

Cognitive impairments by alcohol and sleep deprivation indicate trait characteristics and a
potential role for adenosine A₁ receptors

Short title: Trait Vulnerability to sleep loss and alcohol

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The other authors declare no conflict of interest.

Abstract:

Trait-like differences in cognitive performance after sleep loss put some individuals more at risk than others, the basis of such disparities remaining largely unknown. Similarly, inter-individual differences in impairment in response to alcohol intake have been observed. We tested whether performance impairments due to either acute or chronic sleep loss can be predicted by an individual's vulnerability to acute alcohol intake. Secondly, we used positron emission tomography (PET) to test whether acute alcohol infusion results in an upregulation of cerebral A₁ adenosine receptors (A₁AR), similar to the changes previously observed following sleep deprivation. Sustained attention in the psychomotor vigilance task (PVT) was tested in 49 healthy volunteers (26 ± 5 SD years, 15 females) under 1) baseline conditions, 2) after ethanol intake and after either 3A) total sleep deprivation (TSD, 35 h awake, n=35) or 3B) partial sleep deprivation (PSD, 4 nights with 5 h scheduled sleep, n=14). Ethanol versus placebo induced changes in cerebral A₁AR availability were measured in 10 healthy male volunteers (31 ± 9 years) with [¹⁸F]CPFPX-PET. Highly significant correlations between the performance impairments induced by ethanol and sleep deprivation were found for various PVT parameters including mean speed (TSD r=0.62, PSD r=0.84). A₁AR availability increased up to 26 % in several brain regions with ethanol infusion. Our studies revealed individual trait characteristics for being either vulnerable or resilient to both alcohol and sleep deprivation. Both interventions induce gradual increases in cerebral A₁AR availability pointing to a potential common molecular response mechanism.

Significance statement:

Modern work environments favour working around the clock at the cost of an increased risk for fatigue related human error. Performance impairments caused by sleep loss show great variability among individuals and resemble the effects of ethanol intoxication. We found that there are individual trait characteristics that make individuals either vulnerable or resilient to both alcohol and sleep deprivation. We also provide evidence by molecular brain imaging pointing to the cerebral adenosine system as common molecular response mechanism to ethanol and sleep deprivation. Understanding the mechanistic link between cerebral adenosine and the vulnerability to performance impairment might help to identify countermeasures for fatigue related human error.

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Introduction:

Fatigue due to sleep loss and extended time awake is a major cause of accidents and incidents in the modern 24-h society (1–6). Surveys on sleep habits suggest that ~40% of the German population (7) and ~30% of the U.S. population (2, 8) sleep less than the recommended 7-h minimum sleep duration (9). Chronic sleep restriction is associated with increased sleepiness and cognitive performance impairment (10–13). The extent of impairment varies greatly among individuals, putting some more at risk than others (14, 15), whereas the physiological basis of this variation remains largely unknown. In contrast, performance varies relatively little across repeated exposures within an individual, indicating trait-like behaviour (16) and a substantial genetic influence (17). For a comprehensive review on the inter-individual neurobehavioral variation in the response to sleep deprivation and its involvement of adenosinergic, dopaminergic and cholinergic pathways see Tkachenko and Dinges (18). Similarly, trait-like differences exist in alcohol sensitivity and the vulnerability to performance impairment after alcohol intake (19). Ethanol is a psychoactive drug with depressant effects on the central nervous system. For example, alcohol facilitates sleep onset

and increases deep sleep in the first half of the sleep episode, whereas it disrupts sleep in the second half of the sleep episode (20, 21). It affects multiple neuroreceptor systems in the brain: It is a positive allosteric modulator at GABA receptors, agonist at the $\alpha 7$ nicotinic acetylcholine and serotonin 3 receptors, antagonist at the NMDA, AMPA and Kainate receptors, and an inhibitor of various ion channels. Moreover, ethanol blocks the reuptake of adenosine and increases its formation. Adenosine is a nucleoside that plays a central role in energy transfer as it is formed by breakdown of ATP, the main energy source of cells. In the brain, adenosine acts as a neuromodulator and co-transmitter that inhibits the impact of excitatory neurotransmitters like glutamate. The physiological effect of adenosine is mediated by 4 types of receptors of which the A_1 subtype is most widespread in the human brain. The brain's adenosine system has emerged as a potential candidate mediating both the sleep inducing effects of alcohol as well as the increase in sleep propensity and sleepiness after sleep loss. The impairment of alertness and motor / cognitive performance by alcohol (22) is attributed to the inhibitory effects of increased adenosine concentration and to an increase of inhibitory A_1 adenosine receptor (A_1AR) availability (23–25). The increase in extracellular adenosine has been reported to inhibit wake-promoting cholinergic neurons in the basal forebrain, since blocking the A_1AR as well as lesioning of cholinergic neurons in the basal forebrain resulted in a reduction of the sedative effect of ethanol (26). Ethanol was found to increase extracellular adenosine levels up to 4-fold in cell culture and in vivo microdialysis experiments by stimulating its production and by uptake inhibition (27). Agonists at the A_1AR such as cyclohexyladenosine are known to accentuate the effects of ethanol whereas A_1AR antagonists like caffeine and theophylline attenuate them. Sleep deprivation increases A_1AR availability in the human brain as a function of elapsed time awake (28), thereby impairing brain functioning.

We hypothesized that 1) the individual performance degrading effects of sleep loss and alcohol correlate, i.e. that the response to alcohol predicts individual vulnerability to sleep loss, and that 2) ethanol induces a global increase in A₁AR availability in the human brain resembling the effects of sleep loss.

Three studies were conducted to test these hypotheses. First, 47 participants were exposed to alcohol and 38 h of wakefulness on separate days to compare the impairment of sustained attention on an individual basis. Second, individual impairment was compared in 16 participants who were exposed to alcohol and 4 days of partial sleep deprivation (5 h time in bed) on separate days. Third, cerebral A₁AR availability of 10 participants was measured in vivo using positron emission tomography (PET) comparing the impact of ethanol versus placebo infusion.

Results:

Alcohol intake, total sleep deprivation and 4 days of sleep restriction each resulted in impairment of sustained attention, measured as deviation from baseline in a psychomotor vigilance task (PVT); (Fig. 1). Individuals who showed good performance after alcohol intake proved to be more resistant to the effects of acute and chronic sleep loss, whereas individuals who showed large impairments after alcohol, were more vulnerable to the two types of sleep deprivation (Fig. 1 upper row). A median split was performed based on individuals' performance under the influence of alcohol. The resulting two groups differed significantly in mean speed during TSD and PSD (Fig. 2). While the resilient group recovered from sleep loss after one night of sleep, recovery remained incomplete in the vulnerable group.

Highly significant Spearman correlations (Table 1, Fig. 1 lower row) between performance impairments after alcohol intake and acute or chronic sleep deprivation were found for several PVT measures. In contrast, the impairment in the examined performance parameters under

alcohol or sleep deprivation did not depend on an individual's absolute performance at baseline (i.e. no significant correlation except for 10% slowest reaction times after sleep deprivation: $p=0.048$). Moreover, the small differences in blood alcohol levels (BAC) between subjects did not predict performance impairment ($r<0.36$, $p>0.2$).

