The effect of age of onset on alcohol consumption and loss of control over alcohol use

Simone Goedemans (4075803)
Supervisors: drs. M. Labots and dr. H.M.B. Lesscher

Abstract

Background: Alcohol use disorder (AUD) is a worldwide problem affecting 4,1% of the population aged 15 or older. AUD is characterized by several symptoms amongst which are large alcohol consumption and loss of control over alcohol use. Adolescents, consuming a large amount of alcohol, are therefore at risk. To better understand the effect of age of onset on alcohol consumption and loss of control, this research tested the hypothesis that rats that started alcohol consumption in adolescence show less control loss, possibly due to increased cognitive flexibility, and are therefore less sensitive to develop alcohol addiction, using rats that started drinking at different ages.
Methods: Male Lister Hooded rats were given access to alcohol on an intermittent-every-other-day basis. The groups, aged 35 (adolescent) and 70 days (adult), had a choice from two bottles (water and 20% alcohol solution). Afterwards the rats were divided into tertiles (low, medium and high drinkers) and trained in an operant box to self-administer alcohol. Subsequently, pairing took place, in different boxes, making the rats associate a mild footshock to a tone. Then a conditioned suppression test was executed to study whether the rats press equally when confronted with the tone and assess their loss of control over alcohol use.
Results: A difference in alcohol intake (g/kg) was found, with adolescents having a higher intake in the first two weeks and adults the last four weeksof the voluntary intake period.Adult low drinkers reduce their seeking behaviour during the conditioned suppression test, whereas the high drinkers do not. The adolescent low drinkers show an overall lower press rate and no suppression of their seeking behaviour. High drinking adolescents on the other hand, press more and do adapt seeking behaviour in response to the footshock associated tone.
Conclusion: The results suggest age of onset influences the alcohol consumption in rats, with after only one month adults having a steady higher intake. Adolescent high drinkers show no loss of control, however high drinking adults are more compulsive, which is a part of AUD. Further research on the specific neural background is needed for a better understanding on this behaviour.

Introduction

Alcohol is a widely used substance, with worldwide a total of more than two billion adult users (World Health Organization 2004) and 46,1% of fifteen to nineteen years olds have at least once consumed alcohol (World Health Organisation 2014). Genetics, culture, personality traits but also age of first alcohol use are risk factors to develop alcohol use disorder (AUD)(American Psychiatric Association 2013; DeWit et al. 2000), which concerns 4,1% of the population aged 15 or older (World Health Organisation 2014).AUD is a disorder that can be characterised by several physical and behavioural symptoms. These symptoms include a large level of alcohol intake and the loss of control on alcohol use. This loss of control can be reflected by continued alcohol consumption despite negative consequences(American Psychiatric Association 2013). Consequences of AUD may emerge as absence from work, accidents (work-related and in traffic), violence, feeling sad and annoyed (sometimes resulting in suicide), but also substance-induced health problems (American Psychiatric Association 2013; World Health Organisation 2014; Anderson 2006). The compulsive side, also known as aversion resistance, is also important to consider in the treatment of alcoholism, which currently is mainly focused on reducing reward and/or craving, but not at restoring control over substance use (Spoelder et al. 2015; Hopf & Lesscher 2014).Taken together, it is important to understand the mechanisms underlying loss of control over substance use, including alcohol.

It is also known that adolescents may need to experiment with risky behaviours, which inevitably includes substance use, to develop into flexible adults. However, a study showed drinking during adolescence (specifically at ages 11-14) also increased the risk of developing AUD (DeWit et al. 2000). Since direct comparisons of the consequences of adulthood and adolescent alcohol use are lacking, the question remains whether adolescents are in fact less prone to losing control over alcohol use when compared to individuals that initiate alcohol use in adulthood.

This can be studied with the use of an animal model. For this study, male Lister Hooded rats with individual differences were used to represent the human population. The rats have to consume alcohol in order to research differences in age of onset and high and low drinkers. Previous studies showed that intermittent alcohol access (IAA) results in a higher consumption of alcohol than continuous access, providing a good model to study voluntary alcohol intake, leading to relevant high levels of alcohol use(Spoelder et al. 2015; Simms et al. 2008; Hopf & Lesscher 2014; Loi et al. 2010).Moreover, with the use of conditioned suppression, loss of control over alcohol use can be studied. Rats with a great loss of control will continue seeking during a tone associated with the negative stimulus. It was shown, using this intermittent model for alcohol consumption, that rats can lose control over alcohol use, when alcohol was made aversive by adding a bitter tastant or when the animals were confronted with a tone that predicted mild electrical footshocks(Hopf & Lesscher 2014). These findings show that rats develop loss of control over alcohol use, as would be seen in humans with an addiction (Hopf & Lesscher 2014).High and long-time drinkers were tested to just be less sensitive to make the association between the sound and the shock. As this was not the case, it suggested the animals were in conflict between seeking alcohol on one hand and on the other hand the threat (sound) but still seeking alcohol, and therefore showing loss of control (Hopf & Lesscher 2014).These findings show that footshocks are the better choice to use as a conditioned suppression to show rats develop loss of control over alcohol.

This study was aimed on answering the question ‘what is the relation between age of alcohol consumption, amount of alcohol intake per individual rat and the level of control on the drinking.’ In order to do so, this question can be subdivided into two questions:
1: What is the difference in the level of alcohol consumption between rats that started drinking during adolescence versus rats that started to consume alcohol in adulthood?
2: Is there a difference in loss-of-control over alcohol use between rats that had access to alcohol from adolescence versus rats that had access from adulthood.

