Stress and binge drinking: A toxic combination for the teenage brain

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Abstract

Young adult university students frequently binge on alcohol and have high stress levels. Based on findings in rodents, we predicted that heavy current alcohol use and elevated stress and depression scores would be associated with deficits on high interference memory tasks, while early onset, prolonged binge patterns would lead to broader cognitive deficits on tests of associative encoding and executive functions. We developed the Concentration Memory Task, a novel computerized version of the Concentration card game with a high degree of interference. We found that young adults with elevated stress, depression, and alcohol consumption scores were impaired in the Concentration Memory Task. We also analyzed data from a previous study, and found that higher alcohol consumption scores were associated with impaired performance on another high interference memory task, based on Kirwan and Stark’s Mnemonic Similarity Test. On the other hand, adolescent onset of binge drinking predicted poorer performance on broader range of memory tests, including a more systematic test of spatial recognition memory, and an
associative learning task. Our results are broadly consistent with findings in rodents that acute alcohol and stress exposure suppress neurogenesis in the adult hippocampus, which in turn impairs performance in high interference memory tasks, while adolescent onset binge drinking causes more extensive brain damage and cognitive deficits.

*Keywords:* Stress, Depression, Alcohol, Binge drinking, memory interference, neurogenesis

1. Introduction

The vast majority of psychological studies are conducted on university undergraduates [? ]. While this limits the generality of such findings [? ], in other respects undergraduates are assumed, by many, to be an ideal participant pool: a homogeneous group of high-functioning, and physically and mentally healthy young adults. However, these assumptions may be called into question, considering the high levels of binge drinking, chronic stress and depression in the undergraduate population. In our own studies involving hundreds of undergraduates over the past 10 years, we find that 25-30% score in the mild to severe range on the Beck Depression Inventory II, and engage in regular binge drinking (where a binge is defined by the National Institute on Alcohol Abuse and Alcoholism to be 4 drinks per 2 hours for a female and 5 drinks per 2 hours for a male); similar binge levels have been reported in the literature for this population [e.g. ? ]. Worldwide, rates of binge drinking and dangerous alcohol consumption behaviour are on the rise in adolescents [? ? ? ]. Given the ongoing brain development that occurs in adolescence to early adulthood [? ], it is important to establish the
long-term consequences of exposure to binge drinking and stress during this period.

While the neurotoxic effects of chronic, long-term stress, depression and alcohol on the human brain are well established, acute effects have been less studied. Multiple episodes of major depressive disorder and prolonged alcohol abuse both lead to hippocampal / medial temporal lobe volume loss [? ? ? , e.g.]; long-term alcohol exposure also affects other brain regions including the prefrontal cortex and fronto-striatal reward circuits [? ? ].

The effects of prolonged heavy drinking are even more pronounced in the adolescent brain [? ]. Although less is known about the acute effects on the human brain, there is evidence that periodic binge drinking in adolescence may also cause brain volume loss [? ].

In animal models, the acute effects of stress and alcohol exposure have been studied more extensively. In adult rodents, several days of binge alcohol or stress exposure reduces hippocampal neurogenesis [? ? ]. Adolescent animals are especially vulnerable to the effects of binge alcohol exposure on the inhibition of neurogenesis [? ]; they also exhibit more widespread brain damage than adult-exposed animals, in regions including the temporal and frontal lobes [? ? ]. Based on these findings, we would expect to see parallel effects of acute stress and binge drinking in the human adolescent brain. Unfortunately, we lack a means of assaying neurogenesis non-invasively in humans. In rodents, the effects of neurogenesis knockdown versus broader hippocampal pathology can be distinguished behaviourally. Knockdown of neurogenesis results in selective impairments on a wide range of high interference memory tasks, whether the interference arises from overlapping stimuli,
time delay between learning and retrieval, context effects, or reversal of previously learned responses [...]. In contrast, broader hippocampal pathology leads to more general associative encoding deficits [...]. Consistent with findings from rodent studies, in humans with no current or previous psychiatric diagnosis, elevated depression and stress scores are associated with selective impairments on high interference memory tasks including the CANTAB delayed match to sample at long delays [...], Kirwan and Stark’s[...] Mnemonic Similarity Task and variants (MST, formerly called the Behavioural Pattern Separation Task) [...], and recognition memory across a 2-week delay [...]. Conversely, exercise is an established up-regulator of neurogenesis in animal models [...] and of neurogenesis biomarkers in rodents and humans [...]; exercise causes improved human performance on an MST-like task [...]. Thus, data from humans and animal models consistently point to a selective role for hippocampal neurogenesis in mitigating memory interference. In contrast, as in rodents, in humans hippocampal damage causes more generalized episodic and associative memory deficits [...]. Based on the above findings, in the two experiments reported here, we sought to investigate the relationship between binge alcohol patterns, stress, depression and memory performance in university students. We hypothesized that high current binge drinking and elevated stress and depression levels would be associated with selective deficits on high interference memory tasks, while early onset binge drinking would cause broader deficits in memory and executive functions.
2. Experiment 1.