Using quantitative [^{18}F]CPFPX positron emission tomography (PET), ethanol infusion rapidly increased $A_1\text{AR}$ availability in various brain regions while placebo infusion had no such effect (Fig. 3 and 4A). Comparison between the post-treatment interval (i.e. 110 to 140 min after tracer infusion) and the baseline interval (50 to 90 min) revealed for the ethanol group a relative increase in $A_1\text{AR}$ availability ranging from $20\% \pm 5\%$ SEM (amygdala) to $26\% \pm 5\%$ (cuneus), depending on the brain region. In comparison, under identical control conditions (placebo group) there were no significant differences in any brain region (variation of $3\% \pm 3\%$ across examined regions). Details are displayed in Table 2. The increase in $A_1\text{AR}$ availability showed large interindividual differences that were not correlated with BAC (Fig. 4B).

The biphasic alcohol effect scale (BAES), which is a measure of alcohol's stimulating and sedating effects, showed in the sedating subscale a significant correlation with the treatment-dependent relative changes in $A_1\text{AR}$ in several brain regions (SI Appendix, Table S1). Exemplarily, the striatum (left and right, $r=-0.99$, $p=0.0001$) is depicted in Figure 4C. Importantly, BAES did not show a correlation with BAC ($r=-0.05$, $p=0.91$).

Discussion:

The present study shows that cognitive performance impairments due to ethanol intake and sleep loss correlate strongly across individuals, indicating the presence of an underlying trait vulnerability. The present study also reveals that acute ethanol administration is accompanied

by a rapid increase in A₁AR availability in the human brain and that the subjective sedating effects as assessed by the BAES correlate strongly with cerebral A₁AR availability. Given the previously observed upregulation of A₁AR availability with extended time awake (28, 29), the cerebral adenosine system might therefore represent a common pathway for both ethanol and sleep deprivation effects on performance. Behavioral studies have established a link between both stressors providing evidence for additive or even synergistic detrimental effects on cognitive performance (30-33).

The large inter-individual variation in the susceptibility of cognitive performance to acute and chronic sleep deprivation that we found here substantiates previous reports (15, 34) and is consistent with a genetic contribution (e.g. refs. (35, 36)). Large inter-individual variations have also been reported for the wakefulness and performance promoting effects of caffeine (37), which is known to act via adenosine receptor antagonism (38). Individual differences in caffeine sensitivity have been linked to genetic variations in the adenosine system (39, 40). In a recent study using PET we found that the degree of an individual's performance impairment after extended time awake was related to cerebral A₁AR availability (29). Taken together, the adenosine system appears to be subject to individual variations that in turn contribute to the differences in the vulnerability to sleep loss.

The increase in A₁AR availability in humans after ethanol exposure confirms previous reports of in vitro and in vivo experiments in rodents. Autoradiography experiments in rats showed that 1.5 g/kg ethanol led to a 40% increase in A₁AR availability after only 15 min (23). A 35 to 55% increase in distribution volume was measured 20 min after application of ethanol along with an adenosine kinase inhibitor (25). Recent results from mice suggest that sleep deprivation attenuates the sensitivity to alcohol via reducing extracellular accumulation of adenosine in a long-lasting manner (41). The magnitude of the 35% increase in cerebral

A₁AR availability after alcohol infusion in the present study is comparable to a respective decrease observed after 4 cups of espresso (42) .

Caffeine has been reported to be an effective countermeasure against performance impairment in the PVT during sleep deprivation, sleep inertia (43–45) , as well as circadian misalignment (43–45) . Although numerous studies have investigated caffeine as antidote for alcohol-related performance decrements, outcomes remain equivocal (43–47) . Moreover, there are differences in their neurochemical action, as the antagonist caffeine blocks receptor binding whereas ethanol rapidly increases A₁AR availability through a mechanism that has not been clarified so far. It is possible that alcohol slows down the turnover of cell-surface receptors and increases receptor trafficking to the cell surface. Mechanisms could be an enhancement of an intracellular receptor pool that involve receptor assembly and maturation or sensitization (at least in the basal ganglia) by A₁ adenosine- D₁ dopamin receptor heteromeres interaction (48, 49) . Nonetheless, the present behavioral and PET studies indicate that alcohol and caffeine may represent at least in part antagonistic drugs as they appear to interact with a common underlying pathway.

In conclusion, these findings support the framework that there are individual trait characteristics for being either vulnerable or resilient to both alcohol and sleep deprivation. Based on previous studies on the effects of extended wakefulness (29) and the present findings on ethanol induced changes in cerebral A₁AR availability we provide evidence that both alcohol and sleep deprivation effects are mediated – at least partly – via the adenosine system of the brain. Important related research questions that need to be addressed to corroborate our conclusion are (i) whether individual changes in A₁AR availability after sleep deprivation correlate with those under ethanol influence, (ii) whether chronic sleep restriction induces alterations of A₁AR availability in the human brain, and (iii) whether chronic sleep

restriction modulates the effect of ethanol on neurobehavioral performance. Targeting the adenosine system might therefore help identify new countermeasures against compromised performance induced by both sleep deprivation and alcohol. Possible countermeasures could include individualized fatigue management systems and training. Furthermore, assessing the sensitivity to alcohol might be cautiously used as a means to identify/protect individuals who are at greatest risk to be impaired by sleep loss, e.g. in safety-critical environments (e.g. transportation, healthcare, and the military) that include extended duration shifts or night work. Especially in situations of high workload and stress, sleep loss can result from sleep disturbances (50) or from trading sleep for additional work hours; in such situations alcohol consumption is increased (51), and thus insight into putative cumulative effects of sleep loss, stress, and alcohol is highly relevant. A recent model that predicts cognitive performance after sleep loss based on putative changes of adenosine and the A₁AR (52) could be extended to the influence of ethanol on performance and be evaluated accordingly. Finally, our studies are important from a public health education point of view. Reports indicate that young drivers believe that driving drowsy is less of a serious problem than driving under the influence of alcohol (53). Our finding of a shared physiological basis raises awareness for the still underestimated danger of driving while sleep deprived.

Materials and Methods:

Alcohol, sleep deprivation, and performance (Study 1 and 2):

Participants: The studies were approved by the Ethics Committee of the North Rhine Medical Board. Written informed consent was obtained from all participants. Volunteers' physical and psychological health was confirmed with questionnaires and medical examination. They reported normal sleep-wake rhythmicity and conducted no shift-work. From a total of 63 healthy volunteers, data sets of 35 participants in study 1 (mean age $\pm$ SD 26 ± 5 years, 20

men/15 women) and 14 participants in study 2 (mean age $\pm$ SD 27 ± 4 years, 14 men) were included in the present analysis. 14 participants (12 from study 1/ 2 from study 2) were excluded (see below).

Study Design: The two studies spanned 12 days and 11 nights and were both carried out in the sleep laboratory of the Institute of Aerospace Medicine (German Aerospace Center). Eight participants at a time underwent the experiments, and they were constantly attended by at least two study staff members.