The hypothesis regarding this study is that rats that started alcohol consumption in adolescencewould be less prone to lose control over their alcohol use, possibly due to increased cognitive flexibility, and are therefore less sensitive to develop alcohol addiction. To test this hypothesis and answer the mean question, this study used male Lister Hooded rats that were exposed to a voluntary alcohol consumption paradigm (with IAA) and a conditioned suppression test (with footshocks) to assess their extent of (loss of) control over alcohol use.

Materials and methods

Animals

Male Lister Hooded rats were orderedfrom Charles River (Germany), of which n=84 arrived during adolescence (at the 28th postnatal day) and n=84 adults (at the 63rd postnatal day). Despite the adolescents also being adults when tested, the groups will be named adolescent and adult in this report.The experiments were executed in four batches (batch 1 and 2 consistingof 24 adolescents and 24 adults, performed by a previous student, and batch 3 and 4 of 18 adolescents and 18 adults, performed by me, my supervisor M. Labots and occasionally by other colleagues of the department). In batch 1, one animal from the adult group was euthanized during the voluntary alcohol intake, as his welfare was affected. This rat was therefore excluded from the complete analysis. Before the conditioned suppression test for batch 2 was performed, two rats of the CS+ group were euthanized as their welfare was compromised. Their consumption data were included, however their data was excluded from this point forward. Batch 4 was still in the process of operant training for alcohol consumption during the writing of this thesis. The voluntary alcohol consumption data is included in the analysis.The rats were individually housed, with a temperature of 18-22 C° and a humidity of 50-70%. The 12h light/dark cycle was reversed, with the lights turning off at 7 AM (the schedule changed along with the seasonal time changes). The rats had *ad libitum* access to water and chow. The experiments were executed under red light conditions. The room where the rats were housed had a continuous background sound produced by a radio. After arrival, the rats were acclimatized to the housing for one week, and during the experiment were handled and weighed on a weekly basis.

Alcohol consumption

During two months, the 168 rats were given intermittent access to alcohol and water in a two-bottle choice setup. The adolescent group started at 35 days postnatal and the adult group 70 days postnatal. The first month they were given access for 7 hours a day, on an every other day schedule (Monday, Wednesday, Friday). The second month this time was extended to 24 hours, on the same days. The bottles were closed off with a stainless steel dual ball-bearing drinking spout. On the drinking days the bottles, one containing 20% alcohol and one with tap water, were first weighed, then presented to the rats. After the session, the bottles were weighed again. The two bottles switched sides every session, to avoid side bias. The bottles were cleaned and refilled every week. After every session, the data on alcohol intake, along with preference and water intake were noted per rat and after two months the averages on these data were calculated per rat per week. With the use of weekly ranking, based on average alcohol intake in that week, the sum of ranksper rat could be calculated and the rats were split up into tertiles: low, medium and high drinkers.

Operant training

After two months of alcohol consumption, the rats were first habituated (for either one or two weeks) and trained to press for alcohol in an operant training box (29.5 cm L, 24 cm W, 25 cm H; Med Associates, Georgia VT), which were placed in light and sound proof cubicles equipped with a ventilation fan. In each box two retractable levers (4.8 cm wide) were present, 11.7 cm apart, and 6 cm from the floor. A signal light (28 V, 100 mA) was situated right above each lever and in between the lever was a magazine with a liquid dipper underneath which an alcohol container (containing a 20% alcohol solution, refreshed every session for each rat) was

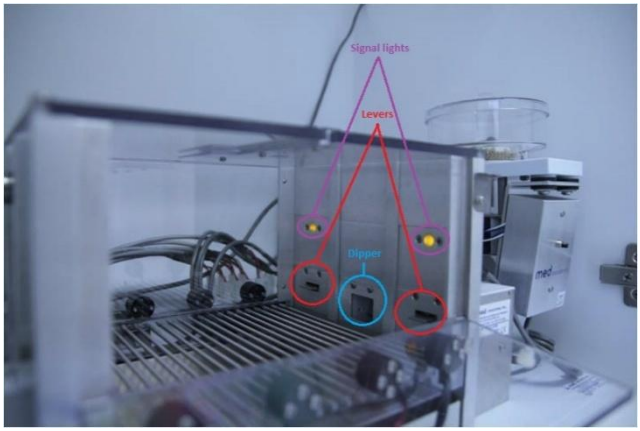


Figure 1. A picture of an operant training box, showing the right side with the levers, signals light and magazine where the dipper rises. The house light is on the opposite side, not shown is this picture. (Source: Service Commun d'Analyse Comportementale)

placed during a session. Furthermore, there was a house light (28 V, 100 mA) and a tone generator (85 dB, 2900 Hz) on the opposite side of the box. Side bias was prevented by counterbalancing the position of the active and inactive lever. Pressing the active lever once, raises the dipper, providing the rat with 0,1 ml of the alcohol solution, whereas pressing the inactive lever had no consequences. Simultaneously with pressing the active lever, the house light was turned off and the signal light above the active lever was turned on. As soon as the rat put his head into the magazine, he had ten seconds before the dipper would drop again, after which the signal light was turned off and a five second inter-trial interval started before the new trial started. Following each30-minute session pressing the active lever, as well as the inactive, the total amount of presses, the amount of trials and amount of rewards was documented. The rats were trained three to five days each week. To check if the rats actuallyconsumed alcohol during the sessions, the alcohol container was weighed before and after the session. The experiment was performed with the use of MED-PC for Windows.

