Supplement

Loss and Frontal Striatal Reactivities Characterize Alcohol Use Severity and Rule-Breaking Behavior in Young Adult Drinkers

Supplementary Methods

Items of Achenbach Adult Self-Report (ASR)

There are a total of 120 items in 12 subscales: anxious/depressed, withdrawn, somatic complaints, thought problems, attention problems, aggressive behavior, rule-breaking behavior, intrusive behavior, other problems, as well as all critical items (items of general clinical concern), an internalizing subscale (consisting of anxious/depressed, withdrawn and somatic complaints), and an externalizing subscale (consisting of aggressive, rule-breaking, and Intrusive behavior). Participants were required to circle 0 (not true), 1 (somewhat or sometimes true), or 2 (very or often true) for each item to describe themselves over the past 6 months.

Fourteen items of the rule-breaking behavior subscale of the Achenbach ASR

1. I use drugs (other than alcohol and nicotine) for non-medical purposes.

2. I damage or destroy my things.

3. I break rules at work or elsewhere.

4. I don't feel guilty after doing something I shouldn't.

5. I hang around people who get in trouble.

6. I am impulsive or act without thinking.

7. I lie or cheat.

8. My behavior is irresponsible.

9. I steal.

10. I drink too much alcohol or get drunk.

11. I do things that may cause me trouble with the law.

12. I fail to pay my debts or meet other financial responsibilities.

13. I have trouble managing money or credit cards.

14. I have trouble keeping a job.

Imaging protocol, gambling task and data preprocessing

MRI was done using a customized 3 T Siemens Connectome Skyra with a standard 32-channel Siemens receiver head coil and a body transmission coil. T1-weighted high-resolution structural images were acquired using a 3D MPRAGE sequence with 0.7 mm isotropic resolution (FOV = 224 × 224 mm, matrix = 320 × 320, 256 sagittal slices, TR = 2400 ms, TE = 2.14 ms, TI = 1000 ms, FA = 8°) and used to register functional MRI data to a standard brain space. FMRI data were collected using gradient-echo echo-planar imaging (EPI) with 2.0 mm isotropic resolution (FOV = 208 × 180 mm, matrix = 104 × 90, 72 slices, TR = 720 ms, TE = 33.1 ms, FA = 52°, multi-band factor = 8).

Participants completed two runs of a gambling task each with 4 blocks (~3 m and 12 s each run) – 2 of punishment and 2 of reward – in a fixed order (run 1: punishment – reward – punishment – reward; and run 2: reward – punishment – punishment – reward) with a fixation period (15 s) between blocks. The participants guessed whether the number of a mystery card (represented by a '?' and ranging from 1 to 9) was larger or smaller than 5 by pressing a corresponding button (1). The feedbacks comprised a green up-pointing arrow for correct guess and $1 win, a red down-pointing arrow for $0.5 loss; or a gray double-headed arrow for a wash (mystery card number = 5). The mystery number was controlled by the program and shown for 1.5 s, followed by the feedback for 1.0 s. There was a 1.0 s inter-trial interval with a "+" shown on the screen. Each block contained 8 trials. In reward blocks, 6 win trials were pseudo-randomly interleaved with either 1 neutral and 1 loss trial, 2 neutral trials, or 2 loss trials. In punishment blocks, 6 loss trials were interleaved with either 1 neutral and 1 win trial, 2 neutral trials, or 2 win trials. Thus, the amount

of money won was the same across subjects.

BOLD data were analyzed with Statistical Parametric Mapping (SPM8, Welcome Department of Imaging Neuroscience, University College London, U.K.), following our published routines (2-4). Images of each individual subject were first realigned (motion corrected). A mean functional image volume was constructed for each subject per run from the realigned image volumes. These mean images were co-registered with the high-resolution structural MPRAGE image and then segmented for normalization with affine registration followed by nonlinear transformation. The normalization parameters determined for the structural volume were then applied to the corresponding functional image volumes for each subject. The voxel is of 2x2x2 mm^3 after spatial normalization. Finally, the images were smoothed with a Gaussian kernel of 4 mm at Full Width at Half Maximum.

