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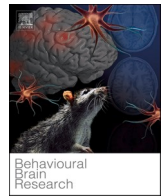
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Research article

Age-related effects on the association between alcohol use, severity and resting-state fMRI: A rat study comparing adolescent-onset and adult-onset drinking

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ABSTRACT

Adolescence is marked by neurodevelopmental changes that increase reward sensitivity, risk-taking, and behavioural control challenges, heightening the risk of alcohol use and dependence. Alcohol's impact on resting-state functional connectivity (RSFC) may explain this risk, though comparisons to adulthood remain limited. This study examined age-related differences in the association between RSFC and alcohol use severity in rats that initiated low or high voluntary alcohol consumption during adolescence or adulthood. Forty-two male rats were selected based on their alcohol consumption levels after two months of exposure: adolescent (PND42) onset low ($N = 12$) and high drinking ($N = 7$) rats, and adult (PND77) onset low ($N = 11$) and high drinking ($N = 12$) rats. RSFC was measured 4–10 days after exposure ceased, and group-ICA identified networks. Permutation tests assessed associations between onset age and use severity on RSFC. Low drinking was associated with increased RSFC within the salience network compared to high drinking. High adolescent onset alcohol consumption was associated with increased Default Mode Network (DMN) connectivity relative to low use. Connectivity in this area was generally stronger in adult compared to adolescent onset groups. An age group effect was observed, with overall higher DMN-thalamic connectivity in adult versus adolescent onset rats. In conclusion, high adolescent alcohol use was associated with increased DMN connectivity compared to low use, potentially reflecting altered self-referential processing or withdrawal susceptibility in adulthood. In adult-onset rats, the observed increases in DMN and DMN-thalamic connectivity may reflect developmental differences rather than alcohol exposure. These findings highlight the need for further research on DMN connectivity and its role in AUD risk and resilience, particularly regarding adolescent alcohol use, and the inclusion of a control group without alcohol access to better isolate the effects of alcohol consumption.

1. Introduction

Adolescence marks a period of neurodevelopmental change, encompassing normative increases in reward sensitivity and risk-taking behavior, alongside challenges in behavioral control. This is suggested

to increase adolescents' risk of developing an Alcohol Use Disorder (AUD) [12,24]. Currently, there is limited evidence directly testing the differential impact of alcohol use during adolescence versus adulthood, as well as exploring the effects of low versus high alcohol consumption. The purpose of the present study was to investigate age-related effects

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on the association between resting-state functional connectivity (RSFC) and alcohol use severity in rats that initiated low or high voluntary alcohol consumption during adolescence or adulthood.

Resting-state functional connectivity measures correlated spontaneous activity between brain regions, which form functional networks [63]. During adolescent development, relative early maturation of the salience network (SN) is thought to relate to increases in sensitivity to rewards and risk-taking behaviour [46]. By contrast, the executive control network (ECN) is developing more protractedly throughout adolescence [2,20]. This imbalance is thought to contribute to adolescents' increased risk of developing an AUD [24,42].

Evidence suggests that RSFC is sensitive to both acute [28,68] and chronic [39,74] alcohol exposure. Most studies have included adult populations, where individuals with AUD and high users show dysconnectivity within and between the default mode network (DMN), SN, ECN and subcortical reward circuitry [8,9,39,64,70], alongside increased connectivity to other regions outside these key networks compared to controls [39]. This is suggested to reflect a compensatory mechanism [10]. Abnormal fronto-striatal connectivity has been linked to impaired decision making in AUD [19], relapse vulnerability [29], and alcohol craving [44]. These fronto-striatal networks also show heightened alcohol cue-reactivity in AUD [41]. To a lesser extent, RSFC has been investigated in adolescents, with moderate-to-high drinkers showing reduced connectivity between the DMN and the limbic network, with the amygdala as the seed region, compared to no/low drinkers [40]. Increased connectivity was also found within the ECN that was associated with increasing age in no/low drinkers only. There was also evidence of age-related DMN and limbic network desynchronization, which negatively correlated with episodic memory performance in the moderate-to-high drinkers. Besides this study, to the best of our knowledge there are no human studies that have investigated age-related effects on the association between RSFC and alcohol use or AUD (See [31] for a review).

However, there is evidence of age differences in neural alcohol cue-reactivity. Higher beer odour-induced activity was found in mesocorticolimbic structures of the limbic and SN in adolescents with an AUD, compared to adults with an AUD [13]. Further, a positive association between social alcohol cue-reactivity in the medial Prefrontal Cortex (part of the SN) and social attunement was found in low-to-high drinking adolescents and a negative association was found in their adult counterparts [32]. Interestingly, an earlier age of substance use has been found to predict stronger connectivity between the ECN and reward networks in a sample of substance (alcohol, tobacco, nicotine) using adolescents [71], as well as increased RSFC within several regions of the prefrontal cortex [71]. This suggests that the normative development of functional networks, which typically evolve from being more internally connected to becoming more segregated, may be disrupted by substance use, including alcohol [40]. This disruption may also result in stronger segregation and connectivity between networks implicated in AUD [71].

Although human studies are informative, they cannot control exposure or fully isolate environmental influences. Animal models help to address these limitations [56]. Accumulating literature supports the existence of rat resting state networks, which have been found to be homologous across rodents and humans. There is evidence of the SN [4, 34,62], DMN [14,23,34,36,54] and somatosensory networks [26,54], however, evidence for the ECN is still in its infancy [53].

Preclinical resting-state studies specific to AUD are still scarce (See [18] for a review). In one study, 30 days of alcohol exposure reduced connectivity between retrosplenial-visual and striatal networks while increasing connectivity between prefrontal-cingulate and striatal networks in adult rats [47]. Similarly, rats exposed to alcohol during adolescence were found to have altered connectivity between the prefrontal cortex and striatal regions in adulthood, however, connectivity was reduced [7]. The dysconnectivity between prefrontal and striatal networks mirrors the findings in human studies, which have been suggested to be linked to heightened cue-reactivity as an important

characteristic of AUD [41]. Rats exposed to alcohol during adolescence were also found to have reduced fronto-limbic connectivity in adulthood [22]. To date, one study investigated age-related differences in the acute effects of 4 g/kg alcohol on RSFC in adolescent versus adult exposed rats, whereby adult exposed rats were found to exhibit greater ethanol induced increases in connectivity within the lateral cortical network (LCN), as well as between the LCN and both the DMN and SN, in a dose-dependent manner compared to adolescent-exposed rats. Lee et al., [34].

This study examined the influence of age on the association between RSFC and the degree of voluntary alcohol consumption in rats. Rats that initiated alcohol consumption during adolescence or adulthood, were subsequently classified into low or high drinkers based on their level of alcohol consumption. We hypothesise that adolescent-onset rats will exhibit greater alcohol effects due to developmental sensitivity, reflected in higher within-network connectivity of the SN, DMN and ECN [20,25], along with stronger segregation and connectivity between the ECN/prefrontal and salience/striatal networks [7,71].

1.1. Methods

1.1.1. Animals

All protocols adhered to the Animal Ethics Committee of Utrecht University guidelines and complied with Dutch laws (Wet op die dierproeven, 2014) and European legislation (Guideline 86/609/EEC; Directive 2010/63/EU). The study included 72 Male Lister Hooded rats (Charles River Laboratories), with 36 rats arriving at postnatal day (PND) 35 (adolescent) and 36 at PND 70 (adult). The facility maintained a reversed 12-hour light/dark cycle (lights off at 7:00 am). Rats were housed in type III Makrolon cages with type III-S lids, provided unlimited access to chow and water, and equipped with polycarbonate tunnels (9 × 9 × 15 cm) and paper tissues. After a one-week acclimatization period, rats were individually housed, handled, and weighed weekly.

1.1.2. Voluntary home-cage alcohol consumption

Rats underwent a two-month intermittent alcohol access (IAA) schedule as previously described [33,60]. The protocol employed a two-bottle choice setup in home cages, offering both water and 20 % (v/v) alcohol (Klinipath, The Netherlands) three days per week (Monday-Wednesday-Friday). Each cage contained one water and one alcohol bottle (250 ml, Techniplast, Germany), with bottle positions alternated between sessions. During non-alcohol days, rats received a single 500 ml water bottle. Adolescent rats began alcohol access at PND 42, corresponding to the adolescent phase based on cognitive milestones [65], while adults started at PND 77. The timing for adolescent onset of drinking prevented individual housing during social play onset, as play deprivation has been linked to increased substance reward sensitivity [65].

