SUPPLEMENTARY MATERIAL

Acute alcohol effects on impulsive choice in adolescents

Nadine Bernhardt[1], Elisabeth Obst[1], Stephan Nebe[1,2], Shakoor Pooseh[1,3], Friedrich M. Wurst[4,5,6], Wolfgang Weinmann[7], Michael N. Smolka[1] and Ulrich S. Zimmermann[1]

[1] Department of Psychiatry and Psychotherapy, Technische Universität Dresden, Dresden, Germany

[2] Laboratory for Social and Neural Systems Research, Department of Economics, University of Zurich, Zurich, Switzerland

[3] Freiburg Center for Data Analysis and Modeling, Albert-Ludwigs-Universität Freiburg, Germany

[4] Paracelsus Medical University, Salzburg, Austria

[5] Psychiatric University Hospital, Basel, Switzerland

[6] Centre for Interdisciplinary Addiction Research, UKE Hamburg, Germany

[7] Institute of Forensic Medicine, Forensic Toxicology and Chemistry, University of Bern, Switzerland

Methods

Determination of phosphatidylethanol (PEth) from venous blood

The most predominant PEth-homologue PEth 16:0/18:1 and also the minor homologue PEth 16:0/18:2 were quantified in dried blood spots (DBS) with D5-PEth 16:0/18:1 and D5-PEth 16:0/18:2 as internal standards. Deuterated standards were synthesized in our laboratory from PC 16:0/18:1, PC 16:0/18:2 and D6-ethanol catalysed by phospholipase D (Schröck et al., 2016). For DBS preparation, 20 µL of whole blood were pipetted on filter cards (GR2261004, PKI 226 Bioanalysis Card, Perkin Elmer, Rodgau, Germany) and dried for a minimum of 3 hours prior to extraction. PEth was extracted from DBS with 500 µL of methanol (10 min). The supernatant was transferred to a vial and evaporated to dryness under a stream of nitrogen at 50 °C. The residue was re-dissolved in 200 µL of mobile phase (ammonium acetate (10 mM)/acetonitrile (30:70, v/v)). An aliquot of 80 µL was injected into the online-SPE-LC-MS/MS system. A previously published validated method for PEth analysis in whole blood samples was modified for DBS by use of a calibration range of 20 – 2000 ng/mL (Schröck et al., 2017). Limits of quantitation (LoQ) for PEth 16:0/18:1 and PEth 16:0/18:2 were 20 ng/mL. The analysis was performed with a QTrap 3200 tandem mass spectrometer with a turbo ionspray source (Sciex, Toronto, Canada). After trapping with a Synergi Polar-RP column (20 x 2 mm, 5 µm) the two homologues were separated with a Luna RP-C5 column (50 mm x 2 mm, 5 µm) (Phenomenex, Brechbühler, Schlieren, Switzerland) by gradient elution.