Study 1 – Total sleep deprivation: After an adaptation night and two baseline nights (sleep opportunity: 23:00-07:00 h) participants underwent three experimental conditions administered in a balanced cross-over design. The experimental conditions were 1) 38-h total sleep deprivation, 2) one night of partial sleep deprivation (i.e. sleep opportunity restricted to 00:00-04:00 h), and 3) alcohol administration at 16:00 h followed by partial sleep deprivation (sleep opportunity: 00:00-04:00 h). The experimental conditions were followed each by two nights of recovery sleep (all recovery nights 23:00-07:00 h except for the first recovery night after total sleep deprivation 21:00-07:00 h).

Study 2 – Partial sleep deprivation: Methodological details of the study were published elsewhere (13, 54). In brief, after an adaptation night and two baseline nights (sleep opportunity: 23:00-07:00 h) participants underwent one day with hypoxia exposure during daytime cognitive testing, one day with alcohol administration at 10:30 h (n=12) or 16:00 h (n=2), one recovery day/night followed by 4 nights with restricted sleep opportunities (00:00-5:00 h) and two recovery nights (sleep opportunity: 23:00-07:00 h) in a sequential design.

Alcohol administration: In study 1 and 2 a drink of vodka whose amount was calculated concerning sex, weight, height, and age was consumed within 5 min. Participants' blood alcohol concentration (BAC) was based on calibrated breath alcohol analyser (ALCOTEST of the Draeger company) results immediately before the cognitive testing session at 18:00 h or

12:00 h. A BAC of more than 0.06% immediately prior to the respective cognitive testing session was chosen as minimum for inclusion in the analyses. Data of 14 participants (i.e. 12 in study 1 and 2 in study 2) had to be excluded due to physical problems like nausea or vomiting or because they did not reach this threshold due to inability to drink the whole amount of vodka. In study 1 the dataset included 35 participants with a mean BAC of 0.074% $\pm$ 0.001% SD (min. 0.064%, max. 0.095%, 25% Quantile 0.065%, 75% Quantile 0.078%). In study 2 the 14 participants included in the present analysis had a mean BAC of 0.076% $\pm$ 0.009% SD (min. 0.060%, max. 0.094%, 25% Quantile 0.069%, 75% Quantile 0.080%).

Performance measurements: Sustained attention was measured with a 10-min PVT (16) at 3-h intervals during baseline, experimental and recovery days. Apart from the cognitive testing session, participants engaged in non-vigorous activities such as reading and watching TV.

Statistics: Impairment of cognitive performance in the experimental conditions was assessed by calculating the difference in the PVT measures (e.g. mean speed, etc.) from the respective values during the first baseline day. Spearman rank correlations were calculated to compare performance impairments due to sleep deprivation/restriction with those due to alcohol intake. Based on participants' impairment in mean speed after alcohol exposure a median split was performed. Unpaired t tests compared the mean speed of the two groups during baseline, sleep deprivation, sleep restriction, and recovery.

Alcohol PET study (study 3):

Participants: The study was approved by the Ethics Committee of the Medical Faculty of the University of Duesseldorf and the German Federal Office for Radiation Protection. The study was performed at the PET laboratory of the Institute of Neuroscience and Medicine (Forschungszentrum Jülich). Written informed consent was obtained from all participants. Physical and psychological health was confirmed by medical examination and questionnaires

before ten healthy male volunteers were assigned to the experimental ($n=7$; mean age $\pm$ SD 31 ± 9 years) or control group ($n=3$; mean age $\pm$ SD 31 ± 12 years) (non-randomized, single blinded).

PET: 3D data acquisition took place on a Siemens ECAT EXACT HR+ scanner (Siemens-CTI, Knoxville, TN, USA). Scan duration was 140 min with radiotracer injection as bolus followed by a constant infusion with a K_{bol} value of 63 min. Realignment, co-registration, segmentation, and normalization of PET data and corresponding MRI (acquired on a 3T Siemens Magnetom Trio with MPRAGE sequence) were done with PMOD (version 3.305, PMOD Group, Zuerich, Switzerland). Repeated arterialized venous blood sampling was scheduled at 2, 5, and 10 min, and every 10 min until 80 min and every 5 min subsequently. The total distribution volume V_T in the equilibrium (between 50 and 140 min) is represented by the formula $V_T = TAC / C_p$ with TAC being the tissue activity concentration and C_p the plasma activity (28).

More details concerning the PET experiment are provided in SI Appendix.

Alcohol administration/subjective effects: From minute 80 to 110 of the radiotracer infusion, 40 g of ethanol in 1 L saline or solely 1 L saline solution as placebo were infused. In order to assess the subjective effects of the infusion we used a German version of the biphasic alcohol effect scale (BAES) (55) with a slightly modified instruction that did not imply that alcohol was consumed. Subjects were asked to rate how they feel right before the ethanol infusion and at the end of the PET scan.

Statistics: The alcohol response in the PET regional A_1AR distribution volumes (V_T ; mL/mL) was quantified in reference to: (i) baseline (pre alcohol infusion) and (ii) post-infusion with a t tests for pairwise comparisons with a Bonferroni-corrected significance level. Spearman rank correlation was used to evaluate associations between adenosine receptor availability and: (i) BAC and (ii) BAES.

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References

1. Lee ML et al. (2016) High risk of near-crash driving events following night-shift work. *Proc Natl.Acad.Sci U.S.A.* 113(1):176-181.
2. Liu Y et al. (2016) Prevalence of Healthy Sleep Duration among Adults--United States, 2014. *MMWR.Morb.Mortal.Wkly.Rep.* 65(6):137-141.
3. Akerstedt T, Philip P, Capelli A, & Kecklund G (2011) Sleep loss and accidents--work hours, life style, and sleep pathology. *Prog.Brain Res.* 190:169-188.
4. Caldwell JA, Caldwell JL, & Schmidt RM (2008) Alertness management strategies for operational contexts. *Sleep Med Rev.* 12(4):257-273.
5. Barger LK et al. (2005) Extended work shifts and the risk of motor vehicle crashes among interns. *N.Engl.J Med.* 352(2):125-134.
6. Vejvoda M et al. (2014) Significance of time awake for predicting pilots' fatigue on short-haul flights: implications for flight duty time regulations. *J Sleep Res.* 23(5):564-7.
7. Schlack R, Hapke U, Maske U, Busch MA, & Cohrs S (2013) Häufigkeit und Verteilung von Schlafproblemen und Insomnie in der deutschen Erwachsenenbevölkerung. Ergebnisse der Studie zur Gesundheit Erwachsener in Deutschland (DEGS1) [Frequency and distribution of sleep problems and insomnia in the adult population in Germany]. *Bundesgesundheitsblatt* 56:740-748. German
8. Krueger PM & Friedman EM (2009) Sleep duration in the United States: a cross-sectional population-based study. *Am.J.Epidemiol.* 169(9):1052-1063.
9. Watson NF et al. (2015) Recommended Amount of Sleep for a Healthy Adult: A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society. *Sleep* 38(6):843-844.
10. Cohen DA et al. (2010) Uncovering residual effects of chronic sleep loss on human performance. *Sci Transl Med* 2(14):14ra3.
11. Kim Y et al. (2012) Decoupling of sleepiness from sleep time and intensity during chronic sleep restriction: evidence for a role of the adenosine system. *Sleep* 35(6):861-869.
12. Belenky G et al. (2003) Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: a sleep dose-response study. *J.Sleep Res.* 12(1):1-12.
13. Elmenhorst D et al. (2009) Performance impairment during four days partial sleep deprivation compared with the acute effects of alcohol and hypoxia. *Sleep Med* 10:189-197.