The GLM and 2nd-level analyses

Briefly, a statistical analytical block design was constructed for each individual subject, using a general linear model (GLM) by convolving the canonical hemodynamic response function (HRF) with a boxcar function in SPM. Realignment parameters in all six dimensions were entered in the model as covariates. We constructed for each individual subject the statistical contrast "reward vs. baseline" and "punishment vs. baseline", with baseline = 15-s fixation period between blocks. In group analyses, we conducted a full factorial analysis (men vs. women $\times$ binger vs. non-binger) of the contrasts with age as a covariate. We also performed a voxel-wise regression against the rule-breaking score for all subjects with sex and age as covariates, and for each of the four groups of subjects separately with age as a covariate. We evaluated the results at voxel $p < 0.001$, uncorrected, in combination with cluster $p < 0.05$, corrected for family-wise error (FWE) of multiple comparisons, on the basis of Gaussian random field theory, as implemented in SPM. We identified brain

regions using the Data Processing & Analysis of Brain Imaging toolbox (DPABI) (5) and an atlas (6), if the peak was not identified by the DPABI.

Mediation analysis

In a mediation analysis, the relation between the independent variable X and dependent variable Y, i.e. X→Y, is tested to see if the relation is significantly mediated by a variable M. The mediation test is performed by employing three regression equations:

$$Y = i_1 + cX + e_1$$
$$Y = i_2 + c'X + bM + e_2$$
$$M = i_3 + aX + e_3$$

where *a* represents X→M, *b* represents M→Y (controlling for X), *c*' represents X→Y (controlling for M), and *c* represents X→Y. The constants i_1, i_2, i_3 are the intercepts, and e_1, e_2, e_3 are the residual errors. In the literature, *a*, *b*, *c* and *c*' were referred as path coefficients or simply paths, and we followed this notation. Variable M is a mediator of the correlation X → Y if (*c* –*c*'), which is mathematically equivalent to the product of the paths *a***b*, is significantly different from zero (7). If the product *a***b* and the paths *a* and *b* are significant, one concludes that X→Y is mediated by M. In addition, if path *c*' is not significant, there is no direct connection from X to Y and that X→Y is completely mediated by M. Note that path *b* is the relation between Y and M, controlling for X, and should not be confused with the correlation coefficient between Y and M.

Path analysis

With path analysis we evaluated the relationships among neural markers (IFG and AG activity), rule-breaking behavior, and the severity of alcohol use. Path analysis involves a set of exogenous variables with variance not accounted for by the model and endogenous variables with variance explained in part by other variables in the model (8, 9). Path analysis is conducted with regression analysis, which predicts the effects of all other

variables on the endogenous variables. The beta weights (β) from these multiple regressions are the path coefficients. Standardized path coefficients convey assumptions about the directionality of interactions between variables. Model fit is typically assessed with fit indices that include the root mean square estimation of approximation ($\leqslant$0.08 for an acceptable fit), chi-square (χ^2/df, $\leqslant$ 3), comparative fit index ($\geqslant$0.9), and standardized root mean square residual ($\leqslant$0.08) (10, 11).

Supplementary Results

4-way (trial $\times$ block $\times$ group $\times$ sex) repeated measures ANOVA of RT

To characterize how individuals reacted to wins and losses, we computed individual RT of trials following loss (post-loss RT) and win (post-win RT) each for reward and punishment blocks in the gambling task. The results of a 4-way repeated measures ANOVA (post-win vs. post-loss $\times$ reward vs. punishment block $\times$ binger vs. non-binger $\times$ men vs. women) showed no significant 4-way interaction effect (F = 0.64, p = 0.423). We removed sex as a factor and observed in a 3-way ANOVA that both within-subject trial (post-win vs. post-loss: F = 6.76, p = 0.010) and block (reward vs. punishment: F = 73.93, p < 0.001) main effects as well as between-subject group main effect (binger vs. non-binger: F = 7.39, p = 0.007) were significant. Further, trial $\times$ group (F = 5.98, p = 0.015) and trial $\times$ block (F = 5.31, p = 0.022) but no other interaction effects were significant (p's > 0.467, **Supplementary Table S3**). Next, we removed block as a factor and conducted a 2-way trial $\times$ group ANOVA. The results showed significant group (F = 8.00, p = 0.005) and trial (F = 71.10, p < 0.001) main and group $\times$ trial interaction (F = 9.66, p = 0.002) effect in RT.