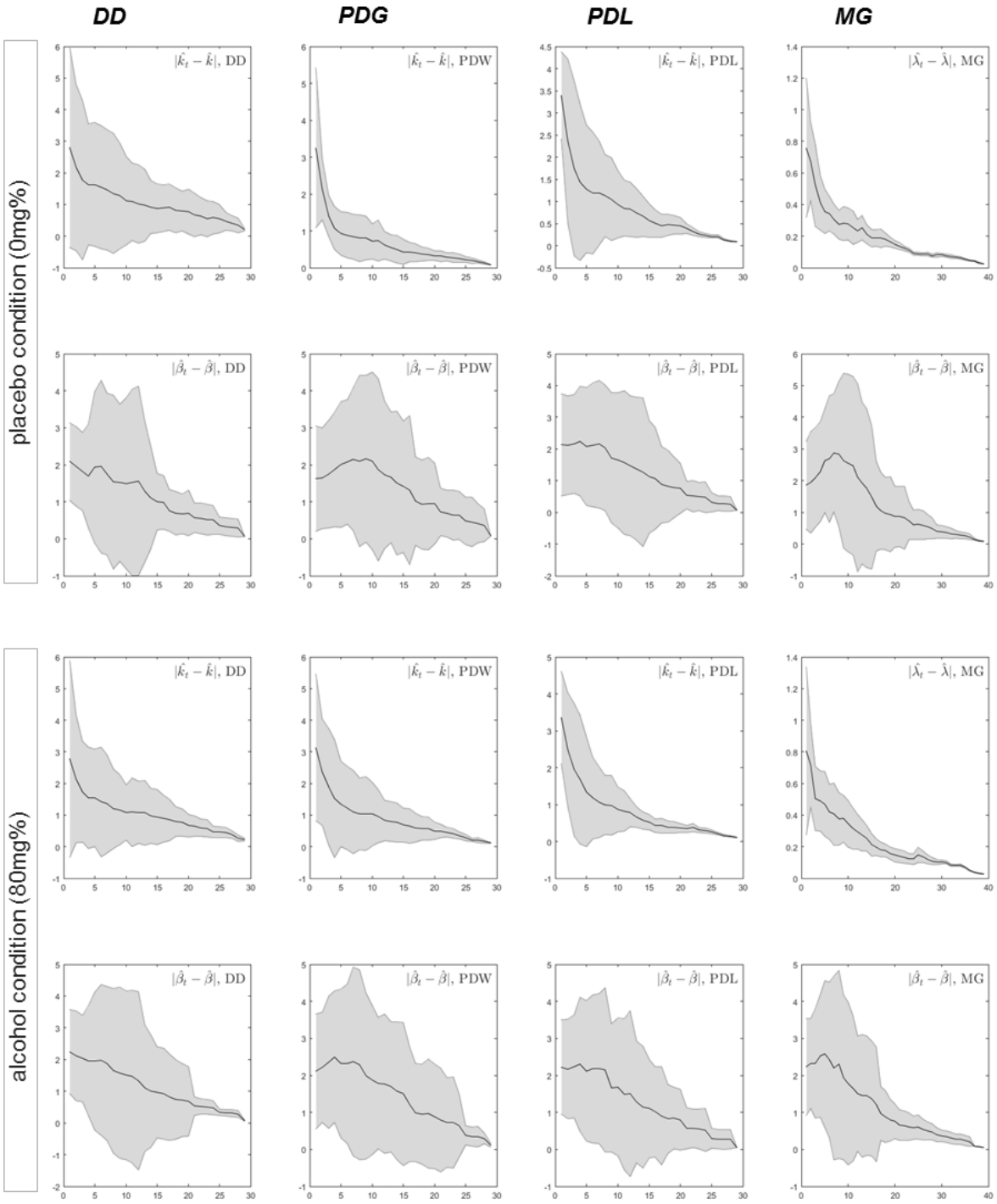

Figure S1: Convergence of parameter estimation in participant sample data for placebo (0mg%) and alcohol (80mg%) condition. The average absolute differences between the estimation at each trial and the final estimation for all participants are shown trial by trial by black lines. The gray area depicts the on standard deviation distance from the average. The decreasing pattern in black lines is a sign of convergence and the same for standard deviations means that this is true for the whole group. The top row depicts $|\hat{k}_t - \hat{k}|$ for DD, PDG, and PDL and $|\hat{\lambda}_t - \hat{\lambda}|$ for MG; the bottom row shows $|\hat{\beta}_t - \hat{\beta}|$ for the same tasks, for each condition respectively.

Table S1: QC of value-based decision-making tasks

	DD	PDG	PDL	MG
n (datasets)	108	108	108	108
# missings	0	0	0	0
wrong choice on catch trial	0	0	0	0
# of datasets with low consistency; ß<.1 (%)	6 (5.55)[a,b]	9 (8.33)[a,b]	3(2.77)[a,b]	5(4.63)[a,b]
# statistical outliers behavioural measure k/λ (%)	0	0	1(.93)[a]	0
# statistical outlier excluded (%)	0	0	0	0
final n	108	108	108	108

[a] <3IQR not excluded

[b] equally distributed over conditions

Table S2: Correlations behavioural measure between tasks

	both			**placebo**			**80mg%**		
Behavioral measure	PDG[a]	PDL[a]	MG[b]	PDG[a]	PDL[a]	MG[b]	PDG[a]	PDL[a]	MG[b]
DD[a]	**.367****	.069	.138	**.254**	.213	.166	**.389****	-.038	.109
	<.001	.481	.156	**.064**	.123	.231	**<.001**	.784	.434
	108	108	108	**54**	54	54	**54**	54	54
PDG[a]		.000	**.272****		.001	**.287****		.000	**0.259**
		.997	**.004**		.994	**.035**		.997	**.058**
		108	**108**		54	**54**		54	**54**
PDL[a]			-.140			-.116			-.160
			.150			.450			.248
			108			54			54

