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Gamma-hydroxybutyrate enhances mood and prosocial behavior without affecting plasma oxytocin and testosterone

Running title: Prosocial effects of GHB

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ABSTRACT

Gamma-hydroxybutyrate (GHB) is a GHB-/$GABA_B$-receptor agonist. Reports from GHB abusers indicate euphoric, prosocial, and empathogenic effects of the drug. We measured the effects of GHB on mood, prosocial behavior, social and non-social cognition and assessed potential underlying neuroendocrine mechanisms. GHB (20 mg/kg) was tested in 16 healthy males, using a randomized, placebo-controlled, cross-over design. Subjective effects on mood were assessed by visual-analogue-scales and the GHB-Specific-Questionnaire. Prosocial behavior was examined by the Charity Donation Task, the Social Value Orientation test, and the Reciprocity Task. Reaction time, memory, empathy, and theory-of-mind were also tested. Blood plasma levels of GHB, oxytocin, testosterone, progesterone, dehydroepiandrosterone (DHEA), cortisol, aldosterone, and adrenocorticotropic-hormone (ACTH) were determined. GHB showed stimulating and sedating effects, and elicited euphoria, disinhibition, and enhanced vitality. In participants with low prosociality, the drug increased donations and prosocial money distributions. In contrast, social cognitive abilities such as emotion recognition, empathy, and theory-of-mind, and basal cognitive functions were not affected. GHB increased plasma progesterone, while oxytocin and testosterone, cortisol, aldosterone, DHEA, and ACTH levels remained unaffected. GHB has mood-enhancing and prosocial effects without affecting social hormones such as oxytocin and testosterone. These data suggest a potential involvement of GHB-/$GABA_B$-receptors and progesterone in mood and prosocial behavior.

Key Words: sodium oxybate, gamma-hydroxybutyric acid, club drug, altruism, fairness, rape drug

1. INTRODUCTION

Gamma-hydroxybutyrate (GHB) is an endogenous short-chain fatty acid neuromodulator which is biosynthetically derived from the major inhibitory neurotransmitter gamma-aminobutyrate (GABA) (Bessman and Fishbein, 1963). It appears to bind to specific GHB- and $GABA_B$-receptors (Snead, 2000). A potent interaction of GHB with extrasynaptic α4βδ $GABA_A$ receptors suggested previously has recently been challenged (Connelly *et al.*, 2013). While physiological concentrations of GHB seem to be insufficient to stimulate $GABA_B$ receptors, this mechanism is discussed to be responsible for its psychotropic effects when administered orally (Andresen *et al.*, 2011). Although the physiological role of endogenous GHB is still unclear, some evidence points to an anti-apoptotic activity (Wendt *et al.*, 2014). Apart from its direct effects on GHB- and $GABA_B$-receptors, GHB has neuromodulatory properties on glutamate, dopamine, serotonin, norepinephrine, and cholinergic transmission (Andresen *et al.*, 2011). Clinically, GHB is internationally registered for the treatment of narcolepsy, and in some European countries for the treatment of alcohol withdrawal and craving (Keating, 2014). Moreover, it was recently proposed as an experimental therapeutic in depression (Bosch *et al.*, 2012).

GHB abusers report enhancing effects on sociability and mood (Sumnall *et al.*, 2008), whereby the drug has gained some notoriety as a “club drug” used by a small but growing part of the population (Carter *et al.*, 2009). In some aspects, the acute effects of GHB resemble the entactogenic effects (i.e. feelings of closeness, desire for physical contact) of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), which has stimulated its most widespread street name “liquid ecstasy” (Uys and Niesink, 2005). MDMA is known to enhance emotional empathy and prosocial behavior (Bedi *et al.*, 2010; Hysek *et al.*, 2014), which was paralleled by increased oxytocin plasma levels (Schmid *et al.*, 2014). Additionally to the MDMA-like entactogenic effects, GHB was reported to enhance sexually connoted affiliative behavior (Lee and Levounis, 2008), indicating an involvement of more neuroendocrine mechanisms than the oxytoninergic pathway. GHB is known to affect levels of several steroidal

hormones such as neurosteroids and cortisol in animals and humans (Bosch *et al.*, 2012), and GABA-B receptors are discussed in the regulation of testosterone secretion (Amikishieva, 2007). Testosterone is a sex steroid hormone which is known to play an important role in human social interaction (Eisenegger *et al.*, 2010; Bos *et al.*, 2012). Taking these evidences together, the androgen system with the primary hormone testosterone and its precursor dehydroepiandrosterone (DHEA) seems another plausible candidate neuroendocrine mechanism of the prosocial effects of GHB.

Anxiolytic and stress reducing effects are attributed to neurosteroids such as progesterone, tetrahydroprogesterone (3α,5α-THP), and tetrahydrodeoxycorticosterone (THDOC), whose syntheses are promoted by GHB in animals (Barbaccia *et al.*, 2002). Moreover, animal and human data show that progesterone release mirrors an individual`s level of social-affiliative motivation (Maner *et al.*, 2010). Also, hypothalamic-pituitary-adrenal (HPA-) axis activity was bidirectionally altered by the drug (Van Cauter *et al.*, 1997; Nava *et al.*, 2007), and it was shown that stress influences social interactions (Tomova *et al.*, 2014). Consequently GHB might elicit its social effects either directly via GHB/GABA receptors or indirectly by increasing plasma levels of hormones such as oxytocin and testosterone, by altering neurosteroidogenesis or through the modulation of the HPA-axis.

In order to characterize the acute effects of GHB on prosocial behavior, social cognition, and mood, we assessed a social decision-making and social cognition test battery, as well as subjective mood ratings in a randomized, placebo-controlled, balanced, cross-over design in 16 healthy males. We decided to focus on male individuals to reduce variance due to steroid hormone fluctuations during menstrual cycle. Potential neuroendocrine parameters mediating GHB effects were investigated by determination of plasma time-courses of oxytocin, testosterone, DHEA, progesterone, and stress hormones such as cortisol, aldosterone, and adrenocorticotropic hormone (ACTH). We hypothesized that GHB enhances mood, emotional empathy, and prosocial behavior, while increasing plasma levels of oxytocin, testosterone, and progesterone and altering HPA axis activity.

2. METHODS AND MATERIALS

2.1 Participants

Sixteen healthy, male, and non-smoking participants with mean age of 23.9 years (±2.9 SD, range 19-29), a mean verbal intelligence quotient (IQ) of 104.2 (±14.6 SD, range 86-145), and a mean weight of 74.4 kg (±8.2 SD, range 60.4-87.0) participated in the study. Exclusion criteria were any Axis-I DSM-IV psychiatric disorder, any form of addiction or regular illegal drug use (lifetime use $\geq$5 occasions) with exception of occasional cannabis use, a lifetime history of GHB use, a neurological disorder or head injury, clinically relevant medical diseases, a family history of schizophrenia or bipolar disorder, and any use of prescription drugs. All participants had to abstain from caffeine on the study days and from alcohol for at least 24 hours before the experiments. In order to ensure drug abstinence on the test days, a urine screening was done using a Dimension RXL Max (Siemens, Erlangen, Germany) immunoassay. The study was approved by the Cantonal Ethics Committee of Zurich and by Swissmedic and registered at ClinicalTrials.gov (NCT02342366). All participants gave written informed consent according to the Declaration of Helsinki and were compensated for their participation.

