitates social behavior and where a social environment can support alcohol use, presumably by emphasizing the positive effects of alcohol. Much research is done towards the underlying mechanisms of alcohol use, social behavior and the relation between the two. Of particular interest in this respect is the research on prairie voles that display strong social bonds and have a high preference for alcohol that is related to social behavior. Based on different studies it turned out that low doses of alcohol can support play behavior, presumably by the release of opioids. One of the brain structures involved in social play and alcohol is the amygdala, which contributes to the rewarding effects of both. The amygdala is also involved in the sex differences of social play, and with respect to alcohol it might also play a role in the positive and negative reinforcing effects of alcohol, which suggest a regulating function of the amygdala. Furthermore, there was an increased expression of the adapter protein 14-3-3ζ found in the amygdala during escalated alcohol intake, which might confirm the regulating role of the amygdala on alcohol intake. However, there is more research needed towards this effect and if the amygdala has indeed a regulating role, it might be a potential target for a treatment of alcoholism, perhaps via the stimulating effects of social play on the amygdala.

Keywords: amygdala, alcoholism, social play, prairie voles, 14-3-3ζ

Introduction
Alcoholism is a causal factor for more than 60 types of injuries and diseases, for example cirrhosis of the liver, brain atrophy, epilepsy, road accidents and violence. These effects of alcohol makes alcohol use is one of the world's major health risks and it results in approximately 2.5 million deaths per year, which is more compared to the deaths caused by HIV/AIDS and tuberculosis[1]. Alcoholism, also called alcohol dependence, is defined as 'a maladaptive pattern of drinking, leading to clinically significant impairment or distress'. It is characterized by long periods of heavy alcohol use, tolerance, all time spent on obtaining and using alcohol, no control over alcohol use, using alcohol despite of physical and psychological problems and withdrawal symptoms. The previous described characteristics confirm that alcohol dependence is an addiction. The development and manifestation of alcoholism can be influenced by genetic, psychosocial and environmental factors[2]. It is important to find out more about these influences and about the neural circuit involved in alcoholism, to prevent and treat this major health problem.

Alcohol addiction is a relapsing disorder and therefore it is hard to overcome. The misuse and negative outcomes depend on the volume, quality and pattern of alcohol consumption and is especially applied to younger age groups (15-24 years) and to males (24-59 years)[1,3-5]. There are two different types of alcoholism, namely type 1 and 2. Type 1 (80% of alcoholics) is characterized by a low genetic load, late onset and few social complications. This type is often developed in response to the inability to cope with stressors. Type 2 (20% of alcoholics) is characterized by a high genetic load, early onset and severe social complications, such as aggressiveness, low socialization, anti-social behavior, poorly cooperative, vengeful and complications at work. The importance of genes, environmental and psychosocial factors can be seen in the two types of alcoholism and personality traits play also an important role in both types of alcoholism. Especially dis-inhibitory traits, such as impulsivity, sensation seeking and psychopathy can contribute the development of alcoholism. Persons with those traits are more sensitive for novel experiences and have a clear association between alcohol drinking and immediate pleasure, which increases the chance to develop a drinking problem[5-7].

Stress and emotional pain can also affect the alcohol consumption, these factors can increase the consumption of alcohol[8-10]. Anxiety also seems to influence alcohol use, however in different studies in humans different influences of anxiety are shown. Some studies showed no effect of anxiety on alcohol use[6,8,9], whereas others show a bidirectional effect. Indicating that anxiety promotes alcohol consuming via acute anxiolytic effects and prolonged consumption of alcohol increases anxiety[11,12]. In rodents the relation between alcohol and anxiety is clearer, because anxiety is related to an increased alcohol consuming, presumably to cope with the anxiety[13-15]. In general, different factors can influence alcohol use and thereby increase the risk to develop an alcohol problem. All these different factors make it hard to find the true cause and as a consequence the optimal treatment for a specific alcohol addicted patient. Therefore different treatments are used to treat alcoholism, for example social support groups, drug therapies and cognitive and behavioral therapies[16]. Most of these treatments are limited in number and efficacy [17,18] and that may be one of the reasons why alcoholism and the associated negative consequences still exist. To find a solution for this global burden, research is needed to learn more about the influence of different factors and to understand the underlying mechanisms of alcoholism.

Social behavior and social context
One important factor related to alcohol use is social behavior, because alcohol use can affect social behavior; individuals found it easier to form social interactions when alcohol is consumed. Especially adolescents and young adult drinkers have powerful expectancies of social facilitation and endorse social motives for drinking. Therefore, the social facilitating effect of alcohol supports alcohol use and may therefore contribute to the development of an alcohol addiction[6,19-21]. However, is this effect also the other way around; contributes a social drinking context to more alcohol use?

There is much research done in adolescents and young adults towards the difference between social and solitary drinking, because alcohol abuse in adolescents is a growing problem that

may be influenced by social interactions and social events. Adolescence is the period between youth and adulthood, where young adults acquire the behavioral skills to live independently. This is characterized by increased exploration, risk taking, peer influences and drug and alcohol use[22]. During adolescence people first experiment with alcohol and this makes the adolescence phase interesting to examine, because early onset drinking is correlated with an increased risk of developing alcohol related problems later in life, including alcoholism[23]. Besides behavioral changes, also important physiological changes occur during adolescence. These changes include changes in brain regions important for the reinforcing effects of alcohol and drugs, which may explain the increased vulnerability towards alcohol and drug use[22]. Adolescents are often with peers and do therefore nearly all of their drinking with their peers. These social drinking contexts may be important for the drinking behavior later in life, because the rewarding effects of alcohol can be altered by the context in which it is consumed[24,25]. It differs per person, but in general alcohol consumption has predominantly positive effects in a social context[19,26]. The positive effects related to alcohol consumption in a social context resulted in a heightened risk for the development and maintaining of a drinking problem. The positive and euphoric effects of alcohol consumption in relation to social circumstances are confirmed by a study of Lindman (1982), where they investigate the amount of alcohol consumed and the mood of social drinkers in a real life social drinking situation and in an artificial solitary drinking situation. It turned out that almost twice as much alcohol was consumed during social drinking and solitary drinking was experienced as aversive[27]. However, Lindman (1982) analyzed only 4 subjects, thus more research was needed to confirm the relation between alcohol and a social drinking context. This relation is among others confirmed by the socio-environmental context model of Ward (2011), where the social environment is defined as ´the extent to which a given environment can create, facilitate and reinforce social interaction´. This study showed that pubs, bars and nightclubs have a unique atmosphere with regard to social interactions and in all these locations plays alcohol an important role[28]. The study of Pliner and Cappell (1974) looked at the mood of 120 subjects in solitary and social drinking contexts and also confirmed the fact that alcohol has mainly positive effects in social circumstances. Because at the same objective level of intoxication, solitary drinkers experienced more physical symptoms compared to social drinkers, who had an affective response to alcohol. This study confirms again that alcohol and the environment interact to determine the affective response to alcohol[25]. All these studies showed that the social context can affect the alcohol consumption, the social context can even overwhelm individual characteristics[29], however personality traits are still considered to have also an effect on the alcohol consumption[6].

As mentioned before, in adolescents there is in particular an influence of friends and peers. These influences can be categorized in *modeling* (adapting the drinking behavior to peers) and *persuasion* (encouraging and persuading to adapt alcohol consumption)[30]. Both influences are important for the alcohol use of adolescents, because they show imitation effects and have context specific drinking norms. The degree of influence depends on the role and relationship with the drinking peer. For example, interactions with unfriendly peer might results in negative emotions which lead to increased drinking to escape the situation. Alternatively, interactions with friendly peers create a convivial atmosphere which is associated with positive affective experiences and this can increase the social drinking[31]. This suggests that both, the influence of friendly and unfriendly peers (social context) can increase the amount of alcohol consumed in adolescents. A study of Kuendig and Kuntsche (2012) confirmed the influence of friends, because in this study young adults drank more frequently in social contexts and drank more when their friends drank more intensively[21]. The effect of a social context is also confirmed by the fact that the mean consumption level of alcohol in social surroundings is almost double (170%) compared to the solitary consumption and that a social context stimulates binge drinking (consuming 5+ drinks on one single occasion)[27]. Binge drinking is becoming normal nowadays, which is problematic, because it increases the risk to develop a drinking problem [32]. In the end, there are suggestions that the effects of a social drinking partner are sex dependent[33], however the data is not consistent, for example the study of Andrews et al. (2002) showed that gender does not play a role in the degree of influence[34]. Thus more

research is needed towards the gender differences in the susceptibility to social influences on alcohol consumption. There are of course inter-individual differences in the susceptibility to social influences due to genetic factors, which will be discussed later in this thesis.

Contrary to all the supportive effects of a social context on drinking behavior, there are also protective consequences of the social environment on alcohol consumption. For example a social context can function as a supportive social network, which can be an aide for abstinent alcoholics. Furthermore, the social control of parents might also lead to less alcohol use. These examples show that social contexts can also have protective effects on alcohol use and even isolation might lead to increased alcohol intake in some cases, an example is the separation from a loved one, which might increase alcohol intake[35].

Besides the effects of social contexts, there are also other factors that can affect the alcohol consumption. In fact there are four motives related to drinking, which are coping motives, enhancement motives, conformity motives and of course social motives. Social motives are often related to infrequent, light and non-problematic alcohol use. Conformity motives indicate drinking to avoid social disapproval, are just like social motives mostly shown during adolescence. Most drinking problems are related to coping motives and enhancement motives. Coping motives indicate that negative emotions are regulated or eliminated due to alcohol use and enhancement motives indicates that positive mood and well-being is enhanced. These two motives can result in heavy problematic drinking[36]. Therefore in alcoholism there is presumably little effect of the social context.

In the end, the social context and social behavior might play an important role in the development of an alcohol addiction, especially during adolescence. In fact, social behavior and alcohol have a bidirectional effect on each other, because the use of alcohol facilitates social behavior and a social context can support the use of alcohol presumably by emphasizing the positive effects of alcohol. Thus, in social circumstances more alcohol is more frequently consumed. Drinking with friends can also affect the consumption of alcohol but this depends on the role and quality of the friendship. The exact biological mechanism by which social circumstances can facilitates drinking and vice versa is unknown. Therefore, the goal of this thesis is to find out what the relation is between social behavior and alcohol, with respect to the neural substrate. The amygdala, a brain region which is important for emotion and memory might play a role in this. The latter suggestion leads to the main question: What is the role of the underlying amygdala mechanism on social behavior and alcoholism? Different topics will be discussed, for example the neural circuits and genes involved in alcoholism, animal models related to alcohol and social behavior, and there will be a focus on play behavior, which is a very characteristic form of social behavior. The consequences of play deprivation on social behavior, on the brain and on alcohol/cocaine consumption are also going to be discussed. In the end, the focus will be on the amygdala in relation to social behavior and alcohol, trying to find an answer on the main question.

