Alcohol-Induced Impairment of Inhibitory Control Is Linked to Attenuated Brain Responses in Right Fronto-Temporal Cortex

Supplemental Information

Supplemental Methods

Recruitment

Recruitment of participants for the Dresden Longitudinal Study on Alcohol Use in Young Adults (D-LAYA) study was carried out by mailing invitation letters (*n* = 3580) for study participation to 18 and 19 year-old residents of Dresden, Germany. Seven hundred and thirty young adults (20%) were interested in study participation. We conducted telephone interviews with all interested people to check inclusion and exclusion criteria (see Methods, participants section). Eligible participants (*n* = 135; 18.5% of all interested persons) were invited to a screening visit for evaluation of the family history of alcoholism (FHA) in more detail using the Family History Assessment Module (1). Additionally, we conducted a detailed interview of mental health using the Mini-DIPS (2), and the substance abuse section of the Munich-Composite International Diagnostic Interview (3), and of physical disorders. None of the participants had a history of alcohol or illicit drug abuse or dependence. Liver enzymes and a full blood count were measured to exclude pre-existing alcohol toxicity. In total, 87 young adults took part in both sessions of the free-access alcohol self-administration experiment (reported by 4, *in preparation*). Of those, 47 participated in the functional magnetic resonance imaging (fMRI) alcohol clamping part.

Sample Characteristics

We present sample characteristics about drinking behavior assessed with free-access alcohol self-administration, a time-line follow-back interview (for 45 days prior to the experiment; 5), and the Alcohol Use Disorders Identification Test (6) in Table S1. Additionally, we present information about smoking, “illicit drug use” and education for the final fMRI sample.

Table S1. Alcohol drinking behavior assessed with the AUDIT, the time-line follow-back interview, and free-access alcohol self-administration for the fMRI sample.

	Mean	± SEM	Minimum	Maximum
AUDIT (total score)	7.5	0.6	1.0	21.0
Time-line follow-back interview				
No. of drinks per drinking day	4.1	0.3	1.3	7.4
No. of drinking days in 45 days	15.4	1.3	2.0	41.0
No. of binge days in 45 days	5.5	0.8	0.0	21.0
Free-access alcohol self-administration of the second session				
Mean BrAC	65.5	3.6	17.3	101.5
Maximum BrAC	92.7	4.5	22.4	133.7
No. of alcohol requests	18.4	0.8	7.0	27.0

AUDIT, Alcohol Use Disorders Identification Test; BrAC, breath alcohol concentration; fMRI, functional magnetic resonance imaging.

Smoking and "Illicit Drug Use"

Twenty-three participants currently smoked cigarettes on a regular or occasional basis. Of those, 12 smoked approximately 2 hours before the start of the infusion. Smokers had a mean Fagerström Test for Nicotine Dependence score of 0.71 (SD = 1.35; maximum = 4) with three smokers meeting the DSM-IV criteria for nicotine dependence. Nineteen were non-smokers.

Thirteen participants reported regular (n = 4) or occasional cannabis use (n = 9). Of those, 12 were also smokers, and three had already consumed illicit drugs (amphetamines, n = 3; ecstasy, hallucinogens, cocaine, n = 2). Twenty-six never consumed illicit drugs. All participants had negative urine drug screenings before study participation (nal von minden, "Drug-screen-multi 10R", Regensburg, Germany; testing for traces of amphetamines, barbiturates, benzodiazepines, cocaine, ecstasy, methamphetamine, opiates/morphine, tricyclic antidepressants, cannabinoids, and tramadol). For 3 participants, information about illicit drug use was not available.

Before data analysis, we verified that neither smoking nor illicit drug use influenced outcome variables (see Supplement "Results – Between-subject variables").

Education

The majority of participants included in the analyses were school or 1st year university students (n = 34), two were employees, and six did not provide information about their current employment status.

