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Effect of GHB-use and GHB-induced comas on dorsolateral prefrontal cortex functioning in humans

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ABSTRACT

Background: Gamma-hydroxybutyric acid (GHB) is a recreational drug associated with increasing numbers of GHB-dependent patients and emergency attendances often related to GHB-induced comas. Working memory (WM) deficits have been reported in association with GHB use, and animal studies have shown that GHB induces oxidative stress in vulnerable WM-related brain areas such as the dorsolateral prefrontal cortex (DLPFC). However, the effects of chronic GHB use and multiple GHB-induced comas on WM-related brain function in humans remains unknown.

Methods: We recruited 27 GHB users with ≥4 GHB-induced comas (GHB-Coma), 27 GHB users who never experienced GHB-induced coma (GHB-NoComa), and 27 polydrug users who never used GHB (No-GHB). Participants performed an n-back WM task during functional magnetic resonance imaging (fMRI) to probe DLPFC functioning.

Results: The GHB-Coma group had lower premorbid IQ (p = .006) than the GHB-NoComa group despite comparable age and education level. There were also group differences in the use of other drugs than GHB. Therefore, all group comparisons were adjusted for IQ and drug use other than GHB. Compared with the GHB-NoComa and the No-GHB groups, the GHB-Coma group showed increased activity in the right DLPFC (p_{SVC} = 0.028) and increased functional connectivity of the right DLPFC with a cluster comprising the left anterior cingulate and medial frontal gyrus (p_{FWE} = 0.003). No significant fMRI differences were observed between the GHB-NoComa and No-GHB groups. Due to technical problems, no behavioural data were collected.

Discussion: These results suggest that multiple GHB-induced comas, but not GHB-use per se, are associated with alterations in WM-related brain function. Public awareness campaigns are required to minimize the potential adverse effects induced by GHB recreational use, and especially GHB-induced comas, even if no immediate side effects are experienced.

1. Introduction

Gamma hydroxybutyric acid (GHB) is a recreational drug that poses a substantial risk for public health (World Health Organization, 2015). A recent increase in the number of individuals seeking treatment for GHB dependence and emergency room attendances often related to GHB-induced comas, are just some of the indicators of the potential public health risks associated with recreational GHB use (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2016; Public Health England, 2015; United Nations Office on Drugs and Crime, 2017). However, despite a disproportional number of severe side effects, GHB remains popular amongst party goers due its effects of euphoria, loss of inhibition, and sexual arousal (Abanades et al., 2006; European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2016; Korf et al., 2014; Miró et al., 2017; Public Health England, 2015; United Nations Office on Drugs and Crime, 2017; Van Amsterdam et al., 2012).

Neverthless, recreational use of GHB poses a high risk of intoxication with severe side effects, resulting from a narrow dose-response window between the desired high and overdose (Abanades et al., 2006; Korf et al., 2014; Miró et al., 2017; Van Amsterdam et al., 2012). Amongst these severe adverse effects GHB-induced coma is one of the most common, lasting between 1 and 4 h and frequently reaching the most critical classification on the Glasgow Coma Scale (Abanades et al.,

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2006; Korf et al., 2014; Miró et al., 2017; Van Amsterdam et al., 2012). Remarkably, GHB users awake from these comas with no apparent negative outcomes, leading them to believe that GHB use is safe and GHB-induced comas are innocent events (Korf et al., 2014; Van Amsterdam et al., 2012).

However, upon its discovery GHB was widely used as a general anaesthetic, being GHB-induced comas compared to a state of pharmacological-induced unconsciousness (Miró et al., 2017; Van Amsterdam et al., 2012). Research on pharmacological induced unconsciousness (anaesthesia) in humans suggest that without oxygen support, even if transient, these states may induce neural deprivation of oxygen (hypoxia) and consequently lead to oxidative stress in vulnerable regions related to the WM-network such as the dorsolateral prefrontal cortex (DLPFC) (Perouansky and Hemmings, 2009). DLPFC is a region particularly rich in GHB-bindings sites and animal studies show this region to be highly vulnerable to GHB-induced neurotoxic effects (Castelli et al., 2000; Johansson et al., 2014; Pedraza et al., 2009). In line with these findings, impairments in cognitive processes such as WM have been associated with GHB administration in animals, but similar impairments have also been reported in humans who regularly used GHB (Abanades et al., 2006; Carter et al., 2009a, 2009b; Johansson et al., 2014; Korf et al., 2014; Pedraza et al., 2009).