2.2 Procedure

The study design consisted of four sessions: screening session, experimental day I, experimental day II, and follow-up session, all separated by an interval of seven days. We used a randomized, double-blind, placebo-controlled, and balanced cross-over design. A trained psychiatrist carried out a Structured Clinical Interview for DSM-IV Axis-I Disorders during the screening session. We assessed drug use with the Interview for Psychotropic Drug Consumption (Quednow *et al.*, 2004). Subjects also performed the Mehrfachwahl-Wortschatz-Intelligenztest (Lehrl, 2005), a standardized German vocabulary test, in order to estimate potential premorbid verbal IQ. Finally, in the screening session subjects performed a brief neuropsychological test battery to assure normal cognitive functions (data not shown). On the experimental days a peripheral venous catheter for blood sampling was placed at

8:30am, and GHB (Xyrem® solution; 20 mg/kg in juice) or placebo (salted juice) was given orally at 9:00am. Each experimental session lasted for 225min (**Supplementary Figure 1**). Subjects had to be fasting during the morning of the experiments. At the follow-up session, the neuropsychological test battery of the screening session was repeated (data will be published elsewhere).

2.3 Measures

2.3.1 Subjective Effects

For the measurement of acute subjective drug effects we used four Visual Analogue Scales (VAS) assessing the *general drug effect*, *sedation*, *stimulation*, and *dizziness* at the time points t-15,+40,+60,+100,+120, and+180min, as well as a GHB Specific Questionnaire (GSQ)(Kim *et al.*, 2008) at t-17,+38,+66,+104,+138,+198min. The GSQ consists of 15 sensory-motor and cognitive items measuring involuntary muscle jerking, silliness, happiness, loss of memory or amnesia, acoustic hallucinations, increased sexuality, visual hallucinations, tendency to talk, disinhibition, heightened sense of touch, increased sensitivity to sound, stimulation, euphoria, and vitality. Subjects rate the occurrence/intensity of each item via five scales, ranging from 0-4 (“not present” to “strong”).

2.3.2 Charity Donation Task

Subjects performed a computer-based Charity Donation Task (CDT, adapted from Hare *et al.* [2010)]) at t+70min. Subjects were asked to read a description of 10 charities (**Supplementary Table 1**), and were then informed that they could donate 0-40 Swiss Francs (CHF) from their study compensation to one of the listed charities. Finally, subjects were asked to rate on a 7-point rating scale (“not at all” to “very much”), how much the charity deserved the donation, and how much pleasure the subject felt about having donated.

2.3.3 Social Value Orientation

The Social Value Orientation (SVO) test was implemented at t+90min. It is a paper-based, resource allocation test to assess social behavior (Murphy *et al.*, 2011). The subjects were instructed to choose their favorite joint distribution between themselves and another person, from six primary and nine secondary SVO slider items with a resource allocation choice over a defined continuum of joint payoffs. The subjects were told that two of their choices would be randomly selected, and that funds would be shared according to these choices. Prosocial behavior – defined as maximizing the total of resources for the self and others and minimizing the difference between the two – is indicated by the wideness of the SVO angle, which increases when subjects maximized the allocation for the others. For the calculation of the SVO angle please see the Supplementary Material.

2.3.4 Reciprocity Task

To investigate effects of GHB on subjects´ positive reciprocity, i.e. their tendency to respond to a positive action with another positive action, we administered a Reciprocity Task at t+160min. This computer-based, simulated task is similar to the Trust Game (Evans and Krueger, 2011). After the decision-making phase, subjects had to rate the amount of pleasure they felt related to their decisions. For details please see Supplementary Material.

2.3.5 Multifaceted Empathy Task

Participants performed the Multifaceted Empathy Task (MET) at t+75 min. This computer test comprises 40 photographs of people in emotionally charged situations (Dziobek *et al.*, 2008), and has been described in detail elsewhere (Preller *et al.*, 2014). The stimuli depict everyday life situations conveying information on emotional mental states via facial expression, body language, and context. Via distinct questions, which are answered on visual analogue scales, cognitive empathy (CE), explicit emotional empathy (EEE), and implicit emotional empathy (IEE) are measured (for details see Supplementary Material).

2.3.6 Movie for the Assessment of Social Cognition

The Movie for the Assessment of Social Cognition (MASC) is an ecologically valid, video-based test of social cognition (Dziobek *et al.*, 2006), which we implemented at t+105min. It has also been described in detail elsewhere (Preller *et al.*, 2014). Participants had to watch a 15-min movie and make inferences about the video characters' mental states requiring the understanding of emotions, thoughts, and intentions, and concepts such as false belief, faux pas, metaphor, and sarcasm in an everyday-life situation, which are measured by Theory-of-Mind-related multiple-choice questions (for details see Supplementary Material).

2.3.7 Cognitive Tests

To study acute GHB effects on visual working memory and reaction time subjects had to perform the Delayed Matching to Sample (DMS, t+28min) and the Reaction Time (RTI, t+52min) tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition, Cambridge, UK). In order to assess immediate (t+25min) and delayed verbal recall (t+57min), we employed a shortened German version of the Rey Auditory Verbal Learning Test (RAVLT) (Helmstaedter et al., 2001), as GHB has a short half-life of 45min (Abanades *et al.*, 2006). Details of these tasks are reported in the Supplementary Material.

2.3.8 Endocrine Parameters and Pharmacokinetics of GHB

We performed blood sampling at -20,+35,+60,+100,+135, and+190min to generate plasma. We determined plasma concentrations of GHB using gas chromatography-mass spectrometry (GC-MS) according to Meyer *et al.* (2011) with some modifications; and testosterone, progesterone, DHEA, cortisol, and aldosterone using liquid chromatography-mass spectrometry (LC-MS/MS) according to (Gachet *et al.*, 2014). Oxytocin levels were measured according to procedures established previously (Neumann *et al.*, 2013). For details see Supplementary Material.

2.4 Statistical Analysis

All data were analyzed using SPSS® 22.0 for Windows. GHB and hormone plasma levels were plotted against time and areas under the curve (AUC) were calculated using the trapezoid rule. Demographic and neuropsychological test data were analyzed by paired t-tests. For the analyses of VAS and GSQ scales as well as GHB and hormone plasma levels, repeated measures ANOVA with drug (2-fold: GHB vs. placebo) and time (GSQ items: 3-fold; oxytocin and ACTH: 4-fold; VAS scales, GHB, and all other hormones: 6-fold) as within-subject factors were applied. Greenhouse-Geisser correction and adjusted p-values were used in models with more than one degree of freedom in the numerator. Paired t-tests were applied for post hoc treatment comparisons (placebo vs. GHB). All confirmatory statistical comparisons were carried out at a significance level of $p<.05$ (two-tailed). Finally, because of the small sample size, Spearman's rho was used for correlation analysis. Here, the significance level was set at $p<.01$ (two-tailed) to avoid accumulation of alpha-error.

3. RESULTS

3.1 GHB plasma levels and subjective effects measured by VAS

GHB reached peak plasma concentration at t+35 min and dropped back to close to the physiological level at t+190min (**Fig. 1a**). A drug*time (2*6) ANOVA showed significant main effects for drug ($F(1,15)=104.0$, $p<10^{-7}$), time ($F(5,11)=55.9$, $p<10^{-7}$), and a significant drug*time interaction ($F(5,11)=55.7$, $p<10^{-7}$), reflecting supraphysiological GHB levels after GHB intake and the time-course of the pharmacokinetic of the drug.

Under GHB, the *general drug* and *sedative effects* peaked at t+40min and vanished at 180min post intake (**Fig. 1b,c)**. Interestingly, subjects rated the effect of GHB as *sedating* and *stimulating* at the same time (**Fig. 1c,d**). *Stimulation* and *dizziness* induced by GHB peaked at t+40min but reached placebo levels already at t+100min (**Fig. 1d,e**). In drug*time (2*6) ANOVAs, all four VAS scales showed significant time effects ($F(5,11)=10.4$-21.5, $p<.0001$). With exception of the *stimulation* scale all other scales revealed significant drug effects ($F(1,15)=4.0$-7.3, $p<.05$) and significant drug*time interactions ($F(5,11)=3.4$-7.2, $p<.05$-$.01$).