The rats started training at a fixed ratio of 1 (FR1) in which the rats gained access to alcohol right after each press (during a trial). After three sessions, the rats were introduced to a random interval (RI) schedule, starting at the RI5. During these sessions pressing the active lever for the first time starts a random interval, in RI5 having an average of five seconds (RI15 having an average of fifteen seconds and so on). In the meantime, the rats could still press the active lever, however this did not result in a reward. As soon as the random interval ended, one press on the active lever would result in a reward. Three sessions with RI5 were executed, followed by three sessions of RI15, three of RI30, three of RI60, and four to five RI120 sessions.

Conditioned suppression

Before performing the conditioned suppression test, the rats were classified into two groups: one group undergoing the conditioning with a footshock-associated tone (CS+) and the other group underwent the same treatment but without the footshocks (CS-). This classification was, besides the tertile split, also based on the average number of active presses in the first fifteen minutes of the final three RI120 sessions, stability of seeking behaviour and randomisation.The seeking behaviour is stable when there was less than 20% variation in active responses over the first fifteen minutes of the last three RI120 sessions.The stable and unstable rats where equally divided to prevent a bias.

Subsequently to the training sessions, all rats were habituated in conditioning boxes, for ten minutes, four days in a row. The pairing would later take place in these boxes. This was done to make the tone the only variable when testing the rats for their seeking suppression in the operant boxes. The pairing consisted of aforty-minute session, including a tone (85 dB, 2900 Hz) and in the CS+ group footshocks (0,40 mA shock for one second). In the first five minutes only the house light was on, followed by ten minutes with the tone on and footshocks, at random moments. The next ten minutes again without tone and shocks, with another ten minutes with tone and shocks. The session ends with five minutes without tone and shocks. Both ten minute frames with shocks gave a sum of 20 shocks in total per CS+ rat. The CS- rats were just exposed to the same session with the tone and without any shocks. After the pairing session, two more RI120 sessions were executed.

A week later, after fear conditioning, the conditioned suppression test was performed. The test lasted fourteen minutes and throughout the session the house light was on. First only the house light was on and after two minutes the two levers were presented and the tone started for two minutes. The following two minutes the tone was off, and these 4 minutes were repeated two more times. Presses on both active and inactive lever had no consequences and were documented. An alcohol container was placed in front of the dipper so the rats could smell the alcohol, to stimulate them to press.

Freezing behaviour

Next another RI120 session was performed, followed by a second pairing session(either CS+ or CS-, according to the same classification) to make sure the association still existed.A freezing test was executed to determine whether the adolescents and adults differed in sensitivity to the conditioning itself, which could have influenced the (results of the) conditioned suppression test. A camera was placed on top of an operant box, to record the rats behaviour. The sessions lasted four minutes, first two minutes with tone one, then two minutes with the tone off. Freezing, meaning no movement besides breathing, was afterwards scored. The previous intern was using Observer 5.1 softwarefor batch 1 and 2, and we used version 12.4for batch 3, and will be using the same version for batch 4 (Noldus Indormation Technology).

Statistics

First the exact one sample Kolmogorov-Smirnov test was performed on all data to check for normal distribution. Univariate ANOVAs, with fixed factor age of onset and category, were used to analyse the mean alcohol intake, preference and mean water intake per day, including the Levene's test that was used to assess whether population variances were equal. *Post-hoc* analyses, by using one sample t-tests, were performed on each variable, separated by age of onset, to study differences in age for alcohol intake. The weekly alcohol intake was analysed with a two-way repeated measures ANOVA, with age of onset and category as in between factors and within subjects variables week 1-8.*Post-hoc* analysis showed in which week the adolescent significantly differed from the adultswith the use of one sample t-tests. The conditioned suppression and latency data were also analysed with repeated measures ANOVA, with tone (off vs. on) as within subject variable and age of onset, category and conditioned suppression classification as in between factors. Mauchly's test of sphericity was used to test the differences in variance between the levels. Whenever the assumption of sphericity was violated, the degrees of freedom needed to be corrected, here using the Huyn-Feldt estimates of sphericity. *Post-hoc* comparisons were performed to show significant differences between the CS+ and CS- groups in each category in the age groups with the use of one sample t-tests. The means along with +/- standard error of the mean (SEM) are presented in the graphs made in Excel 2016. A p-value below 0,05 was determined as significant. The data was analysed with the use of IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA).

Results

Alcohol intake

The average alcohol intake per day increased significantly over de course of weeks ($F_{(2.647,426.157)}$=267.676, p=0.000, η_p^2=0.624; figure 2A). During the 8 weeks, an overall effect of age of onset was found ($F_{(1,161)}$=8.876, p=0.016, η_p^2=0.035). *Post-hoc* comparisons showed that the adolescents consumed more alcohol in week 1 and 2, but adults consumed more during week 5-8 (figure 2B). When comparing the adolescents to the adults in the first month there was no significant difference (*n.s.*), whereas the difference did show in the second month ($F_{(1,161)}$=14.586, p=0.000, η_p^2=0.083) (figure 3). *Post-hoc* analyses of the second month only showed a significant difference between the adolescent low-drinkers and the adult low-drinkers. Neither the first nor the second month showed an age of onset * category effect (*n.s.*).