Neuroimaging research on the effects of GHB in humans is still on its early days with studies only assessing the acute effects of GHB on brain functioning. These studies suggest that acute administration of GHB induces alterations in activity and connectivity of regions of the PFC and the limbic system that are associated with alterations in emotional awareness and prosexual behaviour (Bosch et al., 2017a, 2017b). However, little is known about the neural effects of regular recreational use of GHB and GHB-induced comas on the human brain. In order to investigate the effect of GHB-use and GHB-induced comas on WM related brain function, we used functional magnetic resonance imaging (fMRI) (Owen et al., 2005). The dorsolateral part of the PFC (DLPFC) is the integrative hub of the WM network (D'Esposito and Postle, 2015; Owen et al., 2005). This region is responsible for the storage of task-relevant sensory information, but also for integrating this incoming information into top-down goal-directed behaviour (D'Esposito and Postle, 2015; Niendam et al., 2012; Ranganath and D'Esposito, 2001; Rissman et al., 2008). Interestingly, altered activity and functional connectivity of the DLPFC is regularly seen in alcohol use disorder, another well-known GABA-substance use disorder (Campanella et al., 2013; Desmond et al., 2003; Han et al., 2015; Wilcox et al., 2014).

Based on the WM impairments associated with GHB administration in animals and humans, we expect that regular GHB use will particularly affect DLPFC neural processing during a WM functional magnetic ressonance imaging (fMRI) task. To disentangle the effects induced by GHB use itself from the effects induced by multiple GHB-induced comas, we recruited three groups of participants: (1) GHB users who had ≥4 GHB-induced comas, (2) GHB users who never had a GHB-induced coma, and (3) polydrug users who never used GHB. This allowed us to assess: (a) the GHB-induced coma effect by comparing GHB users who had multiple GHB-induced comas with GHB users who never had a GHB-induced coma and polydrug users who never used GHB; and (b) the effect of GHB use per se by contrasting GHB users who never had a GHB-induced coma with polydrug users who never used GHB.

2. Materials and methods

2.1. Participants

In this cross-sectional study, 81 male participants were recruited trough addiction centers in the Netherlands, flyers, internet advertisement and snowball sampling. To be included in the study, all participants had to be native Dutch speakers, between 18 and 40 years old. We only recruited males as the vast majority of GHB users are men (Miró et al., 2017). We recruited three distinct groups of participants, matched for age and educational level: 27 GHB users who had at least ≥4 GHB-induced comas (GHB-Coma); 27 GHB users without a history of GHB-induced coma (GHB-NoComa); 27 polydrug users who never used GHB (No-GHB). To be included in the GHB groups participants had to use GHB ≥ 25 times in the 2 years preceding the assessment (De Jong and Dijkstra, 2013). The threshold of 4 comas for inclusion in the GHB-Coma group was selected to maximize potential differences with the GHB-NoComa group. Polydrug use comprised the use of alcohol, nicotine, cannabis, cocaine, stimulants other than cocaine, ecstasy, ketamine, and sedatives other than GHB. MRI data from 3 GHB-Coma participants, 2 GHB-NoComa participants, and 1 No-GHB participant had to be discarded due to excessive head movement inside the scanner and/or insufficient brain coverage.

Potential participants were excluded if they had a history of epilepsy; if underwent general anaesthesia on the 2 years preceding the study; if any contra-indication was reported for fMRI scanning (e.g. metal object in the body); if any coma episode not related to GHB use was reported; or if they were currently under treatment for narcolepsy with cataplexy (since treatment involves the use of Xyrem, brand name for GHB) (Abanades et al., 2006; Carter et al., 2009a, 2009b). After an explanation of the study, written informed consent was obtained from all the participants prior to the study initiation. This study was in accordance with the Helsinki Declaration principles (7th revision, 2013), the Medical Research Involving Human Subjects (World Medical Association, 2013), and approved by the Medical Ethics Review Committee of the Academic Medical Centre (Büller et al., 2010; World Medical Association, 2013). The data presented here are part of a larger study investigating the effects of recreational GHB use in humans. The study consisted of an initial urine test, followed by completing questionnaires related to GHB and other drug use, depression, anxiety, stress and impulsivity levels. During the subsequent neuroimaging session, structural and functional scans were collected in the following order: structural; resting-state; long-term memory (paired association task); diffusion weighted imaging, WM (the present n-back task); emotion processing (face matching task). Finally outside the scanner, participants performed digitized neuropsychological testing including verbal memory, spatial memory, intra-extra dimensional set shifting and probabilistic reversal learning. The focus of the current manuscript is solely about WM, and data from other experiments will be presented elsewhere (Raposo Pereira et al., 2018).

2.2. Clinical assessment

Drug use was assessed with the substance use section of the MATE 2:1 questionnaire, and premorbid intellectual functioning as proxy for IQ was assessed with the Dutch version of the National Adult Reading test (Schippers et al., 2011; Schmand et al., 1991).