- Figure 1 about here -

3.2 GHB Specific Questionnaire (GSQ)

Drug*time (2*6) ANOVAs revealed significant drug*time interactions for the items *disinhibition*, *euphoria*, and the *tendency to talk* ($F(5,11)=3.3$-7.7, $p<.05$-$.01$), a significant drug effect for the item *vitality* ($F(1,15)=9.0$, $p<.01$)(**Fig. 2a-c**), and significant time effects for *disinhibition*, *euphoria*, *increased sexuality*, *silliness*, *happiness*, *tendency to talk*, *vitality*, and *increased sensitivity to sound* ($F(5,11)=3.0$-15.4, $p<.05$ - $.001$)(**Fig. 2a-c**,**Supplementary Fig. 2a-d**). Taken together, 20 mg/kg GHB induced an affective state that resembles hypomania.

- Figure 2 about here -

3.3 Charity Donation Task, Social Value Orientation, and Reciprocity Task

To avoid ceiling effects, two highly prosocial subjects who already spend the maximum of 40 CHF under placebo and did not change under GHB were excluded from the analysis of the Charity Donation Task. Under GHB, subjects donated significantly more (+5.36 CHF) than under placebo ($t(13)=-2.3$, $p<.05$, $d=0.60$)(**Fig. 3a**). Moreover, under GHB, subjects also tended to enjoy their donation more (rating range 1-7 [mean±SD]: placebo: 3.1±2.7, GHB: 3.8±2.4; $t(13)=-1.7$, $p=.11$, $d=.46$) and reported that the charity deserved the donation more strongly (placebo: 3.5±2.9, GHB: 4.7±2.7; $t(13)=-1.9$, $p=.08$, $d=.52$). Pleasure of donation was positively correlated with GHB plasma concentration at t+100min (the task was done between t+60 and t+100min; $r=.60$, $p<.01$), indicating that pleasure of donation increased with GHB plasma levels.

For the analysis of the SVO task four subjects were excluded as they displayed an SVO° of >40° in the placebo condition, indicating highly prosocial behavior already at baseline with little chance to be further enhanced. In the GHB condition subjects displayed a significantly wider SVO° compared to placebo ($t(11)=-2.2$, $p<.05$, $d=0.62$), reflecting a preference for prosocial resource allocations (**Fig. 3b**).

In the analysis of the Reciprocity Task, one highly prosocial subject spending more than 500 points under placebo was excluded as no further improvement was expected. Post-acutely (t+160min), GHB administration was associated with slightly elevated positive reciprocity but the effect was far from being significant ($t(14)=-.60$, $p=.56$, $d=0.15$) (**Fig. 3c**). Interestingly, under placebo the reward given to the other player was not significantly correlated with the feeling of pleasure when being prosocial ($r=-.30$, $p=.29$), whereas under GHB the reward given and the pleasure of giving were correlated ($r=-.65$, $p=.008$)(**Supplementary Fig. 3**). When we considered the order of placebo and GHB administration in repeated measures ANOVAs with drug as within-subject factor and order as between-subject factor, the results remained unchanged.

- Figure 3 about here -

3.4 Multifaceted Empathy Task and Movie for the Assessment of Social Cognition

GHB showed no effect on cognitive and emotional empathy in the MET. Furthermore, there was no significant drug effect on mental perspective-taking measured with the MASC (**Tab. 1**).

3.5 Reaction time and memory

At this moderate dose, GHB neither affected reaction and movement time nor verbal or visual memory performance (**Tab. 1**). Thus, the prosocial effects are likely not explained by cognitive effects or increased motor impulsivity.

3.6 Neuroendocrinology

In order to control for potential hormonal baseline differences between test days, hormone levels corrected for baseline levels (t-20min) were analyzed additionally to the uncorrected values (**Fig. 4a,b; Supplementary Fig. 4**). Drug*time (2*4-6) repeated measures ANOVAs revealed significant time effects for progesterone (**Fig. 4a,b**), testosterone, aldosterone, and cortisol ($F(5,11)=3.7\text{-}21.7$, $p<.05\text{-}.001$)(**Suppl. Fig. 4c-l**), reflecting well-known circadian changes of these steroids. For baseline-corrected progesterone, we found a significant drug effect ($F(1,15)=3.4$, $p<.05$), indicating increased progesterone release under GHB, which was significant at+100min in the post hoc test (**Fig. 4a**). In the analysis of AUCs, baseline-corrected progesterone levels showed similar effects (here with moderate effect sizes), pointing to an increase of progesterone under GHB (**Tab. 2**).

GHB plasma levels at+135min were correlated with progesterone change scores at 135min ($r=.69$, $p<.01$) and with AUC change scores ($r=.73$, $p<.001$). We did not observe any significant correlations between progesterone plasma levels and subjective drug effects or behavioral task performance within the GHB condition. We also did not observe significant effects of progesterone change scores [GHB minus placebo] and subjective and behavioral outcomes. Interestingly however, we found that low progesterone levels at baseline (t-20 min) were predictive of donation effects in the charity

donation task (r=-.65, p<.01) and the SVO° (r=-.65, p<.01), indicating that GHB provoked prosocial behavior specifically in individuals with low progesterone levels.

- Figure 4 about here -

4. DISCUSSION

In this study, GHB enhanced mood and induced prosocial effects in humans which were paralleled by increased progesterone plasma levels. Contrary to our hypothesis, GHB did neither alter empathy or mental perspective-taking nor plasma levels of oxytocin, testosterone, cortisol, ACTH and aldosterone.

Surprisingly, the quality of subjective effects showed a simultaneous, mixed stimulating-sedating pattern. The sedating, stimulating, and general drug effects both peaked at 40min. While the stimulating effects had returned to placebo levels already after 100min, the general and sedating effects lasted until 180min post intake, corresponding to the time-course of GHB plasma concentrations. This stands in contrast to a previous study which examined much higher doses of GHB (40-72 mg/kg) reporting a temporally separated mixed stimulant-sedative pattern. The pattern was biphasic with initial stimulation (peak at minute 45 p.i.) and following sedation (peak at minute 60-90 p.i.)(Abanades *et al.*, 2006). In contrast, our dose induced a state with both qualities at the same time, albeit showing more enduring general and sedating effects compared to stimulation (**Fig. 1c,d**). These differing time-courses seem attributable to divergent dose-dependent receptor activation patterns. It has been suggested that lower doses disinhibit thalamocortical neurons and neurons in the ventral tegmental area (VTA) via agonism at presynaptic $GABA_B$ receptors and simultaneously inhibit these neurons via postsynaptic $GABA_B$ receptors, while higher doses additionally involve G protein-gated inwardly rectifying potassium (GIRK) channels, leading to a delayed and more intense neuronal hyperpolarization (Luscher and Slesinger, 2010). Consequently, simultaneous stimulating and sedating effects would be expected at lower doses, while a dissociation of these effects with increased sedation at later time points would be expected at higher doses of the drug.

GHB enhanced mood in our subjects, eliciting an affective state that resembled hypomania, including disinhibition, euphoria, increased vitality, and enhanced tendency to talk. Mood alteration, either as

induction of euphoria or self-treatment of depression symptoms, was reported as a motivation of illicit GHB use (Sumnall *et al.*, 2008). Potential antidepressant effects of the drug were reported in animal models (Zerbib *et al.*, 1992) and early clinical studies (Tanaka *et al.*, 1966; Rinaldi *et al.*, 1967), but never evaluated in-depth according to present clinical trial standards. Consequently, the mood alteration in healthy subjects might be an indicator for a putative therapeutic effect in depressed patients, which we recently proposed because of the unique clinical and pharmacological profile of GHB (Bosch *et al.*, 2012).