Supplementary Table S1. ANOVA of age, performance metrics of the gambling task, and drinking variables, all with age as a covariate.

Characteristic	Men Binger (n=132)	Men NonBinger (n=97)	Women Binger (n=49)	Women NonBinger (n=191)	Two-way ANOVA					
					Group		Sex		Interaction	
					F_{468}	p	F_{468}	p	F_{468}	p
Age	27.9 ± 3.4	27.9 ± 3.9	28.4 ± 3.5	30.2 ± 3.7	5.8	.017	13.6	.000	5.1	.024
RT_{REW}	438 ± 110	441 ± 120	436 ± 97	471 ± 107	2.4	.122	1.1	.288	1.8	.180
RT_{PUN}	412 ± 102	425 ± 120	417 ± 100	458 ± 114	4.8	.029	2.0	.158	1.1	.305
Dp_Sx	1.3 ± 1.1	0.2 ± 0.5	1.1 ± 0.8	0.1 ± 0.4	161.7	.000	2.8	.095	0.6	.450
Ab_Dx	2.6 ± 2.0	1.0 ± 0.0	2.2 ± 1.9	1 .0 ± 0.0	125.4	.000	1.9	.167	1.6	.212
Ab_Sx	0.8 ± 0.8	0 .0 ± 0.0	0.6 ± 0.8	0.0 ± 0.0	160.9	.000	3.1	.081	2.7	.099
Dp_Dx	1.6 ± 1.5	1.0 ± 0.0	1.4 ± 1.2	1.0 ± 0.0	27.9	.000	2.7	.102	2.3	.128
Daily drinks	4.3 ± 1.3	1.2 ± 0.9	3.3 ± 1.5	1.1 ± 0.9	476.7	.000	15.0	.000	12.0	.001
Frq	-2.3 ± 1.0	-5.5 ± 0.9	-2.8 ± 0.8	-5.3 ± 1.2	608.7	.000	2.0	.154	8.7	.003
Frq_5plus	-1.7 ± 0.5	-5 .0 ± 0.0	-2.0 ± 0.0	-5.0 ± 0.0	13487	.000	30.6	.000	29.7	.000
Frq_Drk	-1.9 ± 1.0	-3.7 ± 0.5	-2.4 ± 0.6	-3.8 ± 0.4	467.2	.000	15.5	.000	10.5	.001
Max_Drk	5.2 ± 1.7	1.2 ± 0.8	3.8 ± 1.1	1.1 ± 0.7	707.3	.000	30.2	.000	26.8	.000
Age_Use	2.4 ± 1.0	3.8 ± 1.3	2.6 ± 1.2	3.6 ± 1.4	75.3	.000	0.0	.959	3.0	.086
Hvy_Daily	4.9 ± 1.3	2.5 ± 1.7	4.2 ± 1.6	2.2 ± 1.4	203.9	.000	6.3	.012	1.5	.216
Hvy_Frq	-1.7 ± 1.0	-5 .0 ± 1.4	-2.0 ± 1.1	-4.6 ± 1.5	413.8	.000	0.0	.948	2.5	.116
Hvy_5plus	-1.3 ± 0.5	-4.3 ± 1.1	-1.4 ± 0.6	-4.3 ± 1.0	933.1	.000	0.1	.752	0.2	.639
Hvy_Drk	-1.5 ± 0.9	-3.2 ± 1.0	-1.5 ± 0.8	-3.1 ± 0.9	263.2	.000	0.1	.796	0.0	.825
Hvy_Max	5.5 ± 1.6	2.1 ± 1.5	4.2 ± 1.5	1.7 ± 1.0	391.2	.000	29.7	.000	7.5	.006
PC1	1.2 ± 0.5	-0.7 ± 0.4	0.8 ± 0.5	-0.7 ± 0.3	1376	.000	16.5	.000	14.3	.000