The first month of IAA provided 7-hour daily access (9:00 am to 4:00 pm), extending to 24-hour access in the second month to accommodate higher consumption patterns, particularly in high alcohol-drinking rats [60]. Alcohol intake was monitored by weighing bottles before and after each session, converting measurements to grams of ethanol per kg of rat (g/kg) per session, and calculating weekly averages. After the two-month period, the rats were classified by alcohol use onset (adolescent; adult) and ranked from low to high intake. Weekly rankings were summed to generate total scores, with the lowest and highest tertiles designated as low (LD) and high (HD) drinkers. Following exclusions due to RABIES preprocessing issues (five rats) and timeseries restructuring complications (one rat), the final sample comprised 42 rats: Adolescent LD (N = 12), Adolescent HD (N = 7), Adult LD (N = 11), and Adult HD (N = 12).

1.1.3. fMRI experimental design

After the alcohol exposure period, rats were left undisturbed for 4–10 days. Following this each rat underwent a 7-minute resting-state scan,

with adolescent rats scanned between PNDs 106–112 and adult rats between PNDs 141–147. The protocol included a cue-exposure paradigm that is not discussed in this analysis.

1.1.4. Data acquisition

Rats were anesthetized using 2.0–3.0 % isoflurane induction, maintaining respiration at 30–40 breaths/minute. They were positioned prone in the MRI cradle, secured with bite and ear bars, and received continuous isoflurane in > 90 % oxygen-enriched air via a nose cone. Following positioning, rats received a 0.020 mg/kg bolus injection of dexmedetomidine (0.42 mg/ml undiluted), followed by a 0.040 mg/kg/h maintenance dose. Throughout scanning, vital signs (heart rate, respiration, oxygen saturation, and core temperature) were monitored.

Imaging was performed using a 300 MHz Bruker USR 4.7 T/20 cm scanner. Anatomical scans were acquired at $125^2 \times 20$ slice resolution with a 40^2 mm field of view and 1.0 mm slice thickness. Resting-state fMRI parameters included $600^2 \times 20$ slice resolution, 32.4^2 mm FOV, 1.0 mm slice thickness, and $3^2 \times 1$ mm voxel size.

1.1.5. fMRI data preprocessing

RABIES 20.4-dev software, based on the fMRIprep pipeline [15], processed the MRI data using default preprocessing and spatial normalization parameters. Head motion correction utilized rigid registration to functional data (antsMotionCorr; [3]). Preprocessing steps included non-local mean denoising, bias field correction (N3 Algorithm; [55]), and intensity thresholding for initial masking. Rigid registration aligned a reference atlas-derived brain mask to functional data. Anatomical images underwent similar processing plus non-linear registration, followed by functional data registration to structural images and the Fischer344 atlas [21] using FNIRT. Each functional volume was resampled using cross-modal alignment transforms and head motion parameters, while correcting for EPI distortions through nonlinear registration [67].

Confound correction, also performed via RABIES 20.4-dev, began with voxel-wise detrending to remove first-order drifts, followed by high-pass filtering (0.01 Hz) and removal of 30-second segments from timeseries ends to address edge artifacts [49]. Nuisance regressors (white matter and CSF mean signals, six rigid motion parameters) underwent filtering to prevent confound reintroduction [35]. Ordinary least squared regression modelled nuisance regressors at each voxel before data regression. Finally, spatial Gaussian smoothing (0.3 mm FWHM) was applied. See [supplementary section 1.1 and 1.2](#) for full details.

1.1.6. Behavioural analysis

Alcohol consumption (g/kg) analysis employed a three-way repeated measures ANOVA, with month (1st-7hrs and 2nd-24hrs) as the within-subjects factor, and age (adolescent and adult-onset) and alcohol subgroup (low and high) as between-subjects factors.

1.1.7. fMRI analysis

Group-independent component analysis (group-ICA) was performed on concatenated scan time series. After testing decompositions of 10, 15, 20, and 30 components, a 10-component solution was selected as optimal, as higher numbers caused network fragmentation (See [supplementary section 1.3](#)). Visual inspection classified components as signal (8) or noise (2). Dual regression modelled individualized brain network connectivity from group-ICA results, maintaining signal/noise classifications. Statistical analysis utilized FSL's randomise tool with 5000 permutations, employing Threshold-Free Cluster Enhancement (TFCE) for cluster detection and family-wise error (FWE) correction ($\alpha = .05$). Analyses focused on the following networks of interest: Somatosensory network (Component 1) which encompassed the motor cortex (M1) and primary and secondary somatosensory cortices (S1, S2), with minor activation observed in the pons and superior colliculus. The ECN (Component 6) showed activation in the prefrontal cortex,

including the anterior secondary motor cortex (M2), S1, and granular insula cortex. The DMN (Components 2 & 7), where component 2 showed activation in the orbital cortex, cingulate cortex, retrosplenial cortex, hippocampus, primary visual cortex and posterior parietal cortex, while component 7 showed activation in the prelimbic, cingulate cortex, caudate putamen, hippocampus, retrosplenial cortex, primary visual cortex, posterior parietal cortex, and temporal association cortex. The SN (Components 3, 4 & 10) included three distinct components. Component 3 showed activation in the dysgranular/granular insular cortex, posterior agranular insular cortex, S1, S2, caudoputamen, globus pallidus. Component 4 included activation in the primary and secondary cingulate cortex, prelimbic cortex, infralimbic cortex, lateral orbital cortex, ventral orbital cortex, M2, and dorsal and ventral agranular insular cortex. Component 10 showed activation in the S1, S2, nucleus accumbens, primary and secondary cingulate cortex, prelimbic cortex, infralimbic cortex, agranular insular cortex, globus pallidus, and caudoputamen. And a basal ganglia network (Component 5) showed activation in the nucleus accumbens, caudoputamen and infralimbic cortex.

2. Results

2.1. Age differences in alcohol consumption

Across all rats, alcohol intake was significantly higher in the second month when alcohol access was extended to 24 h, compared to the first month when there was 7 h of access, $F(1, 38) = 183.72, p < .001, \eta^2 = .83$. There was no significant main effect of age on alcohol intake, $F(1, 38) = 0.89, p = .35$, nor was there a significant interaction between month and age of onset, $F(1, 38) = 0.007, p = .93$. Additionally, no interaction was found between month, age of onset, and severity of use, $F(1, 38) = 1.56, p = .22$. Across the two months, the mean alcohol intake for the adolescent HD group was 4.24 g/kg ($SD = 0.81$), while the adolescent LD group consumed on average 1.11 g/kg ($SD = 0.40$). For the adult groups, the HD group had a mean alcohol intake of 3.98 g/kg ($SD = 1.23$), and the LD group consumed on average 0.88 g/kg ($SD = 0.39$). These results suggest a similar increase in total alcohol consumption from month one to two across the two age groups, coinciding with the shift from 7-hour to 24-hour access. There were no significant differences in alcohol consumption between low and high drinkers across ages, and no differences in absolute volumes consumed. [Fig. 1](#) displays the mean alcohol intake of the groups across the two-month period.

2.2. Age differences in alcohol use-related RSFC

There were no significant main effects of age on within and between RSFC. Regarding the main effect of alcohol use severity, LD compared to HD rats showed increased RSFC within a small cluster in the SN (S2). No other significant differences in RSFC within and between the networks were found. See [Fig. 2](#), and [Table 1](#).

2.3. Interactions between age and alcohol use severity on RSFC

The interaction between age and alcohol use severity was significant between RSFC of the DMN and thalamus, as well as RSFC within the DMN (primary visual cortex). No significant interaction effects were found in the BGN, somatosensory network, SN and ECN. See [Fig. 3](#), and [Table 1](#).