Experimental Procedures

Measurement of Breath Alcohol Concentration (BrAC) Inside and Outside of the Magnetic Resonance (MR) Room

The breathalyzer is not MR-compatible and could not be used inside the MR-room. Therefore, we obtained samples (approximately 1.5l) of end-expiratory air using a tube (PVC, 140 cm) connected to a T-piece, and a standard children's toy balloon (diameter of 75 cm) that was attached to the straight outlet of the T-piece. When participants started to blow into the tube, we first discarded dead space and early expiratory air through the second outlet of the T-piece. Then we blocked the second outlet and the balloon was filled with end-expiratory air. The balloon was then quickly detached from the tube, taken outside of the MR room, attached to the breathalyzer and blown through the device, applying gentle manual pressure on the balloon. In pilot experiments, we evaluated that BrAC readings from a balloon (mean: 0.43 g/kg ± 0.06 g/kg; n = 28 samplings) deviated from direct BrAC readings obtained one minute later in the same subject (0.48 g/kg ± 0.06 g/kg; n = 28 samplings) on average by a factor of 1.12. The BrAC readings obtained in the MR room were corrected by this factor prior to being used as feedback for the CAIS software. During the actual experiments reported here, we compared balloon readings inside the MR room with direct BrAC readings at two occasions (i.e., when subjects left the scanner for the bathroom break after the stop-signal task (SST) and at the end of the fMRI experiment, see Figure S1). We averaged over the two direct readings and balloon readings and showed that they did not differ significantly (paired t-test: $t_{(49)}$ = .048; p = .962; direct: 0.582 g/kg ± 0.064 g/kg, corrected balloon: 0.582 g/kg ± 0.061 g/kg) and correlated by r = .82 (Pearson product-moment correlation coefficient, p < .001, n = 50 participants).

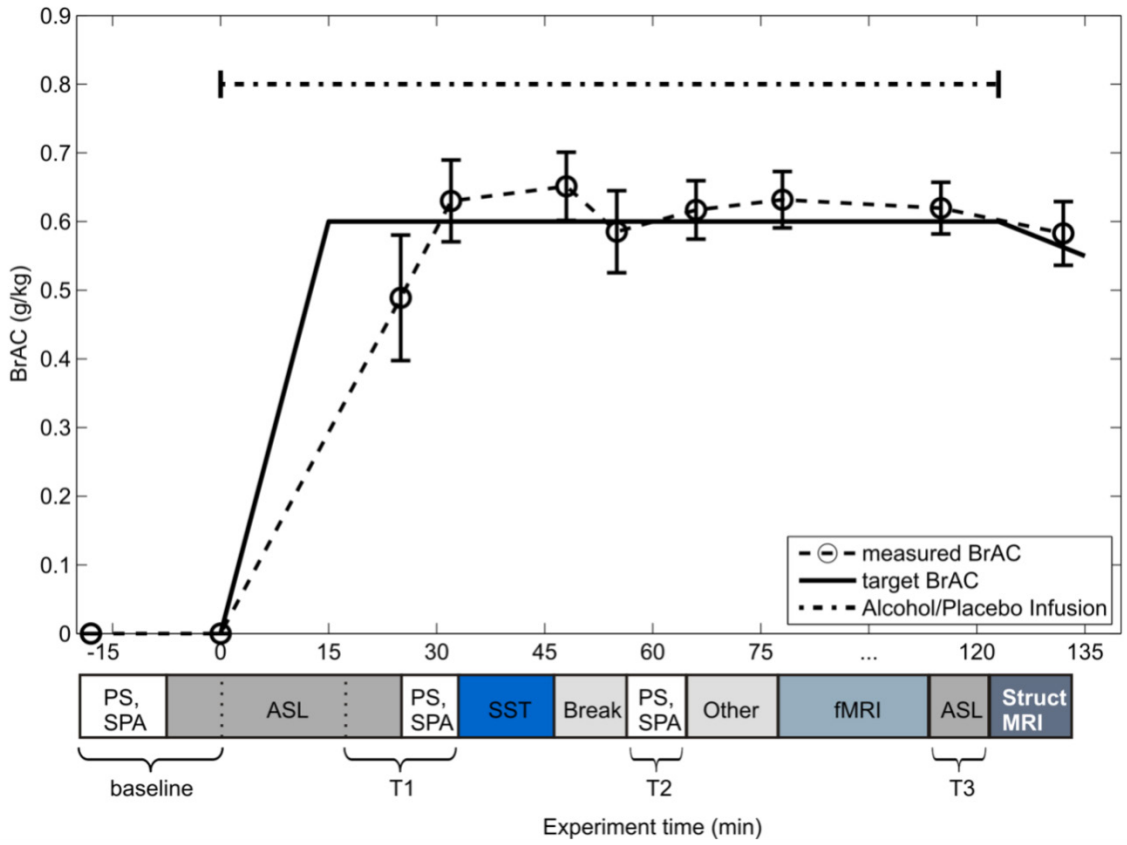

Figure S1. Time course of the complete fMRI alcohol clamping experiment. Anatomical scans were performed at day 1 only. ASL, arterial spin labeling; BrAC, breath alcohol concentration; fMRI, functional magnetic resonance imaging; PS, prosaccades; SPA, subjective perceptions of alcohol; SST, stop-signal task; Struct MRI, structural magnetic resonance imaging.