Note: Age/years, RT_{REW} (RT of reward blocks, ms), RT_{PUN} (RT of punish blocks, ms), Dp_Sx (Number of DSM4 Alcohol Dependence Criteria Endorsed), Ab_Dx (DSM4 Alcohol Abuse Criteria Met), Ab_Sx (DSM4 Alcohol Abuse number of symptoms), Dp_Dx (DSM4 Alcohol Dependence Criteria Met), Daily drinks (Drinks per drinking day in past 12 months), Frq (Frequency of any alcohol use in past 12 months), Frq_5plus (Frequency of drinking 5+ drinks in past 12 months), Frq_Drk (Frequency drunk in past 12 months), Max_Drk (Max drinks in a single day in past 12 months), Age_Use (Age at first alcohol use), Hvy_Daily (Drinks per day in heaviest 12-month period), Hvy_Frq (Frequency of any alcohol use, heaviest 12-month period), Hvy_5plus (Frequency of

drinking 5+ drinks, heaviest 12-month period), Hvy_Drk (Frequency drunk in heaviest 12-month period), Hvy_Max (Lifetime max drinks in single day), PC1 (Severity of alcohol use as quantified by the weight of the first principal component (PC1) of PCA of all 15 drinking measures). Note that Frq, Frq_5plus, Frq_Drk, Hvy_Frq, Hvy_5plus and Hvy_Drk were flipped. Values are mean ± SD. Note that some entries are negative in value because the original scales needed to be reversed in scoring so that across metrics, a higher value reflects more severe alcohol misuse, to be consistent.

Supplementary Table S2. ANOVA (group by sex) of the Achenbach Adult Self-Report measures with age as a covariate.

Characteristic	Men Binger (n=132)	Men NonBinger (n=97)	Women Binger (n=49)	Women NonBinger (n=191)	Two-way ANOVA					
					Group		Sex		Interaction	
					F_{468}	p	F_{468}	p	F_{468}	p
Anxd	5.7 ± 5.0	6.0 ± 5.9	6.3 ± 6.3	5.5 ± 5.2	0.1	.741	0.2	.647	0.6	.437
Witd	2.6 ± 2.3	3.1 ± 2.7	1.5 ± 1.8	1.7 ± 2.1	2.5	.114	24.9	.000	0.2	.651
Soma	2.5 ± 3.0	2.1 ± 2.7	3.3 ± 3.9	2.7 ± 3.1	2.2	.138	4.5	.034	0.1	.765
Thot	2.4 ± 2.4	2.2 ± 2.2	2.3 ± 2.3	1.9 ± 2.2	0.9	.354	0.2	.671	0.0	.979
Attn	6.5 ± 4.2	6.8 ± 4.1	6.0 ± 4.6	5.5 ± 4.0	0.0	.905	2.3	.129	0.3	.582
Aggr	4.5 ± 3.9	4.0 ± 3.6	4.1 ± 3.4	3.0 ± 2.9	4.4	.037	3.4	.065	0.4	.521
Rule	4.7 ± 3.3	2.1 ± 2.3	2.8 ± 2.3	1.3 ± 1.8	**63.5**	**.000**	**24.6**	**.000**	**5.5**	**.020**
Intr	2.8 ± 2.3	2.3 ± 2.3	2.9 ± 2.4	1.7 ± 1.9	12.6	.000	1.0	.313	1.6	.205
Oth	10.0 ± 4.7	9.4 ± 5.0	9.1 ± 4.7	8.4 ± 4.0	1.4	.233	2.1	.145	0.0	.843
Crit	5.4 ± 3.6	3.6 ± 3.1	4.6 ± 3.6	3.0 ± 2.8	22.0	.000	2.3	.133	0.2	.641
Intn	10.9 ± 9.1	11.2 ± 9.7	11.2± 10.6	10.0 ± 8.6	0.1	.758	0.1	.818	0.4	.504
Extn	12.1 ± 7.7	8.4 ± 6.6	9.8 ± 6.2	6.0 ± 5.1	29.5	.000	10.7	.001	0.0	.901

Note: Anxious/Depressed score (Anxd), Withdrawn score (Witd), Somatic complaints (Soma), Thought problems score (Thot), Attention score (Attn), Aggressive behavior score (Aggr), Rule breaking behavior score (Rule), Intrusive score (Intr), Other problems score (Oth), Internalizing score (Intn), Externalizing score (Extn). Values are mean ± SD.

Supplementary Table S3. Four-way repeated measures ANOVA of RT: trial (post-win vs. post-loss) × block (reward vs. punishment) × group (binger vs. non-binger) × sex.