Neural circuits of alcoholism
Alcoholism is like many other behaviors driven by negative and positive reinforcing effects. The negative reinforcing effects often consist of motivational and affective symptoms, such as irritability and emotional pain, which occurs during the absence of alcohol (withdrawal) and this is thought to support and sustain alcohol dependency. The positive reinforcing effects consist of the rewarding effect of previous experiences, for example alcohol-induced euphoria, that results in the re-use of alcohol[4]. The positive reinforcing effects of alcohol are among others mediated by the dopamine reward system, which is involved in the behavioral effects of all drugs of abuse. In general, the dopamine (DA) neurotransmission system is important for the reward of food and sex. This reward system, or so-called mesolimbic pathway consists of the ventral tegmental area (VTA), amygdala, hippocampus, (hypo) thalamus, nucleus accumbens (NAcc) and cortical areas, such as the dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), insula and the cingulate cortices [4,37]. These cortical areas have a modulatory function, which can be clearly seen in figure 1 (next page), where the arrows are pointing from the red box towards the NAcc and the VTA. The DA release of the mesolimbic pathway starts in the VTA, which is the main source of dopamine. The VTA

projects dopamine to the NAcc, the (hypo) thalamus and the cortices (figure 1). The consuming of alcohol is followed by an increased DA release in the NAcc and the frontal cortex. Dopamine is related to reward, thus an increase in the dopamine release results in increased reward and in an increased motivation for alcohol. In alcoholics dopamine can even contributes to a 'wanting' state[38]. The DA release and sensitivity differs per person and is among others based on the different DA receptors and transporters, for example the D2 receptors and DA transporters are lower in the NAcc in type 1 alcoholics compared to type 2 alcoholics, which result in a lower DA level[24,37]. Therefore, a possible explanation why type 1 alcoholics consume alcohol might be to compensate for this lower DA level (dopaminergic deficit), because more dopamine is released when alcohol is consumed. In persons with a higher amount of D2 receptors and DA transporters, less dopamine is needed to receive the same effect. A possible treatment for alcoholics with less D2 receptors and DA transporters is therefore a DA agonist[7,39].
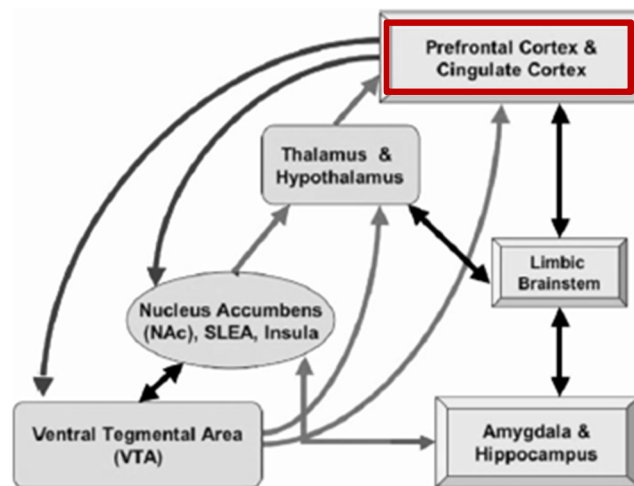

Figure 1. The mesolimbic pathway, where the cortical areas are modulating the Nucleus Accumbens (NAcc) and the Ventral Tegmental Area (VTA).The NAcc has a mediating function and when alcohol is consumed the dopamine release of the VTA is increased which results in a reward feeling and a positive mood. To aid in visualization, not all structures are shown. SLEA: sublenticular extended amygdala. (Figure derived of Makris et al. (2008))

Thus the mesolimbic reward pathway in the brain contributes to the reward and motivation of alcoholism, however the effects are also the other way around; alcohol has major effects on the brain. These effects are visualized with structural and functional magnetic resonance imaging (MRI), which showed that that chronic excessive consumption of alcohol results in neuronal modifications of the brain's structure, function and physiology. Alcoholics have significant deficits in the grey and white matter volumes and there is shrinkage of brain regions, especially in the prefrontal and frontal regions[40]. The shrinkage of the prefrontal and frontal regions might contribute to the increased social behavior and the disinhibiting effects of alcohol, because the frontal regions are normally important for cognitive control[41]. However, when there is shrinkage of these regions, there is presumably less cognitive control; less inhibition of behavior and more social expressions. Furthermore, the cerebellum of alcoholics is also decreased in volume (figure 2) and the fronto-cerebellar neuronal nodes and its connections are degraded. This is considered to contribute to a disruption of the executive functions, visuo-spatial impairments and ataxia. Other characteristics of an alcoholic brain are the enlarged ventricles (figure 2), especially the third ventricle[7,40,42,43].
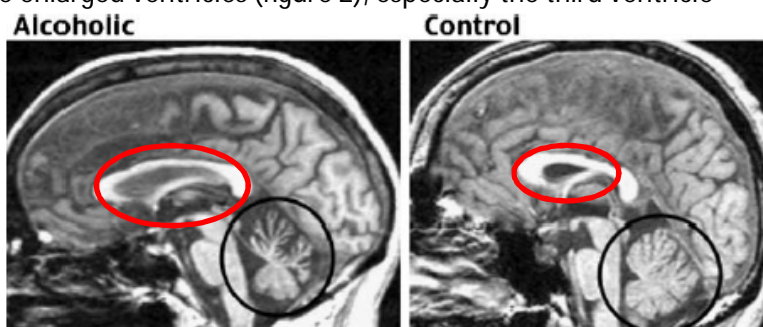

Figure 2. A mid-sagittal view of two male brains, age matched, showing the difference in volume of the cerebellum (black circles), the whole brain and the difference in lateral ventricle size (red circles). (Image derived of Sullivan and Pfefferbaum (2005))

Moreover, other brain regions which are affected by chronic alcohol use are the mammillary bodies, corpus callosum, amygdala, striatum and the hippocampus. These regions are all decreased in volume and there is neuronal loss related to alcohol use[44]. Different studies showed that the small amygdala of alcohol dependent individuals is a predictor for alcohol relapse and lower memory scores[4,5,7,37,42,43,45]. Fortunately for alcoholics, most structural and functional deficits in the brain are partly reversible when they abstain from alcohol. This applies also to the shrinkage of the reward related brain regions[37,40]. The fact that brain regions are affected by alcohol use can support the findings that alcohol affects social behavior, because this is modulated in the brain. Furthermore the relation between alcohol and the brain might contribute also to the fact that adolescents consume more alcohol compared to other age groups, since their brain is developing. Critical functional and structural maturation occurs during adolescence and this might explain why adolescents are less sensitive to alcohol sedation and more sensitive to alcohol related disruptions in memory[46].

Animal models
Animal models are useful to further investigate the underlying mechanism of social behavior and alcohol. In the literature most findings are related to the relation between stress, anxiety and alcohol consumption. However, Randall and Lester investigated the effect of social behavior on alcohol consumption already in 1975. They used two different mice strains, namely C57BL/6, which are well known for their high preference for alcohol and DBA mice, that are known to display an aversion to alcohol. The preference and aversion for alcohol is determined by genes and the results of this study showed that social rearing condition can alter the preference or aversion, because when a C57BL/6 weanling mouse was housed for 7 weeks with a DBA adult mouse, the alcohol consumption of C57BL/6 was decreased. And when a DBA weanling mouse was housed for 7 weeks with a C57BL/6 adult mouse, the alcohol consumption of DBA was increased. These results showed that social behavior affect alcohol consumption; the adult mouse's drinking behavior is presumably an example of how to drink and the weanling mouse will imitate this[47]. Thus in weanling mice social factors can influence the alcohol consumption and this effect is also seen in young rats that learn to consume alcohol in a social context[48]. However, based on these studies it is not sure if the alcohol intake of a weanling is enhance by social learning or by exposure to alcohol in the mother's milk or an alcohol consuming adult's breath. The study of Honey et al. (2004) showed that only social learning of an alcohol consuming adult to a weanling can enhance the alcohol intake and that the other influences, such as the mother's breath and/or milk do not play a role. They found this effect by different housing conditions for weanling rats, for example housing with lactating or virgin adult female rats that consume alcohol or water[48].

The previous findings are based on weanling rodents which suggest that social influences on alcohol intake are related to breeding and imitation. A study of Hunt et al. (2001) reject this, because they investigate the brief interactions with intoxicated rat siblings and it turned out that these sibling interactions increase the intake of alcohol in post-weanling and adolescent animals. The increased alcohol intake was probably achieved by alcohol related cues of the siblings (salivation, urination and perspiration) in combination with social contacts[49]. The effects of social influences on younger animals are also shown in humans, where children of alcoholics are more exposed to alcohol related cues at home and in social environments. These cues can affect the children's learning about the positive responses towards alcohol, because alcohol and alcohol related cues are directly related to home and is part of the normal daily life[50,51].

To come back to the animal research, there is also research done towards adolescents, social behavior and alcohol. For example, a study of Varlinskaya and Spear (2002) studied the effects of alcohol on social behavior (contact behavior, social investigation and play fighting) in adult and adolescent rats. It turned out that adolescent rats were more sensitive to alcohol-induced social facilitation compared to adult rats, when the blood alcohol level in both rats was the same. The adolescent rats were also more sensitive to the rewarding effects of alcohol in general, this suggests that the rewarding properties of alcohol are presumably further enhanced in a social context[52,53]. However, not all studies showed the increased alcohol intake due to social contexts, for example the

study of McCool and Chappell (2009) investigated post-weaning rats and found that social isolation increased alcohol responding presumably due to the increased anxiety like behavior. The increased expression of anxiety like behavior was measured by the elevated plus maze and it turned out that the isolated anxious rats also used more cocaine and amphetamine. Social isolation can also increase the intake of alcohol, cocaine or amphetamine in adulthood[13].

Based on different animal models, it can be concluded that alcohol use can affect social behavior and that a social context can affect alcohol intake. These effects are more promising in adolescents. However, more research is needed towards the exact mechanism by which alcohol and social factors affect each other, some suggestions for further research are focusing on genes, neural circuits, alcohol cues and anxiety. However, one major drawback of mice and rats is that little is known about bond formation and this makes it hard to further investigate the influence of social relations on the drinking behavior, as seen in humans[54]. An interesting species in this respect are the voles, in particular the prairie voles (*Microtus ochrogaster*; figure 3). The prairie vole is monogamous, has heterosexual pair bonds and has lifelong associations between family members, for example 70% of the offspring stays in the natal group and help raising their siblings. Affective behavior of the prairie voles is even high towards strangers, which is favorable for unmated voles[16,55]. Only 3% of the mammalian species is socially monogamous, which makes the prairie voles a very interesting animal model to study in the context of social bond formation. Monogamy in the free living prairie voles is indicated by sharing a home range, a common nest, mate guarding and low incidence of re-mating. In a laboratory setting monogamy is shown by more time spent with a partner than with a stranger in partner preference tests. Prairie voles do not only have social preference for their sexual partner, but also for their same sex cage mates. This can be explained by the natural behavior of the prairie voles, because during juvenile survival in the late autumn become communal groups the predominant social group[56–58]. All these findings make the prairie vole a useful model for studying the formation, effects and maintenance of specific pair bond relationships.

Figure 3. Two prairie voles for the visualization.

During the past decades, the prairie voles have been studied quite extensively for alcohol intake in a social context. In comparison with mice, the prairie voles reach the same intoxicated levels of blood alcohol concentrations when given the same amount of alcohol. However, prairie voles can drink higher levels of alcohol compared to mice, even when they experience noticeable behavioral effects of intoxication. The higher elimination rate of alcohol in prairie voles might play a role in this. The prairie voles drank even more compared to inbred mice with an increased alcohol metabolism and a pilot study of Anacker et al. (2011) suggest that prairie voles have a preference for alcohol. The combination of (high) alcohol intake and social bond formation makes the prairie voles a unique animal model, in particular because these behaviors are naturally. This makes genetic research interesting in the prairie voles, because they still have their original genetic variability and are not selectively bred for these behaviors. However, the genetic variability has also disadvantages since there is more variation in the high alcohol intake and social behaviors. To compensate this effect, relative large sample sizes are needed to observe significant effects[54].

Besides the prairie vole, also other voles have been characterized for their alcohol consumption phenotypes, for example the montane vole (*Microtus montanus*) and the meadow vole *(Microtus pennsylvanicus)*. These voles were not appropriate for alcohol and social behavior research, because the montane voles do not form pair bonds and the meadow voles showed different social behavior, drank lower doses of alcohol and have higher blood alcohol concentrations after the same dose of alcohol, suggesting a different alcohol metabolism as compared to prairie voles[59,60]. The pine vole (*Microtus pinetorum)* shared most properties with the prairie voles; it shows pair bonding behavior and is highly social. However, no literature is found about alcohol and pine voles, therefore more research is needed to find out what the alcohol mechanism of the pine voles is to make clear if the pine voles are an appropriate animal model. Up to now, the prairie voles appear to be the best model for biological mechanisms underlying social factors and alcohol use[60,61].