We administered a battery of cognitive tests, stress and depression inventories and a lifestyle questionnaire to healthy undergraduate participants. The lifestyle questionnaire included questions about recent and remote drinking patterns. The cognitive battery included a paired associate learning task, a visual reverse digit span test, and a novel high interference test of spatial memory, the Concentration Memory Task. We also analyzed data from a previous study, parts of which had been published [?], to assess the effects of recent binge drinking on another high interference memory test.

2.1. Materials and Methods

Participants were brought into a quiet testing room and seated at a desk in front of a touchscreen computer. After reading the letter of information and providing written consent, they completed computerized versions of the Beck Depression Inventory (BDI), Cohen’s Perceived Stress Scale (PSS), and a lifestyle questionnaire developed by our lab. Next, they performed the three memory tests detailed below: a CANTAB-like paired associates learning task, a reverse digit span task, and the Concentration Memory Task.

2.1.1. Participants

84 McMaster University students were recruited online (at “www.experimetrix.com/mac” and “http://mcmaster.sona-systems.com”). Of these, 76 completed our experiment and met inclusion criteria: normal or corrected to normal vision and no history or previous diagnosis of major depression or other psychiatric disorders. Participants received course credit in an Introductory Psychol-
ogy course for their participation. The McMaster Research Ethics Board (MREB) approved all aspects of our study.

2.1.2. Questionnaires

To assess stress, depression, and alcohol consumption levels, we administered Cohen’s Perceived Stress Scale (PSS) (http://www.psy.cmu.edu/cohenscales.html), the Beck Depression Inventory-II (BDI) (Psychological Corporation) and our own lifestyle questionnaire. The PSS is a freely available, 10-item questionnaire that asks participants to rate various measures of their perceived stress level within the past month, on a 5-point scale. The BDI is a standardized, commercially available neuropsychological test including 21 multiple choice questions, each on a 4-point scale, about the individual’s mood during the past week. We have used our lifestyle questionnaire in several previous studies; it probes a number of different variables. The key measures of alcohol consumption included in the analyses reported here were number of drinks consumed on a typical drinking occasion (typical alcohol consumption) and a series of questions probing frequency of binge drinking at ages 13-22. A binge is defined by the United States National Institute on Alcohol Abuse and Alcoholism to be 4 drinks per 2 hours for a female and 5 drinks per 2 hours for a male.

2.1.3. Paired Associate Learning test

The paired associates learning (PAL) task was designed to be similar to the PAL task in the Cambridge Automated Neuropsychological Test Battery (CANTAB), and was implemented in e-prime. The CANTAB PAL is a widely used visuo-spatial associative learning task that was predicted to
be sensitive to major hippocampal pathology. However, as it lacks a high-interference component, it was not hypothesized to be sensitive to acute levels of binge drinking, stress or depression. Indeed, we have shown previously that performance on this task does not vary as a function of BDI score [? ? ]. During each study trial, participants viewed six to eight white boxes displayed in a circle on the screen. The boxes were opened one at a time, in a random order, to reveal concealed patterns. Each box stayed open for 4 seconds. Once all of the white boxes were opened, after a 2 second delay, a test trial began. In a test trial, while the white boxes remained in a circle around the screen, the studied patterns were displayed in the middle of the screen, one at a time for five seconds, during which the participant had to touch the box where the same pattern was located. There were six levels of difficulty in which ‘1, 2, 3, 4, 5, 6, and 8 patterns were presented. For levels one through five there were six boxes, while for the final level there were eight boxes. Participants had up to six trials at each stage before moving on to the next, and automatically moved from one stage to the next by correctly locating all of the patterns. When an error was made, the participant was allowed to finish the test trial before all of the patterns were presented again as a reminder of their locations. The total number of errors across trials at each difficulty level was recorded.