14. Van Dongen HP (2004) Comparison of mathematical model predictions to experimental data of fatigue and performance. *Aviat.Space Environ.Med.* 75(3 Suppl):A15-A36.
15. Van Dongen HP & Belenky G (2009) Individual differences in vulnerability to sleep loss in the work environment. *Ind.Health* 47(5):518-526.
16. Van Dongen HP, Baynard MD, Maislin G, & Dinges DF (2004) Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. *Sleep* 27(3):423-433.
17. Kuna ST et al. (2012) Heritability of performance deficit accumulation during acute sleep deprivation in twins. *Sleep* 35(9):1223-1233.
18. Tkachenko O & Dinges DF (2018) Interindividual variability in neurobehavioral response to sleep loss: A comprehensive review. *Neurosci Biobehav Rev.* 89:29-48.
19. Bartholow BD et al. (2003) Effects of alcohol consumption and alcohol susceptibility on cognition: a psychophysiological examination. *Biol Psychol.* 64:167-190.
20. Ebrahim IO, Shapiro CM, Williams AJ, & Fenwick PB (2013) Alcohol and sleep I: effects on normal sleep. *Alcohol Clin.Exp.Res.* 37(4):539-549.
21. Dijk DJ, Brunner DP, Aeschbach D, Tobler I, & Borbely AA (1992) The effects of ethanol on human sleep EEG power spectra differ from those of benzodiazepine receptor agonists. *Neuropsychopharmacology* 7(3):225-232.
22. Dawson D & Reid K (1997) Fatigue, alcohol and performance impairment. *Nature* 388(6639):235.
23. Clark M & Dar MS (1991) In vitro autoradiographic evidence for adenosine modulation of ethanol-induced motor disturbances in rats. *Alcohol Alcohol Suppl* 1:203-206.
24. Jarvis MF & Becker HC (1998) Single and repeated episodes of ethanol withdrawal increase adenosine A₁, but not A_{2A}, receptor density in mouse brain. *Brain Research* 786:80-88.
25. Paul S et al. (2011) Small-animal PET study of adenosine A₁ receptors in rat brain: blocking receptors and raising extracellular adenosine. *J Nucl.Med* 52(8):1293-1300.
26. Thakkar MM, Sharma R, & Sahota P (2015) Alcohol disrupts sleep homeostasis. *Alcohol* 49(4):299-310.
27. Sharma R, Engemann SC, Sahota P, & Thakkar MM (2010) Effects of ethanol on extracellular levels of adenosine in the basal forebrain: an in vivo microdialysis study in freely behaving rats. *Alcohol Clin.Exp.Res.* 34(5):813-818.
28. Elmenhorst D et al. (2007) Sleep deprivation increases A₁ adenosine receptor binding in the human brain: a positron emission tomography study. *J Neurosci.* 27(9):2410-2415.

29. Elmenhorst D et al. (2017) Recovery sleep after extended wakefulness restores elevated A1 adenosine receptor availability in the human brain. *Proc Natl.Acad.Sci U.S.A.* 114(16):4243-4248.
30. Krull KR, Smith LT, Kalbfleisch LD, & Parsons OR (1992) The influence of alcohol and sleep deprivation on stimulus evaluation. *Alcohol* 9:445-450.
31. Arnedt JT, Wilde GJS, Munt PW, & MacLean AW (2000) Simulated driving performance following prolonged wakefulness and alcohol consumption: separate and combined contributions to impairment. *J. Sleep. Res.* 9:233-241.
32. Banks S, Catcheside P, Lack L, Grunstein RR, & McEvoy RD (2004) Low levels of alcohol impair driving simulator performance and reduce perception of crash risk in partially sleep deprived subjects. *Sleep* 27(6):1063-1067.
33. Lee J, Manousakis J, Fielding J, & Anderson C (2015) Alcohol and sleep restriction combined reduces vigilant attention, whereas sleep restriction alone enhances distractibility. *Sleep* 38(5):765-775.
34. Van Dongen HP, Maislin G, Mullington JM, & Dinges DF (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep* 26(2):117-126.
35. Rupp TL, Wesensten NJ, Newman R, & Balkin TJ (2013) PER3 and ADORA2A polymorphisms impact neurobehavioral performance during sleep restriction. *J Sleep Res.* 22(2):160-165.
36. Reichert CF et al. (2014) Insights into behavioral vulnerability to differential sleep pressure and circadian phase from a functional ADA polymorphism. *J Biol Rhythms* 29(2):119-130.
37. Retey JV et al. (2006) Adenosinergic mechanisms contribute to individual differences in sleep deprivation-induced changes in neurobehavioral function and brain rhythmic activity. *J Neurosci.* 26(41):10472-10479.
38. Urry E & Landolt HP (2015) Adenosine, caffeine, and performance: from cognitive neuroscience of sleep to sleep pharmacogenetics. *Curr.Top.Behav.Neurosci.* 25:331-366.
39. Retey JV et al. (2007) A genetic variation in the adenosine A2A receptor gene (ADORA2A) contributes to individual sensitivity to caffeine effects on sleep. *Clin.Pharmacol.Ther.* 81(5):692-698.
40. Byrne EM et al. (2012) A genome-wide association study of caffeine-related sleep disturbance: confirmation of a role for a common variant in the adenosine receptor. *Sleep* 35(7):967-975.
41. Clasadonte J, McIver SR, Schmitt LI, Halassa MM, & Haydon PG (2014) Chronic sleep restriction disrupts sleep homeostasis and behavioral sensitivity to alcohol by reducing the extracellular accumulation of adenosine. *J Neurosci.* 34(5):1879-1891.