At the behavioral level, GHB induced prosociality mostly in participants who showed low to moderate prosocial behavior under placebo. First, the drug increased the willingness to donate money in a charity donation task. Moreover, by trend, GHB increased the pleasure associated with donating money and reinforced the belief that the charity deserved the donation more strongly. This may be interpreted as an increase in altruism, which is conceptualized as giving up a value with no expectation of compensation. Interestingly, depressed patients show various impairments during social interactions, including both increased (Pulcu *et al.*, 2014) and reduced altruism (Zhang *et al.*, 2012). While depression-related hyper-altruism was attributed to excessive feelings of guilt (Pulcu *et al.*, 2014), increased altruism in our study participants appears to be rather pleasure- and compassion-related. Furthermore, GHB induced a tendency towards prosocial money distributions in our Social Value Orientation (SVO) task, reflecting an increased preference for fairness. Although SVO is commonly viewed as a personality trait, it is also susceptible to pharmacological modulations as shown in drug challenge studies using MDMA (Hysek *et al.*, 2014).

Finally, positive reciprocity was only slightly increased in the post-acute phase without reaching significance, but subjects again experienced significantly more pleasure during prosocial decisions. This might be in line with the so called “warm glow” hypothesis assuming that individuals experience pleasure during prosocial behavior, an effect which has been linked to activity of the mesolimbic

reward system (Phan *et al.*, 2010). GHB seems to have effects on mesocorticolimbic dopaminergic pathways, which are controversially discussed, as the drug has both addictive and anti-craving properties (Keating, 2014). A recent translational model described an enhancement of mesolimbic pathway activity after GHB intake, resulting from a converging disinhibition of dopaminergic projections from the VTA to the nucleus accumbens and prefrontal cortex (Snead and Gibson, 2005). In this regard, the GHB-induced prosociality might be the behavioral correlate of a disinhibited mesolimbic reward system, which increases the sensitivity for hedonia-associated cues. As reaction time and memory performance remained unaffected by the drug, general impairment of task performance as a reason for increased prosociality can be ruled out. Considering the relatively short half-life of GHB of about 40 minutes (Abanades *et al.*, 2006), together with the plasma concentration curve assessed in our subjects (**Figure 1**), it is possible that the decreasing GHB concentrations might explain the decreasing prosocial effects across tasks (Charity Donation > SVO > Reciprocity task).

Currently, there are only few pharmacologic compounds with documented prosocial activity. In several studies, oxytocin was shown to facilitate prosocial behavior including increased charity donations (van Ijzendoorn *et al.*, 2011). Recently, MDMA was shown to have empathy-enhancing and prosocial effects, which were paralleled by an increase of oxytocin plasma levels (Schmid *et al.*, 2014). In contrast, in another study with MDMA-experienced individuals, the drug enhanced emotional empathy, while cognitive empathy, trust and reciprocity remained unaffected. Interestingly, neither oxytocin plasma levels nor exogenously applied oxytocin affected those measures (Kuypers *et al.*, 2014). GHBs' most widespread street name is "liquid ecstasy", as it is reported to share MDMAs empathogenic and prosocial effects (Uys and Niesink, 2005). However, both drugs differ strongly regarding their pharmacodynamic profile. While MDMA is a serotonin and noradrenalin releaser, the pharmacological effects of GHB are primarily mediated by $GABA_B$ receptor stimulation and secondary dopaminergic modulation (Uys and Niesink, 2005), or yet unknown mechanisms. Contrary to MDMA (Hysek *et al.*, 2014; Schmid *et al.*, 2014), GHB had neither

empathogenic effects such as increasing complex emotion recognition, emotional empathy, and mental perspective-taking in our social cognition tasks, nor were oxytocin plasma levels affected in our study. However, as oxytocin plasma levels do not fully reflect central oxytocin release (Neumann, 2007), and GHB was shown to up-regulate hypothalamic oxytocin mRNA in rats (van Nieuwenhuijzen *et al.*, 2010), at least centrally restricted involvement of oxytocin regarding the prosocial effects of GHB cannot be ruled out. Four reasons led to our hypothesis that the androgen system might be involved in the prosocial effects of GHB: the drug seems to enhance sexually connoted affiliative behavior, alters levels of several steroid hormones, GABA-B receptors are discussed in the regulation of testosterone secretion (Amikishieva, 2007), and testosterone is a sex steroid hormone that might be a mediator of prosocial effects (Eisenegger *et al.*, 2010). However, we did not observe any alterations of testosterone (and DHEA) levels in our subjects and hence conclude that GHB-related prosocial effects do not operate via the androgen system.

Interestingly, GHB-induced neuroendocrine changes were limited to an increase in progesterone release here. Progesterone is a steroid hormone primarily synthesized in the testicles and the adrenal glands in males. However, small amounts are also synthesized in the brain, there targeting and influencing neuronal activity, which qualifies it as a neurosteroid (Guennoun *et al.*, 2015). Animal and human data show that progesterone release mirrors an individual`s level of social affiliative motivation (Frye *et al.*, 2000; Maner *et al.*, 2010). For example, affiliative behavior of female rats is most pronounced, when progesterone release peaks during the estrous cycle (Frye *et al.*, 2000). In humans, the desire to affiliate with others correlates positively with basal progesterone levels (Wirth and Schultheiss, 2006), and endogenous fluctuations of the hormone reflect fluctuations of female social-affiliative motivations during the menstrual cycle (Schultheiss *et al.*, 2003). Moreover, interpersonal closeness increased salivary progesterone in a sample of 160 female college students, which predicted altruistic motivations one week later (Brown *et al.*, 2009). Finally, in subjects with high rejection sensitivity, experimental social rejection can lead to heightened progesterone release

when given an opportunity to reaffiliate, which might reflect a desire for compensatory closeness (Maner *et al.*, 2010). These studies support the notion that progesterone release might be a neuroendocrine mechanism that contributes to the prosocial effects of GHB. Although, we did not find direct correlations of progesterone levels and behavioral parameters, which might be due to the small sample size, our finding that GHB provoked prosocial behavior specifically in individuals with low progesterone levels strongly supports this relationship.

Moreover, progesterone is a precursor of the other neurosteroids tetrahydroprogesterone (3α,5α-THP) and tetrahydrodeoxycorticosterone (THDOC), which are both $GABA_A$ receptor agonists and studied as experimental therapeutics in stress-related disorders such as anxiety and depression (Zorumski *et al.*, 2013). In animals, GHB leads to a dose-dependent increase of progesterone, 3α,5α-THP and THDOC in the central nervous system, which significantly contribute to the sedative effects of the drug (Barbaccia *et al.*, 2005). Moreover, central and peripheral increase of neurosteroid plasma levels were closely related (Barbaccia, 2004), thus implicating increased progesterone concentration to be a relevant marker of central neurosteroidogenesis.

Another clinical aspect arises regarding GHB-induced progesterone release: GHB is used for the treatment of alcohol withdrawal and as an anti-craving agent (Keating, 2014), while some studies also point to anti-craving and relapse preventing effects of progesterone in nicotine (Lynch and Sofuoglu, 2010) and cocaine addiction (Yonkers *et al.*, 2014). Therefore, increased progesterone release might contribute to the therapeutic effect of GHB in alcohol withdrawal and abstinence maintenance.

In our subjects, stress hormones such as ACTH, cortisol, and aldosterone plasma levels remained unchanged. Previous studies indicate bidirectional effects of GHB on stress hormone release, depending on the initial activity of the HPA axis: increase at rest (Van Cauter *et al.*, 1997), reduction in stressful conditions (Nava *et al.*, 2007). The fact that stress hormone concentrations were not

increased under placebo suggests that our participants performed the tasks within a psychophysiologically balanced experimental setting.

Nevertheless, the present study has some limitations. First, we only report a single dose of GHB (20 mg/kg). However, when we evaluated a higher dose (35 mg/kg) we observed intense sedative effects and induction of nausea in several subjects, which strongly impaired test performance. Second, GHB is likely a “dirty drug”, acting on distinct neurotransmitter and neuroendocrine systems, thus complicating the interpretation of the results at a molecular level. Third, due to small sample size, the prosocial effects but also the lack of effect in some hormones and tasks have to be considered as preliminary and should be replicated in larger studies. Fourthly, we included only male subjects to avoid controlling for hormonal fluctuations due to menstrual cycle in women.