2.1.4. Reverse Digit Span Task

To assess working memory, we administered a computerized visual reverse digit span task. This task was implemented in e-prime and was adapted from the protocol of Waters and Caplan (2003). Participants were presented with a series of digits and were asked immediately after each sequence to
repeat them back in reverse order using a keyboard. There were five trials for each sequence length. The sequence length started at two and increased to a maximum of eight digits. If a digit sequence of a given length was recalled successfully on at least 3 trials, they were given a longer list (e.g., '9, 2, 4, 0'). Participants were instructed to leave a space as a placeholder in the sequence if they were unsure of a digit in a sequence, but were confident in the digits surrounding that spot in the sequence. The participants digit span was calculated as the sequence length at which they recalled all of the digits in the correct order on at least three out of five trials. An additional 0.5 was added if the participant was correct on two out of five trials at the next level.
Figure 1: The Concentration Memory Task (CMT). Top row: Progression through several trials in one game of the CMT using a single image as an example. A target is briefly revealed at the start of a trial and then hidden. Participants must search the grid until they find the correct match. Second row: Progression through several trials in game 2. Importantly, some images are repeated between games so that these images are experienced in different spatial locations. Third row: Following completion of two full games participants complete a 2-alternative forced choice task in which they select the location they have experienced an object in most recently. Bottom: Participants complete a total of 4 games in which they search for 8 image pair matches within a 4x4 grid of playing cards. Following games 2, 3 and 4, participants complete 2-alternative forced choice tasks consisting of 4 trials each for a total of 12 trials (Game 1 - Game 2 - 2AFC1 - Game 3 - 2AFC2 - Game 4 - 2AFC3).
2.1.5. Concentration Memory Task

The Concentration Memory Task (CMT), illustrated in Figure 1, is a computerized version of the Concentration card game. Participants play multiple games of the CMT interleaved with spatial memory tests. The memory tests require selecting the location where each card appeared in the most recent game. Repetition of the same cards in different locations across games creates proactive interference.

In each game of CMT, using a touchscreen computer, participants perform an exhaustive search through a grid of 16 face down playing cards to find matching image pairs. When a correct choice is made, the card is turned over to reveal the target object. When an incorrect choice is made, the card is not turned over. Thus, participants have an equal number of exposures to each card in each location. After completion of Game 1, three more challenging games are played in which some images from the previous game are repeated at new locations. These repeated images appear in a total of 4 different locations within 2 consecutive games (but never re-appear in later games). After each game, participants complete a 2-alternative forced choice (2-AFC) test of their spatial memory. On each trial of the 2-AFC test, an image appears simultaneously in two locations; their task is to indicate in which of the two locations they saw the image most recently, with 1 image having been presented in the most recent game and the other presented in the game immediately prior. Optimal performance on this task requires the avoidance of interference from multiple similar memory representations, requiring the participant to segregate the memories of identical objects experienced in more than one location. We predict that the high potential for memory interfer-
ence associated with multiple object presentations places a high demand on neurogenesis, consistent with the rodent literature \[ ? ? ? ? \]. Participants played a total of 4 games for a total of 32 image pair searches (8 per game) and completed three, 2-alternative forced choice tasks appearing after games 2, 3 and 4. Each 2-AFC spatial memory test included 4 trials for a total of 12 2-AFC trials. It was predicted that those with elevated depression and alcohol binge scores would have suppressed neurogenesis and exhibit selective performance deficits on the CMT while maintaining normal performance on the two control tasks predicted to be neurogenesis-independent.

2.2. Results

All statistical analyses were performed using SPSS version 23 (SPSS Inc.). All significance levels reported are two-tailed. Non-drinkers were excluded from all analyses of the effects of binge drinking, to allow a comparison between those who indicated that they drink at binge levels to those who did not. In each regression analysis, performance on a task (CMT proportion correct, SSRT proportion correct, PAL number of errors or reverse digit span (all scored as numeric variables) was predicted from one or more categorical predictors. Predictor variables included current binge drinking status (1 = non-binge drinkers, 2 = binge drinkers), age of onset of binge drinking (1 = age 13-14, 2 = age 15-16, 3 = age 17-18, 4 = age 19+), PSS group (1 = below median, 2 = at or above median) and BDI group (1 = below median, 2 = at or above median) which were scored as ordinal variables. Predictor variable sex was scored as a nominal variable. In reporting the results of regression analyses, beta refers to the standardized regression coefficient for the indicated predictor variable.
Because the CMT, PAL and Digit Span tasks were postulated to tap into slightly different mechanisms, we assessed the inter-correlations among these tasks. Performance on the CMT was marginally correlated with PAL errors ($r = -0.207, df = 71, p = 0.079$), and strongly correlated with digit span ($r = 0.337, df = 71, p = 0.004$).