42. Elmenhorst D, Meyer PT, Matusch A, Winz OH, & Bauer A (2012) Caffeine occupancy of human cerebral A1 adenosine receptors: in vivo quantification with 18F-CPFPX and PET. *J Nucl.Med* 53(11):1723-1729.
43. Van Dongen HP et al. (2001) Caffeine eliminates psychomotor vigilance deficits from sleep inertia. *Sleep* 24(7):813-819.
44. Wyatt JK, Cajochen C, Ritz-De CA, Czeisler CA, & Dijk DJ (2004) Low-dose repeated caffeine administration for circadian-phase-dependent performance degradation during extended wakefulness. *Sleep* 27(3):374-381.
45. Drake CL, Roehrs T, Turner L, Scofield HM, & Roth T (2003) Caffeine reversal of ethanol effects on the multiple sleep latency test, memory, and psychomotor performance. *Neuropsychopharmacology* 28(2):371-378.
46. Martin FH & Garfield J (2006) Combined effects of alcohol and caffeine on the late components of the event-related potential and on reaction time. *Biol.Psychol* 71(1):63-73.
47. Ferre S (2016) Mechanisms of the psychostimulant effects of caffeine: implications for substance use disorders. *Psychopharmacology (Berl.)* 233(10):1963-1979.
48. Mundell S & Kelly E (2011) Adenosine receptor desensitization and trafficking. *Biochim.Biophys.Acta* 1808(5):1319-1328.
49. Ferre S & O'Brien MC (2011) Alcohol and Caffeine: The Perfect Storm. *J Caffeine.Res.* 1(3):153-162.
50. Akerstedt T, Fredlund P, Gillberg M, & Jansson B (2012) Work load and work hours in relation to disturbed sleep and fatigue in a large representative sample. *J Psychosom Res.* 53(1):585-588.
51. Buchvold HV, Pallesen S, Oyane NMF, & Bjorvatn B (2015) Associations between night work and BMI, alcohol, smoking, caffeine and exercise – a cross-sectional study. *BMC Public Health.* 15:1112.
52. Phillips AJK, Klerman EB, & Butler JP (2017) Modeling the adenosine system as a modulator of cognitive performance and sleep patterns during sleep restriction and recovery. *PLoS Comput Biol.* 13(10): e1005759.
53. Williams LR, Davies DR, Thiele K, Davidson JR, & MacLean AW (2012) Young drivers' perceptions of culpability of sleep-deprived versus drinking drivers. *J Safety Res.* 43(2):115-122.
54. Elmenhorst EM et al. (2008) Partial sleep deprivation: Impact on the architecture and quality of sleep. *Sleep Med.* 9(8):840-50.
55. Martin CS, Earleywine M, Musty RE, Perrine MW, & Swift RM (1993) Development and validation of the Biphasic Alcohol Effects Scale. *Alcohol Clin.Exp.Res.* 17(1):140-146.

Figure Legends

Figure 1: Comparison of psychomotor vigilance task (PVT) performance impairment by ethanol, total sleep deprivation (left column of diagrams) and partial sleep deprivation (right column).

A, B: Individual impairment in 10% slowest reaction times (RT; means; difference from baseline) after alcohol (diamonds) and sleep deprivation (circle), ordered according to impairment after alcohol. Intraclass correlation coefficients (ICC, two-way mixed effects model, absolute agreement) indicate moderate agreement. Note that degree of impairment did not vary due to differences in blood alcohol concentration (color-coded diamonds ranging from orange (low concentration) to black (high concentration)).

C, D: Significant spearman correlations between the impairment (difference from baseline) in mean speed under alcohol and after sleep deprivation (see also Table 1).

Figure 2: Time course of mean speed during (A) total sleep deprivation (TSD), after (B) repeated partial sleep deprivation (PSD) and after recovery (REC) sleep. Based on performance impairment under alcohol the subjects were divided into two subgroups (median split). Gray vertical bars represent sleep periods. Error bars indicate standard error of the mean (SEM). * $p < 0.05$ for differences between subgroups (unpaired t-tests).

Figure 3: Effects of ethanol infusion on cerebral A₁ adenosine receptor availability. Spatially normalized averaged images of anatomy (A: MRI) and adenosine receptor availability (B+C: PET distribution volume) of the ethanol receiving subjects (n=7) during the baseline interval (average of 50-80 min after start of radioligand infusion) and during the post-treatment interval (110-140 min) are shown. Left column: axial, middle column sagittal and right column coronal view.

Figure 4: Effects of ethanol infusion on cerebral A₁ adenosine receptor availability. A: Time course of the normalized (to the baseline interval) distribution volume in the frontal cortex of two representative subjects during the steady state phase of radioligand delivery. 30-min ethanol infusion (0.53g/kg, black diamonds) or placebo infusion (shaded circles for reference) started at 80 min after start of the PET experiment during the steady state phase and continued for 30 min (33 ml/min infusion rate). B: No correlation between changes in A₁AR binding and ethanol dose. C: Significant correlation between changes in A₁AR binding and changes in ethanol induced sedating symptoms (biphasic alcohol effect scale).

Table 1: PVT performance impairment after total and partial sleep deprivation, and alcohol administration.

PVT parameters	TSD	Ethanol		PSD	Ethanol	
	mean Δ	mean Δ	Spearman	mean Δ	mean Δ	Spearman
	(SEM)	(SEM)	r-value	(SEM)	(SEM)	r-value
			p-value			p-value
Median RT (ms)	36.92 (10.03)	31.96 (4.91)	0.567 0.0004	4.10 (4.73)	23.51 (8.10)	0.862 <0.0001
Mean Speed (1/s)	-0.64 (0.11)	-0.47 (0.07)	0.620 <0.0001	-0.07 (0.10)	-0.41 (0.13)	0.842 0.0002
10th Percentile Speed (1/s)	-1.25 (0.23)	-0.64 (0.10)	0.603 0.0001	-0.16 (0.14)	-0.53 (0.16)	0.886 <0.0001
90th Percentile Speed (1/s)	-0.29 (0.06)	-0.32 (0.06)	0.614 <0.0001	0.05 (0.08)	-0.27 (0.10)	0.833 0.0002
Lapses (#)	5.14 (1.29)	1.60 (0.48)	0.508 0.0018	1.43 (0.72)	2.43 (1.15)	0.281 0.5410
Mean RT (ms)	29.99 (4.20)	33.08 (4.76)	0.659 <0.0001	4.89 (5.19)	24.00 (8.22)	0.846 0.0001
Standard deviation of RT (ms)	19.70 (2.59)	14.99 (2.08)	0.742 <0.0001	6.14 (2.78)	10.12 (3.28)	0.574 0.0320
Mean 10% slowest RT (ms)	74.73 (9.78)	65.58 (8.50)	0.662 <0.0001	17.00 (11.29)	42.49 (13.76)	0.666 0.0093
Mean 10% fastest RT (ms)	8.47 (2.08)	12.97 (2.80)	0.462 0.0052	-1.80 (3.53)	9.72 (4.16)	0.846 0.0001