2.3. Statistical analysis

Demographic and clinical data were analyzed with SPSS24 software (IBM Software Analytics, New York, USA). Normally distributed data were assessed with Analysis of variance (ANOVA). When not normally distributed, data were transformed or assessed using non-parametric tests (see Tables 1and 2). Differences between GHB groups were considered in terms of total exposure to GHB as defined by years of use × daily dose, daily dose during last month (ml/day), years since first use, and prevalence of days using GHB in the previous month. Exposure to other drugs, defined as years of weakly use × daily dose, was assessed for alcohol, nicotine, cannabis, cocaine, stimulants other than cocaine, ecstasy, ketamine, and sedatives (Table 2). Neuroimaging analyses were adjusted for group differences in demographic variables, IQ and exposure to drugs other than GHB.

2.4. Working memory task

Participants performed a visual digit n-back memory task during fMRI scanning, known to probe DLPFC activity and connectivity (Owen et al., 2005). The block design consisted of the alternate presentation of 5 blocks of moderate WM load (2-back) with 5 blocks of low WM load (0-back) conditions, cued by instructions at the beginning of each block. Each condition consisted of the presentation of a random sequence of digits (1–9). During the 0-back condition, participants were requested to press a button every time digit "1" would appear. During the 2-back condition, participants were requested to press a button every time a digit was the same as two positions earlier in the sequence. Each block consisted of 16 stimuli, presented for 400 ms each with an inter-stimulus interval of 1400 ms. Three or four targets were presented in each block (van Wingen et al., 2012). To ensure the task was understood before the actual experiment, participants practiced it beforehand outside the scanner. Due to a technical error by which the button box responses were not properly recorded by the experimental software, we did not obtain behavioural data. Nevertheless, the experimental conditions were chosen such that no behavioural differences were expected (Harvey et al., 2005).

2.5. fMRI data acquisition

MRI data were collected with a 3.0 T Ingenia scanner (Phillips Medical System, Best, the Netherlands), with a 32 channel head coil. For spatial normalization use purposes, T1-weighted 3D MPRAGE structural images [field-of-view = 256 × 240 × 180 mm^3; 256 × 240 acquisition matrix; 180 slices; voxel size = 1 × 1 × 1 mm^3; flip angle = 9°] were acquired. T2* blood oxygenated level-dependent contrast images were collected using an echo-planar sequence, with each volume comprising 37 ascending slices [field-of-view = 240x240x121.8 mm3; 80 × 78 acquisition matrix; slice thickness = 3 mm; slice gap = 0.3; voxel size = 3x3x3.3 mm3; TR/TE = 2000/27 ms; flip angle = 76.1°].

2.6. fMRI data analysis

Statistical parametric mapping 12 (SPM12; Wellcome Trust Centre for Neuroimaging, London UK) was used to analyze the neuroimaging data. Functional images were realigned to the mean image, corrected for slice time acquisition, co-registered to structural scan, spatially normalized into Montreal Neurologic Institute (MNI) space using the default segmentation procedure implemented in SPM12, resampled into 2 × 2 × 2 mm^3 voxels, and smoothed with a 3D Gaussian kernel of 8 mm at full-width-half-maximum.

The 2-back and 0-back conditions were modelled as box-car regressors and convolved with the canonical hemodynamic response function for each subject. Realignment parameters were introduced as covariates of no interest to account for potential movement artefacts. Data were then high-pass filtered (1/128 Hz) and temporal autocorrelation was modelled using an AR(1) process. Contrast images comparing the 2-back and the 0-back conditions were tested for group differences using analysis of covariance. Since significant group differences in IQ and in total exposure to nicotine, cocaine, stimulants other than cocaine, ecstasy, and sedatives other than GHB were observed (see Tables 1 and 2), these variables were introduced as covariates of no-interest throughout the analyses (IQ as a linear variable; differences in exposure to the referenced drugs as 5 dummy variables). Two orthogonal planned comparisons were used to assess the effect of GHB-induced coma [contrast (a)] and the effect of GHB-exposure [contrast (b)].

Voxel-wise statistical tests were family-wise-error (FWE) corrected ($p_{FWE} < 0.05$) for multiple comparisons at the cluster level (using a height threshold of $p < .001$) across the whole brain and at peak level for the region of interest (ROI; DLPFC) using small volume correction (SVC). The DLPFC was defined as a sphere of 10 mm radius around the coordinates (42,32,30) reported in a meta-analysis of n-back studies (Owen et al., 2005).

Finally we calculated the mean framewise displacement per participant (mean of the sum of absolute values of realignment estimates) to test for potential group differences resulting from movement during scanning (Power et al., 2012).