In alcohol studies with prairie voles a social facilitated alcohol intake was found, where the voles prefer alcohol over water when housed with a sibling. The preference and increased intake of alcohol was not present when siblings housed apart, therefore these findings indicate that voles housed together influence each other's alcohol intake[58]. The effects of social housing were also shown in a study with high and low drinkers, where high drinking voles decrease the intake and preference for alcohol when housed with low drinking voles. The difference in drinking level remained during the subsequent isolation period (figure 4) and because of the long lasting effects; this might be an interesting mechanism for the treatment of a drinking problem. Interestingly, there is variability between pairs, because low drinkers can also increase their intake due to housing with a high drinker. Therefore more research is needed to this influencing effect, because it might be related to the dominance of an animal. The same drinkers together (high with high or low with low) showed no influence on each other's drinking behavior[16,54,55]. The influence of a social context on alcohol drinking was only found in same-sex pairs and not in opposite-sex pairs. The influence of the social partner's sex on the drinking behavior is also applicable to humans, where difference in gender can negatively or positivity influence the alcohol drinking. However, the exact effect in humans in unclear due to contrary findings[14,16].
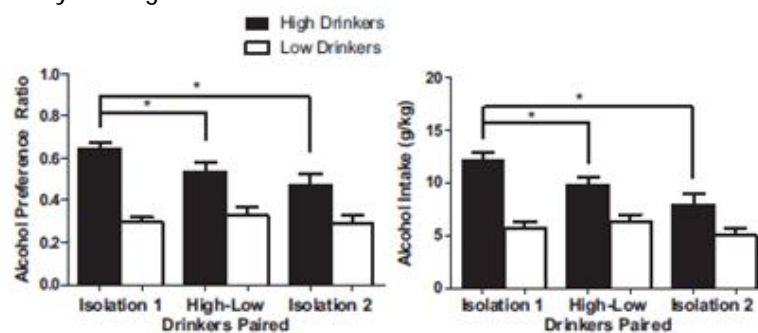

Figure 4. Alcohol preference and intake of high (black) and low (white) drinking prairie voles. After pairing, the alcohol preference and intake of the high drinkers is significant decreased and remains so, whereas the alcohol preference and intake of the low drinkers stays the same. (Figure derived of Anacker, Loftis, Kaur et al. (2011))

Other studies with prairie voles found that an isolation period of 4 days from a social partner leads to anxiety like behavior, which result in an increased alcohol intake. This effect is unique for prairie voles compared to other animal models, presumably because they display strong social bonds[14,54,62]. Therefore this animal model is functional for more specific alcohol research, such as binge drinking and the underling neural circuitry involved in social behavior and alcohol. Studies towards the neural circuitry underlying pair bonding and affiliation showed that this circuitry overlaps with reward and addiction in prairie voles. To be more precise, the dopamine transmission in the NAcc is important for social partner preferences and alcohol reward. This is mediated through circuitries that comprise striatal projections, thus the striatum has a mediating function in both[14,63]. Another similarity between the circuitries involved in pair bonding and alcohol use are the involvement of both dopamine receptor systems (D1 and D2)[55]. Furthermore, the study of Aragona et al. (2006) showed that D1 receptors in the NAcc prevent pair bond formation, whereas D2 receptors facilitate it. Socially housed prairie voles have greater NAcc dopamine D1-like receptor levels compared to isolated voles, which promote pair bond maintenance by selective aggression towards unfamiliar females. The effects of a D1 antagonist support these effect since it increases social behavior. These findings support also the idea that dopamine has not only a positive nature (reward), but also a negative one and is therefore important for motivation[64].

Another study towards the establishment of social bonds in praire voles showed that the neuropeptide arganine vasopressin (AVP) plays also role. This neuropeptide acts on the vasopressin 1a (V1a) receptor which is encoded by the AVPR1A gene. This gene contains polymorphic microsatellite regions (repeating sequence of 2-6 base pairs of DNA) upstream of the transcription site. The microsatellites influence the gene expression of the V1a receptors in many brain regions, which can affects social behavior[16]. Prairie voles have higher densities of V1a receptors in the ventral

pallidum and NAcc, compared to the less social meadow voles. These brain structures are reward related and implicated in drugs and alcohol use and the increase in V1a receptors enhance the social bond formation[59]. These results suggest that the AVP and the associated V1a receptors in the ventral pallidum and NAcc might play a role in social behavior and alcohol, however more research is needed to further confirm this, for example with AVP antagonists.

All these findings in prairie voles are a step further in the research towards the underlying mechanism of social behavior and alcohol use. Moreover, the prairie voles are also used for other studies with alcohol consumption, for example towards anxiety. It turned out that alcohol consumption elevates the mRNA expression of the corticotropin-releasing factor (CRF) receptors (CRFR1 and CRFR2) in the hypothalamus. The increased expression contributes to the 'stop signal' for drinking[54,65]. CRF is among others important in the hypothalamic-pituitary-adrenal (HPA) axis, which is involved in mood disorders such as stress and anxiety[66]. Isolated voles are more anxious and have therefore a hyper-reactive HPA axis, thus when there is increased expression due to alcohol, the 'stop signal' pathway for drinking is more easily activated. These findings indicate that separated prairie voles decrease drinking compared to paired voles[54]. This is contrary to most rodent findings, where the animals drink more in respons to anxiety. However, more research is needed to this topic, because more brain regions and circuits might be involved in anxiety.

In the end, the prairie voles are also a relevant animal model for the research towards different therapies of alcoholism. Naltrexone is studied in prairie voles, which is an opioid receptor antagonist and a pharmacotherapy for alcoholics. The opioid system normally influence the drinking behavior by increasing the positive rewarding effects of alcohol, via interactions with the mesolimbic pathway or by itself[4,67,68]. Naltrexone decreased alcohol preference and intake in prairie voles and it also decreases partner preference[54,61]. This finding suggesting that the same mechanism is involved as in humans, which validates the animal model. Furthermore, naltrexone also confirms that social behavior and alcohol share biological roots (presumably the opioid system), because the partner preference and the alcohol intake was lower in prairie voles.

In sum, the prairie vole animal model is a highly valuable model for the associations between social behavior and alcohol. The voles show high alcohol intake, alcohol preference and are sensitive to social manipulations, which is important for research towards excessive drinking. Based on previous findings, the prairie voles presumably share the same biological roots with respect to alcohol use and social behavior in humans. This makes the prairie voles an important animal model and further research in this specie will probably lead to a better understanding of the mechanisms underlying social behavior and alcohol and hopefully to more clearness about addictive behavior and socio-physiology, which is important for the psycho- and pharmacotherapy of alcoholism.

Genes

Besides the prairie vole animal model there is another way to study the underlying mechanism of social behavior and alcoholism, namely with respect to genes involved, for example towards the AVPR1A gene metioned previously. Nowadays genetics is an important research topic and it is interesting to discuss some genetic findings. As already mentioned in the introduction, genes can affect the development of alcoholism and also twin studies confirmed that alcohol dependency is heritable by 40-60%[69]. There are many genes related to alcohol use, alcohol use disorders and social behavior, since a human genome contains around 20.000 protein encoding genes. For example, in the amygdala are already 83 genes found which differs in expression in response to alcohol and 23 additional genes are found implicated in an addiction[70]. One example of a gene which plays among others a role in the susceptibility to alcohol and alcohol related cues is the dopamine receptor type 4 gene (DRD4)[71]. This gene contains tandem repeats (2 to 11 repeats) at exon 3. When the long allele (≥7 repeats) is present, the ligand binding of the D4 receptor is altered, which result in a blunted inhibiting effect of the D4 receptor (see figure 5, next page). This in turn, results in an attenuated response to dopamine in the ventral striatum and in an addiction related phenotype, because more reward is obtained. Long allele carriers also experience more craving, which makes it hard to overcome the alcoholism[19]. Van der Zwaluw et al. (2012) did an interesting study towards the

interaction between the DRD4 genotype and the influence of friends' drinking behavior. They looked at whether this interaction predicted the development of alcohol use in adolescents. Results showed that when friends consume higher levels of alcohol, this leads to higher levels of alcohol consumption during adolescents. There was no moderation of the DRD4 genotype[72]. This was the first study which observed the effect of alcohol related genes and the influence of social behavior, therefore replication is needed. However, it is an interesting topic, because it might lead to more answers about the underlying mechanism of social

Figure 5. The dopamine D4 receptor gene, with the 7 repeat allele.

behavior and alcohol use. Recently a novel gene associated with alcohol consumption is discovered, namely the YWHAZ gene. This gene encodes for the adapter protein 14-3-3ζ in the amygdala and is important for neurodevelopment and neurodegeneration. The gene expression was up-regulated during escalated alcohol use which suggests that this gene and the amygdala might play a role in the development of alcoholism[73]. This gene is going to be discussed in more detail later on in this thesis.
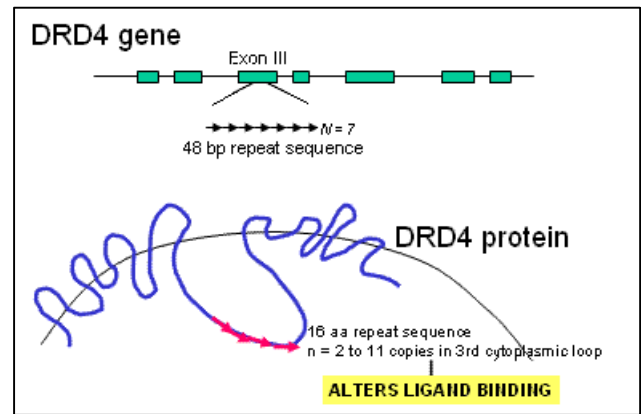
Social play
To find out more about social behavior and the underlying mechanism, the focus of this chapter will be on a very characteristic form of social behavior, which is play behavior. Play behavior is seen in different mammalian species and it occurs most of the time in juveniles. Social play is the earliest form of social behavior which is directed to peers (especially same-age conspecifics) and it indicates a healthy development, because social play is relevant for a normal development of adult social behavior. Play behavior includes rough-and-tumble play, play fighting, climbing and chasing [50,74–78].

At first sight, social play seems to be a purposeless behavior, because it costs a lot of energy, more accidents occur and the individuals are at a higher risk of predation. However, research in juvenile rats (*Rattus norvegicus)* and mice *(Mus musculus)* found different positive effects of play behavior. First of all, play behavior might support plasticity of emotional, motivational and hormonal changes. This effect is derived of the fact that social play occurs most of the time in adolescent animals, which is confirmed by the occurrence of the key element of social play in rats: *pinning*. The pinning behavior follows an inverted U-shaped distribution, starting 18 days after birth, peaks between 4 and 5 weeks of age and decreases till sexual maturity (figure 6)[74,75,77,79–84]. Furthermore,

play behavior might support the male's sexual maturity, such as having sex and establishing dominance by means of aggression. This positive effect of play is derived of the fact that social play, in particular rough-and-tumble play is mainly seen during adolescence and occurs mostly in males. However, when the supporting effect on the male's sexual maturity was true, the animals would show more difficult aggressive moves and intromission during play, but this was not the case. Although aggressive and sexual behavior can be recognized in social play, it differs in form, contextual setting and intensity from adults[74,75,79,82].
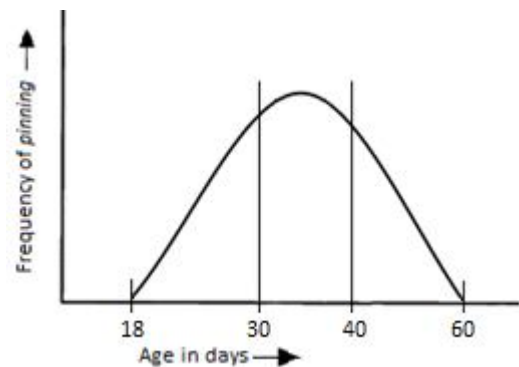

Figure 6. The U-shaped distribution of social play behavior (pinning).