When comparing the alcohol intake over timetwo interactions were found, week * age of onset ($F_{(2.647,426.157)}$=12.314, p=0.000, η_p^2=0.071) and week * category ($F_{(5.294,426.157)}$=39.585, p=0.000, η_p^2=0.330). This shows a significant difference between the age groups and the categories over time.

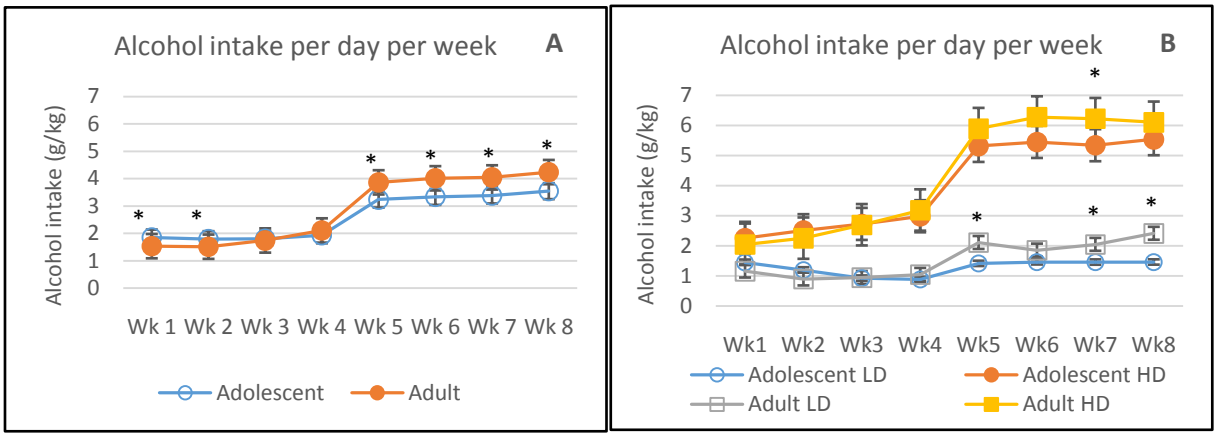


*Figure 2.Alcohol intake (g/kg) increases over the period of 2 months for adolescents (n=84) and adults (n=83) (A), and per category with adolescent LD (n=28), adult LD (n=28), adolescent HD (n=28) and adult HD (n=28) (B). Data are shown as mean +/- SEM. *p<0.05*

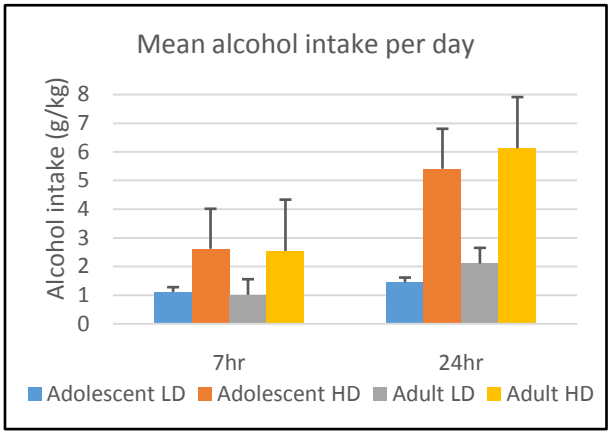


*Figure 3. Alcohol intake per day (g/kg) in each month, per category in age group with adolescent LD (n=28), adolescent HD (n=28), adult LD (n=28) and adult HD (n=28) (B). Data are shown as mean +/- SEM, *p<0.05.*

Preference

An overall age effect on the mean preference per day was found ($F_{(1,161)}$=29.828, p=0.000, η_p^2=0.156) and *post-hoc* analyses showed a significant difference between adolescents and adults in allthe categories (LD and HD are presented). In both the first and second month, an age effect was found

($F_{(1,161)}$=4.861, p=0.029, η_p^2=0.029, $F_{(1,161)}$=32.719, p=0.000, η_p^2=0.169, respectively). *Post-hoc* comparisons of the first month revealed this difference lies only in the high drinking category, where the second month showed significance between adolescents and adults in all categories (figure 4A).

Water intake

The mean water intake per day also showed an overall age effect ($F_{(1,161)}$=87.997, p=0.000, η_p^2=0.353), with a *post-hoc* analyses that showed the effect in all categories (LD and HD are presented) of which the extremes are shown (figure 4B). Age of onset had a significant influence on the water intake in the first month ($F_{(1,161)}$=53.940, p=0.000, η_p^2=0.251), as well as in the second month ($F_{(1,161)}$=52.675, p=0.000, η_p^2=0.247). *Post-hoc* comparisons displayed a significant difference between the age groups in all categories.