In addition, we performed *post-hoc* analyses to assess the relationship between brain function, and the amount of GHB exposure or the number of GHB-induced comas experienced within the GHB-Coma group. Because these clinical variables were positively skewed, we used a median-split approach to divide the GHB-Coma group in a high and a low GHB exposure sub-group, and a high and a low number of GHB-induced comas sub-group. Parameter estimates from peak voxels of significant clusters were extracted and compared between these sub-groups using two-sample *t*-tests. Results were corrected for multiple comparisons using Bonferroni correction.

To assess condition-dependent functional connectivity of the right DLPFC, we performed a generalized psychophysiological interaction (gPPI) analysis (McLaren et al., 2012). The seed region in the right DLPFC was defined as a sphere of 10 mm radius around the peak of activity differences (coordinates: 50,34,22) of the task-based group analysis. PPI images were obtained by multiplying the time series of the right DLPFC with the task regressors, providing differences in right DLPFC functional connectivity between the 2-back and 0-back conditions. Group differences were tested as described above.

3. Results

3.1. Clinical characteristics

Table A.1 and A.2 present the demographic and clinical characteristics of the three groups. There were no significant differences in age (mean ± SD = 26.51 ± 6.8) and education level (mean ± SD = 5.15 ± 1.5) between the groups. However, the GHB-Coma group had a lower premorbid IQ than the GHB-NoComa group ($p = .004$). When considering GHB use, no group differences were observed in the duration of GHB use but, as expected the GHB-Coma group used more GHB than the GHB-NoComa group: higher daily doses of GHB ($U = 103, p < .001$), more daily GHB use during more months ($U = 172, p = .004$), and a tendency to use GHB more often during the last 30 days before the assessment ($U = 209, p = .066$). When assessing the use of other recreational drugs, significant differences between groups were observed in the use of nicotine, cocaine, other stimulants, ecstasy and sedatives other than GHB (all $p < .05$). GHB-Coma participants used more nicotine, cocaine, ecstasy, and sedatives than the other two groups, and the No-GHB group used more cocaine and stimulants other than cocaine than the GHB-NoComa group (Table A.2). No significant differences were observed between groups in the use of alcohol, cannabis, and ketamine.

3.2. Neuroimaging

No significant differences were observed in head motion during scanning (Table 1). The analysis across groups comparing the 2-back and the 0-back conditions (main effect of task) showed activation of different regions of the WM network, including the PFC, anterior cingulate cortex (ACC), and inferior temporal cortex ($p_{FWE} < 0.05$; Fig. A.1).

To evaluate the effect of GHB-induced comas on WM activity we further contrasted the GHB-Coma group with the GHB-NoComa and the No-GHB group. This analysis showed significantly higher recruitment of the right DLPFC ((50,32,24); $z = 3.14$; $p_{SVC} = 0.029$) in the GHB-Coma group compared with the other two groups (Fig. A.2). To evaluate the effect of GHB use per se, we contrasted the GHB-NoComa group with the No-GHB group. This analysis showed no significant group

differences.

We used a gPPI analysis to further investigated whether GHB-induced comas and GHB-use per se were associated with altered functional connectivity of the right DLPFC. With regard to the effect of multiple GHB-induced comas, a whole brain analysis showed an increase in right DLPFC functional connectivity with a large cluster ($p_{FWE} = 0.003$) that included both gray and white matter in the medial frontal gyrus (MFG; (−18,30,18); z = 5.20) and the left ACC (−(20,38,18); z = 4.86) in the GHB-Coma group compared with the other two groups (Fig. A.3). No group differences were observed in functional connectivity of the right DLPFC when considering the effect of GHB use per se.

As the results particularly showed differences in the GHB-Coma group, we performed a *post-hoc* analysis within this group to explore how the outcomes related to the amount of GHB exposure and the number of GHB-induced comas. No significant differences were observed between subgroups with high and low GHB exposure or high and low number of GHB-induced comas in DLPFC activity or connectivity after controlling for multiple comparisons.

4. Discussion

Our results suggest that regular GHB use is associated with alterations in the neural processing of WM, an effect that appears to be driven by multiple GHB-induced comas. We observed an association between multiple GHB-induced comas and hyperactivation of the right DLPFC, but also increased functional connectivity between this region and the left ACC and the MFG, whereas GHB-use per se was not related with alterations in neural processing. Hence it appears that not GHB use per se, but multiple GHB-induced comas are associated with possible deleterious effects on WM processing in humans.