Imaging Data Acquisition and Analysis

MRI Data Acquisition

For functional Imaging, 40 transversal slices were acquired in a descending way using a standard gradient echoplanar imaging (EPI) sequence (voxel size = 3.4 x 3.4 x 2.4 mm, gap = 1 mm (distance factor = 42%), repetition time (TR) = 2200 ms, echo time (TE) = 60 ms, flip angle = 75°, field of view (FOV) = 220 mm tilted by 30°, in-plane resolution = 64 x 64 pixels, bandwidth (BW) = 2004 Hz/Px). Arterial spin labeling (ASL) data were acquired using a pulsed ASL-sequence with a 3D-GRASE readout (7) with 7/8 slice partial Fourier (voxel size = 5 x 5 x 4 mm, #slices = 26, gap = 0, TR = 3060 ms, TE = 18.18 ms, refocusing flip angle = 120°, turbo factor = 23, BW = 2790 Hz/Px). High resolution anatomical scans were acquired with a T1-weighted,

anatomical, 3D, magnetization-prepared rapid gradient echo (MPRAGE) sequence (voxel size = 1 x 1 x 1 mm, #slices = 176, TR = 1900 ms, TE = 2.26 ms, FOV = 256 mm, flip = 9°).

Preprocessing of fMRI Data

The first five scans were discarded from data analysis to ensure tissue steady-state magnetization resulting in 368 volumes per session per participant. We used standard procedures implemented in SPM8 for preprocessing of fMRI data: slice timing (reference: middle slice), spatial realignment to first slice, normalization to the MNI (Montreal Neurological Institute, Quebec, Canada) standard EPI template, and smoothing using an isotropic Gaussian kernel of 8-mm full-width at half-maximum. fMRI data were high-pass filtered (1/128 Hz cutoff) to remove low frequency drifts. If head movements during scanning exceeded a predefined threshold (>3 mm/3° of translation/rotation; n = 7), participants were discarded from fMRI data analysis.

ASL Data-analysis

For both scan sessions, one selective saturation image from each series with inversion time TI = 2100 ms was used for motion correction using SPM8. The determined transformation matrices were applied to all difference images. Images were normalized to MNI space using T2-weighted images and resampled to a 3 x 3 x 3 mm resolution. A 1-compartment perfusion model was applied to the difference data as a function of TI (8). The following parameters were fixed: T1(blood) = 1684 ms, T1(tissue) = 1300 ms, λ = 0.9, bolus length = 1 s (9). The magnetization of arterial blood was determined from cerebrospinal fluid signal from separate scans with long TR and no saturation. Perfusion and bolus arrival time were fitted. For more information, see Marxen *et al.* (10).

Supplemental Results

Between-subject Variables

FHA, gender, drug order, smoking, and illicit drug use might influence alcohol effects on behavioral and brain responses as well as free-access alcohol consumption. Thus, we tested possible effects of these between-subject variables with independent *t*-tests before running statistical analyses with our dependent variables. We did not correct those independent *t*-tests for multiple comparisons to avoid Type-II errors due to unbalanced small group sizes.

FHA, gender, drug order, smoking, and illicit drug use did not significantly affect alcohol-induced impairment of inhibitory control ($t_s < .85$, $p_s > .4$), alcohol effects on inhibition-related brain responses in the right inferior frontal gyrus (RIFG)/insula ($t_s < 1.8$, $p_s > .08$), and in the occipito-temporal cortex ($t_s < 1.9$, $p_s > .079$), and alcohol effects on global and local perfusion (RIFG/insula, occipito-temporal cortex; $t_s < 1.7$, $p_s > .1$).