Other reasonable explanations for social play may include learning to understand the other's intentions, maintaining group cohesion, reducing stress and it is a way of self-assessment, because it needs immediate feedback of the own behavior. For example, rough-and-tumble play has indirect effects on the refinement of the sensory-motor circuits, increases a better awareness and it helps to develop a flexible use of different capacities (physical, social and cognitive) in various circumstances. Furthermore, studies with play deprivation experiments showed among others decreased adult

social interactions. This suggest that social play is essential for the development of normal social, aggressive and sexual behavior in rats[75,77,80,83,84]. In rats, the most prominent social effect of play deprivation is the play rebound, where play deprived rats play more when reunited with other rats. This is the case when rats were play deprived due to isolation and also when play was prohibited. The rebound effect suggests that there might be a mechanism which manages the amount of play, to obtain a sufficient amount which is needed to receive the benefits of play. If this mechanism hypothesis is true, it might be sensitive, because the rebound effects are already seen after 20 hours of play deprivation. However, the rebound effect can also suggest that social play is rewarding and that the rats therefore compensate after a period of reduced opportunity to play. In the end, the rebound effect also occurs in the natural environment, for example to compensate for any play losses due to poor weather, food shortage and illness.[74,75,79,81,84,85]

The fact that social play is necessary for a normal development of adult social behavior is confirmed by a study where rats were isolated and this resulted in a disturbed social development. For example, less anogenital sniffling and less approach behavior due to play deprivation was shown in these adult rats (for example, see figure 7), which indicates deficits of sexual behavior[77]. However, the timing of play deprivation plays an important role in the effects. When play deprived in week 4, less social play is acquired and developed (acquisition phase), but when deprived in week 5 the play acquisition was not affected (maintenance phase). Therefore, these results suggest that there might be a sensitive period for the development of social and sexual behavior in rats at the age of 4 weeks. Play deprivation in this week also results in more aggression to members of different species[79,80].

Figure 7. The effects of isolation during week 4 and 5 on social behavior in rats. The rats were tested in week 6 and data is expressed as mean number ± SEM. Anog. snif = anogenital sniffling of the test partner, approach = approach of the test partner, social expl = social sniffing and grooming of the test partner and non-social = self-grooming, rearing and exploration of the test case. (Figure derived of van den Berg et al. (1999))

In sum, play deprivation affects a great variety of social behaviors, most rats become socially incompetent as adults. Important to note is that all the effects of play deprivation described in this thesis are permanent and that play deprivation experiments should be interpreted with care. Because isolated animals have continuous experiences on top of the play deprivation, such as physiological and behavioral modifications. These experiences can also alter the development, although experiments with drugged or non-playful peers were also performed and those experiments confirm the effects of social incompetent effects due to play deprivation[74,75,81]. To further understand the mechanism behind the effects of social play and play deprivation, the reported effect on the brain are described below, even as the relation with alcohol and cocaine use.

Social play in the brain

Based on the previous described rebound effects in rats, the suggestion was made that social play is rewarding, which can be confirmed by the effects in the brain. Social play is a natural reinforcer and that is why young mammals invest a lot of energy and time in playing together. Rats even show to prefer locations where they have played before[79,86]. The rewarding effects of social play are thought to be mediated by neurotransmitter systems that are widely implicated in reward processes, such as dopamine, opioids, cannabinoids and norepinephrine[76,77,86]. All these neurotransmitters act on subcomponents of reward, for example hedonics (liking), cognition (learning) and motivation (wanting) (table 1, next page). The role of dopamine implicated in reward of social play is confirmed by haloperidol, a dopamine receptor antagonist, which indeed decreases social play and suggest that an optimal level of dopamine function is needed for social play to occur[74,76,86]. The involvement of endogenous opioids on the reward play is also confirmed by an antagonist, namely naloxone, which suppresses social play behavior. In general there are three classes of endogenous opioids;
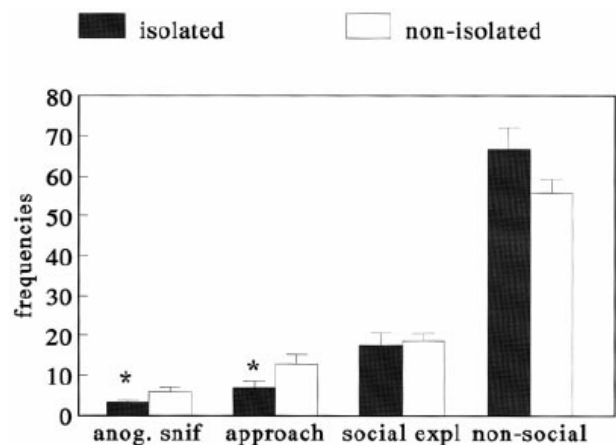
11

endorphins, dynorphins and enkephalins, all with different opioid receptors (μ, κ and δ)[4,67,68]. Naloxone acts competitively on all these receptors. Opioids play an important role in the brain development, such as an increase in neural and glial proliferation, which is relevant for social play behavior during adolescence[75,76]. Therefore, there is also research done toward the specific opioid receptors, for example with morphine, which is a μ-receptor agonist and (as expected) it stimulates social play. An important note is the fact that only mild increases of opioids support playfulness, whereas high levels of opioids reduce play[74,76,82,83,86–88]. The supporting effect of opioids on social play was also found the other way around, because play itself reduces the μ-receptor binding sites in the NAcc. Less binding sites suggest more opioid release, which probably indicates an increased reward, because opioids have rewarding properties and the NAcc is part of the reward pathway[75,89]. The finding that the μ-receptors are involved in social behavior is also confirmed by a study of Vanderschuren et al. (1995), where binding sites of the μ-receptors where measured with autoradiography in isolated and non-isolated animals. After seven days of isolation, more binding sites were available for the μ-receptors in the medial prefrontal cortex (mPFC) and the parafascicular area, suggesting that isolation causes an up-regulation of the μ-receptors in these brain regions or an decrease of opioids, which in turn results in more available binding sites[90]. The study of Van den Berg et al. (1999) confirms the up-regulation of μ-receptors due to isolation in the basolateral amygdala and the bed nucleus of the stria terminalus. These brain regions are also implicated in the reward pathway and therefore support also the involvement of opioids in reward and motivation of play[91]. Further research was done towards the κ- and δ-opioid receptors. The κ-receptor showed the opposite effect of the μ-receptor; during isolation fewer binding sites became available, presumably because activation of the κ-receptor reduces social play. The δ-receptors showed no difference in binding sites. Thus in general, the rewarding effects of social play mediated by the opioid system depends on the μ- and κ-receptors[88,91].

Table 1. Social play reward and the associated neurotransmitter system. (Trezza et al. (2010))

Subcomponent of social play reward	Neurotransmitter
Motivational aspects	Dopamine
Pleasurable effects (hedonics)	Opioids and cannabinoids (depend on neural site of action)
Cognitive aspects	Dopamine, opioids, cannabinoids, norepinephrine (different neural levels)

As shown in table 1, cannabinoids also mediates the reward related to social play. However, cannabinoids can both, increase and decrease social play due to different mechanisms of action. The effect of cannabinoids depends on how the endocannabinoid system in the brain is stimulated. Direct stimulation by cannabis, inhibits social play, however release of endocannabinoids on demand facilitates social play[76,92]. Most cannabinoid receptors are found in the NAcc and the amygdala and those regions are both involved in emotion, motivation and reward of play (part of the mesolimbic pathway). Therefore the Nacc and the amygdala are thought to mediate the stimulatory effects of endocannabinoids on social play[86,93,94]. Furthermore, norepinephrine (noradrenaline) has also modulatory effects on play, which is in particular confirmed by the noradrenaline reuptake inhibitor atomoxetine. Atomoxetine suppresses social play and this might suggest that norepinephrine itself suppresses play, since there is more norepinephrine available due to the inhibited reuptake[86,95]. This effect of noradrenaline is also supported by the fact that activation of alpha-2 noradrenergic receptors suppresses play[76,78].

The previous findings with neurotransmitters support the fact that social play is rewarding and therefore it can be suggested that play deprivation does not only alters the normal adult social behavior, but also affect the normal development of motivational processes. Besides disturbances in social competences, it can also result in cognition and emotion disturbances[75,96]. Furthermore, the fact that social play is rewarding is also confirmed by lesion studies, where especially lesions to the ventromedial hypothalamus, dorsomedial thalamus decrease the motivation to play. An explanation

for this effect might be that the thalamus is part of the mesolimbic pathway (see figure 1), therefore lesions to this brain region can disturb the pathway and might results in less reward and thus less motivation to play[97]. Lesions of the neocortex do not seem to influence social play, but lesions to somatosensory cortex in particular to the parietal cortex do affect social play. For example in the study of Panksepp et al. (1994) where a substantial part of the parietal cortex was bilaterally removed, which resulted in a 65% decrease in the pinning behavior during social play[97,98].

Besides lesion studies, the involvement of specific brain regions in social play can also be confirmed by an increasing in *c-Fos* levels. *C-Fos* is an immediately early gene which is expressed relatively early (of mRNA) when neurons are highly active[75]. The measuring of *c-Fos* activity is widely known as a neuronal activity marker, for example growth factors, depolarization and receptor activity induce *c-Fos* expression. However, it is important to keep in mind that neuronal activity can also occur in the absence of *c-Fos* expression and that chronically activated neurons not show *c-Fos* expression[94]. When social play was performed, the *c-Fos* activity was increased in different brain regions (table 2). Based on the *c-Fos* activity, van Kerkhof et al. (2013) suggested that the projections from the medial prefrontal cortex to the striatum are involved in social play behavior, even as the projections from the amygdala to the frontal cortex and the striatum. In this study rats were isolated for 24 hours to enhance their motivation to play, thereafter the rats were placed in pairs or alone for 15 minutes and the *c-Fos* activity was defined 30 minutes after that[94].

Table 2. *C-Fos* gene is more activated in these regions in animals that had played compared to those who did not play. The increased *c-Fos* gene activity indicates more plasticity and thus more development due to social play. [37,75,86,94,96]

Brain region	Function	Play
Medial prefrontal cortex	Connections with motor and rewarding brain areas	Social self-monitoring during play
Parietal cortex (primary somatosensory cortex)	Somatosensory areas	Refined during play
Medial and ventral orbitofrontal cortex	Cognitive processing of decision making	Fast decisions have to be made
Ventromedial nucleus (hypothalamus)	Involved in fear, feeding and sexual activity	Social play reduces fear and support the development of sexual behavior
Dorsomedial parafascicular (thalamic nucleus)	Influences behavioral flexibility and processes somatosensory inputs and outputs to frontal cortex and striatum	Flexible behavior is needed to reacts on the changeable circumstances
Hippocampus	Part of mesolimbic pathway and involved in spatial memory	Reward and memory are important for social play
Amygdala	Part of limbic system and involved in memory and emotion	Reward, memory and emotion are important in play
Dorsolateral and deep tectum	Important for defensive behaviors	Defensive behavior and awareness is needed during play
Dorsal striatum	Essential for sensory-motor integration	Important for the body movements
Ventral striatum (nucleus accumbens)	Reward seeking and appetitive eagerness	Involved in the reward aspects of play
Dorsal periaqueductal gray	Mediating aversive stimuli	Arouses defensive reactions
Inferior colliculus	Receives auditory and somatosensory information	Mediates social play

The involvement of the medial prefrontal cortex (mPFC) and the orbitofrontal cortex (OFC) in social play is not only confirmed by increased *c-Fos* activity, but also by a study of Bell et al. (2010) where they looked at the complexity of the neurons. This study showed that the neurons of the mPFC and the OFC are both in a different way related to social behavior and play. The neurons of the OFC were more complex when juveniles were exposed to multiple animals, whereas the neurons of the mPFC were more complex in response to play experiences. This suggests that the OFC is more sensitive to

the number of individuals exposed during adolescence, whereas the mPFC is more sensitive to the type of interaction, for example social play. This finding showed among others that play deprivation can change brain regions, resulting in deviations of social behavior in adulthood. It also showed that social play can have an influence on the plasticity of the brain, because the social experiences can affect the neurons[81].