In summary, the primary finding of this study is that GHB enhances mood and induces prosocial behavior in healthy subjects paralleled by an increase in progesterone release but without affecting oxytocin and testosterone plasma levels. The study thus confirms recreational GHB users´ self-reported effects on prosociality on an objective, experimental level. Mood enhancement and potentially increased neurosteroidogenesis make GHB an interesting experimental therapeutic agent to treat forms of depression and anxiety. On the other hand, the prosociality observed may be elicited by a direct activation of the mesolimbic reward system due to GHB- and/or $GABA_B$ receptor stimulation and/or indirectly due to increased progesterone secretion. Recently, arbaclofen, another GABA-B agonist with prosocial effects, has been suggested as new treatment for autism spectrum disorders (Insel, 2012). The clinical use of GHB as a means to improve interpersonal contact was noted early on in studies showing an improvement of patient-doctor alliance (Danon-Boileau *et al.*, 1962). Thus, the prosocial compound GHB might be useful to increase social engagement in patients with autism spectrum disorders or depression.

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6. AUTHOR CONTRIBUTIONS

O.G. Bosch[1,2,3,5,6,7], C. Eisenegger[1,3,4,6,7], J. Gertsch[3,4,6,7], D. Dornbierer[2,6,7], M. S. Gachet[3,6,7], M. Heinrichs[1,4,6,7], T. C. Wetter[1,3,6,7], E. Seifritz[1,4,6,7], B. B. Quednow[1,3,4,5,6,7]

[1]conception and design of the study; [2]acquisition of data; [3]analysis of data; [4]interpretation of data; [5]drafting of the article; [6]revising the article, [7]final approval of the version to be submitted

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Table 1: Neuropsychological test performance under placebo and GHB (means and SD). MASC: Movie for the Assessment of Social Cognition, MET: Multifaceted Empathy Test, RTI: Reaction Time (CANTAB), DMS: Delayed Matching to Sample (CANTAB), RAVLT: Rey Auditory Verbal Learning Test.

		Placebo	GHB	t	df	p	Cohens dz
MASC*	Correct answers	34.9	33.6	1.67	13	0.12	0.45
	(sum)	*(3.6)*	*(3.9)*				
	Correct control items	4.6	4.9	-1.59	13	0.14	0.42
	(sum)	*(1.2)*	*(0.9)*				
	Recognition of emotions	73.3	71.4	0.59	13	0.57	0.16
	(%)	*(14.3)*	*(14.2)*				
	Recognition of thoughts	85.7	76.8	1.44	13	0.17	0.38
	(%)	*(18.9)*	*(20.7)*				
	Recognition of intentions	71.9	72.4	-0.14	13	0.89	0.04
	(%)	*(10.2)*	*(6.3)*				
MET	Cognitive Empathy	25.0	24.3	0.75	15	0.47	0.19
	(correct items)	*(4.5)*	*(4.5)*				
	Explicit Emotional Empathy	5.1	5.0	0.67	15	0.51	0.17
	(mean rating)	*(1.3)*	*(1.4)*				
	Implicit Emotional Empathy	4.5	4.5	-0.04	15	0.97	0.01
	(mean rating)	*(1.3)*	*(1.5)*				
	Mean reaction time	3965	3728	0.84	15	0.41	0.21
	(ms)	*(1053)*	*(682)*				
RTI	Simple reaction time	275	276	-0.08	15	0.94	0.02
	(ms)	*(43.4)*	*(47.7)*				
	Simple movement time	279	280	-0.24	15	0.82	0.06
	(ms)	*(64.9)*	*(71.9)*				
	Simple accuracy	14.6	14.7	-0.37	15	0.72	0.09
	(score)	*(1.1)*	*(0.7)*				
	5-choice reaction time	301	299	0.38	15	0.71	0.09
	(ms)	*(49.7)*	*(49.7)*				
	5-choice movement time	282	279	0.69	15	0.50	0.19
	(ms)	*(65.5)*	*(69.1)*				
	5-choice accuracy score	14.9	14.6	0.89	15	0.39	0.28
	(score)	*(0.3)*	*(1.1)*				
DMS	Correct simultaneous	4.8	4.9	-0.81	15	0.43	0.20
	(sum)	*(0.5)*	*(0.3)*				
	Correct all delays	13.1	13.2	-0.10	15	0.92	0.02
	(sum)	*(1.5)*	*(1.7)*				
RAVLT	Immediate recall	9.3	10.1	-1.65	15	0.13	0.41
	(number of words)	*(1.9)*	*(2.4)*				
	Delayed recall	7.1	7.8	-1.55	15	0.14	0.39
	(number of words)	*(2.1)*	*(2.5)*				
	Recognition	0.88	0.90	-0.65	15	0.52	0.16
	(p(A))	*(0.1)*	*(0.1)*				

*****Due to technical problems with the MASC, only data from 14 subjects were available.

Table 2: Area under the curve (AUC) of hormones under placebo and GHB (means and SD). ACTH: Adrenocorticotrophic hormone, AUC: Area under the curve, corr: corrected, DHEA: Dehydroepiandrosterone.

	Placebo	GHB	t	df	p	Cohens dz
ACTH AUC	132.4	133.0	-0.07	14	0.95	0.02
(4 time points)	*(41.2)*	*(34.4)*				
ACTH_corr AUC	-44.0	-15.0	-0.94	14	0.36	0.24
(4 time points)	*(109.4)*	*(44.5)*				
Oxytocin AUC	46.0	32.3	1.53	14	0.15	0.40
(4 time points)	*(53.5)*	*(30.7)*				
Oxytocin_corr AUC	-7.0	-2.7	-0.18	14	0.86	0.05
(4 time points)	*(84.7)*	*(45.3)*				
Testosterone AUC	72.3	74.5	-0.75	15	0.47	0.19
(6 time points)	*(14.8)*	*(19.1)*				
Testosterone_corr AUC	3.7	2.6	0.63	15	0.54	0.16
(6 time points)	*(6.7)*	*(8.9)*				
Progesterone AUC	1.11	1.20	-0.88	15	0.39	0.22
(6 time points)	*(0.44)*	*(0.63)*				
Progesterone_corr AUC	-0.14	0.03	-1.81	15	0.09	0.45
(6 time points)	*(0.22)*	*(0.40)*				
Aldosterone AUC	32.8	32.0	0.68	15	0.51	0.17
(6 time points)	*(7.0)*	*(5.1)*				
Aldosterone_corr AUC	-7.7	-9.1	0.78	15	0.45	0.20
(6 time points)	*(5.7)*	*(8.0)*				
DHEA AUC	151.0	161.4	-1.52	15	0.15	0.38
(6 time points)	*(33.9)*	*(44.3)*				
DHEA_corr AUC	-1.0	-15.8	1.71	15	0.11	0.43
(6 time points)	*(16.2)*	*(35.6)*				
Cortisol AUC	1164.5	1192.7	-0.32	15	0.76	0.08
(6 time points)	*(311.8)*	*(375.7)*				
Cortisol_corr AUC	-392.3	-519.2	1.03	15	0.32	0.26
(6 time points)	*(408.8)*	*(464.8)*				

Figures

Fig. 1: GHB plasma (means and SEM) concentration (a), and visual analogue scales (VAS) general drug effect (b), sedation (c), stimulation (d), and dizziness (e) after GHB and placebo administration. Paired t-tests: *p<.05, **p<.01, ***p<.001.