Pearson 2-tailed

[a] log(k)

[b] log(λ)

Table S3: Correlations reaction times between tasks

	both			**placebo**			**80mg%**		
Deliberation time[a]	PDG	PDL	MG	PDG	PDL	MG	PDG	PDL	MG
DD	**.481****	**.272****	**.501****	**.424****	.209	**.532****	**.512****	**.289***	**.501****
	<.001	**.004**	**<.001**	**.001**	.129	**<.001**	**<.001**	**.004**	**<.001**
	108	**108**	**108**	**54**	54	**54**	**54**	**54**	**54**
PDG		**.682****	**.516****		**.677****	**.568****		**.686****	**.456****
		<.001	**<.001**		**<.001**	**<.001**		**<.001**	**.001**
		108	**108**		**54**	**54**		**54**	**54**
PDL			**.437****			**.470****			**.334***
			<.001			**<.001**			**.014**
			108			**54**			**54**

Spearmen-Rho

[a] Mean RT

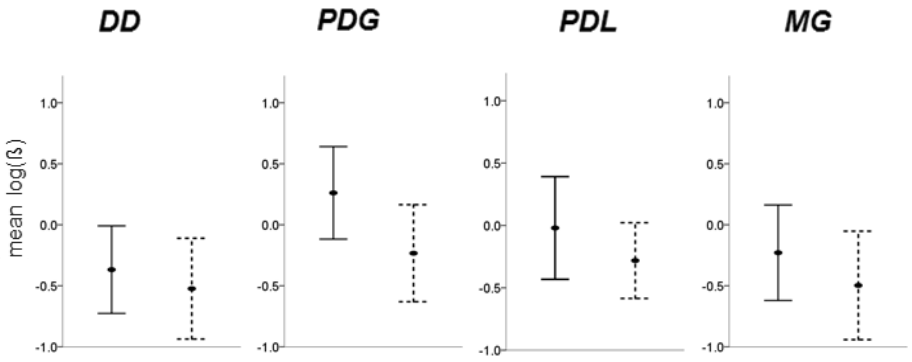

Figure S2: Alcohol effects on task performance Consistency estimates during alcohol infusion as compared to placebo DD: placebo *Mean*=-.367, *SD*=1.31; alc Mean=-.523, *SD*=1.51; *T*(53)=.695, *p*=.490; PDG: placebo *Mean*=.261, *SD*=1.39; alc *Mean*=-.232, *SD*=1.45; *T*(53)=2.182, *p*=.034 ; PDL: placebo *Mean*=-.02, *SD*=1.51; alc *Mean*=-.281, *SD*=1.11; *T*(53)=1.074, *p*=.288; MG: placebo *Mean*=-.229, *SD*=1.44; alc *Mean*=-.497, SD=1.53; *T*(53)=1.119, *p*=.268; Consistency of choice parameters correlated between placebo and alcohol infusion within each task (DD: *r*(54)=.327, *p*=.016, PDG: *r*(54)=.317 *p*=.020, PDL: *r*(53)=.251 *p*=.070, MGA: *r*(54)=.345 *p*=.011). DD = delay discounting, PDG = probability discounting for gains, PDL = probability discounting for losses and MG = mixed gambles Significance codes: **<.01, *<.05

Explorative split group analysis

Methods Individual differences on behavioural choice between infusion conditions were computed as Diff_scores subtracting values obtained during placebo condition from alcohol condition. Thus, participants were grouped according to positive versus negative Diff_scores for subsequent analysis. Group by condition interactions were tested with repeated-measures ANOVAs. To test for session effects (alcohol first versus placebo first), additional analyses were done including session as a moderating and mediating factor within the model. Subsequent explorative group comparisons were performed using independent 2-sample t-tests, or nonparametric Exact Mann-Whitney U-tests for non–Gaussian distributed variables. The significance level for all analyses was set to α=.05 (two-tailed) with False Discovery Rate (FDR) correction (Benjamini and Hochberg, 1995) to account for multiple comparisons.