Participants who performed significantly below chance on the CMT were excluded from all analyses, assuming that they may have misunderstood the instructions or did not attend to the main task, the CMT. There were 12 trials of two-alternative forced choice. If a participant was guessing with $p = 0.5$ on all 12 trials, the cumulative probability of getting 2 of 12 correct would be 0.0161, and the cumulative probability of getting less than 2 correct would be 0.003. Therefore, participants were considered outliers if they got less than 2 correct. On this basis, three participants’ data were excluded, resulting in 73 participants’ data included in the final analysis (26 males, 45 females, 2 did not specify; mean age=18.5 years, SD=1.35).

53 of the 73 participants indicated on the lifestyle questionnaire that they do consume alcohol; each of these ”drinkers” were assigned to one of two groups, those who currently binge drink and those who do not. Participants were labeled as current binge drinkers if, in response to a question about their typical alcohol consumption (”When you drink alcohol, how many drinks do you typically consume within a single occasion? 1 = 1 -2 drinks, 2 = 3-4 drinks, 3 = 5-6 drinks, 4 = 7-8 drinks, 5 = 9 or more drinks”), they were female and responded with a 2 or higher, or male and responded 3 or higher. This definition of binge drinking roughly corresponds to the definition used widely in the literature: 4 or more drinks for a female and 5 or more drinks
for a male. Some, but not all, definitions also specify a particular time frame. Based on these criteria, 31 of the 53 participants (12 males, 19 females) who drink were labelled as current binge drinkers, while the remaining 22 (9 males, 11 females) were labelled as not binge drinkers.

Participants who were binge drinkers were also assigned an age of onset of binge drinking based on their answers to a series of questions about their drinking patterns at different ages from age 13 to 22 (or up to their current age), of the form "At age 13, approximately how often did you engage in HEAVY drinking? (Note a period of heavy drinking is defined as 4 drinks within a single time period of 2 hours or less for women and 5 drinks for men). 1: daily, 2: every 2 days, 3: every 4 days, 4: once per week, 5: once every 2 weeks, 6: once per month, 7: < once per month, 8: never, 9: age not applicable.” Participants were identified as binge drinkers at a particular age if they gave any response other than an 8 or 9 (i.e. any amount of binge drinking) for that particular age. They were then assigned an age of onset category based on the earliest age at which they engaged in binge drinking: 1 for age 13-14, 2 for age 15-16, 3 for age 17-18, and 4 for age 19 or higher. Figure 2.2 shows the distribution of participants of each sex who began binge drinking at each age.

To obtain an estimate of high interference memory performance, percent correct on the CMT 2-AFC was analyzed as a function of depression, stress and drinking scores (current binge drinking, and age of onset of binge drinking). During the 2-AFC task, participants were required to select the location where they had seen each image most recently. This proved to be relatively difficult, as evidenced by mean performance of 71.1% (SD=15.1%).
Figure 2: Histogram showing the age of onset of binge drinking, grouped by sex (1 = males, 2 = females).
To assess the reliability of the CMT 2-AFC task, split-half reliability was used. The trials were split into odd and even trial groups. There was a significant positive correlation between performance on the even and odd trials ($r(72)=.409, p < .001$). This reliability estimate was then adjusted for full test length using the Spearman-Brown prediction formula resulting in a predicted reliability ($P^*_{xx'}$) of 0.581.

Independent samples t-tests were conducted to determine whether there were sex differences in any of the measures. These revealed a marginally significant sex difference in typical alcohol consumption (on a 5-point scale, excluding non-drinkers, mean score for males = 2.7, mean score for females = 2.0, $t(df=32.24 \text{ corrected for unequal variances}) = 2.014, p = .052$) and a trend toward a sex difference in age of onset of binge drinking (mean age for males = 15.9, mean age for females = 16.7, $t(34) = -1.88, p = .069$). There were no significant sex differences in PAL, CMT, Digit Span, BDI or PSS scores.

To examine the relationship between stress, depression, binge alcohol patterns and CMT performance, we separated individuals into those scoring either low or high on the BDI and PSS based on median splits, and into current binge-drinkers and current non-binge drinkers, as well as assigning binge drinkers an age of onset category as described above.

Binge drinking negatively impacted performance on the CMT. An independent samples t-test revealed a significant difference between the scores of those who currently binge drink and those do not (mean performance for binge drinkers = 0.64, SE = .023, mean for non-binge drinkers = 0.72, SE = .024, $t(49) = 2.32, p = .024$ two-tailed). There were no significant dif-
ferences in performance between the binge-drinking and non-binge-drinking groups on either control task - Digit span or PAL.