As mentioned before, social play occurs more often in males. Many of the brain regions with increased *c-Fos* expression due to social play are sensitive to sex hormones[75] and research towards this effect showed that the sex differences in rough-and-tumble play are partly due to the neonatal exposure to androgens[83]. This was confirmed in a study of Thor and Holloway (1986) where female rats were exposed to androgens and they engaged in social play at the same level as males[99]. The amygdala is suggested to be the site of action, especially the medial amygdala, because lesion to this region suppress social play in male rats and unaffected the play behavior in female rats[75,83,100]. Furthermore it is found that the release of testicular androgens during play is also necessary to perform male-like levels of play behavior[75,101]. Based on the supporting effects androgens on social play in adolescence, it can be suggested that social play is in particular important for the development of a typical male brain and typical male behavior. However, more research towards the underlying mechanism of this effect is needed to confirm it[75].

In the end, all these findings on brain level are mostly found in rats and mice; however social play might also acts on the maturation of the human brain. Some evidence for the effects of social play in humans is shown by the fact that '*deficits in social play*' is a symptom of different neuropsychiatric disorders in childhood and adolescence, such as attention deficit/hyperactivity disorder (ADHD), autism and early onset of schizophrenia. Nonetheless, more research is needed towards the effects of social play on the human brain (and the maturation), for example with special imaging techniques[76,96]. In sum, based on the findings in rats and mice social play is mediated by neural networks involved in reward, cognition, emotion and motivation. Social play can have in its turn an effect on the development of specific behaviors and brain regions.

Alcohol, cocaine and social play

To return to the main topic of this thesis: social behavior and alcoholism, it might be interesting to see if there is any research done towards the effects of alcohol (and/or cocaine) on the characteristic form of social behavior 'social play' and vice versa. As seen in the previous chapter social play is sexually dimorphic; it occurs more often in males. However, when juveniles were prenatally exposed to alcohol this dimorphism was abolished, resulting in females who showed more masculinized behavior (more social play) and males who showed more feminized behavior of play (less social play). This finding suggest that prenatal alcohol exposure affects social play later in life[102].

Not only prenatally, but also during life has alcohol effects on social play. Biphasic effects on social play were shown, which was also seen in social behavior where low doses of alcohol supports social behavior and high doses of alcohol suppresses (social) behavior. This effect is the same for social play, because a lower dose of alcohol (0.25 g/kg and 0.50 g/kg) increases the play behavior, whereas a higher dose of alcohol suppresses social play (figure 8)[87]. Social play is more sensitive to alcohol than other social behaviors, which indicates that social play is a separate category of behavior. Furthermore, social play and alcohol are both mostly seen during adolescence which might make it more easier to influence each other[87].
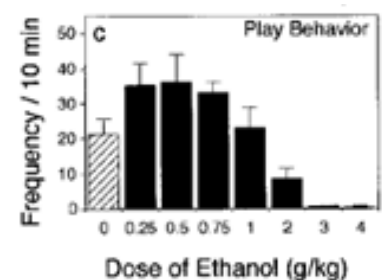
Figure 8. Frequency of play behavior during 10 minutes test session after acute alcohol administration at day 30 of age. (Figure derived of Varlinskaya et al. 2001)

Another point which can make clear that alcohol and social play can affect each other is with respect to the neurotransmitter level. For example, opioids are involved in the rewarding effects of social play and alcohol[76]. Acute alcohol administration increases the release of endogenous ligands for the μ opioid receptor in the hypothalamus and pituitary gland. The previous chapter showed that activation of the μ-receptor increases social play, thus this results in more play due to alcohol use. However, as mentioned before, it depends on the alcohol dose, because a low dose of alcohol has a

stimulatory effect on the opioid release and a high dose an inhibitory effect. Therefore this might be the underlying mechanism of the biphasic effects of alcohol on social play and social behavior[87]. Thus alcohol and social play share the same opioid mechanism, however one major difference is that play is biological relevant for the brain whereas alcohol is not; it disturbs the natural reward pathway[81]. Another effect of alcohol on social play is the suppressing of the development and refinement of the somatosensory cortex. The neural activity in this brain region is decreased due to alcohol exposure (lower *c-Fos* levels) and this affects social play[84]. However, this occurs only at high doses of alcohol, which support the biphasic effects of alcohol on social play.

Some studies focused also on the effect of cocaine on social play and play deprivation. Cocaine has, just like other addictive substances, rewarding properties and it acts on the natural reward pathway. The rewarding effects of cocaine are thought to involve dopamine released from the nucleus accumbens. A difference with alcohol is that cocaine prolongs the dopamine activity by an increase in release and by blocking the reuptake of dopamine, which results in a decrease in social play[103]. Cocaine also stimulates the release and inhibits the reuptake of serotonin and norepinephrine, which also affects play[76,78]. Only low doses of cocaine suppress social play and no other social behaviors, thus some effects of cocaine are (just like alcohol) dose dependent. Studies with dopamine, serotonin and norepinephrine receptor antagonists showed that a combination of antagonists is needed to affect social play[78]. This presumes that a combination of the monoamine receptors is involved in the suppressing effect of cocaine on social play. However, the exact mechanism of the suppressing effect of cocaine on social play is still unclear. Different explanations are devised, for example that cocaine evokes locomotor hyperactivity by itself and this might result in behavioral competition with social play. Nonetheless, the latter explanation is unlikely, because the low dose of cocaine which affects social play is too low to induce psychomotor hyperactivity. Another explanation for the suppressing effect of cocaine on social play is that the rewarding effects of cocaine may substitute for the rewarding effects of social play or that cocaine enhances the attention for non-social stimuli and thereby reduces social play. Another hypothesis is that simple behavior is preferred over complex behavior (social play) due to cocaine[78]. In sum, cocaine reduces social play probably by an increasing in dopamine, serotonin and norepinephrine. However, the exact explanation for the reduction in social play is unknown. Different hypotheses were made and more research towards the exact mechanism is needed to confirm or reject the hypotheses.

To conclude, alcohol and cocaine both influence social play and are behaviorally specific; they do not affect other social behaviors. Alcohol has a biphasic effect on social play, where low doses of alcohol increases social play and high doses suppress it. In contrast to this effect, only low doses of cocaine can affect social play. The relation between in alcohol and social play can also be seen in humans; however no biphasic effect was shown. For example children exposed to alcohol, such as children with fetal alcohol syndrome, show alterations in play due to different sensory stimulations.

The amygdala

The title of this thesis is '*The role of the amygdala in social behavior and alcoholism*' and based on the previous chapters, it is shown that the amygdala is part of the mesolimbic pathway, which is important for the reward derived of alcohol and social play. Furthermore, the amygdala is important for the sexual differences in play; the androgens act mainly on the amygdala. This chapter will discuss in more detail the exact role of the amygdala in social play and alcoholism.

First of all, the amygdala, which is widely known for its involvement in memory, fear and emotional processes, consists of different nuclei. The lateral (LA), basolateral (BLA), basomedial (BMA), the central (CeA), the medial (MeA) and the cortical (CoA) nucleus (figure 9). The basolateral, central, basomedial and medial amygdalae are also called the *extended amygdala* and the cortical nucleus is also called the *olfactory amygdala*, due to its
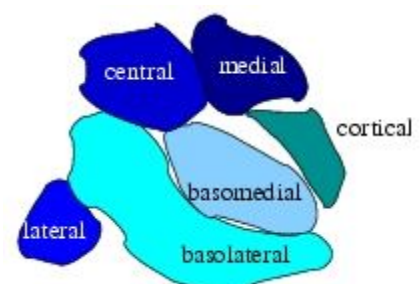
Figure 9. The different nuclei of the amygdala

15

input. The central and medial nuclei are connected to the bed nucleus of the stria terminalis (BNST; part of the hypothalamus) by a strand of fibers called the stria terminalis. These nuclei are also connected to the nucleus accumbens shell and VTA[104,105]. All these different nuclei of the amygdala can have a different function in the involvement in social play and alcoholism.

Play
One of the previous chapters about social play and brain showed that the mesolimbic pathway is important for normal social play behavior. This pathway includes the amygdala and because the amygdala is also related to positive emotions involved in play, it might be important for social play. The importance of the amygdala in social play is confirmed among others by the correlation of the size of the amygdala with social play (and alcoholism), because a larger amygdala is correlated to more time spent on social play in non-human primates. For example, the MeA in males, which is probably the site of action of the androgens and regulates many socio-sexual behaviors, is 10% larger compared to the MeA of age-matched females[86,94]. Furthermore, studies with neonatal and electrolytic lesions of the amygdala show that the amygdala indeed affects play behavior, because these animals were not responsive towards social stimuli anymore. This effect was only seen in juvenile males where it decreases social play (no other social behavior) and no effect was observed in juvenile females [74,83,86,94]. All these results support the fact that the amygdala is important for normal social play, especially in males. However, the amygdala can also be affected by social play, which is shown in a study of Trezza et al. (2012). This pharmacological study focused on the endocannabinoids and, as mentioned before, those act mainly on the amygdala and NAcc. Experiments with inhibitors, blockades and infusions of endocannabinoids showed that endocannabinoids are important for the stimulation of play and for the modulation of the reward of social play. Trezza et al. (2012) showed that social play can also increase the level of endocannabinoids neurotransmitters (anandamide) by itself. In particular in the amygdala and NAcc and it can increase the phosphorylation of the cannabinoid receptors in the amygdala, these effects supports social play[93]. In sum, social play and the amygdala have presumably bidirectional effects on each other; social play might be stimulated by the amygdala and social play can increase the anandamides in the amygdala itself (which increases play).

Furthermore, the importance of the amygdala for social play was also confirmed by an increasing in the *c-Fos* activity (table 2). However, the *c-Fos* activity was not increased in the whole amygdala but only in the LA and the BNST after a play session in rats. The *c-Fos* activity was also increased in the BLA after social play, but this was correlated to the activity in the striatum (where the BLA connects to). This suggests that amygdala-striatal projections are involved in social play behavior, in particular in the rewarding aspects of social play[83,94]. The *c-Fos* activity in the BLA was also correlated to the mPFC and the OFC, which suggests also a role for the amygdala-frontal connections in social play. These findings are supported by increases in brain derived neurotropic factor (BDNF) in the dorsolateral frontal cortex and in the amygdala in playful rats[75,86,94]. The associations between the frontal regions and the amygdala might suggest that cortical (controlling) regions are needed for the reward control of social play. However, the latter is just a suggestion and needs further research.

To conclude, based on the findings described here, the amygdala presumably plays a role in the modulation of social play. This might be done via the rewarding properties of play; however, social play can also affect the modulation of reward by itself, via the release of anandamides in the amygdala and this result in increased play behavior.

Alcohol
Based on the chapter *Alcohol, cocaine and social play* there are some suggestions that the amygdala plays a role in alcohol use and alcoholism, for example because of the involvement of the amygdala in the mesolimbic pathway, which mediates the reward of alcohol (just like social play). Other findings about the amygdala and alcohol are discussed below, to further understand the relation between the amygdala and alcohol.