The DLPFC is a region responsible for top-down goal-directed behaviour and considered the main integrative hub of the so-called WM network (Carter and van Veen, 2007; D'Esposito and Postle, 2015; Niendam et al., 2012). During WM processing DLPFC stores and updates task-relevant sensory information, and guides neural activity to produce task appropriate responses (Carter and van Veen, 2007; D'Esposito and Postle, 2015; Niendam et al., 2012; Wesley et al., 2017). So far neuroimaging research assessing the effects of GHB on humans has been restricted to the acute effects of this substance, showing alterations of neural activity of brain regions related with emotion awareness and sexual arousal, such as the ACC and the anterior insula (Bosch et al., 2017a, 2017b). Although too date, apart from the current study, no neuroimaging studies have assessed WM processing on regular recreational users of GHB. However, several studies have assessed WM processing in patients with another well-known GABA-substance use disorder, i.e. alcohol use disorder (Campanella et al., 2013; Desmond et al., 2003; Han et al., 2015; Wesley et al., 2017; Wilcox et al., 2014). Similar to these patients, GHB users with multiple GHB-induced comas show increased DLPFC activity while performing higher WM loads (Campanella et al., 2013; Desmond et al., 2003; Wesley et al., 2017). Since interference between active WM processes is higher at a 2-back than at a 0-back memory load, it is likely that the higher DLPFC activity seen in both alcohol use disorder patients and in our GHB-Coma group, represents a compensatory attempt to sustain adequate levels of WM performance (Campanella et al., 2013; Desmond et al., 2003; Wesley et al., 2017). Although, once task demand exceeds the capacity of the compromised WM system, performance of WM is expected to decline (Desmond et al., 2003).

GHB-Coma participants also showed increased right DLPFC functional connectivity with the left ACC and the MFG when compared with the other two groups. During WM processing, ACC and MFG are primarily responsible for conflict monitoring and provide error feedback (Carter and van Veen, 2007; Niendam et al., 2012; Wesley et al., 2017; Wilcox et al., 2014). When a response conflict is detected by these regions, the DLPFC is engaged to execute a relevant response correction and provide the appropriate error conflict resolution (Carter and van Veen, 2007; Niendam et al., 2012). Results from resting state research assessing the acute effects of GHB on the human brain are consistent with our findings, showing a correlation between increased levels of sedation and increased spontaneous functional connectivity between regions similar to the dorsal medial PFC and the DLPFC (Bosch et al., 2018). Furthermore in alcohol addiction increased DLPFC functional connectivity with ACC and MFG has been suggested to result from the same compensatory mechanism described in the previous paragraph (Han et al., 2015; Wesley et al., 2017; Wilcox et al., 2014). When considering GHB-abuse, it is likely that GHB-users that undergo multiple GHB-induced comas become more sensitive to external interference than the other two groups. Hence, similarly to alcohol addicts, we suggest that the GHB-Coma group increases connectivity between the DLPFC and the ACC and MFG in order to strengthen goal-directed behaviour, required to compensate the greater degree of conflict experienced while maintaining high WM loads (Han et al., 2015; Wesley et al., 2017; Wilcox et al., 2014).

Moreover, the findings discussed above were observed exclusively in the GHB-Coma group. A possible explanation might result from the fact that GHB-induced coma is often a profound state of unconsciousness similar to a state of general anaesthesia (Abanades et al., 2006; Korf et al., 2014; Perouansky and Hemmings, 2009). However, the former is not accompanied by oxygen support and can lead to hypoxia and consequent oxidative stress (Perouansky and Hemmings, 2009). Studies assessing both the effects of transient coma episodes on the human brain and the GHB-induced effects on animals, show the occurrence of oxidative stress predominantly in the DLPFC and the MTL with consequent disruption of WM processing (Johansson et al., 2014; Kueh et al., 2008; Pedraza et al., 2009; Perouansky and Hemmings, 2009). Based on this evidence, we suggest that GHB-Coma participants exposed to higher doses of GHB and to a larger number of GHB-induced comas are exposed to greater putative neurotoxic effects than the other two groups and consequently show more pronounced impairments in the WM network. However, the scope of our study prevents us to confirm this hypothesis and further studies are required to support this theory in humans.