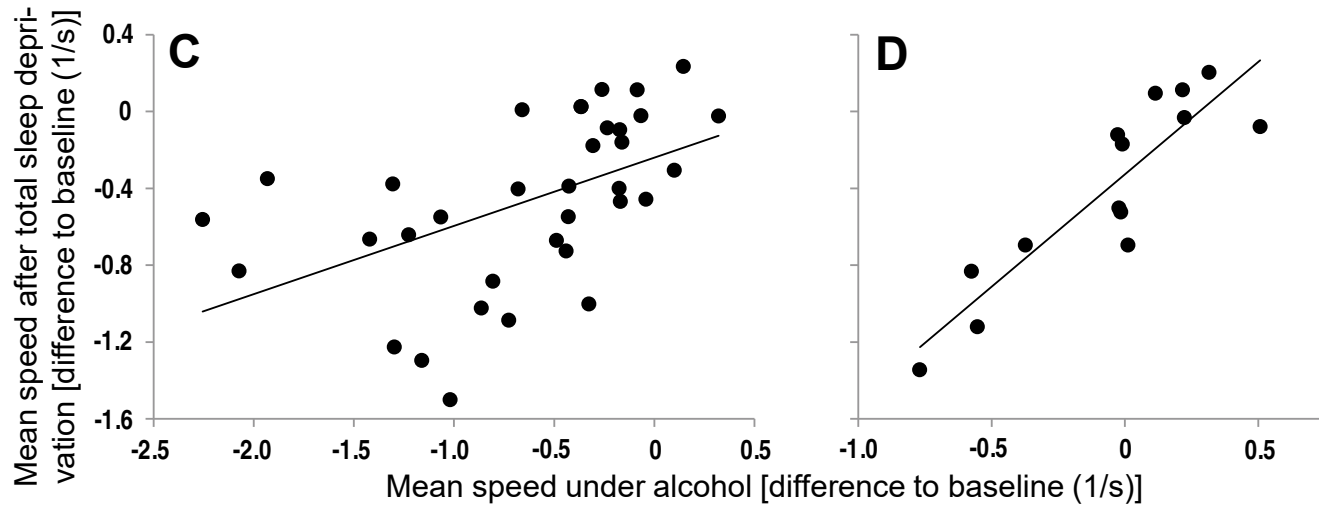
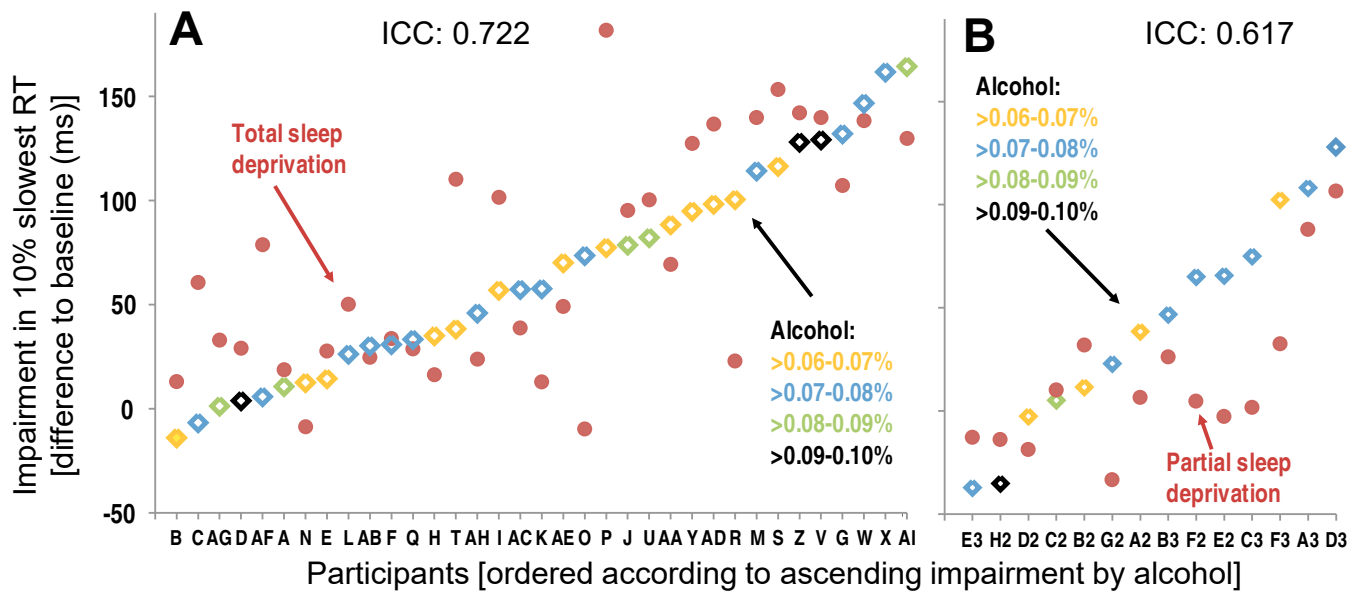
PVT, psychomotor vigilance task; RT, reaction time; TSD, total sleep deprivation (i.e. 35 h awake); PSD, partial sleep deprivation (i.e. after 4 nights with time in bed restricted to 5 h).

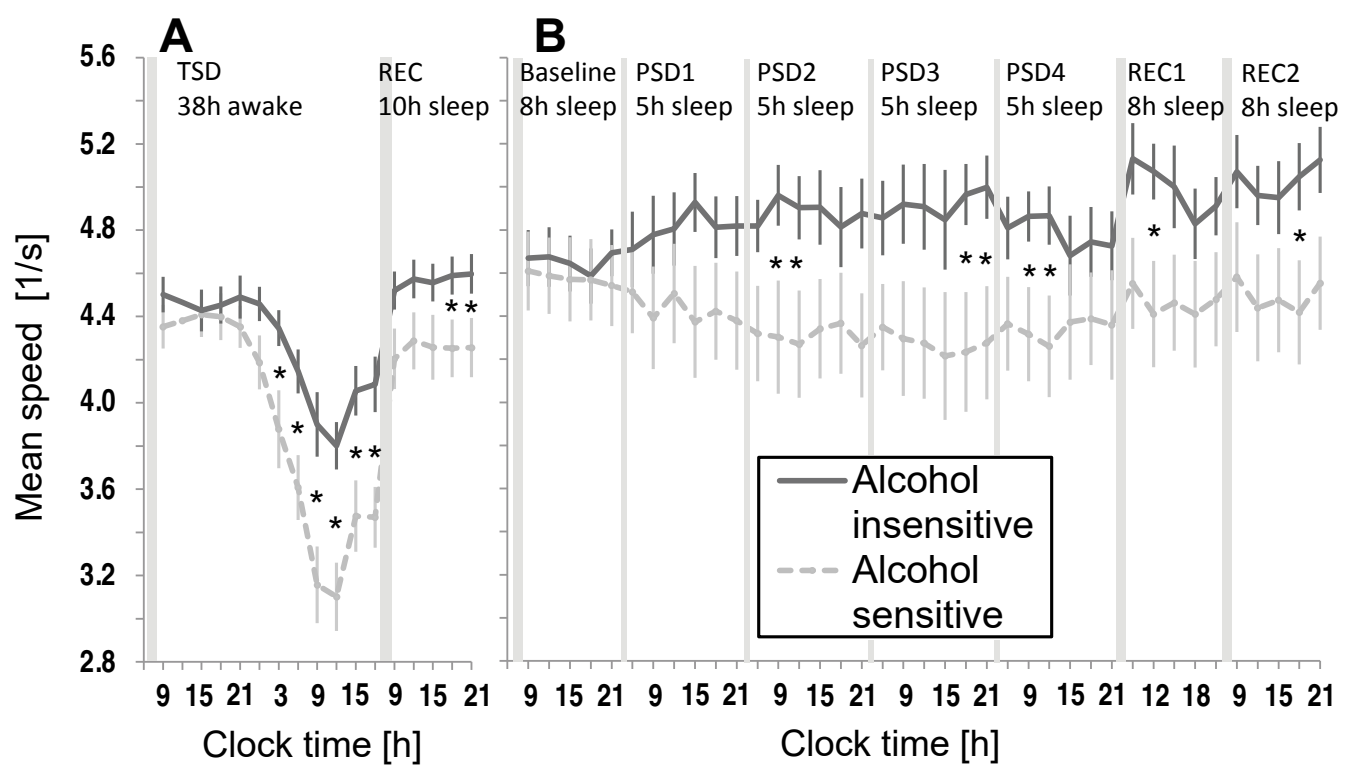
Median RT and parameters of speed include all RTs, Lapses are RTs ≥ 500 ms, other PVT parameters are based on RT excluding lapses. Mean Δ is the difference between experimental condition (TSD, PSD, Ethanol) and baseline at respective daytimes. 10th percentile refers to difference in slow response speed, 90th percentile to difference in fast response speed.