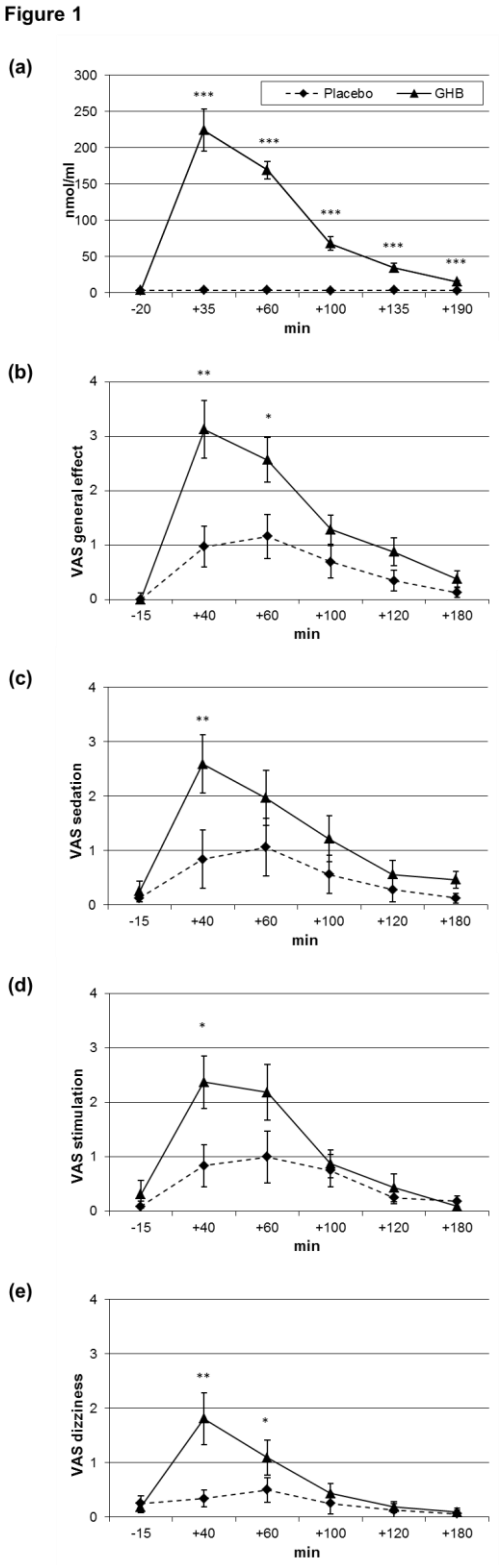


Fig. 2: Baseline corrected ratings (means and SEM) of the items disinhibition (a), euphoria (b), tendency to talk (c) and vitality (d) of the GHB specific questionnaire (GSQ). The values represent changescores from baseline (t-10min) on the respective test day. Paired t-tests: *p<.05, **p<.01.

Figure 2

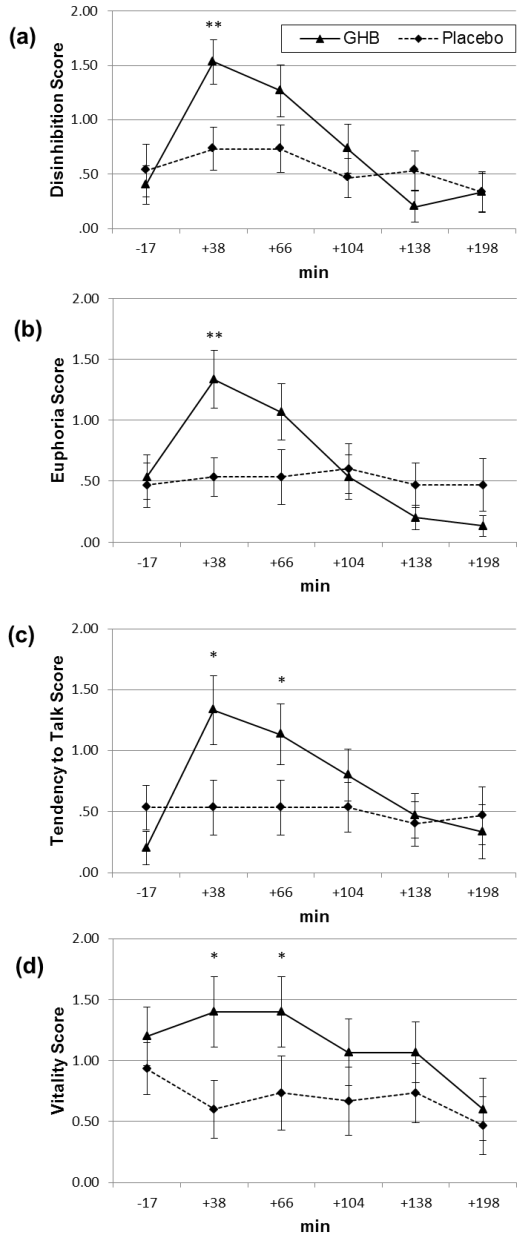


Fig. 3: Prosocial behavior measures under GHB and placebo (means and SEM): Money donated in the Charity Donation Task (a), and Social Value Orientation (SVO) angle (b), and points given to the other player in the Reciprocity Task (c). Paired t-tests: *p<.05.

Figure 3

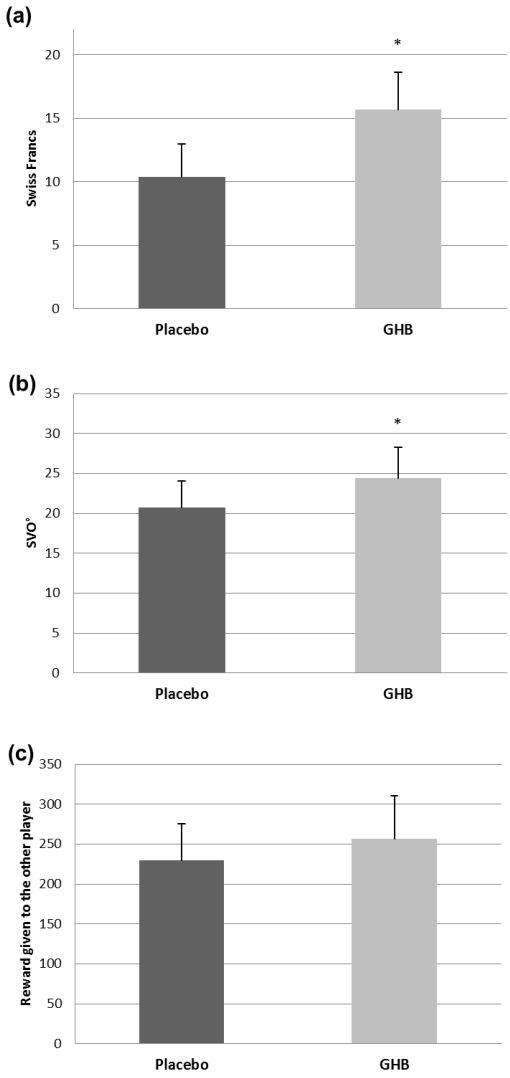
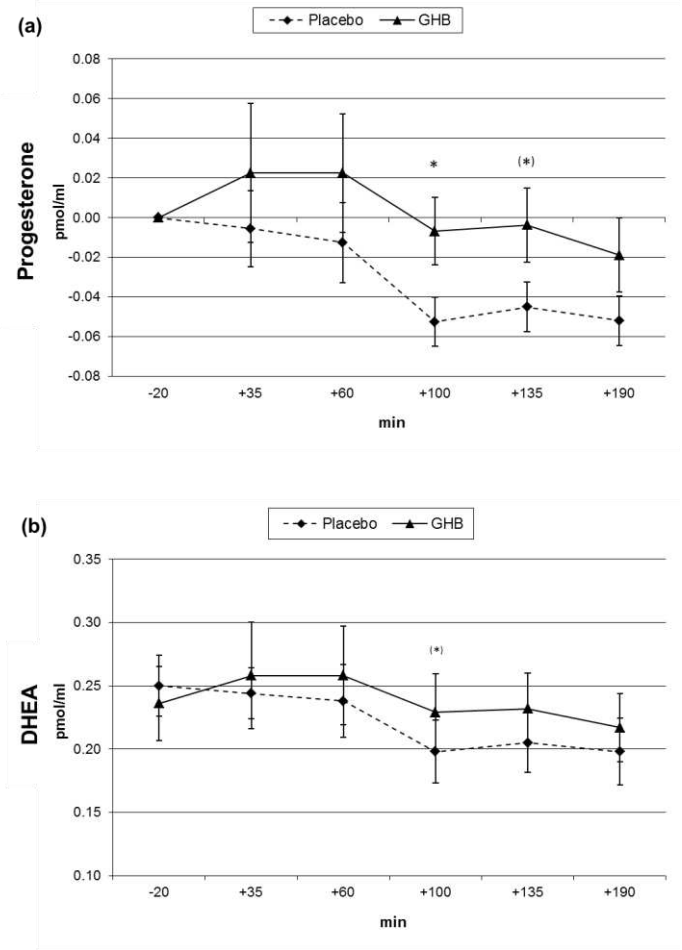