To further assess the separate and combined impacts of binge drinking and mood on task performance, a series of categorical regression analyses were conducted. A model that included only one predictor, current binge status, revealed that binge drinking alone accounts for 31% of the variance in CMT performance (model $R^2 = .096, F(1, 49) = 5.228, p = .027; \beta = -0.311, p = .016$).

Adding sex to the regression model with current binge status did not improve the model fit ($R^2 = 0.101, F(2, 48) = 2.68, p = 0.079$). While binge drinking remained a significant predictor of CMT performance ($\beta = -0.318, p = .016$) there was no significant effect of sex ($\beta = .065, p = .45$). Sex was therefore not included as a predictor in subsequent regression analyses. No models significantly predicted performance on either of the control tasks, digit span and PAL, whether current binge, PSS or BDI group was used as the predictor. No models that included age of onset of binge drinking as a predictor were significant in accounting for performance on any of the tasks.

A regression model including both BDI group and current binge status was highly significant in predicting CMT performance. Depression group and binge status jointly predicted 43% of the variance (model $R^2 = 0.182, F(2, 48) = 5.33, p = .008$), with each of the two variables being a significant predictor ($\beta$ for binge status = $-0.355, p = .005$, $\beta$ for BDI = $-0.295, p = .017$). The combined effects of depression and binge drinking on CMT performance are illustrated in Figure 2.2. Similarly, a regression model including PSS and
current binge status as predictors accounted for 39% of the variance in CMT performance (model $R^2 = 0.152, F(2, 48) = 4.313, p = .019, \beta$ for binge status $= -0.327, p = 0.012, \beta$ for PSS $= -0.237, p = .055$).

In a previously published study \[7\], we found a negative relationship between stress and depression scores and performance on another high interference memory task, a version of Kirwan and Stark’s Mnemonic Similarity
Task [? ]. The MST requires participants to study a set of images of distinctive everyday objects, and then perform a 3-alternative recognition memory task in which test items are judged to be "old" if identical to a previously studied item, "similar" to a previously studied item, or "new". The similar lures create a high degree of interference on this task. In that study we also collected data, previously unpublished, using an earlier version of our lifestyle questionnaire which included questions about recent alcohol consumption. We therefore analyzed MST performance on old versus similar items, as well as performance on the "most similar" versus "least similar" of the similar items, as in our previous study [? ]. In that study, the "most similar" items had been judged by a group of participants to have the greatest overlap/visual similarity, relative to the visual patterns judged to be "least similar". While median split analyses on MST performance of participants with below-versus above-median alcohol consumption scores revealed no significant differences, there was a significant negative correlation between performance on the most similar lures [bias corrected, as per ? ] and participants' typical alcohol consumption levels (Pearson's r = -.215, df = 100, p < .05 two-tailed).

2.3. Discussion

Taken together, our findings in experiment 1 with the Concentration Memory Task (CMT), and our analysis of unpublished data from our previous study with the Mnemonic Similarity Task (MST), are consistent with the hypothesis that performance on high interference memory tests is particularly sensitive to the effects of recent depression levels and binge drinking. Our failure to observe significant differences in PAL or digit span based on above-versus below-median mood scores or alcohol binge status suggests that
participants in this study did not exhibit broader hippocampal or prefrontal pathology that could have accounted for the impairments on the CMT. However, it is possible that with a larger sample including more participants who had higher binge alcohol rates or earlier onset binge drinking, PAL or digit span performance may have been affected also.

Our two high interference tasks, MST and CMT, have different sources of interference. The MST creates interference by testing recognition memory for objects using highly visually overlapping lures, while the CMT creates interference by testing object location memory for identical copies of the same object when it has appeared in more than one location. However, the CMT did not tax spatial memory systematically at varying levels of spatial separation.

3. Experiment 2.

To assess the impacts of alcohol and depression on spatial recognition memory more systematically, at varying spatial separations, we developed the Spatial Separation Recognition Task. We hypothesized that performance at the finest spatial separations would depend upon high fidelity encoding mechanisms in the dentate gyrus of the hippocampus, including neurogenesis. These functions should be most vulnerable to the acute effects of stress and alcohol. On the other hand, performance at coarser separations should not be dependent upon high fidelity representations in the dentate gyrus, but may require the overall integrity of the hippocampus including other hippocampal sub-regions. Thus, general associative memory functions (including memory for finely detailed high interference items) may be more
robust against acute binge or stress exposure but should be affected by broad hippocampal pathology, which may be caused by prolonged or early onset binge drinking. A second goal of this experiment was to collect data from a larger sample of participants, to assess more fully the impact of different patterns of binge drinking (early versus late onset) on high interference tasks versus more general cognitive functions including associative encoding and working memory.