For the significant interaction effect found within the DMN *post hoc* t-tests indicated that while there was no significant difference between adult LD and HD rats, adolescent HD rats had lower connectivity compared to the adult LD ($t(15) = 2.87, p > .01, d = 1.30$) and HD rats ($t(13) = 2.36, p = .034, d = 1.11$), but higher connectivity compared to the adolescent LD rats ($t(16) = 2.15, p = .047, d = 0.93$) (see [Figure 3a](#)). Similarly, for the significant interaction effect found between the DMN and thalamus *post hoc* t-tests revealed only significant age group effects

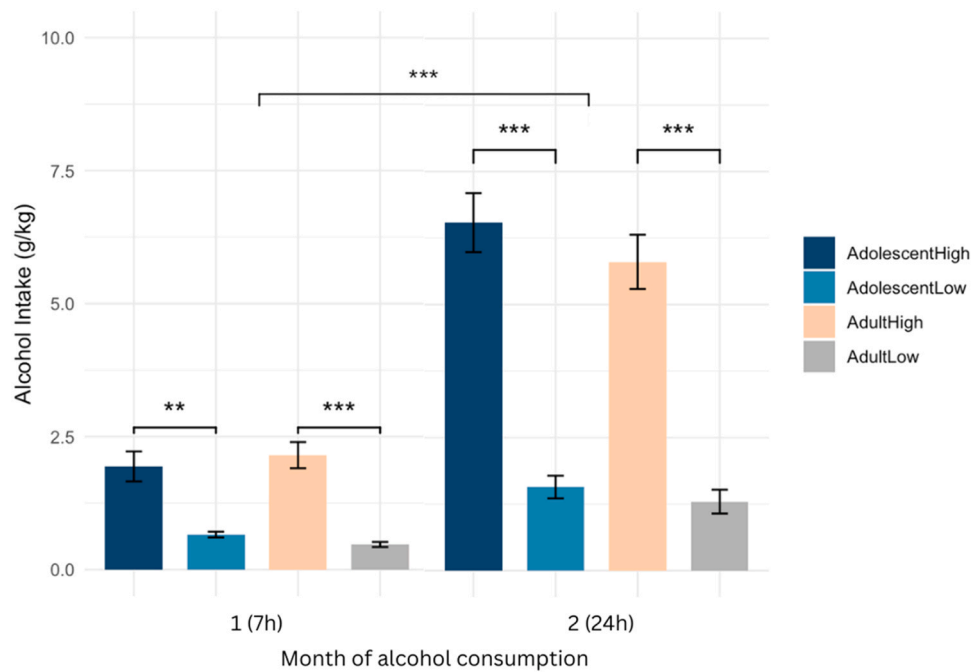


Fig. 1. Mean alcohol intake (g/kg) in adolescent and adult rats, selected as part of the low drinking or high drinking groups of each age group. The data shown is mean + standard error of the mean.

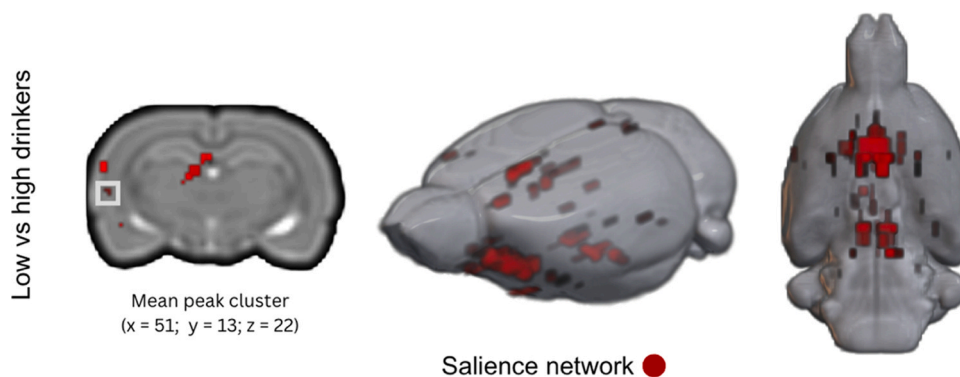


Fig. 2. Higher resting state functional connectivity in the salience network of low vs high drinking rats. Full network as identified by Independent Component Analyses is visualised for descriptive purposes. The mean peak cluster (co-ordinates $x = 51$; $y = 13$; $z = 22$ in native space) found in the secondary somatosensory area of the salience network is visualised with a grey square.

with none of the within age group comparisons being significant; Adult LD rats had increased connectivity compared to adolescent LD rats ($t(21) = 5.11$, $p < .001$, $d = 2.12$), and adult HD rats had increased connectivity compared to adolescent LD rats ($t(21) = 3.26$, $p < .01$, $d = 1.33$).

3. Discussion

The purpose of the current study was to investigate age-related differences in the association between RSFC and alcohol use severity in rats that initiated low or high voluntary alcohol consumption during adolescence or adulthood. Regardless of age of onset of voluntary alcohol exposure, increased RSFC within the S2 of the SN was found in low compared to high alcohol drinking rats, suggesting a potential association between alcohol consumption levels and connectivity in this region. Initiation of high levels of voluntary alcohol consumption during adolescence was associated with increased connectivity within the primary visual area of the DMN relative to low levels of voluntary alcohol consumption during adolescence. Connectivity in this area was

generally stronger in adult compared to adolescent onset groups. Further, an age effect was observed for connectivity between the DMN and thalamus, with higher connectivity in the adult compared to adolescent onset rats. These findings should be interpreted cautiously, given the correlational nature of the study and the absence of a non-drinking control group.

An association between alcohol use and connectivity in the salience network was found, although the direction of this relationship was not as expected. Low drinking rats were found to have increased RSFC within the SN compared to high drinking rats. While we hypothesised that high drinking rats would exhibit dysconnectivity within and between the DMN and SN, as well as hypoconnectivity within the ECN, the opposite pattern emerged, with differential RSFC found in low drinking rats. Previous preclinical studies have focused on RSFC differences between alcohol-exposed rats and control rats with no alcohol exposure, consistently finding dysconnectivity in alcohol-exposed rats [7,22,47]. However, this study is unique in examining differences between low- and high-drinking rats. To the best of our knowledge, there are no existing studies for direct comparison. The increased connectivity within

Table 1
Comparison of connectivity differences in functional connectivity networks based on Independent Component Analysis. Abbreviations: DMN Default Mode Network.

Age of alcohol exposure						
Network	Direction	Size (voxels)	Coordinates			P-value FWR corrected
			X	Y	Z	
<i>Adolescent > adult</i>						
All networks	ns	ns	ns	ns	ns	ns
<i>Adult > adolescent</i>						
All networks	ns	ns	ns	ns	ns	ns
Severity of alcohol use						
Network	Direction	Size (voxels)	Coordinates			P-value FWR corrected
			X	Y	Z	
<i>Low > high</i>						
Within Salience Network	↑	3	51	13	22	0.04
<i>High > low</i>						
All networks	ns	ns	ns	ns	ns	ns
Age of exposure x severity of alcohol use						
Network	Direction	Size (voxels)	Coordinates			P-value FWR corrected
			X	Y	Z	
<i>Adult high > low vs adol high > low</i>						
Between DMN and thalamus	↑	4	40	12	19	0.05
Within DMN	↑	1	38	10	37	0.03
<i>Adol high > low vs adult high > low</i>						
All networks	ns	ns	ns	ns	ns	ns

the SN observed in low drinking rats also contrasts with human literature, which has consistently reported greater dysconnectivity in high drinkers or individuals with AUD [9,25,40,64,70]. Although speculative, this may indicate that the relationship between alcohol consumption and RSFC is not linear. In an exploratory analysis, we investigated associations with individual drinking levels of the rats, but no significant correlations with RSFC were found. Future studies that include a sample of rats with no alcohol exposure could help to further clarify this relationship.