To the best of our knowledge, this is the first neuroimaging study investigating the effects of GHB-induced coma and GHB use per se on WM processing. The study has both strengths and limitations. The inclusion of two control groups that were matched for age and education level, and the additional statistical control for remaining differences in IQ and the exposure to different recreational drugs, allowed us to isolate the effects of GHB use per se from the effects of GHB-induced comas. Another important strength is the use of an n-back task with moderate working memory load, is being executable by individuals with different cognitive capacities. A wider range of memory loads might however be considered in the future to allow the assessment of larger differences in memory capacity between groups. All groups were matched for age and education level, yet GHB-Coma group scored significantly lower on premorbid intellectual functioning. This might suggest a more generalized impairment of neurocognitive functions and therefore, IQ was introduced as a covariate in all the analyses. Moreover, the inclusion of males only does not allow us to extrapolate these results to females. Also, the absence of a non-drug use control group prevented us to explore the effects associated with regular use of drugs other than GHB. Though, GHB users often use GHB in a polydrug fashion and we considered a polydrug user control group to more realistically mirror the patterns of use of this population. Nevertheless, despite statistical control for variation in exposure to different recreational drugs, residual confounding cannot be fully excluded. Another limitation of this study is the lack of behavioural data that was not collected due to a technical error. However, behavioural differences at this moderate WM load are not anticipated (Harvey et al., 2005). Finally, the cross-sectional design of this study prevented us to establish any causal conclusions.

5. Conclusion

To conclude, our results suggest that not the effect of GHB use per se but only the effect of multiple GHB-induced comas is associated with alterations in the WM network on heavy regular recreational users of GHB. In light of both the increasing numbers of emergency attendances related to GHB overdose and increasing numbers of individuals seeking treatment for GHB dependence, as well as the predominant erroneous belief amongst recreational users that GHB use is safe, understanding the potential adverse effects of recreational use of GHB is of paramount importance (Abanades et al., 2006; European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2016; Korf et al., 2014; Public Health England, 2015; United Nations Office on Drugs and Crime, 2017). Our results evoke the need of more coherent awareness campaigns directed to regular GHB users, highlighting the potentially severe adverse effects of multiple GHB-induced comas on the brain and cognition, even if no immediate side effects are experienced.

Declaration of interest

None.

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The authors have no conflicts of interest to declare.

Appendix A

Table A.1
Demographic and clinical data.

	GHB-Coma (N = 27)		GHB-NoComa (N = 27)		No-GHB (N = 27)		Difference
	Mean	± SD	Mean	± SD	Mean	± SD	P
Age	25.67	5.54	26.40	4.64	27.38	9.32	0.675[a]
Educational level	6.50	1.62	6.84	1.21	6.65	1.38	0.756[a]
Premorbid verbal IQ	89.63	10.59	98.04	7.51	93.73	8.19	0.006*,a,1
Years since first use	5.92	0.78	4.44	0.42	–	–	0.290[b]
Daily dose (ml/day)	47.57	38.03	18.65	11.22	–	–	< 0.001*,b
Days of GHB use in preceding 30 days	11.50	2.62	2.84	0.45	–	–	0.066[b]
Months of daily use	24.65	43.79	0.14	0.39	–	–	0.004*,b
Framewise displacement	0.23	0.14	0.17	0.08	0.22	0.22	0.331[a]

Abbreviations: SD = Standard Deviation.
[a] Analysis of variance (ANOVA).
[b] Mann-Whitney U.
[1] Post-Hoc Tukey HSD: GHB-Coma < GHB-NoComa p = .004.
* p < .05.

Table A.2
Exposure to different recreational drugs (MATE2.1).

	Exposure to recreational drugs						
	GHB-Coma		GHB-NoComa		No-GHB		Difference
	Mean	± SD	Mean	± SD	Mean	± SD	P[a]
Alcohol	10.17	30.21	11.94	23.25	12.00	34.25	0.385
Nicotine	115.31	138.18	40.31	61.70	39.60	83.61	0.026*,1,2
Cannabis	4.81	8.71	3.36	5.45	4.00	6.40	0.829
Cocaine	1.96	5.12	0.20	0.50	0.03	0.12	0.012*,2,3
Stimulants	3.36	7.60	0.57	2.15	0.16	0.38	0.013*,2,3
Ecstasy	1.95	4.84	0.09	0.32	0.42	1.36	0.028*,1
Ketamine	0.16	0.46	0.24	0.91	0.06	0.20	0.745
Sedatives	1.52	7.49	0.16	0.80	0.00	0.00	≤0.001*,1,3

Abbreviations: SD = Standard Deviation.
[a] Kruskal-Wallis.
[1] Post-Hoc analysis Mann-Whitney U: GHB-Coma > GHB-NoComa; nicotine, p = .030; ecstasy, p = .0009; sedatives, p = .005.
[2] Post-Hoc analysis Mann-Whitney U: GHB-Coma > No-GHB; nicotine, p = .015; cocaine, p = .003; stimulants, p = .009; sedatives, p < .001.
[3] Post-Hoc analysis Mann-Whitney U: GHB-NoComa > No-GHB; cocaine, p = .037; stimulants, p = .016.
* p < .05.