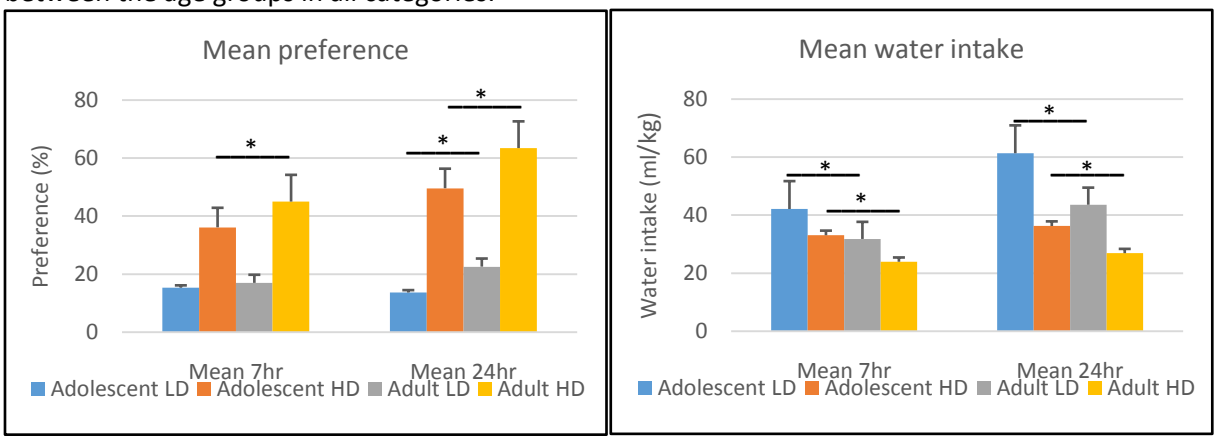
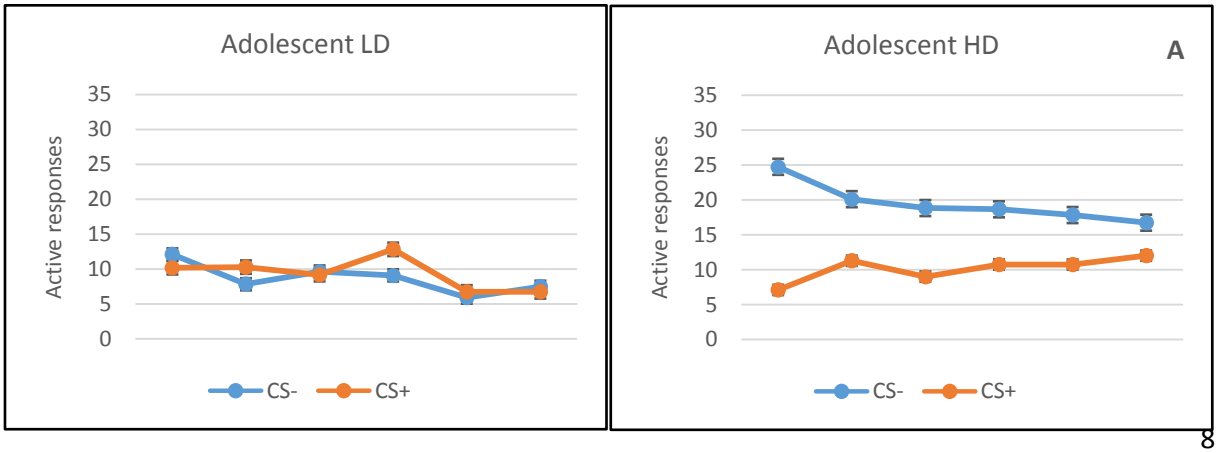


*Figure 4. Mean preference (%) (A), and mean water intake per day (ml/kg) (B) in each month, per category in age group with adolescent LD (n=28), adolescent HD (n=28), adult LD (n=28) and adult HD (n=28) (B). Data are shown as mean +/- SEM, *p<0.05.*

Conditioned suppression

The amount of active presses during the conditioned suppression test were compared for the CS+/- for the categories in both age groups. A significant effect of time was found ($F_{(2.969,347.381)}$=10.339, p=0.000, η_p^2=0.081), showing a decrease in active presses as time progresses (figure 4A-D). The age groups overall showed a similar response during the conditioned suppression test. Adolescents and adults did respond in a different pattern over time, as a significant interaction between time and age of onset was found($F_{(2.969,347.381)}$=3.558, p=0.015, η_p^2=0.030). Also, an overall CSeffect was found over time ($F_{(2.969,347.381)}$=10.283, p=0.000, η_p^2=0.081). Furthermore an interaction between age of onset, category and conditioned suppression classification was found ($F_{(2,117)}$=5.005, p=0.008, η_p^2=0.079).*Post hoc* analysis reveals the possible difference in CS+ and CS- in each category per age group. In the adolescent low drinkers group, no significant difference was found (figure 5A). In the adolescent high drinking group a significance between the CS- and CS+ group was found on three points in the test (figure 5B). In the adult group the analysis showed a significant difference in the low drinkers, on five points (figure 5C), and no difference in the high drinkers (figure 5D).