Contrary to expectations, no main effects of age on RSFC were found. However, when considering both age and severity of use, differences were apparent. Initiation of high levels of voluntary alcohol consumption during adolescence was associated with increased connectivity within the primary visual area of the DMN relative to low levels of alcohol consumption. Connectivity in this area was generally stronger in adult versus adolescent onset groups. Given the absence of a non-drinking control group or baseline RSFC measurements, these findings should be interpreted with caution, and further research is needed to better understand causation in this interaction. Nevertheless, these findings partially support our hypotheses, as dysconnectivity was found to a larger extent in high drinking adolescent-onset rats compared to their lower drinking counterparts. However, contrary to expectations, the largest increases in connectivity within the DMN were observed in adult-onset groups, raising the possibility that factors beyond alcohol exposure, such as developmental differences, may contribute to these patterns. Due to our study design, we cannot determine whether this

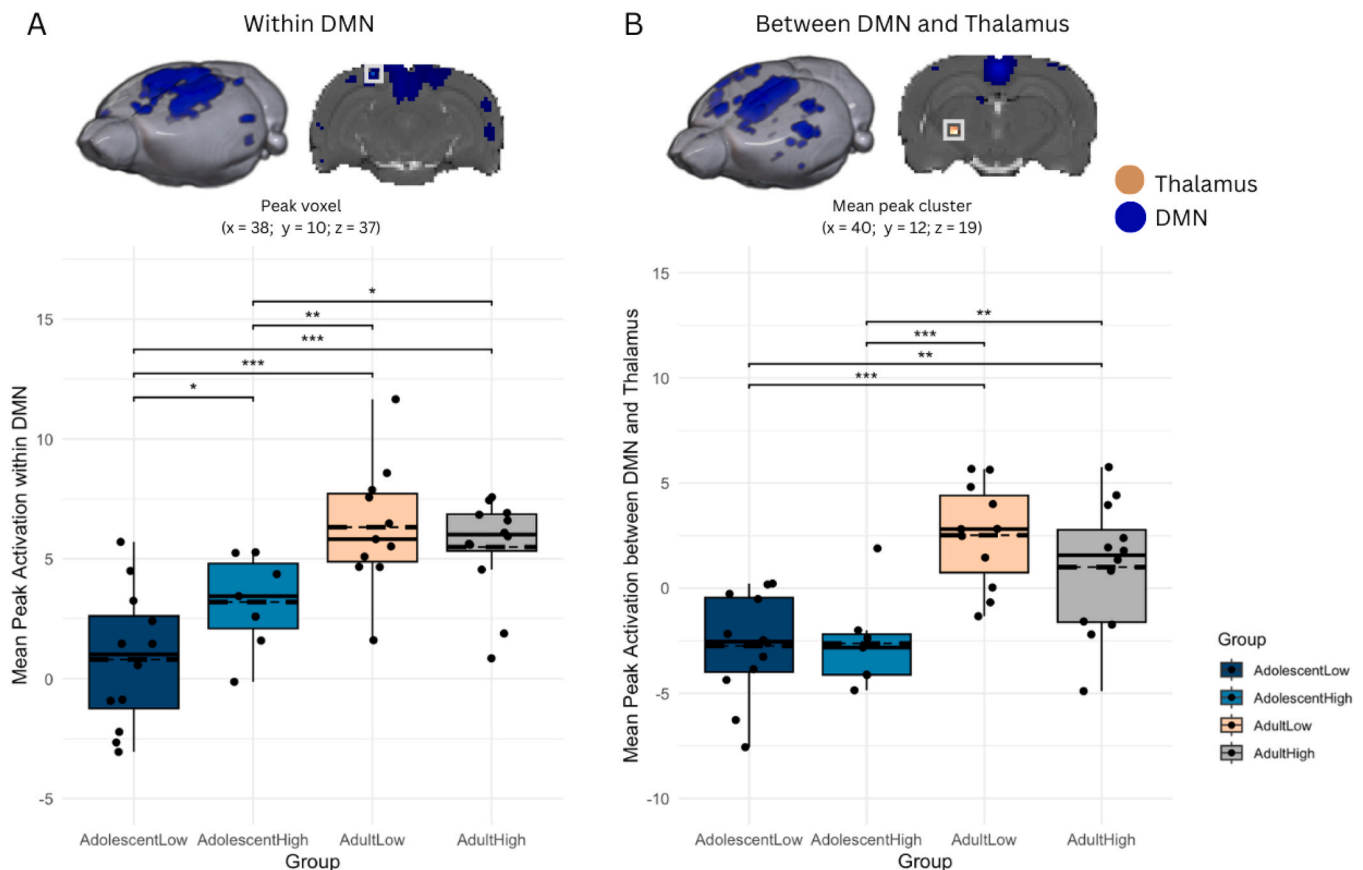


Fig. 3. a. Group differences in mean peak activation in the significant cluster (within the primary visual area of the default mode network) found for the interaction contrast. The full network as identified by Independent Component Analyses is visualised for descriptive purposes, with peak voxel coordinates (in native space) listed and visualised within the grey square. Error bars depict the standard error. The mean is depicted as a dotted line, and the median a solid line. b. Group differences in mean peak activation within the significant cluster (between the default mode network and thalamus) identified for the interaction contrast. Full network as identified by Independent Component Analyses is visualised for descriptive purposes, with peak cluster coordinates (in native space) listed, and visualised within the grey square. Error bars represent the standard error. The mean is depicted as a dashed line, and the median as a solid line. P-values: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

increase in connectivity stems from alcohol exposure or developmental factors. However, one of the only studies to date to investigating age differences in RSFC, independent of alcohol exposure, found that adult rats (PND 80) had increased connectivity within the DMN compared to adolescents (PND 45) [34]. Evidence from human studies also suggests age-related differences in DMN connectivity [16,50], with increased connectivity generally found in older individuals. Moreover, the lack of differences between low- and high- drinking adult-onset rats in our study further suggests that these effects may reflect developmental differences rather than alcohol exposure. In their study, Lee and colleagues scanned rats at baseline and after cumulative doses of ethanol and found increases in connectivity within the LCN, and between the LCN and both the DMN and SN in all rats in a dose-dependent manner. They subsequently found age moderated this association, with adult-exposed rats showing greater ethanol-induced increases within and between these networks compared to adolescent-exposed rats. Notably, they did not find ethanol-induced age differences in within DMN connectivity, but rather between the DMN and LCN. This distinction suggests that some connectivity differences may stem from developmental changes, while others may be driven by alcohol exposure. It will be important for future preclinical studies to include a control group and/or baseline measurements to continue to better isolate the factors driving these connectivity differences.

These findings suggest that the level of alcohol consumption may influence RSFC, particularly when initiated during adolescence, with high alcohol intake associated with increased connectivity within the DMN network compared to low alcohol intake. However, this contrasts with Lee et al., [34], who found no ethanol-induced age dependent effects within the DMN, but rather between the DMN and LCN. Additionally, we observed increased connectivity between low and high adolescent-onset rats, but not between low and high drinking adult-onset rats, whereas Lee et al., [34] found effects in both groups. While the current findings cannot establish causality, they suggest that the DMN may be sensitive to both alcohol exposure and severity of use in an age-dependent manner. The DMN plays a crucial role in self-referential processing and the integration of prior and future experiences [51], functions that are critical for regulating behaviours related to addiction. Dysconnectivity in this network has been associated with alcohol use and AUD, correlating with craving and relapse (See review by [72]), as well as impaired self-awareness in SUD, where individuals may continue substance use without reflecting on the consequences [66]. Furthermore, DMN connectivity has been shown to predict AUD severity as measured by the AUDIT [17], and is associated with withdrawal-related negative affect in the addiction model of neurobiology [30]. When reflecting on the paradox of risk and resilience during adolescence and adulthood, our findings suggest that initiation of high levels of alcohol use in adolescence may be associated with pronounced alterations in RSFC than alcohol use initiated in adulthood. However, it is important to note that causality cannot be inferred from these correlations, especially given the absence of a non-drinking control group. Therefore, future research should aim to build on these findings by including a control group without alcohol access to better isolate the specific effects of alcohol consumption on RSFC.

Importantly, prior research does suggest that high alcohol consumption during adolescence may impair self-awareness in adulthood or increase susceptibility to withdrawal, potentially contributing to continued escalation of use, and increased risk of AUD [66]. Both adolescent- and adult- onset rats were scanned four to 10 days after alcohol cessation and so were in protracted withdrawal by the time of scanning [6,27]. The increased connectivity in high alcohol drinking adolescent-onset rats could potentially suggest accelerated aging, as the connectivity in these rats shifted toward patterns more typically found in adult-onset rats. However, this remains speculative, and further research is needed to determine whether this shift reflects a true acceleration of an adult-like phenotype or a distinct, maladaptive trajectory of neurodevelopment. Notably, behavioural studies have often shown

that adolescent alcohol exposure leads to the retention of adolescent-typical response into adulthood rather than convergence with adult-exposed patterns (See [59] for a review). Integrating both neural and behavioural findings will be essential to fully understand the long-term impact of alcohol use on brain function and behaviour. Alternatively, the increased connectivity in the DMN might also reflect resilience, as higher connectivity in adulthood has been associated with increased efficiency [16]. In a separate study from our group, high drinking adolescent onset rats exposed to the exact same regime as in the current study showed greater control over alcohol seeking compared to the adult-onset rats, even when consuming high levels of alcohol [33]. This could be attributed to the greater plasticity and adaptability known to be present during adolescence [58]. Therefore, it remains unclear whether high alcohol use initiated in adolescence serves to increase the risk of AUD or may instead contribute to resilience in adulthood. Investigating the underlying mechanisms or outcomes that may be associated with the changes in DMN connectivity, particularly with the inclusion of a non-drinking control group, would be crucial in clarifying its role in the development of AUD risk and resilience.