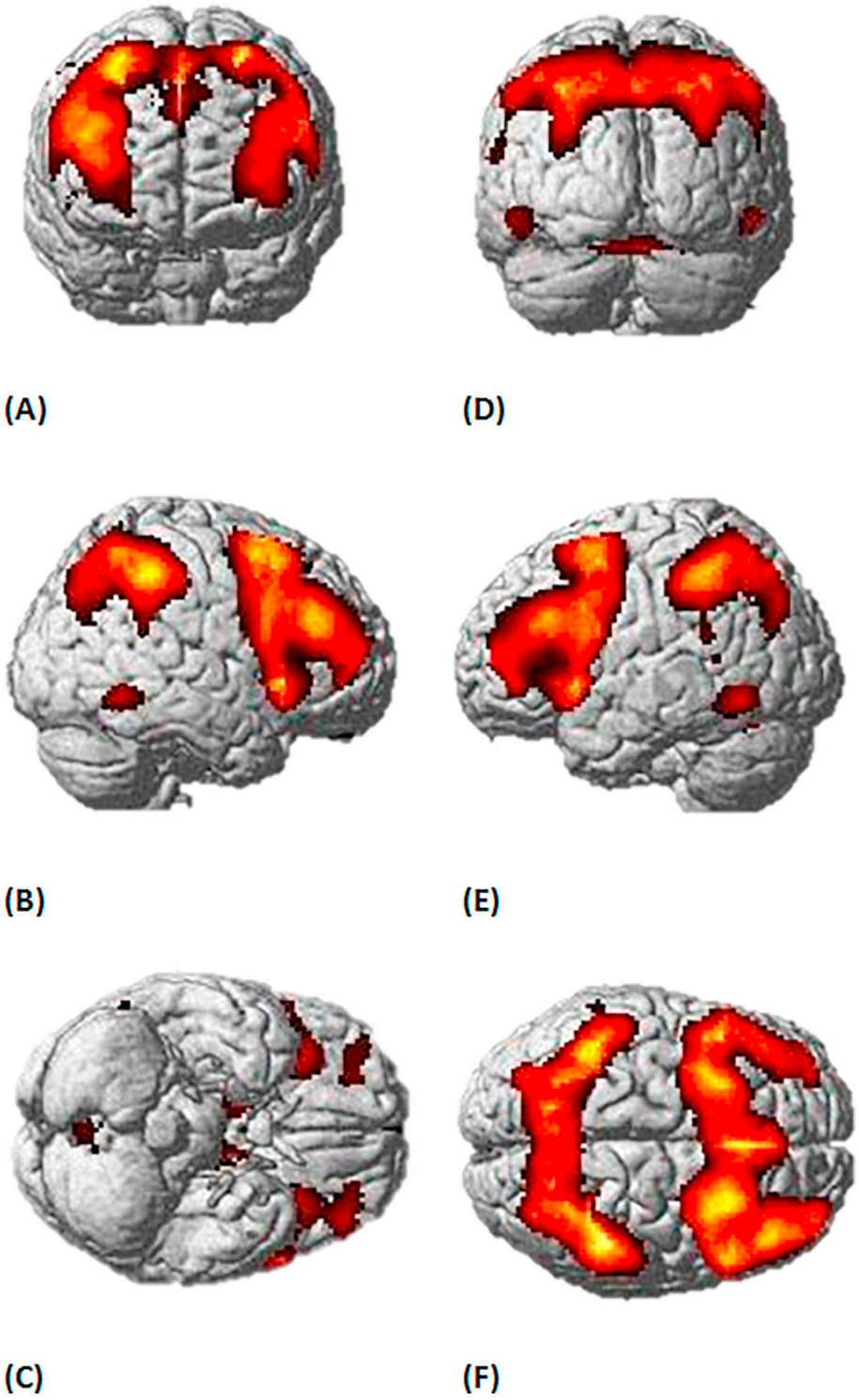

Fig. A.1. Main effect of task across the GHB-Coma, GHB-NoComa, and No-GHB groups. 3D renderings of the n-back task activation pattern on the right (A)(B)(C) and left (D)(E)(F) hemispheres of the brain ($p_{FWE} < 0.05$).