Fig. 4: Progesterone plasma concentrations (means and SEM) corrected for individual baseline (-20min) levels on each test day (a) and baseline-corrected DHEA plasma levels (b). Paired t-tests: $^{(*)}$p<.10, *p<.05.

Figure 4



SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Social Value Orientation

Mean allocations for self and others were calculated for the primary SVO slider items. The subjects SVO index was then calculated from the inverse tangent of the ratio of the two mean allocations for self and the other from the primary items. The subjects' SVO index is gained through an angle, which is produced by the inverse tangent of these two means.

Reciprocity Task

In this task, a virtual player A delegates, in three out of five instances, a decision about the distribution of money to another subject, player B. Player A thus either choses the status quo, i.e. keeping 20 points and leaving 20 points to player B, or trust player B by delegating the decision about the distribution of money to him/her. Delegating the decision will add 20 points to the total amount of distributable points. Being trusted, player B can either betray player A in keeping 55 points and giving 5 back, or show positive reciprocity in giving back 30, and keeping 30 points.

Multifaceted Empathy Test

To measure cognitive empathy (CE) subjects are asked to infer the mental state of a given person in a photograph and choose which of four words provided along with the picture describes best what the person in the picture is feeling. Explicit emotional empathy (EEE) is assessed by ratings of empathic concern ("How concerned are you for this person?") on a visual-analogue scale within a range of 1-9 (1=not concerned to 9=very concerned) while viewing the photograph. Implicit emotional empathy (IEE) is measured analogously by arousal ratings ("How calm/aroused does this picture make you feel?", 1=very calm to 9=very aroused).

Movie for the Assessment of Social Cognition

The film interrupts briefly for 45 times to ask questions about the actors' feelings, thoughts, and intentions ("How is Michael feeling?"). In detail, the MASC asks 15 questions demanding the interpretation of emotions, 14 questions for intentions, and 4 questions for measuring thoughts. These questions are asked in a multiple-choice format with one correct answer and three distractors reflecting three different types of mistakes: a) insufficient mental state inferences (undermentalizing: reduced ToM), b) excessive (overmentalizing), and c) non-mental state inferences (physical causation, no-ToM). Therefore, the MASC provides a sum score for the errors and three subscales for different error types. To control for non-social inference, memory, and general comprehension effects, six control questions referring to physical events instead of a character's mental state are asked during the test. In addition, the MASC allows for a separate quantification of the extent to which emotional mental states, thoughts, and intentions are inferred correctly. Both the MET and MASC were implemented in Presentation (Version 14.1, Neurobehavioral Systems, Albany, CA).

Cognitive Tests

Cambridge Neuropsychological Test Automated Battery (CANTAB):

Delayed Matching to Sample (DMS): The DMS test was implemented at t+28 min. A stimulus consisting of a square with different patterns and colors was presented on the screen, which the subject had to memorize. Subsequently, four patterned squares were presented from which the subject had to recognize the one that was shown before. The test consists of 20 counter balanced trials of five simultaneous presentations of the stimulus and the recognition squares, as well as five trials of subsequent presentation with a delay time of 0 msec, 4000 msec and 12000 msec. The duration of the test is 6.5min.

Reaction Time (RTI): The RTI test was implemented at t+52 min. Subjects had to push down a button until a yellow dot appeared in a circle on the screen (simple choice) or in one of five circles (5-choice), which they then had to try to touch as fast and adequate. The five choice trials started if the

subject performed nine out of ten trials of the simple choice correctly. After nine of ten correctly performed trials, the test was stopped. Total duration of the task was about 5min.

Verbal memory:

A word list of 15 nouns (learning list) was presented once and subsequently participants were asked to recall as many words as possible (t+25min, immediate recall). Participants were then asked to recall the word list again 30min later (t+57min, delayed recall). After that a third list of 50 nouns including all words of the first list as well as 35 new, but semantically- and phonetically-related words were presented (t+58min, recognition,). The task yields the three dependent variables immediate recall, delayed recall, and adjusted recognition performance (p[A] according to (Forrester and Geffen, 1991)]), whereas p(A) is comparable to the discrimination performance (d-prime) in signal detection theory (Green and Swets, 1966).

Endocrine parameters and pharmacokinetics of GHB (Details)

Two types of blood samples were collected: A) samples for the assessment of plasma GHB, testosterone, aldosterone, progesterone, DHEA, ACTH, and cortisol and B) samples for the assessment of oxytocin. Time points of sampling were t-20,+35,+60,+100,+135, and +190min. Blood for the assessment of oxytocin (B) was collected in 5 ml BD Vacutainer K2EDTA tubes for plasma (Vacutainer Systems, becton Dickinson, Plymouth, UK), while blood for the assessment of plasma GHB, testosterone, aldosterone, progesterone, DHEA, ACTH, and cortisol (A) was collected in 10 mL BD Vacutainer K2EDTA tubes for plasma (Vacutainer Systems, becton Dickinson, Plymouth, UK) and spiked with 1 mM PMSF solution in ethanol (to avoid enzymatic activity of serine proteases). Plasma was obtained approx. 3 h after blood collection by centrifugation at 1800 rpm (A) and 4000 rpm (B) for 10 min at 4°C. Blood samples were kept on ice until plasma generation. Plasma was stored at -80°C prior the analysis. Prior to the analysis of GHB and the steroids, plasma was thawed inside the refrigerator at 6°C for 3-4 h. Then, an aliquot of 50 µL plasma was used for the analysis GHB by GC-MS (see below) while an aliquot of 0.5 mL plasma was used for the analysis of bioactive lipids by LC-

MS/MS according to (Gachet *et al.*, 2014). Both analyses were performed in parallel. Plasma ACTH was analyzed using a commercially available ELISA kit for ACTH (IBL International, Hamburg, Germany). The analytical sensitivity was 0.22 pg/mL, intra-assay and interassay coefficients of variation were 7.1% (Nyuyki *et al.*, 2012).

Analysis of GHB in human plasma by GC-MS

Materials

GHB (Xyrem®) as analytical standards was purchased from UCB Pharma GmbH, Monheim, Germany. GHB-d_6 (internal standard (IS)), HPLC-grade methanol, HPLC-grade acetonitrile (ACN), *N,N*-dimethylformamide (DMF) and *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Sigma-Aldrich, Steinheim, Germany. Deionized water (18.2 MΩ x cm) was obtained from an ELGA Purelab Ultra Genetic system (VWS (UK) Ltd, ELGA LabWater, UK).