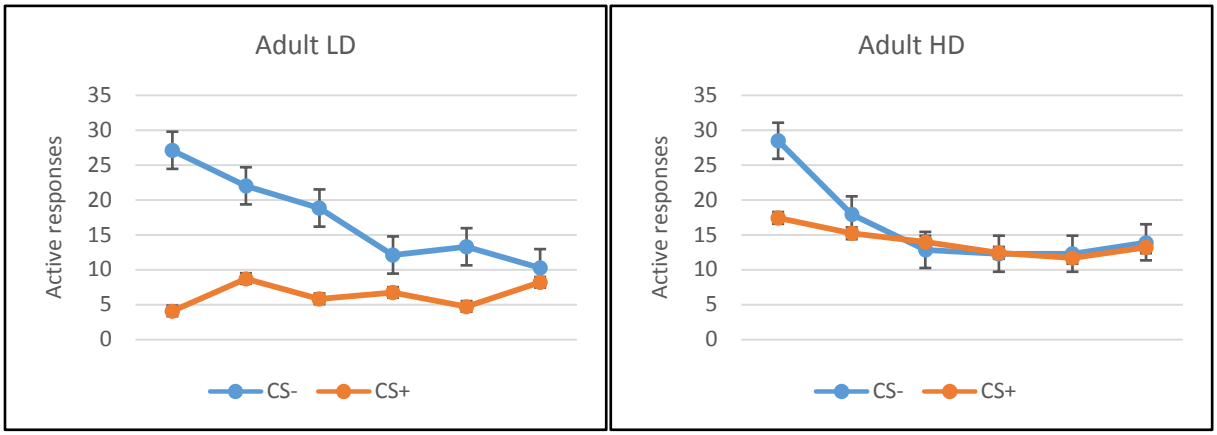
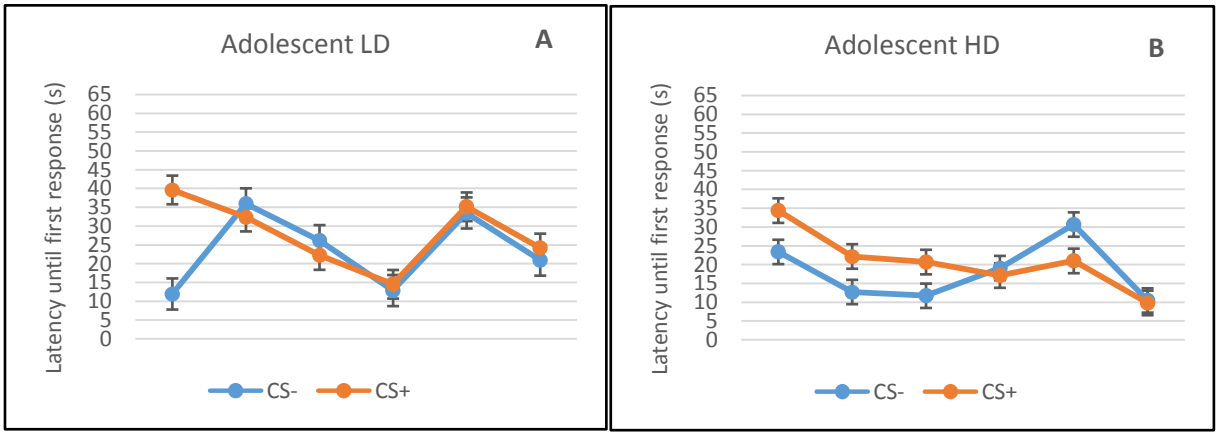


*Figure 5. Number of active responses during the conditioned suppression test, per CS+/CS- group. Adolescent LDCS+ (n=11) and CS- (n=11) (A), adolescent HD CS+ (n=11) and CS- (n=11) (B). Adult LD CS+ (n=11) and CS- (n=11) (C), adult HD CS+ (n=10) and CS- (n=11) (D). Data are shown as mean +/- SEM, *p<0.05.*

Latency to first press

During the conditioned suppression test, latency to first press (s) was measured and is presented per CS+/CS- in low and high drinking adolescents and adults. Again an overall time effect is seen ($F_{(4.458,521.558)}$=4.553, p=0.001, η_p^2=0.037). For latency, nooverall age of onset effect was found (*n.s.*) as well as no time and age of onset interaction(*n.s.*), unlike in the active response. Furthermore, there was an overall CS effect ($F_{(1,117)}$=15.282, p=0.000, η_p^2=0.038) and the CS+ and CS- animals reacted differently over time ($F_{(4.458,521.558)}$=8.306, p=0.000, η_p^2=0.066). In agreement with the active presses shown above, *post-hoc* comparisons showed no significant difference in latency to first press between CS- and CS+ in the adolescent low drinkers, as well as in the adolescent high drinkers (figure 6A and 6B, respectively). In the adult low drinking group it did show a significant difference on two points in the test (figure 6C). The high drinking adults differed on one point in the test (figure 6D).



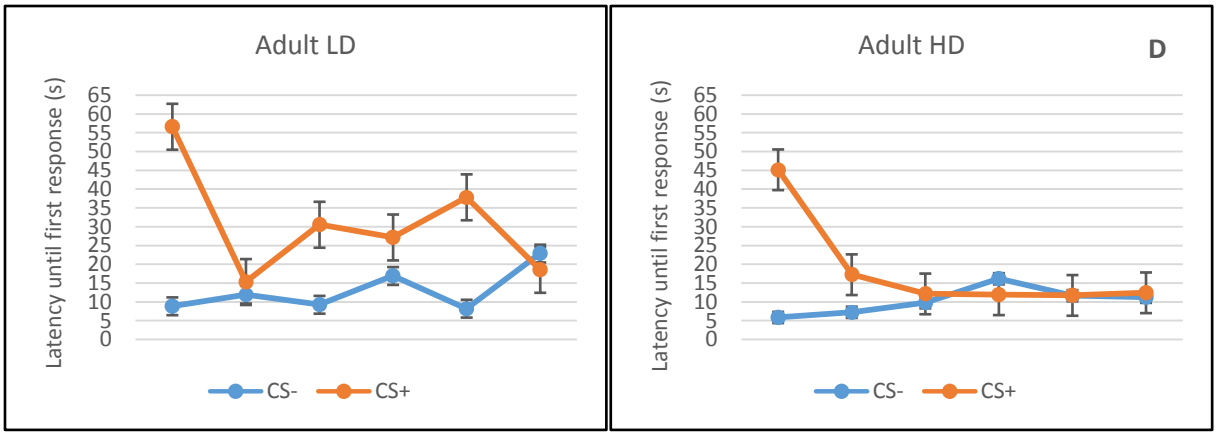


*Figure 6. Latency to first press (s) during the conditioned suppression test, per CS+/CS- group. Adolescent LD CS+ (n=11) and CS- (n=11) (A), adolescent HD CS+ (n=11) and CS- (n=11) (B). Adult LD CS+ (n=11) and CS- (n=11) (C), adult HD CS+ (n=10) and CS- (n=11) (D). Data are shown as mean +/- SEM, *p<0.05.*