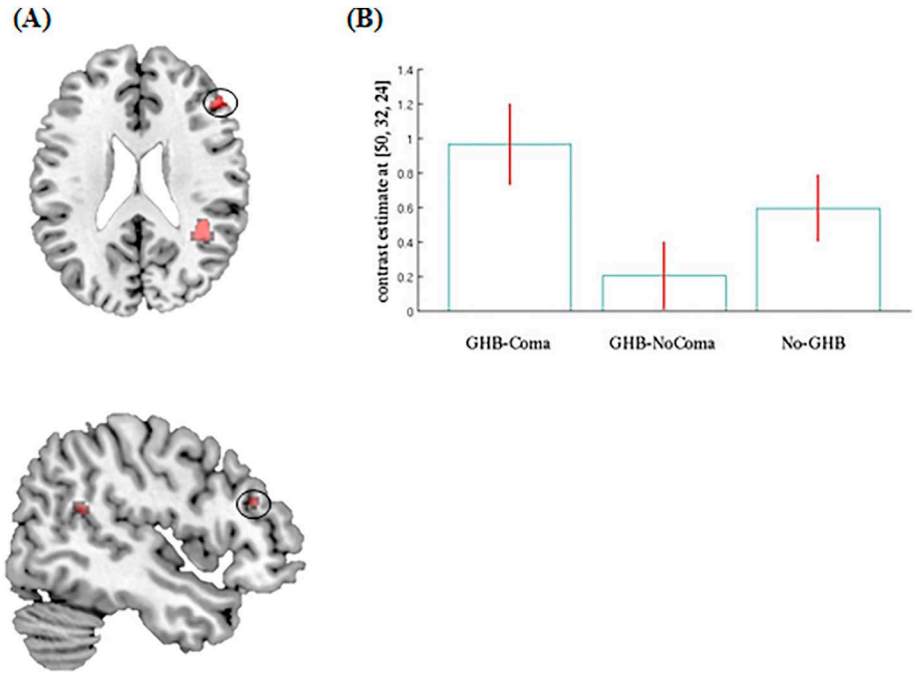

Fig. A.2. Neural response during working memory (2-back > 0-back). Higher neural response of the right dorsolateral prefrontal cortex (DLPFC: 50, 32, 24) in the GHB-Coma group compared to the GHB-NoComa group and the No-GHB group (A), results were controlled for IQ and exposure to nicotine, cocaine, other stimulants, ecstasy, and sedatives. Displayed at $p < .001$ uncorrected for visualization. The bar graph represents the contrast estimate with 90% confidence interval of the GHB-Coma, GHB-NoComa group and No-GHB group on the right DLPFC (B).

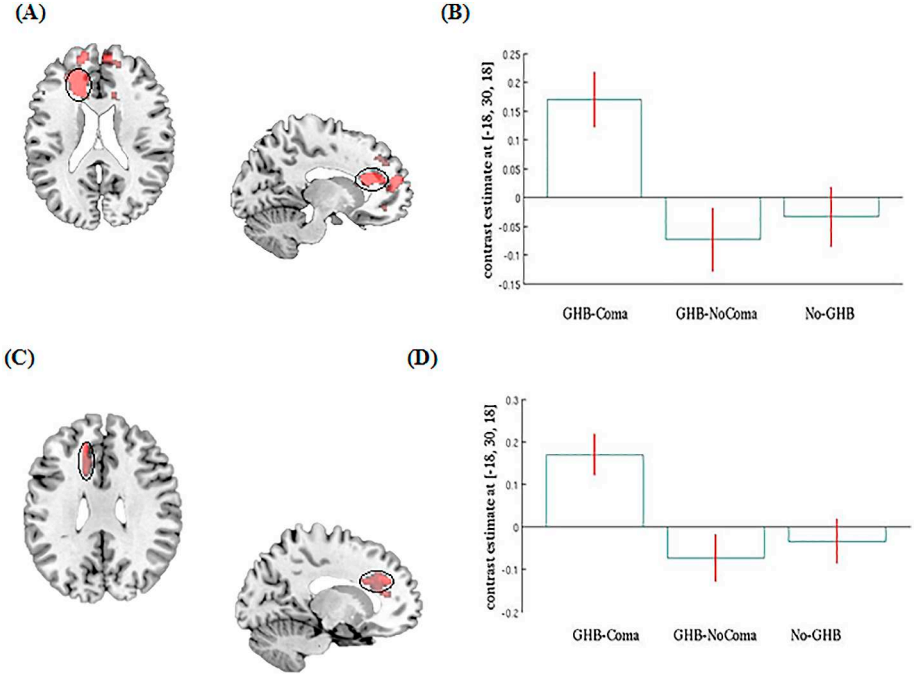

Fig. A.3. Functional connectivity of the right DLPFC. Increased functional connectivity between the right dorsolateral prefrontal cortex and (A) a cluster in the left anterior cingulate and (C) in the middle frontal gyrus of the GHB-Coma group when compared with the GHB-NoComa group and the No-GHB group. Results were controlled for IQ and exposure to nicotine, cocaine, other stimulants, ecstasy, and sedatives. Displayed at $p < .001$ uncorrected for visualization purposes. The bar graphs represent the contrast estimate with 90% confidence interval for the GHB-Coma, GHB-NoComa and No-GHB groups on the left anterior cingulate (B) and medial frontal gyrus (D).

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