Results

DD

Individual tendencies towards a more impulsive or less impulsive pattern of choices during alcohol compared to placebo were found and plotted as the difference score (Diff) (log(k) alcohol condition- log (k) placebo condition; Figure S2A). Accordingly, subjects could be grouped in either exhibiting higher discounting (i.e. more impulsive behaviour, n=24) or lower discounting (i.e. less impulsive behaviour, n=30) during alcohol administration. A factorial repeated-measures ANOVA with these groups (more impulsive vs. less impulsive during alcohol) and condition (placebo vs. alcohol) revealed a significant interaction (group x condition, $F(1,52)=72.177$ $p<.001$; Figure S2A) which remained significant when controlling for session order ($F(1,51)=68.919$ $p<.001$). Individuals grouped according to their change in choice behaviour under alcohol thus form discrete groups also during placebo condition as signified by a significant group difference in their discounting parameter ($t(53)=2.124$, $p=.034$; Figure S2A, Table S3). Additionally, we performed explorative analyses of differences between groups (more vs. less impulsive during alcohol) in subjectively experienced alcohol effects, measures of prior alcohol consumption, and related questionnaires. The group exhibiting higher rates of discounting (i.e. more impulsive choice behaviour) during alcohol administration was found to exhibit generally higher subjectively experienced alcohol effects with stimulation reaching significance ($t(53)=-2.038$, $p=.047$; all other ps > .1; Table S3).

PDG

Individual tendencies towards a more or less risk-seeking choice behaviour during alcohol administration were plotted as the Diff score (log(k) alcohol condition- log(k) placebo condition; Figure S2B). Accordingly, subjects could be grouped in either exhibiting higher discounting (i.e. more risk averse behaviour, n=29) or lower discounting (i.e. more risk seeking behaviour, n=25) during alcohol. Factorial repeated-measures ANOVAs with these groups and condition revealed a significant interaction

(F(1, 52)=115.626 p<.001; Figure S2B) which remained significant when controlling for session (F(1, 51)=110.136 p<.001). Again, individuals grouped according to their change in choice behaviour during alcohol administration depict discrete groups also in the placebo condition (t(53)=3.26, p=.002; Figure S2B, Table S3). Explorative analyses of group differences in regard to assessed subjectively experienced alcohol effects, measures of prior alcohol consumption and questionnaires revealed generally higher subjective alcohol effects with sedation (t(53)=2.110, p=.040) and feeling drunk (t(53)=1.993, p=.052) in the group showing more risk-seeking choice behaviour in the alcohol condition (Figure S3A). While scores on past alcohol consumption (PEth: Z(50)=-2.795, p=.005), alcohol-related questionnaires (AUDIT: Z(50)=-2.336, p=.02; ADS: Z(50)=-2.188, p=.029; OCDS: Z(50)=-2.329, p=.02) as well as positive alcohol expectancies (AEQ: Z(50)=-2.336, p=.02) and drinking-motive-related measures were found to be higher in the group showing more risk-averse choice behaviour during alcohol administration (Table S3, Figure S3B).

PDL

We found individual tendencies towards a more or less risk seeking choice behaviour (Figure S2C). Accordingly, subjects could be grouped in either exhibiting higher discounting (i.e. more risk-seeking behaviour, n=25) or lower discounting (i.e. more risk-averse behaviour, n=29) during alcohol administration as compared to placebo. Factorial repeated-measures ANOVAs with these groups and condition revealed a significant interaction (F(1,52)=66.998, p<.001; Figure S2C) which remained significant when controlling for session (F(1,51)=60.092, p<.001). Again, individuals grouped according to their change in choice behaviour during alcohol depict discrete groups also in the placebo condition (t(53)=2.706, p=.009; Figure S2C, Table S3). Explorative analyses revealed differences in the groups with respect to subjectively experienced alcohol effects with stimulation yielding significance (t(53)=2.368, p=.022) with higher values in the group that showed more risk-averse behaviour in the alcohol condition. While the group exhibiting more risk-seeking behaviour in the alcohol condition showed higher scores on alcohol related questionnaires for dependency (ADS: t(53)=-1.909, p=.056) and craving (OCDS: t(53)=2.639, p=.008; Table S3).