3.1. Methods

We recruited 129 participants in the same manner as in Experiment 1. Participants completed the Spatial Separation Recognition Task (SSRT), detailed below. Participants also completed the PSS, BDI-II, our lifestyle questionnaire, and the CANTAB-like PAL tasks as in Experiment 1. The digit span task was not completed by many participants and is therefore not included in the analyses here.

3.1.1. The Spatial Separation Recognition Task

The SSRT is illustrated in Figure 4. During the presentation phase, participants view images of objects, the locations of which vary along the horizontal axis of the computer screen while the vertical axis is held constant at 50 percent of the screen. Each image is displayed for 1.5 seconds. Following the presentation phase there is a brief delay of 10 seconds. Participants are not given any special instructions during the delay. During the testing phase, participants are shown the same images, one at a time, in either the exact same location as the presentation trial (20% of the time) or a different location. The “different” trials are divided into 4 groups consisting of 5 sep-
arations. Separations ranged from 1-20% of the screen. Separations 16-20% were grouped as the large-, 11-15% as the moderate-, 6-10% as the medium-, and 1-5% as the low-separation conditions, each with varying potential for memory interference. Despite being labelled as the high separation trials (therefore trials with relatively weakest potential for interference), the 16-20% trials are still challenging and have a high potential for interference. It is only relative to the low separation trials that these trials are characterized as low-interference. Participants respond by pressing the 1-key if the image is in the same location or the 2-key if the image is in a different location. Performance during the testing phase is self-paced. Optimal performance on this task requires the participant to create distinct memory representations of each image location during the presentation phase so as to avoid interference when presented with the same image during the testing phase. The experiment included 24 blocks of SSRT trials; each block consisted of 7 presentation and 7 testing trials for a total of 168 trials.

3.2. Results

Of the 129 participants tested, 103 participants (27 male, 75 female) indicated on the lifestyle questionnaire that they do consume alcohol. As in Experiment 1, each of these "drinkers" were assigned to one of two groups, those who currently binge drink and those who do not, based on their response to the typical alcohol consumption question. Those who do binge drink were also categorized by age of onset, as in Experiment 1. Of the 103 participants who drink, 57 (12 males, 45 females) were labelled as current binge drinkers, while the remaining 45 (15 males, 30 females) were labelled as not binge drinkers.
Figure 4: The Spatial Separation Recognition Task (SSRT). The two trial types, **same** (separation of 0%) and **different** (separations of 1-5%, 6-10%, 11-15%, 16-20%), for the Spatial Separation Recognition Task. “P” represents the presentation phase. “T” represents the test phase.

To assess the reliability of the Spatial Separation Recognition Task (SSRT), split-half reliability was used. The separations were grouped into odd and even groups (separations of 2, 4, 6%, etc. vs. 1, 3, 5, etc.). There was a significant positive correlation between performance on the even and odd separation trials ($r(10)=.743$, $p = .014$). This reliability estimate was then adjusted for full test length using the Spearman-Brown prediction formula resulting in a predicted reliability ($P^*xx'$) of 0.853.
Independent samples t-tests were used to assess whether there were sex differences on any measures. These revealed a non-significant trending sex difference in typical alcohol consumption (on a 5-point scale, excluding non-drinkers, mean score for males = 2.56, mean score for females = 2.01, $t(df = 35.179$ corrected for unequal variances) = 1.798, $p = 0.081$) and no sex difference in age of onset of binge drinking (mean age for males = 15.8, mean age for females = 16.3, $t(74) = -1.069, p = 0.289$). There were no sex differences in performance on SSRT or PAL. There were significant sex differences in stress levels (mean PSS score = 12.8 for males, 15.7 for females, $t (119) = -2.07, p = 0.041$) but not in depression scores.

A repeated measures analysis of variance (ANOVA) was used to assess the effect of spatial similarity (separation) on SSRT performance. The assumption of sphericity was found to be violated ($\chi^2(9) = 125.04, p < .001$), therefore we used the Huynh-Feldt correction for degrees of freedom. A main effect of spatial separation was found ($F(2.713, 347.25) = 257.75, p < .001$) as well as a significant linear trend, ($F(1, 128) = 6.35, p = 0.013$), indicating that as separation increased, performance increased proportionately. Participants were separated into high (BDI above 8, $N = 62, M = 15.73$) and low (BDI at or below 8, $N = 63, M = 4.40$) BDI groups using a median split. An ANOVA with between-subjects variable depression status (above or below median) and within-subjects variable spatial separation revealed a significant main effect of spatial separation ($F(2.774, 341.226) = 244.32, p < 0.001$), and a trending effect of depression ($F(1, 123) = 2.988, p = .086$)). The relationship between SSRT performance, spatial separation and depression status can be seen in Figure 3.2. Performance on the SSRT degrades consid-
erably at the finer separations, and masks any effect of depression. Similarly, previous work by [?] showed that the effect of depression status on performance of the MST was carried by performance on the easier / relatively less similar items. That is, the difference in performance between high and low BDI groups was restricted to visual stimuli pairs that were relatively less similar in terms of their visual characteristics. We therefore focused our regression analyses on performance at the largest spatial separation of 15-20%. We expected to find performance differences between individuals with high and low depression scores, and on those who engage in binge drinking versus those who do not.