First of all, the amygdala might play an important role in the regulation of alcohol behavior, because dysfunction or lesions of the amygdala causes impulsivity (due to the positive reinforcing effects of alcohol) and compulsivity (due to the negative reinforcing effects of alcohol dependence). The involvement of the amygdala in positive emotions was surprising, because the amygdala is widely known to be involved in negative emotions. However, more research nowadays confirmed the involvement of the amygdala in positive emotions[69]. Based on the involvement of the amygdala in emotions, it is not surprising that the amygdala plays probably a role in the emotional aspects of craving experienced by alcoholics[4,106]. Furthermore, the amygdala might also play a role in the withdrawal of alcohol, because withdrawal leads to impaired fear conditioning and exaggerated anxiety response and the amygdala is among others involved in fear and anxiety. The underlying mechanism of impaired fear conditioning due to the withdrawal of alcohol is presumably as follows; when alcohol is consumed, the GABA activity is increased and to compensate this, the glutaminergic activity is also increased. The latter causes tolerance to alcohol and is only shown in addicts. When there is withdrawal of alcohol, there is less GABA activity but still increased glutaminergic activity, which indicates an imbalance. This overactivity of glutamate is also found in the process of Long-Term Potentiation (LTP), which causes enhancements in signal transmission between two neurons that fire synchronously. Thus due to withdrawal, there might be more LTP in the amygdala and this elicits more anxious responses. Therefore withdrawal can affect associative fear learning and this effect can have major consequences for an alcohol treatment, because associative (fear) learning is important in some treatments for alcoholism with respect to alcohol related cues[107].

Moreover, as mentioned earlier, the amygdala can also be anatomically affected by chronic alcohol use, where a smaller amygdala (due to neuronal loss) might even predict alcohol relapse. The effect of alcohol on the size and volume of the amygdala is also seen in humans (alcohol dependent versus non-dependent humans) using imaging techniques (figure 10)[4,37]. There is also evidence that a distinction can be made between the different nuclei of the amygdala with respect to alcohol. For example the extended amygdala is important for the modulation of behavioral motivation and reward of alcohol and it plays also a role in avoidance learning and somatosensory activities, which are important factors to develop an alcohol dependency[70]. Furthermore, acute and chronic alcohol intake can result in physiological changes in the central nucleus of the amygdala, because of the increase in GABA transmission[4].

In sum, the amygdala might be important for the negative and positive emotions involved in alcohol use, which in its turn is important for the regulation of alcohol behavior. The withdrawal of alcohol can also be affected by the
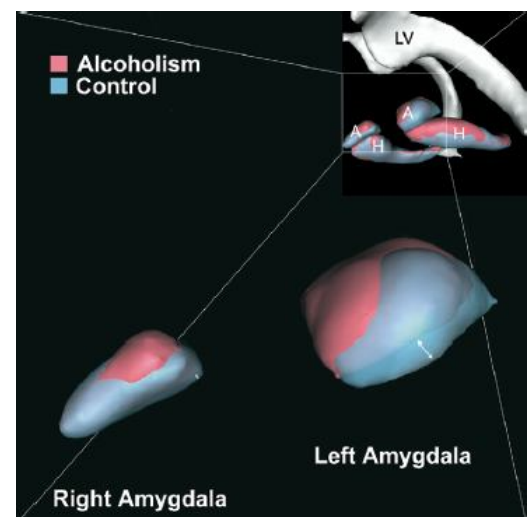

Figure 10. The average shape and volume of the amygdala (A) and the hippocampus (H) of controls and alcoholics (blue minus red). Showing the volume reductions in alcoholism, which is more pronounced in the left amygdala. (LV = lateral ventricle). (Figure derived of Makris et al. (2008))

amygdala, especially the associative learning. On the other hand, alcohol itself has also major effects on the amygdala, because chronic alcohol use can result in physiological changes. More research towards the amygdala in relation to alcoholism is important, because the amygdala might even be of greater importance. For example the connections of the amygdala among others to the ventral tegmental area, nucleus accumbens and prefrontal cortex (figure 1) suggest that there is a possibility that the amygdala modulates alcohol-motivated behavior. Additionally, connections of the amygdala to the hypothalamus and brainstem suggest also that there might be an involvement of the amygdala in stress and arousal, which can promote alcohol consumption[73]. To found out more about these possible functions of the amygdala, it might be useful to look at gene expressions, because for example the activation of the amygdala during social play is among others confirmed by the expression of the earl *c-Fos* gene. Lesscher et al. (2012) studied gene expression in the amygdala and looked at the combination of different genes and alcohol consumption. Most gene expressions occurred one week after the start of alcohol consumption, when the alcohol intake escalates. One

gene was clearly up-regulated during the transition from low to high alcohol intake, namely the adapter protein 14-3-3ζ[73]. The gene which encodes for this adapter protein is the YWHAZ gene, which is located on chromosome 8q23.1 in humans. The YWHAZ gene is for 99% identical to the gene in mice, rats and sheep and this gene expression is involved in the regulation of kinases, proteases and intracellular transport[105,108]. 14-3-3ζ is furthermore related to neurodevelopment and is a biomarker for different neuropsychiatric disorders, such as depression, autism and epilepsy[109,110]. For example, 14-3-3ζ is present in the CSF of rats after seizures and the amount which is present depends on the severity of the seizure. 14-3-3ζ can be demonstrated by the use of the sc-1019 antibody (figure 11)[111].
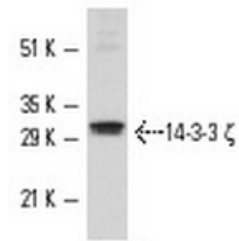

Figure 11. Western blot of the 14-3-3 ζ expression with sc-1019 antibodies. (Figure derived of Santa Cruz Biotechnology Inc.)

The 14-3-3ζ protein which belongs to the 14-3-3 protein family has different binding partners, such as signaling molecules, transcription factors and ionotropic receptors. Those binding partners can also be involved in alcoholism and therefore 14-3-3 proteins are probably able to affect the addiction. A knockdown of the protein 14-3-3ζ in the amygdala, using RNA interference, supports this effect, because the alcohol intake was dramatically increased due to the knockdown and it promotes inflexible alcohol consumption. This effect was only observed for alcohol and not for other natural rewards. The increased and inflexible alcohol intake due to the knockdown in the amygdala suggest that the adapter protein 14-3-3ζ is involved in the control over alcohol intake[73]. Another study might confirm this regulation effect, because a study Carnahan et al. (2013) also showed an up-regulation of YWHAZ in relation to higher doses of alcohol, however this study was performed with neurospheres[108]. Therefore, more studies are needed to confirm the theory that the adaptor protein 14-3-3ζ in the amygdala has a controlling/regulating effect on the alcohol intake.

One study in humans by MacKay et al. (2011) confirms the fact that 14-3-3ζ is also in humans related to extended alcohol use, however there was a significant lower expression found in the motor cortex. This finding is contrary to earlier findings, probably because there are brain region dependent changes in the expression of 14-3-3ζ. Furthermore, they only looked at the dorsolateral prefrontal cortex and the motor cortex; no measures were done in the amygdala. The decreased expression of 14-3-3ζ can suggest that there might be less inhibition of the motor cortex (less control) due to increased alcohol intake, which might also be the result of neurodegeneration due to increased alcohol use[112]. However, the latter is just a speculation based on the other findings, because the exact mechanism by which 14-3-3ζ affects (or is affected by) alcohol intake is still unknown. Further research towards the mechanisms of 14-3-3ζ might focus on the signaling molecule protein kinase C (PKC), because PKC can interact with 14-3-3ζ. Or a research focus can be on the glutamate receptors, since 14-3-3ζ can also interacts with these receptors and is able to affect the activity of it. The interactions of 14-3-3ζ with the glutamate receptors might also be derived of the fact that the genes GABRB3 and GRIA3 (encoding for glutamate receptors) were also up-regulated in the amygdala during the escalated alcohol intake[73]. Furthermore, future research can also focus on the GABA receptors, because 14-3-3ζ can also bind to these receptors which result in an affected GABA neurotransmission[73,112]. In sum, the exact reason for the up-regulation of the adapter protein 14-3-3ζ in relation to increased alcohol intake is unknown. Different explanations are devised, for example a controlling function of 14-3-3ζ or an involvement in the reward of alcohol, however all these explanations need further research to find out what the exact function of 14-3-3ζ is. Finally, it is clear that the amygdala plays an important role in alcoholism and the findings of the adapter protein 14-3-3ζ show that the amygdala might play a more important regulating role in alcoholism compared to what we know so far.

Conclusion

Social behavior and alcohol use have bidirectional effects on each other; alcohol can facilitate social behavior and a social environment can stimulate alcohol use. These effects are probably caused by an increased reward derived of the combination of social behavior and alcohol use. This finding

especially applies to adolescents and it is relevant for the prevention of an alcohol problem, because an alcohol problem often starts during adolescence. However, the relation between alcohol and social behavior is not very relevant for most alcoholics, because they have often other drinking motives. Social environments can only contribute to increase alcohol intake in alcoholics, just like other persons and are (often) not the real cause of the problem. On the other hand, the social environment can function as a supportive network for abstinent alcoholics. However, alcoholism is nowadays mostly a solitary problem and alcoholics are often ashamed of their problem. Therefore it might be useful to create more openness about this problem in the society, so that the social environment can provide help and support to abstinent alcoholics. To come back at the supporting effects of social behavior on alcohol use and the related risks in adolescents (increased alcohol use), it might be worthwhile to delay the alcohol use in adolescents, to decrease the risk. The government is supporting this effect, since the age to legally consume alcohol is increased from 16 to 18 years at 1 January 2014. This is a step in the right direction, but the problems will probably still exist since adolescence continues till the age of 21 and young adolescents might still consume alcohol at home. However, this measure makes is less easy to use alcohol at a younger age.

Furthermore, this thesis was also focusing on the underlying mechanism of alcohol and social behavior and the main question was: What is the role of the underlying amygdala mechanism on social behavior and alcoholism? Based on all the findings, a clear answer can be given to this question: the amygdala is involved in the reward derived of alcohol and social behavior. However, further research also showed that there are more functions related to the amygdala, for example the amygdala is important for the sexual dimorphism of play, and social play can increase neurotransmitters in the amygdala which stimulates social play. Moreover, with respect to alcohol, the amygdala is involved in the positive and negative reinforcing effects which suggest a regulating role of the amygdala. This regulation function might be confirmed by the fact that an increased expression of the adapter protein 14-3-3ζ was shown in the amygdala during escalated alcohol intake. However, this effect needs further research and I think that research towards this topic can be useful, since different findings point to a regulating role for the amygdala. And if there is indeed a regulation of the amygdala on alcohol intake, it might be a relevant target for alcohol treatments. Social behavior might even play a role in this potential treatment, since it can affect the amygdala. However, for now it is important to focus on the exact role of the amygdala in alcohol use.

Acknowledgements

References

1. WHO. *Global status report on alcohol and health.* 85 (2011).
2. Association American Psychiatry. *Diagnostics and Statistical Manual of Mental Disorders.* 4th edition (2000).
3. Moreira, M. T., Smith, L. A. & Foxcroft, D. Social norms interventions to reduce alcohol misuse in University or College students. *Cochrane Libr.* 1–3 (2010). doi:10.1002/14651858.CD006748.pub2.Copyright
4. Gilpin, N. W. & Koob, G. F. Neurobiology of Alcohol Dependence: focus on motivational mechanisms. *Alcohol Res. Heal.* **31,** 185–195 (2008).
5. Campanella, S. *et al.* Chronic alcoholism: insights from neurophysiology. *Neurophysiol. Clin.* **39,** 191–207 (2009).
6. Grau, E. & Ortet, G. Personality traits and alcohol consumption in a sample of non-alcoholic women. *Pers. Individ. Dif.* **27,** 1057–1066 (1999).
7. Tupala, E. & Tiihonen, J. Dopamine and alcoholism: neurobiological basis of ethanol abuse. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **28,** 1221–47 (2004).

8. Beck, K. H., Caldeira, K. M., Vincent, K. B. & Arria, A. M. Social contexts of drinking and subsequent alcohol use disorder among college students. *Am. J. Drug Alcohol Abuse* **39,** 38–43 (2013).

9. Bacon, A. K. & Thomas, S. E. Stress reactivity, social anxiety, and alcohol consumption in people with alcoholism: a laboratory study. *J. Dual Diagn.* **9,** (2013).