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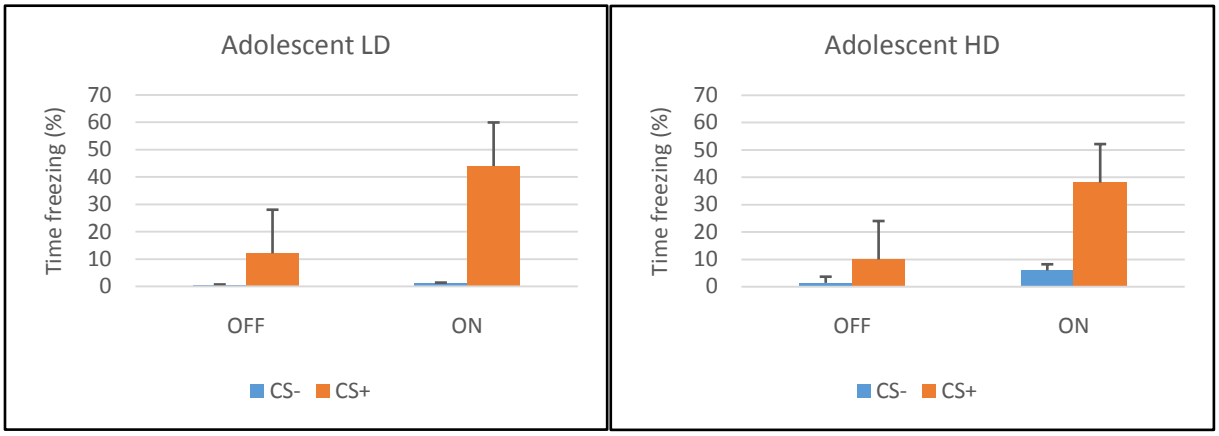
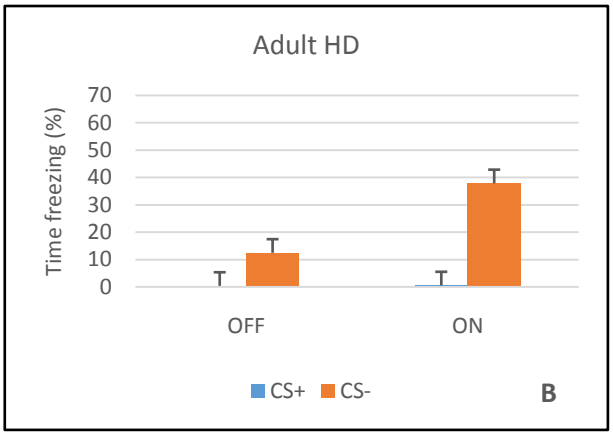


Freezing test

An overall tone effect on the mean percentage of time a rat freezed ($F_{(1.000,\ 117.000)}$=139.465, p=)0, η_p^2=0.544) ; ... as a tone * CS effect was found ($F_{(1.000,\ 117.000)}$=128.661, p=0.000, η_p^2=0.524). *Post-hoc* analyses showed a significant difference between the CS- and CS+ group within each category for both ages of onset (figure 7A-D).



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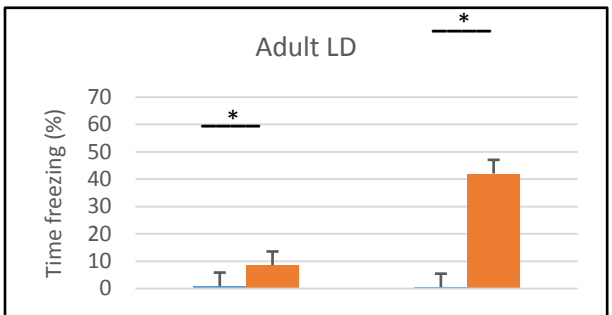

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*Figure 7. Time freezing (%) during freezing test, per CS+/CS- group. Adolescent LD CS+ (n=11) and CS- (n=11) (A), adolescent HD CS+ (n=11) and CS- (n=11) (B). Adult LD CS+ (n=11) and CS- (n=11) (C), adult HD CS+ (n=10) and CS- (n=11) (D).Data are shown as mean +/- SEM, *p<0.05.*