To assess the effects of binge drinking, stress and depression on SSRT performance, a series of categorical regression models were run, using the same predictor variables as in Experiment 1, to predict performance at the highest separations on SSRT. Regression models with current binge status as the predictor did not significantly predict performance on any of these tasks. Including sex as an additional predictor did not result in any significant effects in these regression analyses.

In contrast to current binge status, age of binge onset was a highly significant positive predictor of SSRT performance at the highest (16-20%) separations. That is, a later age of onset of binge drinking was associated with higher task performance. A regression model with the single predictor age of binge onset was highly significant in predicting SSRT, accounting for 36% of the variance ($R^2 = 0.129, F(3, 72) = 3.54, p = 0.019; \beta = 0.359, p < 0.001$). Including both sex and age of binge onset as predictors in the model decreased its significance (model $R^2 = 0.131, F(4, 71) = 2.67, p = 0.039$),
and while age of binge onset remained a highly significant predictor ($\beta = 0.364, p < 0.001$), sex was not a significant predictor ($\beta = 0.048, p = 0.487$).

Including both depression status (above or below median) and age of binge onset jointly accounted for 39% of the variance in SSRT performance (model $R^2 = 0.155, F(4,69) = 3.168, p = .019$), with the age of binge onset being a highly significant predictor ($\beta = 0.352, p < .001$) and that for depression status (BDI below or above median) trending as a negative predictor ($\beta = -0.207, p = 0.063$). A regression model including PSS group (above or below median) and age of binge onset as predictors explained 36% of the variance (model $R^2 = 0.127, F(4,68) = 2.31, p = .053$) but only the age of onset coefficient was significant (age of onset $\beta = 0.364, p < 0.001$; PSS group $\beta = 0.078, p = 0.484$). On the PAL tasks, age of binge onset was a marginally significant predictor of performance ($R^2 = .093, p = .1284; \beta = -0.3059, p = .07$). Performance on the SSRT was significantly correlated with PAL errors ($r = -0.435, df = 120, p < .001$). The effect of age of onset of binge drinking versus performance on the SSRT and PAL can be seen in Figure 3.2.

### 3.3. Discussion

The findings in experiment 2 with the SSRT were somewhat surprising in light of those in Experiment 1. We expected to see an effect of current binge status, stress and depression levels on SSRT performance. In contrast to our findings with the Concentration Memory Task in the first experiment, performance on the Spatial Separation Recognition Test was not related to current binge drinking patterns. Instead, SSRT memory scores were significantly impacted by age of onset of binge drinking. Also, unlike in Experiment
Figure 5: Experiment 2 results. Top: SSRT performance versus spatial separation for those with below- and above-median depression (BDI) scores. Bottom left: Performance on the low interference SSRT trials versus age of onset of binge drinking. Bottom right: Performance on paired associate learning (PAL) versus age of onset of binge drinking.
1, error rates on the paired associate learning task (CANTAB-like PAL) were marginally associated with age of onset of binge drinking. This pattern of results is consistent with our original hypothesis that many years of binge drinking and/or early onset of binge drinking will have broader impacts on the hippocampus and other brain regions. There were some important differences in the participant samples in the two experiments that may account for these discrepancies in findings. Figure 2.2 shows that there was only one participant with a binge onset age of 13, one at 14 and one at 19. In Experiment 2 we had nearly twice the number of participants, so the extreme ends of the age of onset categories were better represented. Importantly, the effect of age of binge onset on SSRT was carried largely by the few participants who began binge-drinking very early (see Figure fig:SSRTresults). This explains why we did not see a significant effect of age of onset in Experiment 1. It remains to be explained why we did not see a significant effect of current binge status in Experiment 2. One possibility is that our spatial separation task was simply not as sensitive as the Concentration task at picking up memory for fine details and high overlap. We hypothesized that at the finer spatial separations the SSRT would be particularly sensitive to mechanisms for encoding high interference memory items, including hippocampal neurogenesis.