Additionally, a significant interaction between age and severity of use was observed in DMN-thalamic connectivity. However, post hoc tests only revealed significant differences between age groups, with higher connectivity in adult-onset compared to adolescent-onset rats. Dysconnectivity between the DMN and thalamus has been reported previously in a sample of nondependent adult drinkers and was found to negatively correlate with AUDIT scores (ie., lower connectivity with higher AUDIT scores)[73]. In a second study, thalamic and DMN engagement was found to be associated with AUDIT scores for both reward/loss anticipation and RSFC within these regions [17]. Additionally, across the thalamus and regions of the DMN, a stronger relationship was found between engagement of these regions and higher alcohol use in younger individuals (age < 41) compared to older individuals (age > 41). Although the sample was older it does point to age-related differences and thus emphasizes the need for replication in younger samples. Regions of the DMN network and thalamus are widely connected, both on a structural and functional level, and it is suggested that the thalamus modulates activity of the DMN and contributes to network integration and resilience [1]. However, it remains speculative whether the altered DMN thalamic connectivity found here is related to risk or resilience of problematic alcohol use or is solely a reflection of developmental differences. Regardless, these findings highlight an important network and connection that warrants further investigation in future studies.

While the findings emphasise the importance of DMN connectivity, it is also crucial to consider the absence of significant differences within and between other networks previously implicated in alcohol use risk and AUD, such as the SN, sensorimotor network and ECN [8,39,70]. Despite consistent associations between these networks and AUD, no significant differences were found when considering both age of initiation and severity of alcohol use. As stated, our findings partially align with previous research that found increased between network DMN connectivity of adults-exposed (PND 80) compared to adolescent-exposed (PND 45) rats following acute alcohol exposure [34]. However, this study reported increased connectivity between the DMN and LCN, whereas we found increased connectivity within the DMN and between the DMN and thalamus. They additionally reported increased connectivity within the LCN, and between the LCN and SN in adult rats compared to adolescent rats. These differences may be attributed to key methodological differences. Specifically, their study focused on acute alcohol exposure (2×1 g/kg, 1×2 g/kg ethanol), over a short interval, with RSFC measured in 15-minute blocks. Furthermore, the use of pancuronium bromide in addition to the combination of isoflurane and dexmedetomidine for anaesthesia may have influenced RSFC outcomes [11]. Prior studies investigating adolescent alcohol exposure have reported long-term RSFC alterations in adulthood. For example, adolescent rats exposed to 5 g/kg of ethanol intragastrically

from PND 25–54 were found to have reduced frontostriatal connectivity in adulthood (PND 100–110) compared to non-exposed control rats [7]. A similar pattern of dysconnectivity was later observed at PND 119 following the same exposure protocol [22]. In contrast, our study identified different patterns of connectivity, likely due to methodological differences. Both aforementioned studies implemented a binge-ethanol exposure paradigm using intragastric administration, whereas we employed IAA. Moreover, these studies included 30 days of exposure and assessed RSFC after prolonged withdrawal periods (5–9 weeks), whereas our adolescent-onset group began exposure at PND 42, continuing for two months into adulthood and were scanned 4–10 days after alcohol cessation. These differences in affected networks could stem from both the method and length of alcohol administration and the timing of the withdrawal assessment. Given evidence that RSFC increases up to a week after alcohol cessation [48], it is possible that our findings capture an earlier phase of connectivity changes, whereas these previous studies identified longer-term effects after an extended period of abstinence. Future research incorporating longer withdrawal periods would help clarify the trajectory of these changes. Further, a study employing a similar administration protocol to our study, this being the IAA, administered ethanol to adolescent rats from PND 45–90 and also reported decreased connectivity, but specifically between the amygdala and cerebellum in male rats scanned after a 35-day withdrawal period [52], suggesting the differences in connectivity were not due to the method of administration. Another key distinction lies in the analytical approach. These studies pre-selected ROIs from the prefrontal cortex and striatum regions whereas we applied group-ICA to assess whole-brain network connectivity. This methodological difference may explain why our findings align more closely with those of Lee et al., [34], who also used a network-based approach and similarly observed increased between DMN connectivity. Importantly, while we did not find differences in prefrontal striatal connectivity, our network-based approach revealed broader patterns of altered connectivity that might have been overlooked in ROI-based analyses.

Overall, a key advantage of our study is the direct comparison between adolescent- and adult-onset alcohol use while also accounting for severity of use, which previous studies have not examined in the same way. While much of the existing literature has focused on adolescent vulnerability, our findings explicitly compare adolescents and adults to assess whether adolescents are uniquely more vulnerable, or whether the severity of use plays a more critical role across both developmental stages. Our results uniquely demonstrate that both factors must be considered together and highlight that adolescent- and adult-onset groups should be treated separately in future research, rather than collapsed into a single category. Furthermore, given that increased within or between DMN connectivity has been observed following both acute and prolonged exposure, replication in studies with longer withdrawal periods and a non-using control group will be crucial for distinguishing alcohol-related effects from broader developmental processes.

While the novelty in our study lies in the controlled length of exposure of alcohol use across adolescence and adulthood, as well as the voluntary procedure, enabling us to differentiate between high and low drinkers, several limitations should be considered. Firstly, while there do appear to be both age of exposure and severity of alcohol use interaction effects on RSFC, we did not include a control group with no exposure. Due to this it is difficult to establish how the effects compared to no exposure to alcohol, and this would allow the comparison of developmental differences to be investigated to a larger extent. Secondly, when interpreting the findings we cannot conclusively say whether the increase in connectivity found within and between the networks are necessarily detrimental as we did not investigate functional outcomes and relations to behaviour. Thirdly, functional connectivity is known to be inherently noisy [5], and within groups large variances in connectivity were found. This may have impacted the findings, especially due to the low sample sizes in some groups (lowest

$N = 7$), which also reduced the power of the study. Only male rats were included in the current study. However, ethanol exposure can alter functional connectivity in a sex dependent manner [61]. Therefore, it is crucial for future studies to include female rats to determine whether similar associations are found across sexes. While isoflurane is commonly used for induction and positioning, followed by dexmedetomidine during MRI [45,69], evidence suggests that these anaesthetics can induce distinct patterns of functional connectivity inhibition within several resting-state networks, differing from patterns observed in awake rats [11,43]. Additionally, these effects may be time dependent (Magnuson et al., 2014). However, a study comparing different anaesthesia protocols found no significant differences in within DMN connectivity between awake rats and those anaesthetised with a combination of isoflurane and dexmedetomidine [43]. Nevertheless, caution is warranted when comparing our findings to human studies conducted in awake subjects without anaesthesia. In our study, alcohol exposure in the adult group began during early adulthood (PND 77) and continued into full adulthood (PND 137) [57,38]. While this range falls within the established definition of adulthood, evidence suggests that neural maturation continues beyond early adulthood. For example, RSFC between the striatum and several resting state networks has been found to undergo continued changes into full adulthood [37]. Therefore, the observed connectivity differences in our study may reflect this ongoing developmental process rather than the more stable connectivity patterns typically seen in full adulthood.

In conclusion, this study highlights the complex interplay between age of voluntary alcohol exposure, severity of use, and RSFC, particularly within the DMN. Although no differences in RSFC were found when solely considering the age of alcohol exposure, differences emerged when considering drinking severity. Specifically, increased RSFC within the S2 of the SN was found in low compared to high drinking rats, which may suggest a potential effect of alcohol use. High levels of alcohol consumption initiated during adolescence were associated with increased DMN connectivity compared to low use, potentially reflecting altered self-referential processing, increased susceptibility to withdrawal or signs of accelerated aging in adulthood. Conversely, increased within DMN connectivity, and connectivity with the thalamus in adult-onset rats may point to developmental differences rather than alcohol exposure effects. These findings highlight the need for further research to explore the role of DMN connectivity in both risk and resilience to AUD, particularly concerning adolescent alcohol use. Importantly, the absence of a non-drinking control group limits our ability to isolate the specific effects of alcohol consumption on RSFC, and future research should aim to include such groups for a clearer understanding of these dynamics.