Discussion

The first sub question wason whether adolescents consume more alcohol than adults was tested with the first part of the experiment measuring alcohol intake. The results actually show a contradictory image to the hypothesis, as adolescents consumed more alcohol (g/kg) in the first two weeks, which is similar to a previous study where adolescent initially consumed more (Schramm-sapyta et al. 2015). However, the adults showed a higher intake (g/kg) in the second month. Previous studies did show a higher intake in adolescents either from the start on throughout the experiment or when they reach adulthood (which would here show escalation in the second month) (Broadwater et al. 2011; Pascual et al. 2009; Walker & Ehlers 2009; Yoshimoto et al. 2002; Vetter-O'Hagen et al. 2009). One study, for adolescents being 31 days old and adults 71 days old at the onset, also resulted in adults having a higher alcohol intake (Siegmund et al. 2005). However this was only at the start of the experiment, so again different from the findings in this research (Siegmund et al. 2005). The categories showed a different pattern over time, as the HD groups (both adolescent and adult) showed an increase in alcohol intake, whereas the LD groups only slightly increased their intake, which is in agreement with a previous study (Spoelder et al. 2015). In the first month, a significant difference in the mean preference was found between the adolescent and adult HD, but not in in the LD. This difference was evident in the second month in both adolescent versus adult HD and adolescent versus adult LD. The adolescents had a significant higher water intake (ml/kg) in both months and both categories. The higher water intake can explain the lower preference calculated in adolescents.

Furthermore, it was stated that adolescents show more cognitive flexibility and less control loss, and are therefore less sensitive to develop loss-of-control over alcohol use. This study shows a significant difference in the active presses between the CS- and CS+ group of the adolescent HD, but not in the LD. It could be the adolescent LD just show a low interest and motivation, separate from the CS classification. These rats were obviously categorised in the low drinking group based on them drinking small amounts of alcohol, which is not in line with (human) addiction. The adolescent HD CS+ showed a significant lower active response during the conditioned suppression test, indicating that they exhibit more flexible behaviour and more control over their behaviour. The adult LD did respond to the tone, with CS- lowering their active presses and CS+ not, which couldbe explained by less motivation, again as they were selected to be in the LD group. They do not lose control over their behaviour and do not rely on alcohol, even though they have a similar pressing rate as the adult HD. The adult HD show a loss of control bycontinuing to press despite hearing the footshock associated tone. Thus, it seems that the adults are less in control over their alcohol use compared to the animals that started drinking in their adolescence.Previous studies used taste aversion in alcohol and cocaine experiments, which resulted in the adults showing more control over alcohol or cocaine use than adolescents, by lowering their intake when confronted with this unpleasant taste(Schramm-sapyta et al. 2015; Schramm-sapyta et al. 2011; Badalà et al. 2008).This is in contradiction with our study involving footshocks, which suggests adults losing control.

When looking at the latency until the first press in the conditioned suppression test only significant differences between the CS+ and CS- in the adult categories became evident during the first tone on. The LD adolescents do differ in latency during the first tone, however this was not significant (p=0,068). The adolescent HD CS+ and CS- groups have a very similar pattern,which could imply that the tone is not be as negative as expected. However, this is not the case when observing the number of active responses between the CS- and CS+ groups. Comparing the active presses to the latency here mostly shows latency is not a sensitive parameter for loss of control, as both age groups reduce their alcohol seeking behaviour during the footshock associated tone. A previous study, on cocaine and sucrose, showed an almost identical pattern for active presses and change in latency as found in this study (Limpens et al. 2014) and another study on cocaine also reported a significant difference in latency between CS+ and CS- (Vanderschuren & Everitt 2004). This confirms a more

generaleffectiveness of footshock associated tones on seeking behaviour, based on active presses and latency. Limpens et al however did find a significant latency response simultaneous with a significant suppression ratio, in both sucrose and cocaine, when comparing different shock intensities. Therefore, latency seems to be a more reliable parameter in cocaine and sucrose experiments than in alcohol experiments.

After the first tone one, extinction of the high latency is seen, bringing the CS+ and CS- groups in both ages and categories on the same latency until first response. This suggests that the effect of the shock may have disappeared over time, however it may also be the shock had an insufficient impact on the rats. The freezing test however indicates a successful association between the tone and shocks, as all CS+ groups had a higher freezing time than de CS- groups. It is therefore unlikely the shock was insufficient enough.

Even though an association was successful, it might be rats do not associate the seeking behaviour (by pressing the levers) to the shocks. Therefore,a research with shocks during the conditioned suppression test should be executed. During these type of tests the researchers can be certain the rats associate the shock with the pressing, instead of just the tone. This could give a more distinctive result. However, shocks during the conditioned suppression test would also mean the rats get a direct consequence. This is not in line with addiction consequences people experience, as the consequences are long term. This should therefore be considered when using the animal model. Moreover, studies have shown differences in response to the footshocks between the age groups (Jones 2015; Brunell & Spear 2005). In this research that age effect can also be present creating the different patterns in active response and latency to first press. Furthermore, research on the mA height of the shocks should be performed. Now 0,4 mA was used, which is a safe choice regarding research on sucrose and cocaine suppression (Limpens et al. 2014), where the found an aversion for sucrose using 0,35 mA and with 0,40 mA for cocaine. It is important to visualise the response of alcohol consuming animals on different heights of mA. It may be possible to reduce this level, and therefore reducing the animals discomfort but keeping the fear response.

In conclusion, some interesting results were found in this research. First of all, itin this research age of onset influences the alcohol consumption, with adults drinking higher amounts over time. Furthermore, the adolescents show no loss of control, indicating them being more flexible than adults (who show loss of control). When the fourth batch is included, the results may be even more distinctive. These findings point to differences in AUD development within different ages of onset. Adolescents being more in control over their behaviour regarding alcohol use can change their individual treatment of AUD. Knowledge on a patients background will therefore be needed to find a suited treatment and extent the full picture of the problem. Further research on the neural background will clarify the picture of AUD and its treatment.

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