Effects	*F*	*p*
Trial	3.66	0.056
Block	60.90	0.000
Group	3.91	0.049
Sex	2.45	0.118
Trial × Block	6.05	0.014
Trial × Group	4.62	0.032
Trial × Sex	0.00	0.995
Block × Group	0.03	0.865
Block × Sex	1.65	0.200
Group × Sex	2.59	0.108
Trial × Block × Group	0.03	0.875
Trial × Block × Sex	0.61	0.436
Block × Group × Sex	0.03	0.857
Trial × Block × Group × Sex	0.64	0.423

Supplementary Table S4. Statistics of the mediation analysis to examine the inter-relationship between IFG/MFG activity, rule-breaking behavior score, and severity of alcohol use.

	Path a (X→M)	Path b (M→Y)	Path c (X→Y)	Path c' (X→Y)	Mediation Path (c-c')
Model 1: X (PC1)→Y (Rule-Breaking) mediated by M (IFG/MFG)					
β	0.186	0.196	1.274	1.237	0.037
p	0.000	0.178	0.000	0.000	0.201
Model 2: X (PC1)→Y (IFG/MFG) mediated by M (Rule-Breaking)					
β	1.274	0.021	0.186	0.160	0.027
p	0.000	0.193	0.000	0.000	0.193
Model 3: X (Rule-Breaking)→Y (PC1) mediated by M (IFG/MFG)					
β	0.047	0.184	0.162	0.153	0.009
p	0.002	0.000	0.000	0.000	0.014
Model 4: X (Rule-Breaking)→Y(IFG/MFG) mediated by M (PC1)					
β	0.162	0.160	0.047	0.021	0.026
p	0.000	0.000	0.002	0.193	0.001
Model 5: X (IFG/MFG)→Y(Rule-Breaking) mediated by M (PC1)					
β	0.264	1.237	0.523	0.196	0.326
p	0.000	0.000	0.001	0.178	0.000
Model 6: X (IFG/MFG)→Y(PC1) mediated by M (Rule-Breaking)					
β	0.523	0.153	0.264	0.184	0.055
p	0.001	0.000	0.000	0.000	0.009

Note: Rule-Breaking = rule-breaking behavior score; IFG/MFG = beta estimates of the contrast "punishment – baseline"; PC1 of drinking metrics = weight of the first principal component identified from PCA of the 15 drinking metrics. The mediation analyses were conducted for all subjects (n=469) with age as a covariate.

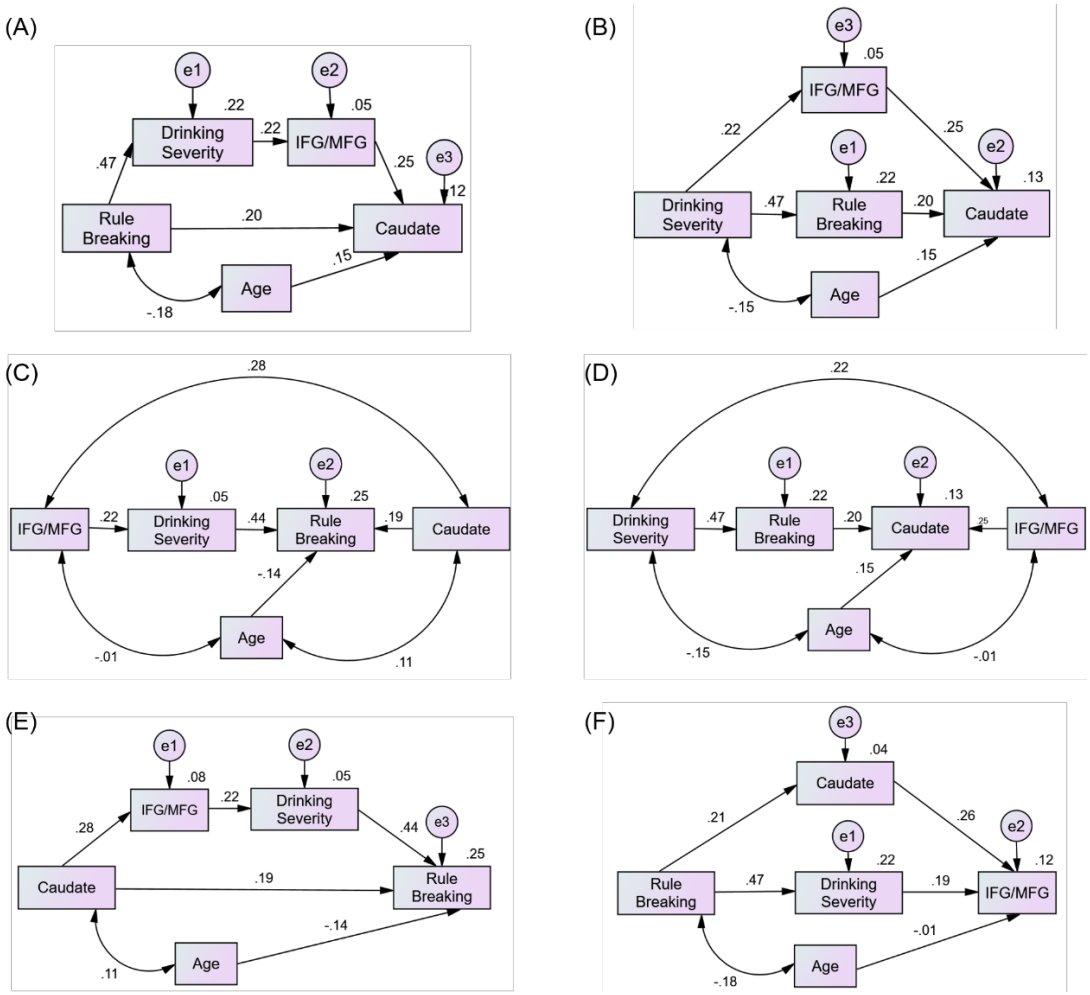
Supplementary Table S5. Statistics of the mediation analysis to examine the inter-relationship between caudate activity, rule-breaking behavior score, and severity of alcohol use.