Contributors

JC, HL, IW contributed to the concept and design of the work. MD performed the experiments. KCP and SS undertook the statistical analysis, and author KCP wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

CRediT authorship contribution statement

Janna Cousijn: Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Heidi M.B. Lesscher:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Karis Colyer-Patel:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Steven H. Scholte:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Maik Derksen:** Methodology, Data curation. **Ingo Willuhn:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bbr.2025.115719](https://doi.org/10.1016/j.bbr.2025.115719).

Data availability

Data will be made available on request.

References

- P.N. Alves, C. Foulon, V. Karolis, D. Bzdok, D.S. Margulies, E. Volle, M. Thiebaut de Schotten, An improved neuroanatomical model of the default-mode network reconciles previous neuroimaging and neuropathological findings, *Commun. Biol.* 2 (1) (2019), <https://doi.org/10.1038/s42003-019-0611-3>.
- M. Arain, M. Haque, L. Johal, P. Mathur, W. Nel, A. Rais, R. Sandhu, S. Sharma, Maturation of the adolescent brain, *Neuropsychiatr. Dis. Treat.* 9 (2013) 449–461, <https://doi.org/10.2147/NDT.S39776>.
- B.B. Avants, N. Tustison, H. Johnson, Adv. Norm. Tools (ANTS) Release 2. x (2014). (<https://brianavants.wordpress.com/2012/04/13/updated-ants-compile-instructions-april-12-2012/>).
- D. Bajic, M.M. Craig, C.R.L. Mongerson, D. Borsook, L. Becerra, Identifying rodent resting-state brain networks with independent component analysis, *Front. Neurosci.* 11 (DEC) (2017), <https://doi.org/10.3389/fnins.2017.00685>.
- A.M. Bastos, J.M. Schoffelen, A tutorial review of functional connectivity analysis methods and their interpretational pitfalls, *Issue JAN2016*. Frontiers Research Foundation, *Front. Syst. Neurosci.* 9 (2016), <https://doi.org/10.3389/fnsys.2015.00175>.
- H.C. Becker, 2000, *Animal Models of Alcohol Withdrawal*, 242.
- M.A. Broadwater, S.H. Lee, Y. Yu, H. Zhu, F.T. Crews, D.L. Robinson, Y.Y.I. Shih, Adolescent alcohol exposure decreases frontostriatal resting-state functional connectivity in adulthood, *Addict. Biol.* 23 (2) (2018) 810–823, <https://doi.org/10.1111/adb.12530>.
- N. Canessa, G. Basso, I. Carne, P. Poggi, C. Gianelli, Increased decision latency in alcohol use disorder reflects altered resting-state synchrony in the anterior salience network, *Sci. Rep.* 11 (1) (2021), <https://doi.org/10.1038/s41598-021-99211-1>.
- S. Chanraud, A.L. Pitel, A. Pfefferbaum, E.V. Sullivan, Disruption of functional connectivity of the default-mode network in alcoholism, *Cereb. Cortex* 21 (10) (2011) 2272–2281, <https://doi.org/10.1093/cercor/bhq297>.
- S. Chanraud, E.V. Sullivan, Compensatory recruitment of neural resources in chronic alcoholism, *Handb. Clin. Neurol.* 125 (2014) 369–380, <https://doi.org/10.1016/B978-0-444-62619-6.00022-7>.
- S. Chen, B. Li, Y. Hu, Y. Zhang, W. Dai, X. Zhang, Y. Zhou, D. Su, Common functional mechanisms underlying dynamic brain network changes across five general anesthetics: a rat fMRI study, *CNS Neurosci. Ther.* 30 (7) (2024), <https://doi.org/10.1111/cns.14866>.
- J. Cousijn, M. Luijten, S.W. Feldstein Ewing, Adolescent resilience to addiction: a social plasticity hypothesis, *Lancet Child Adolesc. Health* 2 (1) (2018) 69–78, [https://doi.org/10.1016/S2352-4642\(17\)30148-7](https://doi.org/10.1016/S2352-4642(17)30148-7).
- J. Cousijn, G. Mies, N. Runia, M. Derksen, I. Willuhn, H. Lesscher, The impact of age on olfactory alcohol cue-reactivity: a functional magnetic resonance imaging study in adolescent and adult male drinkers, *Alcohol. Clin. Exp. Res.* 47 (4) (2023) 668–677, <https://doi.org/10.1111/acer.15037>.
- T. Dai, B.J. Seewoo, L.A. Hennessy, S.J. Bolland, T. Rosenow, J. Rodger, Identifying reproducible resting state networks and functional connectivity alterations following chronic restraint stress in anaesthetized rats, *Front. Neurosci.* 17 (2023), <https://doi.org/10.3389/fnins.2023.1151525>.
- G. Desrosiers-Grégoire, G.A. Devenyi, J. Grandjean, M.M. Chakravarty, A standardized image processing and data quality platform for rodent fMRI, *Nat. Commun.* 15 (1) (2024), <https://doi.org/10.1038/s41467-024-50826-8>.
- D.A. Fair, A.L. Cohen, N.U. F Dosenbach, J.A. Church, F.M. Miezin, D.M. Barch, M. E. Raichle, S.E. Petersen, B.L. Schlaggar, 2008, The Maturing Architecture of the Brain's Default Network, (www.pnas.org/cgi/doi/10.1073/pnas.0800376105).
- S.J. Fede, E.N. Grodin, S.F. Dean, N. Diazgranados, R. Momenan, Resting state connectivity best predicts alcohol use severity in moderate to heavy alcohol users, *NeuroImage Clin.* 22 (2019), <https://doi.org/10.1016/j.nicl.2019.101782>.
- M. Fritz, A.M. Klawonn, N.M. Zahr, Neuroimaging in alcohol use disorder: from mouse to man, *J. Neurosci. Res.* 100 (5) (2022) 1140–1158, <https://doi.org/10.1002/jnr.24423>.