FHA, gender, smoking and illicit drug use did also not influence free-access alcohol consumption in the fMRI sample ($t_s < .75$, $p_s > .45$). We confirmed in a larger sample of 87 people, who all participated in the free-access experiment including participants who were not selected for fMRI ($n = 40$) because of low free-access BrAC levels (<0.5 g/kg), that in our young sample family-history positive (FHP) participants did not differ from family-history negative (FHN) people regarding free-access alcohol consumption (4). In this larger sample, women consumed less alcohol than men. Missing effects of gender on free-access alcohol consumption in the fMRI sample might be explained by the fact that we pre-selected people for fMRI measurements based on moderate to high alcohol consumption levels in the free-access experiment (maximum BrAC ≥ 0.5 g/kg in one of the two sessions) to avoid untoward alcohol effects at the target BrAC level during fMRI. This might have reduced the bandwidth necessary to detect group differences.

To acknowledge that unbalanced small group sizes in the fMRI sample (FHP/FHN: 15/27; female/male: 11/31) might blur potential group effects, we recomputed all statistical analyses including FHA, gender, smoking, illicit drug use, and additionally drug order for SST analyses as covariates. Inclusion of those covariates did not change behavioral and neural results presented in the current manuscript, and there was no increase of the proportion of variance explained in the model. Thus, covariates were discarded from statistical analyses. Moreover, we believe that simple statistical models are preferable compared to over-specified models for reasons of clarity and for potential replication of findings.

Supplemental Imaging Data

Alcohol Effects on Inhibition-Related Brain Responses

In Table S2, we present alcohol effects on inhibition-related brain responses separately for StopInhibit and StopFail, and the main alcohol effect (alcohol < placebo without conjunction) to show that the activation pattern observed for the conjunction analysis of "alcohol<placebo", "StopInhibit", and "StopFail" (Figure 4, Table 2D, main document) was not affected by stopping conditions.

Table S2. Conjunction analysis of "alcohol < placebo" separately with StopInhibit and StopFail, and main effect of alcohol without conjunction ("alcohol < placebo"). The significance threshold was set to $p < .001$ (uncorrected with at least 50 connected voxels). *P*-values corrected for multiple comparisons (FWE-corr at cluster and peak level), *t*-values and MNI coordinates are shown.

			MNI Coordinates			Cluster Level		Peak Level	
Brain Area		**BA**	**x**	**y**	**z**	**FWE-corr.**	**k**	**FWE-corr.**	***t***
Conjunction of alcohol < placebo in StopInhibit trials									
R	IFG	45	36	26	7	0.015	103	0.103	4.56
R	Insula	-	33	14	7			0.140	4.47
R	IFG	47	51	20	1			0.773	3.76
R	MTG	21	57	-52	4	0.054	72	0.372	4.13
R	MTG	21	66	-49	4			0.950	3.51
R	MOG	37	51	-70	1			0.994	3.30
Conjunction of alcohol < placebo in StopFail trials									
R	IFG	45	36	26	7	0.012	109	0.103	4.56
R	Insula	-	33	14	7			0.140	4.47
R	IFG	47	51	20	1			0.773	3.76
R	MTG	21	57	-52	4	0.056	71	0.372	4.13
R	MTG	21	66	-49	4			0.950	3.51
R	MOG	37	51	-70	1			0.994	3.30
Alcohol < placebo (main effect, no conjunction)									
R	IFG	45	36	26	7	0.010	113	0.103	4.56
R	Insula	-	33	14	7			0.140	4.47
R	IFG	47	51	20	1			0.773	3.76
R	MTG	21	57	-52	4	0.054	72	0.372	4.13
R	MTG	21	66	-49	4			0.950	3.51
R	MOG	37	51	-70	1			0.994	3.30

BA, Brodmann area; FWE-corr, family-wise error correction; IFG, inferior frontal gyrus; MNI, Montreal Neurological Institute; MOG, middle occipital cortex; MTG, middle temporal gyrus; R, right.

Alcohol Effects on Global/Local Perfusion, and Its Effects on Regional BOLD Alcohol Effects, and Alcohol-induced Impaired Inhibitory Control

First, we computed paired *t*-tests to confirm that global and local alcohol effects on cerebral perfusion are significantly affected by alcohol (Table S3).

Table S3. Global and local alcohol effects on cerebral perfusion.