10. Hilakivi-clarke, L. & Lister, R. G. Psychopharmacology Social status and voluntary alcohol consumption in mice : interaction with stress. *Psychopharmacology (Berl).* **108,** 276–282 (1992).

11. Kushner, M. *et al.* Hybrid cognitive behavioral therapy versus relaxation training for co-occurring anxiety and alcohol disorder: a randomized clinical trial. *J. Consult Clin. Psychol.* **81,** 429–442 (2013).

12. Kushner, M., Maurer, E., Menary, K. & Thuras, P. Vulnerability to the Rapid ("Telescoped") Development of Alcohol Dependence in Individuals with Anxiety Disorder. *J. Stud. Alcohol Drugs* **72,** 1019–1027 (2011).

13. McCool, B. a & Chappell, A. M. Early social isolation in male Long-Evans rats alters both appetitive and consummatory behaviors expressed during operant ethanol self-administration. *Alcohol. Clin. Exp. Res.* **33,** 273–82 (2009).

14. Hostetler, C. M., Anacker, A. M. J., Loftis, J. M. & Ryabinin, A. E. Social housing and alcohol drinking in male-female pairs of prairie voles (Microtus ochrogaster). *Psychopharmacology (Berl).* **224,** 121–32 (2012).

15. Varlinskaya, E. I. & Spear, L. P. Increases in anxiety-like behavior induced by acute stress are reversed by ethanol in adolescent but not adult rats. *Pharmacol. Biochem. Behav.* **100,** 440–50 (2012).

16. Anacker, A. M. J. & Ryabinin, A. E. Identification of subpopulations of prairie voles differentially susceptible to peer influence to decrease high alcohol intake. *Front. Pharmacol.* **4,** 84 (2013).

17. Van Amsterdam, J. & Van den Brink, W. Reduced-risk drinking as a viable treatment goal in problematic alcohol use and alcohol dependence. *J. Psychopharmacol.* **11,** 987–997 (2013).

18. Van den Brink, W. Evidence-based pharmacological treatment of substance use disorders and pathological gambling. *Curr Drug Abus. Rev.* **5,** 3–31 (2012).

19. Creswell, K. G. *et al.* DRD4 polymorphism moderates the effect of alcohol consumption on social bonding. *PLoS One* **7,** e28914 (2012).

20. Chartier, K. G., Hesselbrock, M. N. & Hesselbrock, V. M. Development and vulnerability factors in adolescent alcohol use. *Child Adolesc. Psychiatr. Clin. N. Am.* **19,** 493–504 (2010).

21. Kuendig, H. & Kuntsche, E. Solitary versus social drinking: an experimental study on effects of social exposures on in situ alcohol consumption. *Alcohol. Clin. Exp. Res.* **36,** 732–8 (2012).

22. Spear, L. Modeling adolescent development and alcohol use in animals. *Alcohol Res. Heal.* **24,** 115–123 (2000).

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

24. Brunelle, C. & Pihl, R. O. Effects of conditioned reward and nonreward cues on the heart rate response to alcohol intoxication in male social drinkers. *Alcohol. Clin. Exp. Res.* **31,** 383–9 (2007).

25. Pliner, P. & Cappell, H. Modification of affective consequences of alcohol: a comparison of social and solitary drinking. *J. Abnorm. Psychol.* **83,** 418–425 (1974).

26. aan het Rot, M., Russell, J. J., Moskowitz, D. S. & Young, S. N. Alcohol in a social context: findings from event-contingent recording studies of everyday social interactions. *Alcohol. Clin. Exp. Res.* **32,** 459–71 (2008).

27. Lindman, R. Social and solitary drinking: effects on consumption and mood in male social drinkers. *Physiol. Behav.* **28,** 1093–5 (1982).

28. Ward, B. W. Identifying Environmental Effects on Alcohol Use and Social-Norms: The Socio-environmental Context Model. *J. Hum. Behav. Soc. Environ.* **21,** 502–520 (2011).

29.	Kuendig, H. & Kuntsche, E. Beyond personality-experimental investigations of the effects of personality traits on in situ alcohol consumption in social and solitary drinking contexts. *Addict. Behav.* **38,** 1635–8 (2013).

30.	Bot, S. M., Engels, R. C. M. E., Knibbe, R. a & Meeus, W. H. J. Friend's drinking behaviour and adolescent alcohol consumption: the moderating role of friendship characteristics. *Addict. Behav.* **30,** 929–47 (2005).

31.	Fromme, K. & Dunn, M. E. Alcohol expectancies, social and environmental cues as determinants of drinking and perceived reinforcement. *Addict. Behav.* **17,** 167–77 (1992).

32.	White, A. M., Kraus, C. L. & Swartzwelder, H. Many college freshmen drink at levels far beyond the binge threshold. *Alcohol. Clin. Exp. Res.* **30,** 1006–10 (2006).

33.	Mrug, S. & McCay, R. Parental and peer disapproval of alcohol use and its relationship to adolescent drinking: age, gender, and racial differences. *Psychol. addicted Behav.* **27,** 604–614 (2013).

34.	Andrews, J. a., Tildesley, E., Hops, H. & Li, F. The influence of peers on young adult substance use. *Heal. Psychol.* **21,** 349–357 (2002).

35.	José, B., Van Oers, H., Van de Mheen, H., Garretsen, H. & Mackenbach, J. Stressors and alcohol consumption. *Alcohol Alcohol.* **35,** 307–312 (2000).

36.	Lyvers, M., Hasking, P., Hani, R., Rhodes, M. & Trew, E. Drinking motives, drinking restraint and drinking behaviour among young adults. *Addict. Behav.* **35,** 116–22 (2010).

37.	Makris, N. *et al.* Decreased volume of the brain reward system in alcoholism. *Biol. Psychiatry* **64,** 192–202 (2008).

38.	Wise, R. A. Addictive drugs and brain stimulation reward. *Annu. Rev. Neurosci.* **19,** 319–340 (1996).

39.	Tupala, E. *et al.* Dopamine receptors and transporters in the brain reward circuits of type 1 and 2 alcoholics measured with human whole hemisphere autoradiography☆. *Neuroimage* **19,** 145–155 (2003).

40.	Sullivan, E. V & Pfefferbaum, A. Neurocircuitry in alcoholism: a substrate of disruption and repair. *Psychopharmacology (Berl).* **180,** 583–94 (2005).

41.	Miller, E. K. & Cohen, J. D. An integrative theory of prefrontal cortex function. *Annu. Rev. Neurosci.* **24,** 167–202 (2001).

42.	Wobrock, T. *et al.* Effects of abstinence on brain morphology in alcoholism: a MRI study. *Eur. Arch. Psychiatry Clin. Neurosci.* **259,** 143–50 (2009).

43.	Pitel, A.-L., Chanraud, S., Sullivan, E. V & Pfefferbaum, A. Callosal microstructural abnormalities in Alzheimer's disease and alcoholism: same phenotype, different mechanisms. *Psychiatry Res.* **184,** 49–56 (2010).

44.	Brooks, P. J. Brain atrophy and neuronal loss in alcoholism: a role for DNA damage? *Neurochem. Int.* **37,** 403–12 (2000).

45.	Laakso, M. P. *et al.* A volumetric MRI study of the hippocampus in type 1 and 2 alcoholism. *Behav. Brain Res.* **109,** 177–86 (2000).

46.	Silveri, M. M. Adolescent brain development and underage drinking in the United States: identifying risks of alcohol use in college populations. *Harv. Rev. Psychiatry* **20,** 189–200 (2012).

47.	Randall, C. L. & Lester, D. Social Modification of Alcohol Consumption in Inbred Mice. *Science (80-.).* **189,** 149–151 (1975).

48.	Honey, P. L., Varley, K. R. & Galef, B. G. Effects of ethanol consumption by adult female rats on subsequent consumption by adolescents. *Appetite* **42,** 299–306 (2004).

49.	Hunt, P. S., Holloway, J. L. & Scordalakes, E. M. Social interaction with an intoxicated sibling can result in increased intake of ethanol by periadolescent rats. *Dev. Psychobiol.* **38,** 101–9 (2001).

50.	Hunt, P. S. & Hallmark, R. a. Increases in ethanol ingestion by young rats following interaction with intoxicated siblings: a review. *Integr. Physiol. Behav. Sci.* **36,** 239–48 (2001).

51.   Mennella, J. a. & Garcia, P. L. Children's Hedonic Response to the Smell of Alcohol: Effects of Parental Drinking Habits. *Alcohol. Clin. Exp. Res.* **24,** 1167–1171 (2000).

52.   Varlinskaya, E. I. & Spear, L. P. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol. Clin. Exp. Res.* **26,** 1502–1511 (2002).

53.   Spear, L. P. Rewards, aversions and affect in adolescence: emerging convergences across laboratory animal and human data. *Dev. Cogn. Neurosci.* **1,** 392–400 (2011).

54.   Anacker, A. M. J., Loftis, J. M., Kaur, S. & Ryabinin, A. E. Prairie voles as a novel model of socially facilitated excessive drinking. *Addict. Biol.* **16,** 92–107 (2011).

55.   Bales, K. L. The value of unusual animal models for alcohol research: a commentary on: Anacker, Loftis, and Ryabinin, "alcohol intake in prairie voles is influenced by the drinking level of a peer". *Alcohol. Clin. Exp. Res.* **35,** 1379–80 (2011).

56.   Getz, L. L., Mcguire, B., Pizzuto, T., Hofman, J. E. & Frase, B. Social organization of the prairie voles (microtus ochrogaster). *J. Mammal.* **74,** 44–58 (1993).

57.   Mcguire, B. & Getz, L. L. The nature and frequency of social interactions among free-living prairie voles (Microtus ochrogaster). *Behav. Ecol. Sociobiol.* **43,** 271–279 (1998).

58.   Anacker, A. M. J., Loftis, J. M. & Ryabinin, A. E. Alcohol intake in prairie voles is influenced by the drinking level of a peer. *Alcohol. Clin. Exp. Res.* **35,** 1884–90 (2011).

59.   Anacker, A. M. J., Ahern, T. H., Young, L. J. & Ryabinin, A. E. The role of early life experience and species differences in alcohol intake in microtine rodents. *PLoS One* **7,** e39753 (2012).

60.   Salo, A., Shapiro, L. & Dewsbury, D. Affiliative behavior in different species of voles (Microtus). *Psychol. Rep.* **72,** 316–318 (1993).

61.   Anacker, A. M. J. & Ryabinin, A. E. Biological contribution to social influences on alcohol drinking: evidence from animal models. *Int. J. Environ. Res. Public Health* **7,** 473–93 (2010).

62.   Bosch, O., Nair, H., Ahern, T., Neumann, I. & Young, L. The CRF System Mediates Increased Passive Stress-Coping Behavior Following the Loss of a Bonded Partner in a Monogamous Rodent. *neuropsychopharmacology* **34,** 1406–1415 (2009).

63.   Liu, Y. *et al.* Nucleus accumbens dopamine mediates amphetamine-induced impairment of social bonding in a monogamous rodent species. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 1217–22 (2010).

64.   Aragona, B. J. *et al.* Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat. Neurosci.* **9,** 133–9 (2006).

65.   Pickering, C., Avesson, L., Liljequist, S., Lindblom, J. & Schiöth, H. B. The role of hypothalamic peptide gene expression in alcohol self-administration behavior. *Peptides* **28,** 2361–71 (2007).

66.   Koob, G. F. Addiction is a Reward Deficit and Stress Surfeit Disorder. *Front. psychiatry* **4,** 72 (2013).

67.   Bodnar, R. J. Endogenous opiates and behavior. *Peptides* (2013).

68.   Edenberg, H. J. & Foroud, T. The genetics of alcoholism: identifying specific genes through family studies. *Addict. Biol.* **11,** 386–96 (2006).

69.   Tessner, K. D. & Hill, S. Y. Neural circuitry associated with risk for alcohol use disorders. *Neuropsychol. Rev.* **20,** 1–20 (2010).