	Path a (X→M)	Path b (M→Y)	Path c (X→Y)	Path c' (X→Y)	Mediation Path (c-c')
Model 1: X (PC1)→Y (Rule_Breaking) mediated by M (Caudate)					
β	0.086	0.653	1.274	1.217	0.056
p	0.023	0.000	0.000	0.000	0.056
Model 2: X (PC1)→Y (Caudate) mediated by M (Rule-Breaking)					
β	1.274	0.070	0.086	-0.003	0.089
p	0.000	0.000	0.023	0.944	0.000
Model 3: X (Rule_Breaking)→Y (PC1) mediated by M (Caudate)					
β	0.070	-0.004	0.162	0.162	0.000
p	0.000	0.944	0.000	0.000	0.945
Model 4: X (Rule_Breaking)→Y(Caudate) mediated by M (PC1)					
β	0.162	-0.003	0.070	0.070	0.000
p	0.000	0.944	0.000	0.000	0.945
Model 5: X (Caudate)→Y(Rule_Breaking) mediated by M (PC1)					
β	0.127	1.217	0.808	0.653	0.155
p	0.024	0.000	0.000	0.000	0.030
Model 6: X (Caudate)→Y(PC1) mediated by M (Rule-Breaking)					
β	0.808	0.162	0.127	-0.004	0.131
p	0.000	0.000	0.024	0.944	0.000

Note: Rule-Breaking = rule-breaking behavior score; Caudate = beta estimates of the contrast "punishment – baseline"; PC1 of drinking metrics = weight of the first principal component identified from PCA of the 15 drinking metrics. The mediation analyses were conducted for all subjects (n=469) with age as a covariate.

Supplementary Table S6. Statistics of path analyses (**Supplementary Figure S2**) of IFG/MFG activities, caudate activities, rule-breaking behavior score and drinking severity, with age as a covariate.

	CFI	RMSEA	SRMR	Chi square/df
Models with age as covariate				
Figure S1A	0.992	0.030	0.025	1.43
Figure S1B	0.967	0.062	0.035	2.77
Figure S1C	0.962	0.077	0.041	3.74
Figure S1D	0.963	0.075	0.034	3.63
Figure S1E	0.962	0.066	0.045	3.05
Figure S1F	0.953	0.074	0.043	3.55



Supplementary Figure S1. Path analyses of IFG/MFG activities, caudate activities, rule-breaking behavior score and drinking severity, with age as a covariate.

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