	Placebo		Alcohol		Paired *t*-tests
Perfusion (1/s)	**Mean**	**(SEM)**	**Mean**	**(SEM)**	**alcohol vs. placebo**
Global**	0.0094	(0.0002)	0.0102	(0.0003)	$t_{(41)} = 3.77$, $p = .001$
RIFG/insula**	0.0125	(0.0005)	0.0115	(0.0002)	$t_{(41)} = 3.59$, $p = .001$
occ/temp**	0.0118	(0.0004)	0.0108	(0.0004)	$t_{(41)} = 3.47$, $p = .001$

RIFG, right inferior frontal gyrus; occ/temp, occipito-temporal cortex.
**p <.01.

Second, we conducted separate path analyses for the RIFG/Insula and the occipito-temporal cortex with alcohol-induced impairments of inhibitory control as dependent variable and alcohol effects on regional BOLD responses and global/local perfusion as predictors to assess whether increased perfusion under alcohol affected alcohol-induced impaired inhibitory control directly or via influencing regional BOLD alcohol effects (see Table S4). Increased perfusion (global/local) under alcohol did neither significantly influence BOLD alcohol effects in the RIFG/Insula, or occipito-temporal cortex, nor alcohol-induced impairments of inhibitory control. Only alcohol effects on regional BOLD responses significantly affected alcohol-induced impairments of inhibitory control (displayed exemplary for alcohol effects on local perfusion and BOLD responses in the RIFG/Insula in Figure S2).

Table S4. Path coefficients and statistics for path analyses between alcohol effects on perfusion (separate analyses for local/global perfusion), BOLD brain responses (separate analyses for RIFG/insula, and occipito-temporal cortex) and behavioral alcohol-induced impaired inhibitory control.

			RIFG/Insula			Occipito-Temporal Cortex		
Alcohol-induced		**Alcohol-induced**	**β**	**C.R.**	***p***	**β**	**C.R.**	***p***
decreased brain responses	<---	increased local perfusion	-.14	-.88	.38	.03	.16	.87
impaired inhibitory control	<---	increased local perfusion	-.01	-.06	.95	.06	.40	.69
impaired inhibitory control	<---	decreased brain responses	-.35	-2.36	.02*	-.33	-2.26	.02*
decreased brain responses	<---	increased global perfusion	-.11	-.74	.46	.00	.02	.98
impaired inhibitory control	<---	increased global perfusion	.04	.27	.79	.08	.55	.58
impaired inhibitory control	<---	decreased brain responses	-.34	-2.33	.02*	-.33	-2.26	.02*

β, estimate of standardized regression weight; BOLD, blood oxygenation level-dependent; C.R., critical ratio for regression weight; RIFG, right inferior frontal gyrus.
*$p < .05$

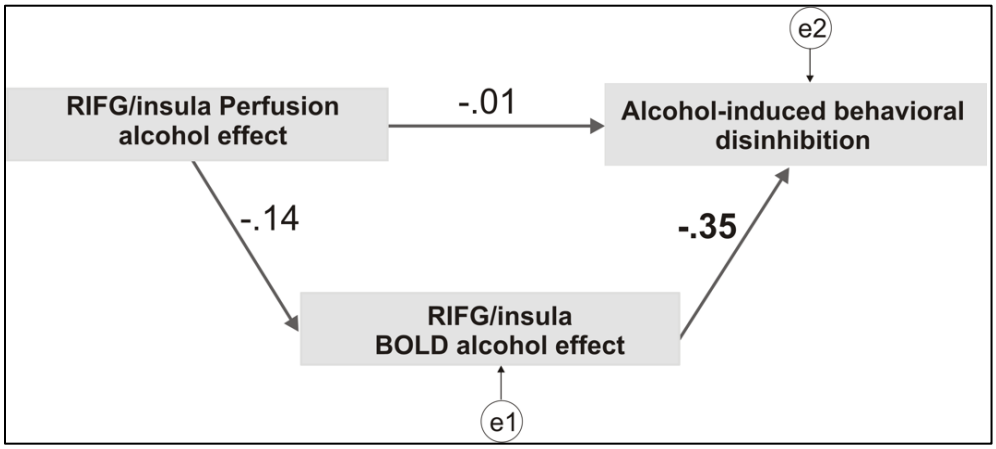

Figure S2. Path analysis comprising connections between alcohol effects on local perfusion, and BOLD brain responses in the RIFG/Insula, and alcohol-induced impairment of inhibitory control. The plots were comparable for the same analyses with global perfusion, and for the BOLD alcohol effect in the occipito-temporal cortex (cf. Table S4). See Table S4 for abbreviations.