Similar tests of recognition memory for objects presented at varying spatial separations have been employed in previous studies to investigate the effects of ageing on high interference memory (Stark et al, 2010, Holden et al, 2012). Both of these studies reported age-related deficits on spatial separation tasks, broadly consistent with the literature on age-related declines in hippocampal function. These data are also broadly consistent.
with the findings of the current study, and suggest that in addition to binge drinking, stress and depression, ageing also impairs hippocampal function on high interference memory tasks.

4. General Discussion

It has been reported previously that as many as 44% of college students binge drink every two weeks, while as many as 19% binge more than 3 times per week [? ]. In addition to the memory deficits described here, long-term effects of alcohol use during adolescence include increased risk of alcohol dependence, learning deficits, and other memory impairments [? ]. Given the cognitive impacts illustrated here and elsewhere as well as the high prevalence of binge drinking, it is imperative that the risks associated with alcohol consumption become more widely appreciated by youths at an age when they are most impressionable. In this way, it may be possible to reduce the prevalence of this physically and cognitively destructive behaviour.

Given that high stress and drinking levels seem to be a part of the typical undergraduate experience, educational efforts should be targeted at this group. For example, lifestyle changes in diet and exercise may mitigate the negative effects of stress and alcohol. In rodents, exercise lessened the effects of binge alcohol exposure [? ], while exercise combined with a diet supplement rich in anti-inflammatory and anti-oxidant components mitigated the effects of stress on the brain [? ].

The present study has several limitations. One is in our definition of a binge, based solely on amount of alcohol consumed per occasion (as is often done in the literature) rather than taking into account the time over which
the alcohol was consumed. The frequency with which someone engages in binge drinking is another critical factor that has not been examined here.

From a research standpoint, it is also important that those dealing with undergraduate populations understand the types of cognitive deficits associated with non-alcoholic adolescents who do tend to binge drink, as well as the high prevalence and impact of stress-related psychiatric disorders. Researchers may want to screen for these factors in future studies.

A key hypothesis in both of our experiments was that high acute levels of alcohol consumption, stress, and depression would lead to selective deficits on high interference memory tasks. This hypothesis was based on past studies, mainly in rodents, indicating that binge drinking and acute stress both potently suppress neurogenesis [? ? ]. Thus one of our key hypotheses hinged on the assumption of an underlying neurogenesis deficit. Converging evidence from human and non-human animal studies implicates neurogenesis in the generation of high fidelity memory representations, protecting them from interference. While our findings are broadly consistent with this assumption, other explanations cannot be ruled out. A limitation in translating such findings to humans is the lack of a non-invasive in vivo measure of neurogenesis. It is possible that one or more additional variables were affected by stress, depression, or alcohol consumption and that these variables may have caused or influenced the memory deficits observed here. For example, depressive episodes in humans are associated with decreased serum levels of brain derived neurotrophic factor (BDNF) [? ], a neurotrophin important for plasticity and long-term potentiation [? ? ]. However, given a reduction in BDNF, one might expect to find more widespread learning and memory
deficits in domains like working memory and paired associates learning. The results of the current study fail to show such deficits in association with acute binge drinking, stress and depression levels. Thus, the deficits we observed in Experiment 1 on the CMT are likely not the result of general plasticity changes via BDNF expression. Direct assessment of neurogenesis would be required to dissociate neurogenesis-dependent and -independent effects on memory. On the other hand, the broader memory deficits observed in Experiment 2 on the SSFT and on PAL in association with early onset binge drinking are consistent with broader hippocampal pathology.

5. Conclusions

We have described two novel memory tests for assessing spatial interference: the Concentration Memory Task (CMT) and the Spatial Separation Recognition Task (SSRT). Our findings indicate that acutely elevated stress, depression and alcohol consumption levels are associated with specific deficits on the CMT, while early onset binge drinking causes broader memory impairments, as reflected by deficits on the SSRT and on paired associate learning; these deficits may indicate reduced hippocampal neurogenesis versus broader hippocampal pathology, respectively. The effects of heavy alcohol consumption on global brain volume loss are reversed partially after a period of abstinence [? ]. An important avenue for further research is to determine to what extent the damage caused by early onset binge drinking can be reversed. We were hoping to address this question in the present study by examining memory deficits in those who began binge drinking early and continued this pattern of drinking into their university years, in comparison to those who
began binge drinking early and then stopped. Unfortunately, in our sample of nearly 200 university students across the two experiments reported here, there were no participants in the latter group. Perhaps by studying a more mature sample we might find participants who started binge drinking early but gave it up by the time of their working years in later adulthood.

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