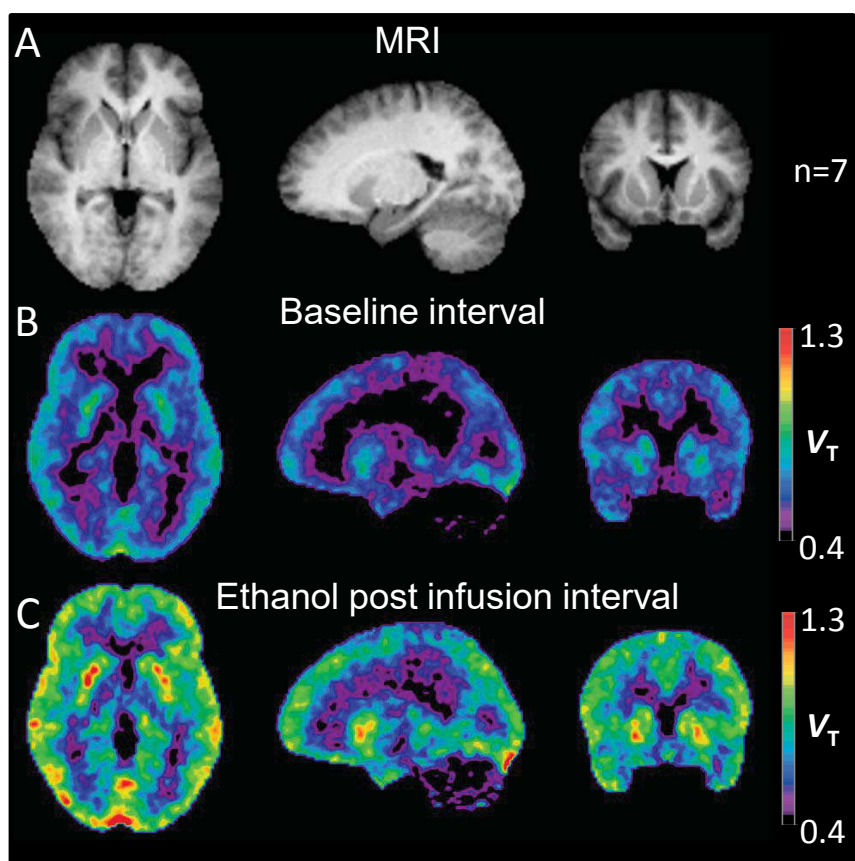
Table 2: Relative regional changes of cerebral A₁AR availability after ethanol or placebo administration.

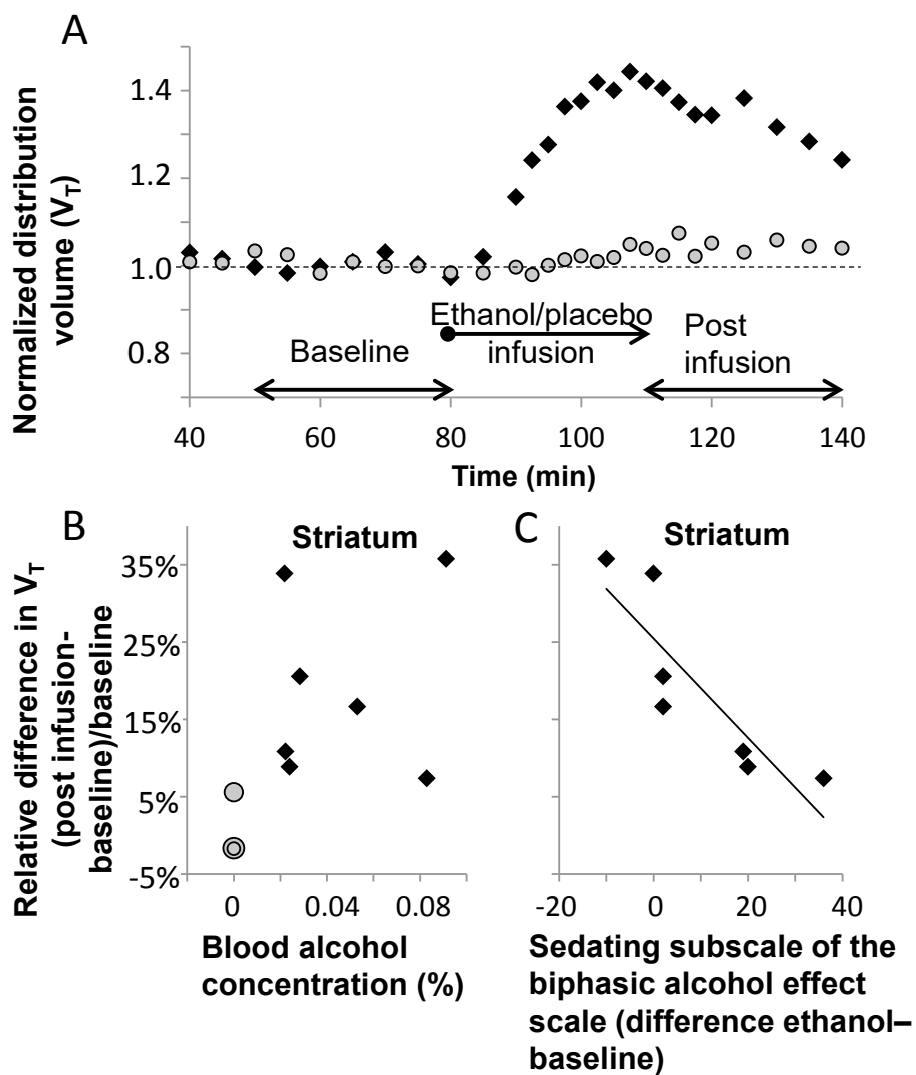
Brain regions	Relative increase in A ₁ AR availability (in %)			
	Ethanol group		Control group	
	mean relative Δ (SEM)	p-value	mean relative Δ (SEM)	p-value
Supp Motor Area	24 (5)	0.001	6 (2)	0.047
Frontal inf	23 (5)	0.001	3 (2)	0.269
Insula	23 (5)	0.002	0 (3)	0.905
Cingulum ant	25 (5)	0.001	2 (2)	0.434
Hippocampus	20 (4)	0.002	5 (4)	0.287
Amygdala	20 (5)	0.002	5 (6)	0.431
Cuneus	26 (5)	0.001	8 (3)	0.063
Occipital	23 (5)	0.002	3 (2)	0.124
Postcentral	24 (5)	0.001	3 (2)	0.288
Striatum	19 (5)	0.002	1 (3)	0.775
Pallidum	25 (6)	0.003	1 (3)	0.742
Thalamus	22 (5)	0.003	2 (2)	0.440
Temporal	23 (5)	0.002	3 (2)	0.185
Parietal	22 (5)	0.001	3 (2)	0.261

A₁AR, A₁ adenosine receptor; ant., anterior; Supp., supplementary. Significant p-values after Bonferroni correction (0.05/14=0.0035) are bold. Mean relative Δ is (post infusion-baseline)/baseline.