MG

Differences in individual choice tendencies (Figure S2D) split participants in either more risk taking (n=27) or more loss averse (n=27) during alcohol administration as compared to placebo. Factorial repeated-measures ANOVAs with these groups and condition revealed a significant interaction (F(1,52)=54.417,p<.001; Figure S2D) remaining significant when controlling for session (F(1,51)=53.552, p<.001). Explorative analyses revealed that nearly all FH positive participants showed more loss averse behaviour in the alcohol condition (χ2(53)=-11.169, p=.001) and higher self-reported scores of sensation seeking (SURPS; t(53)=-2 .411, p=.016) while no other measures were significantly different between the two groups (all *p-values* > .1; Table S3).

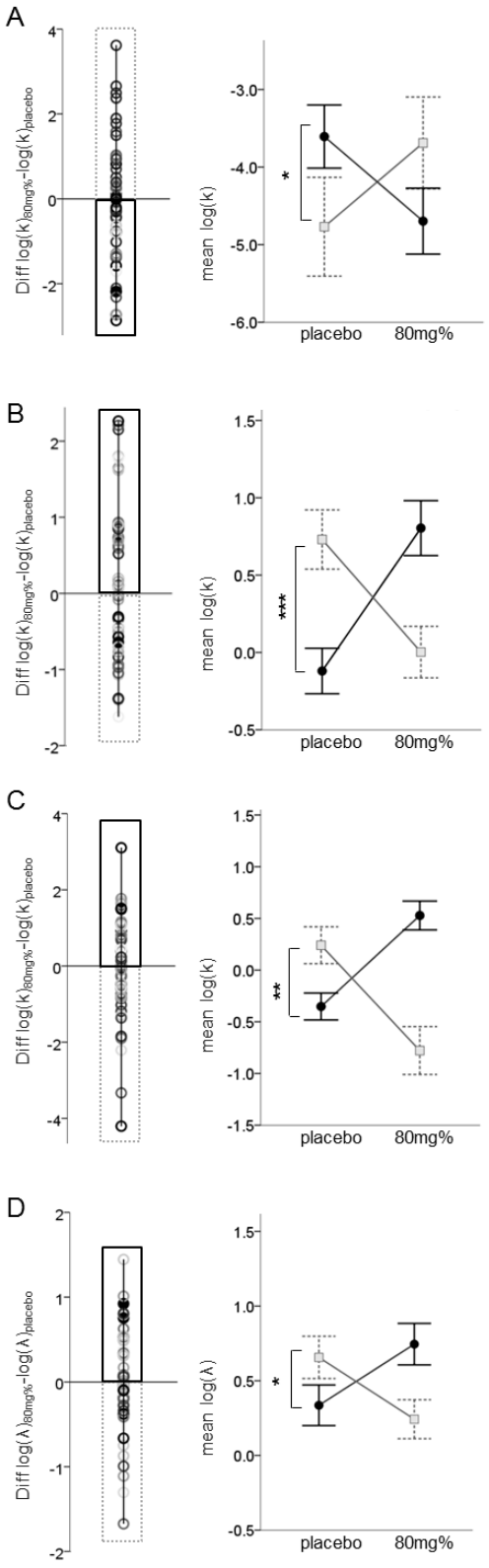

Figure S3: Alcohol effects on impulsive choice Individual preferences towards altered choice behaviour exist and divide participants in two groups either exhibiting increased or decreased behavioural estimates when intoxicated (Diff = behavioural parameter alcohol administration - behavioural parameter placebo condition) A) Distribution of Diff scores for Delay Discounting. Light grey frame indicates individuals (*n*=24) exhibiting increased impulsivity following alcohol administration, Black frame indicates individuals (*n*=30) with decreased impulsivity following alcohol administration. Individuals form discrete groups during placebo condition B) higher or lower risk aversion for probabilistic gains C) more versus less risk seeking for probabilistic losses, and D) Higher risk seeking versus higher degree of loss aversion under alcohol. Light grey labelled groups in accordance with discrepancy from common choice tendencies of general population and direction of significant altered parameters in AUD (Bernhardt et al., 2017).