70.   Contet, C. *et al.* Identification of genes regulated in the mouse extended amygdala by excessive ethanol drinking associated with dependence. *Addict. Biol.* **16,** 615–619 (2011).

71.   Luo, X. *et al.* ADH4 gene variation is associated with alcohol and drug dependence: results from family controlled and population-structured association studies. *Pharmacogenet. Genomics* **15,** 755–68 (2005).

72.   Van der Zwaluw, C. S., Larsen, H. & Engels, R. C. Best friends and alcohol use in adolescence: the role of the dopamine D4 receptor gene. *Addict. Biol.* **17,** 1036 – 1045 (2012).

73.   Lesscher, H. M. B., Houthuijzen, J. M., Groot Koerkamp, M. J., Holstege, F. C. P. & Vanderschuren, L. J. M. J. Amygdala 14-3-3ζ as a novel modulator of escalating alcohol intake in mice. *PLoS One* **7,** e37999 (2012).

74. Thor, D. H. & Holloway, W. R. Social play in juvenile rats: a decade of methodological and experimental research. *Neurosci. Biobehav. Rev.* **8,** 455–64 (1984).

75. Cooke, B. M. & Shukla, D. Double helix: reciprocity between juvenile play and brain development. *Dev. Cogn. Neurosci.* **1,** 459–70 (2011).

76. Trezza, V., Baarendse, P. J. J. & Vanderschuren, L. J. M. J. The pleasures of play: pharmacological insights into social reward mechanisms. *Trends Pharmacol. Sci.* **31,** 463–9 (2010).

77. Van den Berg, C. L. *et al.* Play is indispensable for an adequate development of coping with social challenges in the rat. *Dev. Psychobiol.* **34,** 129–38 (1999).

78. Achterberg, E. J. M. *et al.* Amphetamine and cocaine suppress social play behavior in rats through distinct mechanisms. *Psychopharmacology (Berl).* (2013). doi:10.1007/s00213-013-3272-9

79. Holloway, K. S. & Suter, R. B. Play deprivation without social isolation: housing controls. *Dev. Psychobiol.* **44,** 58–67 (2004).

80. Hol, T., Van den Berg, C. L., Van Ree, J. M. & Spruijt, B. M. Isolation during the play period in infancy decreases adult social interactions in rats. *Behav. Brain Res.* **100,** 91–7 (1999).

81. Bell, H. C., Pellis, S. M. & Kolb, B. Juvenile peer play experience and the development of the orbitofrontal and medial prefrontal cortices. *Behav. Brain Res.* **207,** 7–13 (2010).

82. Panksepp, J., Siviy, S. & Normansell, L. The psychobiology of play: theoretical and methodological perspectives. *Neurosci. Biobehav. Rev.* **8,** 465–92 (1984).

83. Vanderschuren, L. J., Niesink, R. J. & Van Ree, J. M. The neurobiology of social play behavior in rats. *Neurosci. Biobehav. Rev.* **21,** 309–26 (1997).

84. Charles Lawrence, R., Cale Bonner, H., Newsom, R. J. & Kelly, S. J. Effects of alcohol exposure during development on play behavior and c-Fos expression in response to play behavior. *Behav. Brain Res.* **188,** 209–18 (2008).

85. Hole, G. The effects of social deprivation play in the laboratory on levels of social rat Ratfus norvegicus. **25,** 41–53 (1991).

86. Siviy, S. M. & Panksepp, J. In search of the neurobiological substrates for social playfulness in mammalian brains. *Neurosci. Biobehav. Rev.* **35,** 1821–30 (2011).

87. Varlinskaya, E. I., Spear, L. P. & Spear, N. E. Acute effects of ethanol on behavior of adolescent rats: role of social context. *Alcohol. Clin. Exp. Res.* **25,** 377–85 (2001).

88. Vanderschuren, L. J., Niesink, R., Spruijt, B. & Van Ree, J. Mu- and kappa-opioid receptor-mediated opioid effects on social play in juvenile rats. *Eur. J. Pharmacol.* **276,** 257–266 (1995).

89. Vanderschuren, L. J., Niesink, R., Spruijt, B. & Van Ree, J. Effects of morphine on different aspects of social play in juvenile rats. *Psychopharmacology (Berl).* **117,** 225–231 (1995).

90. Vanderschuren, L. J., Stein, E., Wiegant, V. & Van Ree, J. Social isolation and social interaction alter regional brain opioid receptor binding in rats. *Eur. Neuropsychopharmacol.* **5,** 119–127 (1995).

91. Van den Berg, C. L., Van Ree, J., Spruijt, B. & Kitchen, I. Effects of juvenile isolation and morphine treatment on social interactions and opioid receptors in adult rats: behavioural and autoradiographic studies. *Eur. J. Neurosci.* **11,** 3023–3032 (1999).

92. Trezza, V. & Vanderschuren, L. J. Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology (Berl).* **197,** 217–227 (2008).

93. Trezza, V. *et al.* Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. *J. Neurosci.* **32,** 14899–908 (2012).

94. Van Kerkhof, L. W. M. *et al.* Cellular activation in limbic brain systems during social play behaviour in rats. *Brain Struct. Funct.* (2013). doi:10.1007/s00429-013-0558-y

95. Vanderschuren, L. J. M. J. *et al.* Methylphenidate disrupts social play behavior in adolescent rats. *Neuropsychopharmacology* **33,** 2946–56 (2008).

96. Aguilar, R. Infantile experience and play motivation. *Soc. Neurosci.* **5,** 422–40 (2010).

97. Gordon, N. S., Kollack-Walker, S., Akil, H. & Panksepp, J. Expression of c-fos gene activation during rough and tumble play in juvenile rats. *Brain Res. Bull.* **57,** 651–9 (2002).

98.   Panksepp, J., Normansell, L., Cox, J. F. & Siviy, S. M. Effects of neonatal decortication on the social play of juvenile rats. *Physiol. Behav.* **56,** 429–43 (1994).

99.   Thor, D. H. & Holloway, W. R. Social play soliciting by male and female juvenile rats: effects of neonatal androgenization and sex of cagemates. *Behav. Neurosci.* **100,** 275–279 (1986).

100.   Meaney, M., Dodge, A. & Beatty, M. Sex-dependent effects of amygdaloid lesions on the social play of prepubertal rats. *Physiol. Behav.* **26,** 467–472 (1981).

101.   Cooke, B. & Woolley, C. Effects of prepubertal gonadectomy on a male-typical behavior and excitatory synaptic transmission in the amygdala. *Dev. Neurobiol.* **69,** 141–152 (2009).

102.   Meyer, L. S. & Riley, E. P. Social play in juvenile rats prenatally exposed to alcohol. *Teratology* **34,** 1–7 (1986).

103.   Wise, R. A. Neural mechanisms of the reinforcing action of cocaine. *NIDA Res. Monogr.* **50,** 15–33 (1984).

104.   Aggleton, J. P. *The amygdala: neurobiological aspects of emotion, memory and mental dysfunction.* (1992).

105.   Bell, R. L. *et al.* Protein expression changes in the nucleus accumbens and amygdala of inbred alcohol-preferring rats given either continuous or scheduled access to ethanol. *Alcohol* **40,** 3–17 (2006).

106.   Schneider, F. *et al.* Subcortical correlates of craving in recently abstinent alcoholic patients. *Am. J. Psychiatry* **158,** 1075–1083 (2001).

107.   Little, H. J. *et al.* Alcohol Withdrawal and Conditioning. *Alcohol. Clin. Exp. Res.* **29,** 453–464 (2005).

108.   Carnahan, M. N. *et al.* Identification of cell-specific patterns of reference gene stability in quantitative reverse-transcriptase polymerase chain reaction studies of embryonic, placental and neural stem models of prenatal ethanol exposure. *Alcohol* **47,** 109–20 (2013).

109.   Kékesi, K. A. *et al.* Altered functional protein networks in the prefrontal cortex and amygdala of victims of suicide. *PLoS One* **7,** e50532 (2012).

110.   Cheah, P. S. *et al.* Neurodevelopmental and neuropsychiatric behaviour defects arise from 14-3-3ζ deficiency. *Mol. Psychiatry* **17,** 451–66 (2012).

111.   Murphy, N., Yamamoto, A. & Henshall, D. C. Detection of 14-3-3zeta in cerebrospinal fluid following experimentally evoked seizures. *Biomarkers* **13,** 377–84 (2008).

112.   MacKay, R. K., Colson, N. J., Dodd, P. R. & Lewohl, J. M. Differential expression of 14-3-3 isoforms in human alcoholic brain. *Alcohol. Clin. Exp. Res.* **35,** 1041–9 (2011).

Laymen summary
The role of the amygdala in social behavior and alcoholism
Richelle C. Kosse

People with alcoholism are addicted to alcohol. This is dangerous, because drinking large amounts of alcohol can result in injuries, diseases and even death. Different factors can contribute to the development of an alcohol problem, for example genetic, psychosocial and environmental factors. The consumption of alcohol results in reward, which is a pleasurable feeling and this, can also contribute to the development of an addiction. Alcohol consumption supports social behavior in humans, for example people found it easier to start conversations when alcohol is consumed. Furthermore, drinking in a social environment, such as a bar or with friends, stimulates alcohol use, probably by an increased reward derived of alcohol. Therefore, the relation between alcohol and social behavior is important, since it can stimulate alcohol use and vice versa. The relation between social behavior and alcohol is most clearly seen in adolescents, because they have their first experience with alcohol and are socially active (engage in a lot of social activities). However, most drinking problems are not related to social influences, but more to coping motives (dealing with problems) and enhancement motives (enhance positive mood). In the end, the relation between social behavior and alcohol is still important, because especially in adolescents it can alter the experience of alcohol in a positive way, which stimulates alcohol use. The reward derived of alcohol is mediated by different brain regions; one of these brain regions is the amygdala, which is important for memory, fear and emotions. Therefore the main question is: What is the role of the underlying amygdala mechanism on social behavior and alcoholism?

To answer this question, findings based on the relation between alcohol and social behavior in animals was needed. Studies with prairie voles are worthwhile, because those animals show clear social bonds and a preference for alcohol. Especially the social bond forming is unique in rodents, because this is not naturally seen in rats and mice. Research in the prairie voles showed that there is indeed an influence of social circumstances on alcohol use, for example voles that drink high amounts of alcohol were placed in a cage with voles that drink less alcohol. The high drinking voles adapt to the low drinking voles; decreases their alcohol consumption. This finding suggest that social behavior might be a useful way to decrease alcohol intake, however some prairie voles that drank low amounts of alcohol increased their consumption when placed with a high drinking vole. Therefore more research is needed to the exact effect, but it seems that social behavior can affect alcohol consumption. Other research towards alcohol consumption, confirms that the amygdala plays a role in the reward feeling derived of alcohol, since it is part of the mesolimbic reward pathway in the brain. To found out more about social behavior, the focus was on a very characteristic form of social behavior, which is social play. Social play is important for the development of normal social, aggressive and sexual behavior. Social play results also in a reward feeling and is more performed in males compared to females. Low doses of alcohol can support social play through signals in the brain, probably by opioids. The amygdala is related to social play, because of the involvement of the reward pathway and the amygdala is among others responsible for the sex differences in play. In alcoholism is the amygdala, besides the reward feeling, also important for the positive and negative reinforcing effects of alcohol, which suggest a regulation role for the amygdala.

Furthermore, there was a gene found in the amygdala, which was highly expressed when more alcohol was consumed. This gene is responsible for the adapter protein 14-3-3ζ and this protein is known for the regulation effects on enzymes. Therefore, the increased expression might support the regulating function of the amygdala during alcohol intake. However, more research is needed towards this effect, but if the regulating function of the amygdala is true, the amygdala might be a potential target for alcoholism, perhaps via the effects of social behavior on the amygdala. However, in the end more research is also needed towards the exact mechanism by which alcohol and social behavior can affect each other.