Level of Alcohol Intoxication

Subjective perceptions of alcohol (visual analogue scales for stimulation, sedation, unpleasant effects, wanting more alcohol, feeling good/drunk, estimated number of drinks; Table S5), and saccadic eye-movements were recorded during fMRI alcohol clamping at baseline and at T1 (see Figure S1), as well as at the beginning of the second part of the experiment (T2) that was not the focus of the present manuscript.

Table S5. Items of the questionnaire asking for subjective perceptions of alcohol.

No.	Item
1	I feel stimulating alcohol effects right now.
2	I feel sedating alcohol effects right now.
3	I feel unpleasant alcohol effects (like nausea, vertigo) right now?
4	I want more alcohol right now.
5	I feel good right now.
6	I feel as if I had *XX* drinks.
7	I feel drunk right now.

Alcohol effects on saccadic eye-movements are a robust marker of alcohol intoxication (e.g., 11; 12; 13) with increasing saccadic latencies indicating increasing impairment under alcohol. We recorded prosaccades at 1000 Hz using a mr-compatible eye-tracker (EyeLink 1000, SR Research, Mississauga). Participants were instructed to focus on a central fixation cross and to look as fast and accurate as possible to a white square as soon as it appeared on the right or left horizontally of a fixation cross (n = 48 trials). We discarded trials with blinks, wrong saccade directions, and latencies faster than 80 ms and slower than 500 ms from the analysis (cf., 14).

Results

Statistical comparisons (paired t-tests, and Wilcoxon-signed rank tests) of alcohol and placebo responses showed that participants perceived significantly more subjective alcohol effects and were slower in the saccadic eye-movement task under alcohol (see Table S6).

Table S6. Effects of alcohol on behavioral variables measuring the level of alcohol intoxication (saccadic latency, subjective perceptions of alcohol). Alcohol and placebo responses were compared with paired *t*-tests (*t*), or Wilcoxon-signed rank tests (*Z*), as applicable.

	Placebo		Alcohol		Test Statistics
	Mean	**(SEM)**	**Mean**	**(SEM)**	**placebo vs. alcohol**
Eye-movement data					
saccadic latency (ms)***	8.1	(1.9)	22.0	(2.4)	$t_{(39)}$[a] = -5.47, $p < .001$
Subjective perceptions of alcohol					
stimulation (mm)***	-2.4	(2.1)	15.0	(3.0)	$Z = 4.32$, $p < .001$
sedation (mm)***	5.2	(1.8)	28.8	(4.6)	$Z = 4.49$, $p < .001$
unpleasant effects (mm)*	0.5	(0.4)	2.0	(0.7)	$Z = 2.04$, $p = .041$
wanting more (mm)**	-0.6	(0.9)	7.8	(2.1)	$Z = 3.41$, $p = .001$
feeling good (mm)**	-3.0	(1.5)	3.1	(2.0)	$Z = 2.60$, $p = .009$
estimated number of drinks (*n*)***	0.2	(0.1)	1.9	(0.3)	$Z = 4.38$, $p < .001$
feeling drunk (mm)***	2.2	(1.0)	25.3	(4.0)	$Z = 4.87$, $p < .001$

***$p < .001$.
**$p < .01$.
*$p < .05$.
[a] $n = 2$, missing data due to technical problems.

Post-hoc Backward Regression Analysis

We computed a post-hoc backward regression analysis with the number of alcohol requests of the second free-access session as dependent variable and alcohol-induced impaired inhibitory control, alcohol effects (alcohol-placebo) on subjective perceptions (7 scales, see Table S5), and saccadic latency, and baseline inhibitory control ($SSRT_{placebo}$) as predictors. Alcohol-induced impaired inhibitory control was the only predictor that survived the removal criterion of $p < .1$ and predicted number of alcohol requests significantly ($\beta = .34$, $t_{(35)} = 2.07$, $p = .023$ [one-tailed], $R^2 = .11$).

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