Table S4: Explorative analysis in groups split by divergent choice behaviour during alcohol and placebo condition in regard to subjective measures, questionnaire impulsivity and alcohol related experiences (personal or familiar)

			DD				PDG				PDL				MG			
		Conzeptualization of behaviour under alcohol	less impulsive (n=30) *Mean (SD)*	more impulsive (n=24) Mean (SD)	statistical difference test value	*p*	more risk seeking (n=25) *Mean (SD)*	more risk averse (n=29) *Mean (SD)*	statistical difference test value	*p*	more risk averse (n=29) *Mean (SD)*	more risk seeking (n=25) *Mean (SD)*	statistical difference test value	*p*	more risk taking (n=27) *Mean (SD)*	more loss averse (n=27) *Mean (SD)*	statistical difference test value	*p*
VBDM	placebo	behavioural parameter [1]	-3.61 (2.22)	-4.76 (3.12)	2.124	.034	.73 (.96)	-.12 (.79)	3.573	.001	.24 (.91)	-.35 (.69)	2.706	.009	.66 (.75)	.33 (.70)	1.773	.076
		deliberation time (s) [2]	2.2 (.75)	2.18 (.66)	.226	ns	1.39 (.43)	1.37 (.32)	.183	ns	1.46 (.59)	1.64 (.75)	-.995	ns	1.25 (.27)	1.21 (.33)	.558	ns
		consistency [1]	2.14 (.66)	2.19 (.74)	-.679	ns	-.16 (1.4)	.62 (1.3)	-2.11	.040	-.14 (1.63)	.09 (1.41)	-.553	ns	-.772 (1.21)	.315 (1.46)	-2.983	.004
	80mg%	behavioural parameter [1]	-4.7 (2.32)	-3.68 (2.9)	-1.132	ns	.002(.83)	.804 (.95)	-3.26	.002	-.778 (1.18)	.528 (1.18)	-4.916	<.001	.243 (.67)	.745 (.72)	-2.641	.011
		deliberation time (s) [2]	1.98 (.56)	2.31 (.94)	-1.097	ns	1.35 (.44)	1.41 (.41)	.660	ns	1.45 (.47)	1.26 (.41)	1.512	ns	1.15 (.16)	1.07 (.23)	1.343	ns
		consistency [1]	2.31 (.94)	1.97 (.57)	2.054	.041	-.09 (1.37)	-.36 (1.55)	.575	ns	.089 (1.19)	-.625 (.929)	2.47	.017	-.598 (1.53)	-.395 (1.75)	-.455	ns
Subjective measures[§]		sedation[1]	23 (24.37)	28.36 (26.95)	-.776	ns	32.73 (25.22)	18.56 (24.13)	2.110	.040	29.21 (26.96)	20.94 (23.33)	1.195	ns	29.85 (28.19)	20.91 (22)	-1.299	ns
		stimulation[1]	32.28 (22.59)	46.35 (28.18)	-2.038	.047	44.54 (24.01)	32.96 (26.87)	1.664	ns	44.34 (24.77)	31.8 (26.16)	2.368	.022	36.3 (27.08)	40.77 (25.1)	-.630	ns
		negative effects[1]	6.15 (10.93)	11.8 (17.85)	-1.39	ns	10.91 (16.56)	6.39 (12.11)	1.11	ns	10.15 (17.08)	7.3 (11.89)	.693	ns	6.56 (10.95)	10.91 (17.55)	-1.067	ns
		feeling drunk[1]	42.29 (21.85)	45.21 (25.36)	-.455	ns	49.97 (25.14)	37.66 (20.10)	1.993	.052	44.16 (24.88)	42.92 (21.81)	.193	ns	40.58 (23.9)	46.59 (22.71)	-.945	ns