- C. Galandra, G. Basso, S. Cappa, N. Canessa, The alcoholic brain: neural bases of impaired reward-based decision-making in alcohol use disorders, *Neurol. Sci.* 39 (3) (2018) 423–435, <https://doi.org/10.1007/s10072-017-3205-1>.
- J.H. Gilmore, R.C. Knickmeyer, W. Gao, Imaging structural and functional brain development in early childhood, *Nat. Rev. Neurosci.* 19 (3) (2018) 123–137, <https://doi.org/10.1038/nrn.2018.1>.
- D. Goerzen, C. Fowler, G.A. Devenyi, J. Germann, D. Madularu, M.M. Chakravarty, J. Near, An MRI-derived neuroanatomical Atlas of the Fischer 344 Rat Brain, *Sci. Rep.* 10 (1) (2020), <https://doi.org/10.1038/s41598-020-63965-x>.
- A. Gómez-A, C.A. Dannenhoffer, A. Elton, S.H. Lee, W. Ban, Y.Y.I. Shih, C. A. Boettiger, D.L. Robinson, Altered Cortico-Subcortical network after adolescent alcohol exposure mediates behavioral deficits in flexible decision-making, *Front. Pharmacol.* 12 (2021), <https://doi.org/10.3389/fphar.2021.778884>.
- L.M. Hsu, X. Liang, H. Gu, J.K. Brynildsen, J.A. Stark, J.A. Ash, C.P. Lin, H. Lu, P. R. Rapp, E.A. Stein, Y. Yang, Constituents and functional implications of the rat default mode network, *Proc. Natl. Acad. Sci. USA* 113 (31) (2016) E4541–E4547, <https://doi.org/10.1073/pnas.1601485113>.
- P. J. Conrod, K. Nikolaou, Annual Research Review: On the developmental neuropsychology of substance use disorders, *J. Child Psychol. Psychiatry Allied Discip.* 57 (3) (2016) 371–394, <https://doi.org/10.1111/jcpp.12516>.
- M.M. Jacobson, L.M. Jenkins, D.A. Feldman, N.A. Crane, S.A. Langenecker, Reduced connectivity of the cognitive control neural network at rest in young adults who had their first drink of alcohol prior to age 18, *Psychiatry Res. Neuroimaging* 332 (2023) 111642, <https://doi.org/10.1016/j.PSYCHRESNS.2023.111642>.
- E. Jonckers, J. van Audekerke, G. de Visscher, A. van der Linden, M. Verhoye, Functional connectivity fMRI of the rodent brain: comparison of functional connectivity networks in rat and mouse, *PLoS ONE* 6 (4) (2011), <https://doi.org/10.1371/journal.pone.0018876>.
- S. Kattimani, B. Bharadwaj, Clinical management of alcohol withdrawal: a systematic review, *Ind. Psychiatry J.* 22 (2) (2013) 100, <https://doi.org/10.4103/0972-6748.132914>.
- N. Khalili-Mahani, R.M.W. Zoethout, C.F. Beckmann, E. Baerends, M.L. de Kam, R. P. Soeter, A. Dahan, M.A. van Buchem, J.M.A. van Gerven, S.A.R.B. Rombouts, Effects of morphine and alcohol on functional brain connectivity during “resting state”: a placebo-controlled crossover study in healthy young men, *Hum. Brain Mapp.* 33 (5) (2012) 1003–1018, <https://doi.org/10.1002/hbm.21265>.
- M. Kohno, L.E. Dennis, H. McCreedy, W.F. Hoffman, Executive control and striatal resting-state network interact with risk factors to influence treatment outcomes in alcohol-use disorder, *Front. Psychiatry* 8 (SEP) (2017), <https://doi.org/10.3389/fpsy.2017.00182>.
- G.F. Koob, N.D. Volkow, Neurobiology of addiction: a neurocircuitry analysis, *Lancet Psychiatry* 3 (8) (2016) 760–773, [https://doi.org/10.1016/S2215-0366\(16\)00104-8](https://doi.org/10.1016/S2215-0366(16)00104-8).
- L. Kuhns, E. Kroon, H. Lesscher, G. Mies, J. Cousijn, Age-related differences in the effect of chronic alcohol on cognition and the brain: a systematic review, *Transl. Psychiatry* 12 (1) (2022), <https://doi.org/10.1038/s41398-022-02100-y>.
- L. Kuhns, G. Mies, E. Kroon, I. Willuhn, H. Lesscher, J. Cousijn, Alcohol cue reactivity in the brain: age-related differences in the role of social processes in addiction in male drinkers, *J. Neurosci. Res.* 101 (10) (2023) 1521–1537, <https://doi.org/10.1002/jnr.25206>.
- M. Labots, J. Cousijn, L.A. Jolink, J. Leon Kenemans, L.J.M.J. Vanderschuren, H.M. B. Lesscher, Age-related differences in alcohol intake and control over alcohol seeking in rats, *Front. Psychiatry* 9 (SEP) (2018), <https://doi.org/10.3389/fpsy.2018.00419>.
- S.H. Lee, T.A. Shnitko, L.M. Hsu, M.A. Broadwater, M. Sardinias, T.W.W. Wang, D. L. Robinson, R.P. Vetreno, F.T. Crews, Y.Y.I. Shih, Acute alcohol induces greater dose-dependent increase in the lateral cortical network functional connectivity in adult than adolescent rats, *Addict. Neurosci.* 7 (2023), <https://doi.org/10.1016/j.addicn.2023.100105>.
- M.A. Lindquist, S. Geuter, T.D. Wager, B.S. Caffo, Modular preprocessing pipelines can reintroduce artifacts into fMRI data, *Hum. Brain Mapp.* 40 (8) (2019) 2358–2376, <https://doi.org/10.1002/hbm.24528>.
- H. Lu, Q. Zou, H. Gu, M.E. Raichle, E.A. Stein, Y. Yang, Rat brains also have a default mode network, *Proc. Natl. Acad. Sci. USA* 109 (10) (2012) 3979–3984, <https://doi.org/10.1073/pnas.1200506109>.
- Z. Ma, Y. Ma, N. Zhang, Development of brain-wide connectivity architecture in awake rats, *NeuroImage* 176 (2018) 380–389, <https://doi.org/10.1016/j.neuroimage.2018.05.009>.
- J.E. McCutcheon, M. Marinelli, Age matters, *Eur. J. Neurosci.* 29 (5) (2009) 997–1014, <https://doi.org/10.1111/j.1460-9568.2009.06648.x>.
- E.M. Müller-Oehring, Y.C. Jung, A. Pfefferbaum, E.V. Sullivan, T. Schulte, The resting brain of alcoholics, *Cereb. Cortex* 25 (11) (2015) 4155–4168, <https://doi.org/10.1093/cercor/bhu134>.