Supplemental information PET imaging

This supporting information gives additional methodological details about the PET scans.

Participants: Detailed interviews ensured that volunteers did not have a history of neurological and psychiatric diseases, sleep disorders, shift work, jet-lag, night work, head injury, and alcohol or substance abuse. Only non-smoking subjects without any medication were included. Caffeine intake was restricted 36 h prior to the arrival at the laboratory which was controlled by blood plasma samples at the time of the PET scans (below detection limits in all subjects - < 0.5 mg/L). The average BMI of the subjects was 25.8 ± 4.8 kg/m². Daytime of scanning was $11:15 \pm 1:23$ h.

Average amount of injected radioactivity was 264 ± 41 MBq corresponding to an average dose of 2.4 ± 1.2 nmol.

The free fraction of the parent compound in plasma (f_p) was determined 10 min before tracer injection and after 140 min (in 5 subjects additionally at 80 and 110 min). No significant differences were detected between time points, ethanol and placebo group and an independent group of previously investigated subjects. On average the free fraction difference between -10 and 140 min was 3% ($p = 0.87$) in the ethanol group.

The time course of the concentration of the parent compound of all subjects is displayed in Fig. S1.

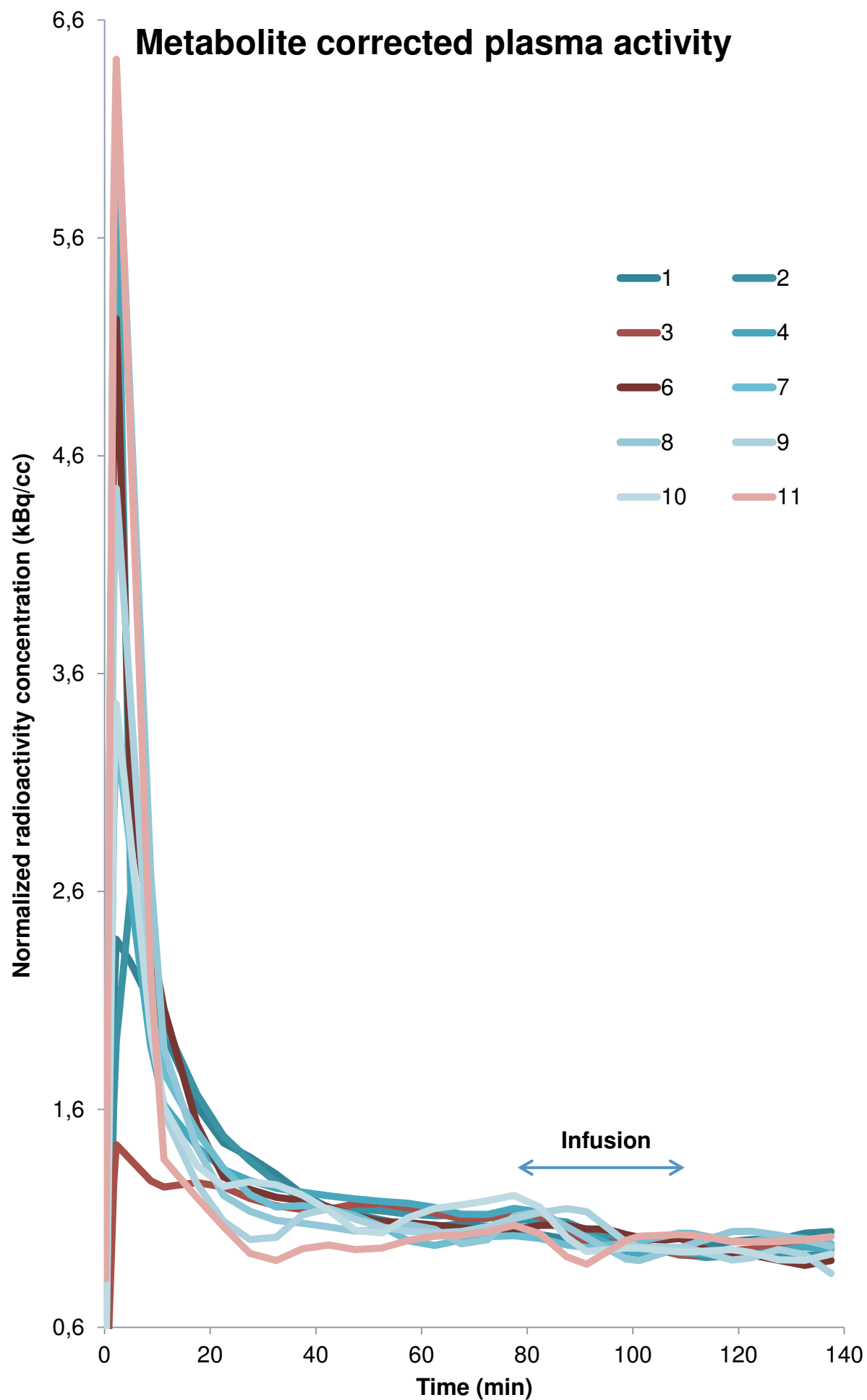


Figure S1: Overview of metabolite corrected plasma activity (normalized to the time span 60-140 min). Red colored lines represent the subjects which received a placebo infusion.

Table S1: Correlation of regional relative difference of cerebral A₁AR availability after ethanol with difference the sedating subscale of the biphasic alcohol effect scale.

Brain regions	r-value	p-value
Supp Motor Area	-0.76	0.0489
Frontal inf	-0.87	0.0120
Insula	-0.87	0.0120
Cingulum ant	-0.81	0.0269
Hippocampus	-0.99	0.0001
Amygdala	-0.85	0.0162
Cuneus	-0.90	0.0056
Occipital	-0.96	0.0008
Postcentral	-0.96	0.0008
Striatum	-0.99	0.0001
Pallidum	-0.68	0.0897
Thalamus	-0.96	0.0008
Temporal	-0.96	0.0008
Parietal	-0.96	0.0008

Significant p-values after Bonferroni correction ($0.05/14=0.0036$) are bold.