		Bis-15 Sum [1]	28.67 (4.3)	30.04 (5.46)	-1.036	ns	29.8 (4.55)	28.82 (5.13)	.731	ns	29.84 (4.9)	28.75 (4.84)	.827	ns	29.9 (5.44)	28.67 (4.2)	.309	ns
SURPS		Anxiety sensitivity [1]	11.10 (1.69)	10.38 (2.06)	1.422	ns	10.64 (1.87)	10.90 (1.92)	-.496	ns	10.38 (1.81)	11.14 (1.9)	-1.498	ns	10.78 (1.78)	10.79 (2.01)	.001	ns
		Hopelessness [1]	12.20 (2.67)	12.46 (3.5)	-.308	ns	12.8 (3.39)	11.90 (2.69)	1.091	ns	12.04 (2.76)	12.57 (3.31)	-.640	ns	12.59 (3.42)	12.04 (2.64)	.668	ns
		Impulsivity [1]	9.27 (1.53)	9.67 (2.04)	-.824	ns	9.4 (1.87)	9.48 (1.73)	-.170	ns	9.38 (1.9)	9.5 (1.67)	-.238	ns	9.41 (1.95)	9.48 (1.36)	-.153	ns
		Sensation seeking [1]	15.83 (2.59)	16.17 (3.33)	-.414	ns	16.2 (3.06)	15.79 (2.83)	.508	ns	16.27 (3.22)	15.71(2.64)	.659	ns	15.22 (2.81)	16.74 (2.88)	-2.411	.016
Alcohol related measures		Age of 1st drink (years) [2]	14.2 (1.32)	14.29 (1.33)	-.055	ns	14.48 (.96)	14.04 (1.55)	1.028	ns	14.65 (1.2)	13.86 (1.32)	2.596	.009	14.2 (1.26)	14.33 (1.39)	-1.025	ns
		PEth [2]	134 (242)	115 (117)	1.007	ns	57.62 (37.41)	186 (252)	-2.795	.005	151 (260)	99.4 (82.5)	.574	ns	98.12 (140)	154 (238)	-1.199	ns
		AUDIT total score [2]	7.05 (2.89)	8.39 (5.75)	-.13	ns	5.98 (2.11)	9.27 (5.4)	-2.306	.021	7.35 (5.2)	7.98 (3.58)	-1.437	ns	7.65 (4.29)	7.67 (4.61)	-.009	ns
		OCDS-G total score [2]	2.77 (1.75)	4.04 (3.78)	-.242	ns	2.64 (1.89)	3.93 (3.44)	-2.188	.029	2.58 (2.51)	4.04 (3.06)	-2.639	.008	2.59 (4.81)	4.07 (3.41)	-1.77	ns
		AEQ-G total score [2]	29.3 (4.46)	29.54 (5.3)	-.253	ns	28.32 (3.97)	30.34 (5.32)	-2.329	.02	29.15 (4.58)	29.96 (5.08)	-.574	ns	29.07 (4.81)	29.74 (4.87)	-.321	ns
		DMQ Enhancement [2]	11.27 (4.16)	13.42 (4.91)	-1.598	ns	11.28 (3.83)	13.03 (5.09)	-1.866	.068	11.92 (4.97)	12.5 (4.28)	-.556	ns	12.3 (5.12)	12.15 (4.08)	.009	ns
		DMQ Social [2]	13.8 (4.08)	13.71 (4.95)	.087	ns	12.8 (1.41)	14.59 (4.38)	-1.733	.089	13.5 (4.59)	14 (4.37)	-.608	ns	14.15 (5)	13.37 (3.86)	.607	ns
		DMQ Conformity [2]	5.73 (1.66)	5.63 (1.14)	.23	ns	5.84 (1.99)	5.55 (.67)	.587	ns	5.88 (1.97)	5.5 (.64)	.119	ns	5.48 (1.09)	5.89 (1.71)	-1.855	ns
		DMQ Coping [2]	6.83 (2.38)	6.71 (2.16)	.434	ns	5.96 (1.14)	7.48 (2.73)	-1.876	.061	6.42 (1.88)	7.11 (2.56)	-1.026	ns	7.22 (2.62)	6.33 (1.78)	1.358	ns
		FHP (%)	7	8	-.042	ns	7	8	.001	ns	9	6	1.169	ns	2	13	-11.169	.001