- [40] E.M. Müller-Oehring, D. Kwon, B.J. Nagel, E.V. Sullivan, W. Chu, T. Rohlfing, D. Prouty, B.N. Nichols, J.B. Poline, S.F. Tapert, S.A. Brown, K. Cummins, T. Brumback, I.M. Colrain, F.C. Baker, M.D. De Bellis, J.T. Voyvodic, D.B. Clark, A. Pfefferbaum, K.M. Pohl, Influences of age, sex, and moderate alcohol drinking on the intrinsic functional architecture of adolescent brains, *Cereb. Cortex* 28 (3) (2018) 1049–1063, <https://doi.org/10.1093/cercor/bhx014>.
- [41] H. Myrick, R.F. Anton, X. Li, S. Henderson, P.K. Randall, K. Voronin, Effect of Naltrexone and Ondansetron on alcohol Cue-induced activation of the Ventral Striatum in alcohol-dependent people, *Arch. Gen. Psychiatry* 65 (4) (2008) 466, <https://doi.org/10.1001/archpsyc.65.4.466>.
- [42] N.L. Nock, S. Minnes, J.L. Alberts, Neurobiology of substance use in adolescents and potential therapeutic effects of exercise for prevention and treatment of substance use disorders, *Birth Defects Res.* 109 (20) (2017) 1711–1729, <https://doi.org/10.1002/bdr2.1182>.
- [43] J. Paasonen, P. Stenroos, R.A. Salo, V. Kiviniemi, O. Gröhn, Functional connectivity under six anesthesia protocols and the awake condition in rat brain, *NeuroImage* 172 (2018) 9–20, <https://doi.org/10.1016/j.neuroimage.2018.01.014>.
- [44] S.Q. Park, T. Kahnt, A. Beck, M.X. Cohen, R.J. Dolan, J. Wrase, A. Heinz, Prefrontal cortex fails to learn from reward prediction errors in alcohol dependence, *J. Neurosci.* 30 (22) (2010) 7749–7753, <https://doi.org/10.1523/JNEUROSCI.5587-09.2010>.
- [45] C.P. Pawela, B.B. Biswal, A.G. Hudetz, M.L. Schulte, R. Li, S.R. Jones, Y.R. Cho, H. S. Matloub, J.S. Hyde, A protocol for use of medetomidine anesthesia in rats for extended studies using task-induced BOLD contrast and resting-state functional connectivity, *NeuroImage* 46 (4) (2009) 1137–1147, <https://doi.org/10.1016/j.neuroimage.2009.03.004>.
- [46] M. Peeters, T. Oldehinkel, W. Vollebergh, Behavioral control and reward sensitivity in adolescents' risk taking behavior: a longitudinal TRAILS study, *Front. Psychol.* 8 (FEB) (2017), <https://doi.org/10.3389/fpsyg.2017.00231>.
- [47] U. Perez-Ramirez, A. Diaz-Parra, R. Ciccocioppo, S. Canals, D. Moratal, Brain functional connectivity alterations in a rat model of excessive alcohol drinking: a resting-state network analysis. 39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2017, pp. 3016–3019, 2017/10.1109/EMBC.2017.8037492.
- [48] Ú. Pérez-Ramírez, V.J. López-Madróna, A. Pérez-Segura, V. Pallarés, A. Moreno, R. Ciccocioppo, P. Hyttiä, W.H. Sommer, D. Moratal, S. Canals, Brain network allostasis after chronic alcohol drinking is characterized by functional dedifferentiation and narrowing, *J. Neurosci.* 42 (21) (2022) 4401–4413, <https://doi.org/10.1523/JNEUROSCI.0389-21.2022>.
- [49] J.D. Power, A. Mitra, T.O. Laumann, A.Z. Snyder, B.L. Schlaggar, S.E. Petersen, Methods to detect, characterize, and remove motion artifact in resting state fMRI, *NeuroImage* 84 (2014) 320–341, <https://doi.org/10.1016/j.neuroimage.2013.08.048>.
- [50] P.J. Pruiitt, L. Tang, J.M. Hayes, N. Ofen, J.S. Damoiseaux, Lifespan differences in background functional connectivity of core cognitive large-scale brain networks, *Neurosci. Res.* (2022), <https://doi.org/10.1016/j.neures.2022.09.005>.
- [51] M.E. Raichle, The brain's default mode network, *Annu. Rev. Neurosci.* 38 (2015) 433–447, <https://doi.org/10.1146/annurev-neuro-071013-014030>.
- [52] Rasgado-Toledo, J., Angeles-Valdez, D., Carranza-Aguilar, C.J., Lopez-Castro, A., Trujillo-Villarreal, L.A., Medina-Sánchez, D., Serrano-Ramirez, M.S., Débora Elizarrarás-Herrera, A., Alcauter, S., Delint-Ramirez, I., Gutierrez, R., Devenyi, G. A., Mallar Chakravarty, M., & Garza-Villarreal, E.A. (2025). The Effect of Chronic Stress and Chronic Alcohol Intake on Behavior, Brain Structure, and Functional Connectivity in A Rat Model. <https://doi.org/10.1101/2025.02.13.638122>.
- [53] A.J. Schwarz, N. Gass, A. Sartorius, C. Risterucci, M. Spedding, E. Schenker, A. Meyer-Lindenberg, W. Weber-Fahr, Anti-correlated cortical networks of intrinsic connectivity in the rat brain, *Brain Connect.* 3 (5) (2013) 503–511, <https://doi.org/10.1089/brain.2013.0168>.
- [54] A. Sierakowiak, C. Monnot, S.N. Aski, M. Uppman, T.Q. Li, P. Damberg, S. Brené, Default mode network, motor network, dorsal and ventral basal ganglia networks in the rat brain: comparison to human networks using resting state-fMRI, *PLoS ONE* 10 (3) (2015), <https://doi.org/10.1371/journal.pone.0120345>.
- [55] J.G. Sled, A.P. Zijdenbos, A.C. Evans, A nonparametric method for automatic correction of intensity nonuniformity in MRI data, *IEEE Trans. Med. Imaging* 17 (1) (1998) 87–97, <https://doi.org/10.1109/42.668698>.
- [56] Spanagel, R. (2017). Animal Models of Addiction. (www.dialogues-cns.org).
- [57] Spear, L.P. (2000). The Adolescent Brain and Age-related Behavioral Manifestations. (www.elsevier.com/locate/neubiorev).
- [58] L.P. Spear, Adolescent neurodevelopment, *J. Adolesc. Health* 52 (2) (2013), <https://doi.org/10.1016/j.jadohealth.2012.05.006>.
- [59] L.P. Spear, H.S. Swartzwelder, Adolescent alcohol exposure and persistence of adolescent-typical phenotypes into adulthood: a mini-review, *Neurosci. Biobehav. Rev.* 45 (2014) 1–8, <https://doi.org/10.1016/j.neubiorev.2014.04.012>.
- [60] M. Spoelder, K.T. Tsutsui, H.M.B. Lesscher, L.J.M.J. Vanderschuren, J.J. Clark, Adolescent alcohol exposure amplifies the incentive value of reward-predictive cues through potentiation of phasic dopamine signaling, *Neuropsychopharmacology* 40 (13) (2015) 2873–2885, <https://doi.org/10.1038/npp.2015.139>.
- [61] S. Tang, S. Xu, J. Waddell, W. Zhu, R.P. Gullapalli, S.M. Mooney, Functional connectivity and metabolic alterations in medial prefrontal cortex in a rat model of fetal alcohol spectrum disorder: a resting-state functional magnetic resonance imaging and in vivo proton magnetic Resonance Spectroscopy study, *Dev. Neurosci.* 41 (1–2) (2019) 67–78, <https://doi.org/10.1159/000499183>.
- [62] P.J. Tsai, R.J. Keeley, S.A. Carmack, J.C.M. Vendruscolo, H. Lu, H. Gu, L. F. Vendruscolo, G.F. Koob, C.P. Lin, E.A. Stein, Y. Yang, Converging structural and functional evidence for a rat salience network, *Biol. Psychiatry* 88 (11) (2020) 867–878, <https://doi.org/10.1016/j.biopsych.2020.06.023>.
- [63] M.P. van den Heuvel, H.E. Hulshoff Pol, Exploring the brain network: a review on resting-state fMRI functional connectivity, *Eur. Neuropsychopharmacol.* 20 (8) (2010) 519–534, <https://doi.org/10.1016/j.euroneuro.2010.03.008>.
- [64] V.M. Vergara, J. Liu, E.D. Claus, K. Hutchison, V. Calhoun, Alterations of resting state functional network connectivity in the brain of nicotine and alcohol users, *NeuroImage* 151 (2017) 45–54, <https://doi.org/10.1016/j.neuroimage.2016.11.012>.
- [65] C.S. Vetter-O'Hagen, L.P. Spear, Hormonal and physical markers of puberty and their relationship to adolescent-typical novelty-directed behavior, *Dev. Psychobiol.* 54 (5) (2012) 523–535, <https://doi.org/10.1002/dev.20610>.
- [66] N.D. Volkow, M. Morales, The brain on drugs: from reward to addiction, *Cell* 162 (4) (2015) 712–725, <https://doi.org/10.1016/j.cell.2015.07.046>.
- [67] S. Wang, D.J. Peterson, J.C. Gatenby, W. Li, T.J. Grabowski, T.M. Madhyastha, Evaluation of field map and nonlinear registration methods for correction of susceptibility artifacts in diffusion MRI, *Front. Neuroinf.* 11 (2017), <https://doi.org/10.3389/fninf.2017.00017>.
- [68] A.M. Weber, N. Soreni, M.D. Noseworthy, A preliminary study on the effects of acute ethanol ingestion on default mode network and temporal fractal properties of the brain, *Magn. Reson. Mater. Phys. Biol. Med.* 27 (4) (2014) 291–301, <https://doi.org/10.1007/s10334-013-0420-5>.
- [69] R. Weber, P. Ramos-Cabrera, D. Wiedermann, N. Van Camp, M. Hoehn, A fully noninvasive and robust experimental protocol for longitudinal fMRI studies in the rat, *NeuroImage* 29 (4) (2006) 1303–1310, <https://doi.org/10.1016/j.neuroimage.2005.08.028>.
- [70] B.J. Weiland, A. Sabbini, V.D. Calhoun, R.C. Welsh, A.D. Bryan, R.E. Jung, A. R. Mayer, K.E. Hutchison, Reduced left executive control network functional connectivity is associated with alcohol use disorders, *Alcohol. Clin. Exp. Res.* 38 (9) (2014) 2445–2453, <https://doi.org/10.1111/acer.12505>.
- [71] D.G. Weissman, R.A. Schriber, C. Fassbender, O. Atherton, C. Krafft, R.W. Robins, P.D. Hastings, A.E. Guyer, Earlier adolescent substance use onset predicts stronger connectivity between reward and cognitive control brain networks, *Dev. Cogn. Neurosci.* 16 (2015) 121–129, <https://doi.org/10.1016/j.dcn.2015.07.002>.
- [72] R. Zhang, N.D. Volkow, Brain default-mode network dysfunction in addiction, *NeuroImage* 200 (2019) 313–331, <https://doi.org/10.1016/j.neuroimage.2019.06.036>.
- [73] S. Zhornitsky, J.S. Ide, W. Wang, H.H. Chao, S. Zhang, S. Hu, J.H. Krystal, C.S.R. Li, Problem drinking, alcohol expectancy, and thalamic resting-state functional connectivity in nondependent adult drinkers, *Brain Connect.* 8 (8) (2018) 487–502, <https://doi.org/10.1089/brain.2018.0633>.
- [74] X. Zhu, C.R. Cortes, K. Mathur, D. Tomasi, R. Momenan, Model-free functional connectivity and impulsivity correlates of alcohol dependence: a resting-state study, *Addict. Biol.* 22 (1) (2017) 206–217, <https://doi.org/10.1111/adb.12272>.