Groups according to Diff (behavioural parameter under alcohol condition - behavioural parameter under placebo condition). Light grey colour in accordance with discrepancy from common choice tendencies of general population and direction of significant altered parameters in AUD (Bernhardt et al 2017). VBDM value-based decision-making battery with four independent task DD = delay discounting, PDG = probability discounting of gains, PDL = probability discounting of losses and MG = mixed gambles. AUDIT = Alcohol Use Disorders Identification Test. ADS = Alcohol Dependence Scale (Skinner & Horn, 1984). BIS-15 = Barratt Impulsiveness

Scale, German short version (Meule et al., 2011). OCDS-G = Obsessive Compulsive Drinking Scale, German version (Mann & Ackermann, 2000). AEQ-G = Alcohol Expectancy Questionnaire,German short version (Demmel and Hagen, 2002). DMQ-R with the 4 motives Enhancement, Social, Conformity and Coping, German version (originally developed by Cooper, 1994, validated by Kuntsche et al., 2006). FH = Family history of alcohol problems if on first- or second-degree biological relative was reported.

[§] Difference 80mg% condition-placebo condition

[1] normal distribution of this measure, t (t-test)

[2] Shapiro-Wilk test implied non-normal distribution of this measure ($p<.05$), Z (Exact Mann-Whitney U-test)

[3] categorical data, Pearson $\chi 2$ (Exact $\chi 2$ test)

[a] log(k)

[b] log(λ)

ns = all $p < .1$

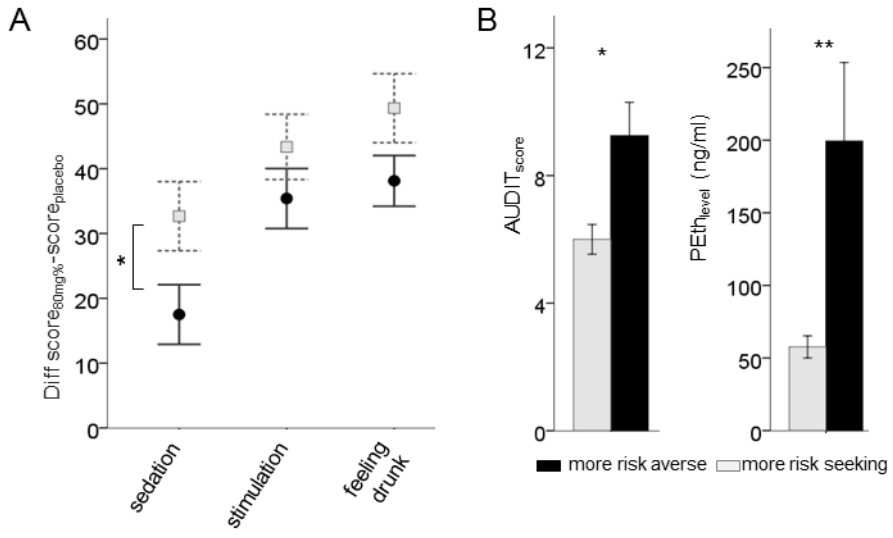

Figure S4: Individual differences in alcohol effects on impulsive choice associate with real-life alcohol consumption and subjective intoxication. Groups defined by choice alterations in probability discounting of gains under moderate dose of alcohol exhibit significant differences in A) subjectively experienced alcohol effects and B) measures of alcohol consumption blood levels of PEth = phosphatidylethanol and questionnaire score on the AUDIT = Alcohol Use Disorders Identification Test.

References

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.

Bernhardt, N., Nebe, S., Pooseh, S., Sebold, M., Sommer, C., Birkenstock, J., Zimmermann, U.S., Heinz, A., Smolka, M.N., 2017. Impulsive Decision Making in Young Adult Social Drinkers and Detoxified Alcohol-Dependent Patients: A Cross-Sectional and Longitudinal Study. Alcohol. Clin. Exp. Res. 41, 1794–1807. https://doi.org/10.1111/acer.13481

Schröck, A., Redondo, A.H., Fabritius, M.M., König, S., Weinmann, W., 2016. Phosphatidylethanol (PEth) in blood samples from "driving under the influence" cases as indicator for prolonged excessive alcohol consumption. Int. J. Legal Med. 130, 393–400. https://doi.org/10.1007/s00414-015-1300-5

Schröck, A., Thierauf-Emberger, A., Schürch, S., Weinmann, W., 2017. Phosphatidylethanol (PEth) detected in blood for 3 to 12 days after single consumption of alcohol—a drinking study with 16 volunteers. Int. J. Legal Med. 131, 153–160. https://doi.org/10.1007/s00414-016-1445-x