Standard solutions

Standard solutions used as calibrators were prepared in methanol covering a concentration range 0.001 to 0.5 mg/mL. For constructing calibration curves and method performance analysis within the study, 10 or 20 µL of standard solutions were applied to plasma samples (instead of only methanol) and followed by sample preparation (see below) to reach the analytical range of 4.5 to 4500 pg on column. The calibration curve used for the analysis of plasma samples was build using 8 calibration points. A GHB-d_6 solution of 0.2 µg/mL prepared in water was used as internal standard (IS)(i.e. 900 pg on column).

Sample preparation and GC-MS analysis

The analysis of GHB was performed according to Meyer *et al.* (2011) with slight modifications. In brief, 50 µL of plasma were added to a 2 mL Eppendorf containing 10 µL of IS, 50 µL ACN and 20 µL methanol (with [i.e. control and calibration samples] or without analytical standard). The mixture

was vigorously vortexed and then centrifuged at 16,100 g at 4°C for 5 min. For derivatization, an aliquot of 20 µLof the supernatant was transferred to into conic amber vial and mixed with 20 µL DMF and 300 µL BSTFA. The vial was immediately closed, vortex and derivatized at 70°C for 15 min. After cooling, samples were analyzed by gas chromatography (GC) electron ionization (EI) mass spectrometry (MS) using an Agilent 6890 N GC equipped with a 30 m HP-5MS column and a 5975 C EI-MS with triple-axis detector. Helium was used as carrier gas at a flow rate of 1.3 mL/min. The inlet temperature was kept at 250°C and 1 µL of the sample was injected in splitless mode. The oven temperature program was as follows: initial temperature of 60°C (hold time 3 min), increased by 10°C/min to 120°C, increased by 30°C/min to 280°C (hold time 15 min). Total analysis time 30min. The MS conditions were: Ionization energy 70eV, ion source temperature 230°C, MS Quant temperature 150°C and Aux temperature 280°C. Specific ions were used for selected ion monitoring (SIM). For the analysis of GHB, one target ion (*m/z*= 233) and two qualifier ions (*m/z*= 204 and 117) were used. For the analysis of GHB-d_6, *m/z*=239 was used. The performance of the method was evaluated using external calibrations (i.e. spiking calibration solutions [see standard solutions] into plasma and processing as stated above [n=3]) and control samples. The results were analyzed based on the peak area ratio between analyte and IS. All the calibration curves had a correlation coefficient (R^2) better than 0.999. The inter-day variation coefficient (CV) was bellow to 10% and accuracy was between 90 and 115%. The recovery of control samples was in the range of 90-100%.

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Nyuyki, K. D., Beiderbeck, D. I., Lukas, M., Neumann, I. D. & Reber, S. O. (2012) 'Chronic subordinate colony housing (CSC) as a model of chronic psychosocial stress in male rats', *PLoS One*, **7**(12), p. e52371.

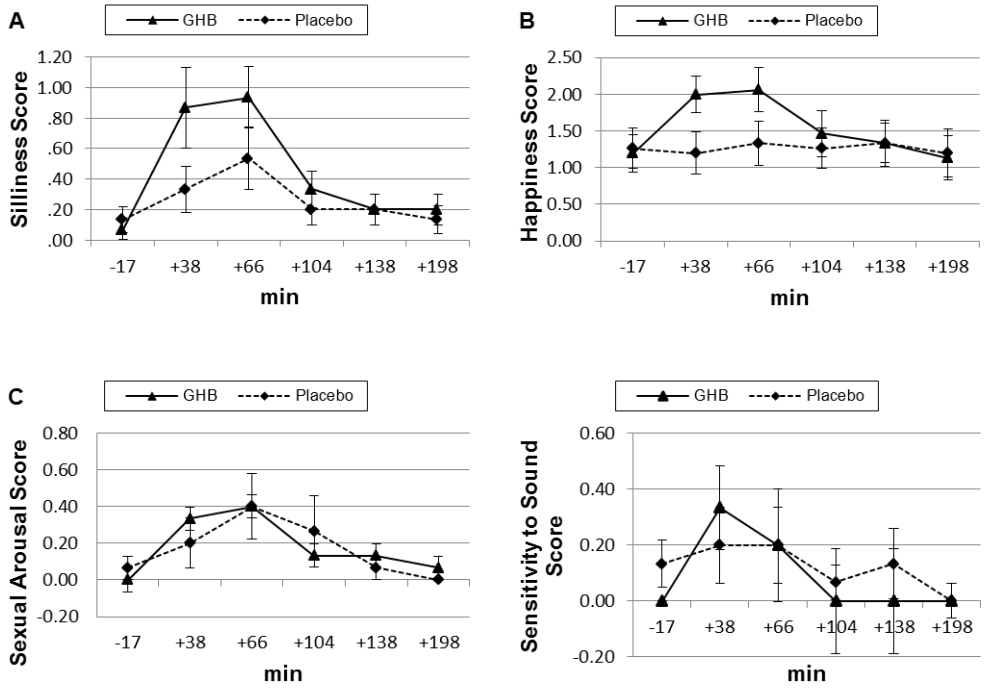
Supplementary Table 1: List of charities used in the Charity Donation Task and percentage of individuals donating and total amount donated in Swiss francs (CHF) under placebo and GHB (without exclusion of subjects highly prosocial under placebo).

Charity	**%**		**CHF**	
	Placebo	**GHB**	**Placebo**	**GHB**
Gesellschaft für bedrohte Völker (Society for Threatened Peoples)	18.8	18.8	65	75
Médecins Sans Frontiers (Doctors Without Borders)	12.5	18.8	30	65
Schweizerisches Rotes Kreuz (Swiss Red Cross)	12.5	6.3	55	20
Stiftung für wissenschaftliche Forschung an der Universität Zürich (Foundation for Research in Science and the Humanities at the University of Zurich)	12.5	6.3	45	10
Kinderkrebshilfe Schweiz (Swiss Children's Cancer Aid	6.3	18.8	15	55
Swiss World Wide Fund For Nature (WWF)	6.3	12.5	15	35
Greenpeace	0	0	0	0
AIDS-Hilfe Schweiz (Swiss AIDS Federation)	0	0	0	0
Brot für Alle (Bread For All)	0	0	0	0
Amnesty International	0	0	0	0
No donation	31.3	18.8	0	0
Total Donation	**68.7**	**81.2**	**225**	**260**

Supplementary Figure 1: Flowchart of the study. Abbreviations: CANTAB, Cambridge Neuropsychological Test Automated Battery; DMS, Delayed Matching to Sample; GSQ, GHB Specific Questionnaire; MASC, Movie for the Assessment of Social Cognition; MET, Multifaceted Empathy Task; POMS, Profile of Mood States; RTI, Reaction Time; SADI, Sexual Arousal and Desire Inventory; VAS, Visual Analogue Scale; VLMT, Verbal Learning and Memory Test. *= data will be published elsewhere.

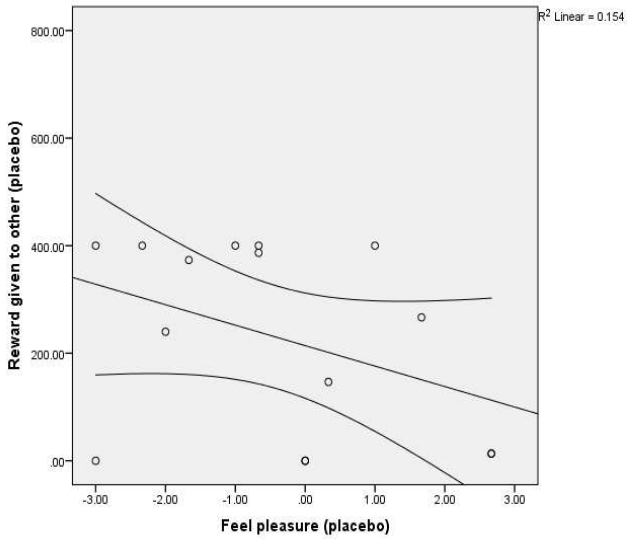
Experimental Session	
Time point	**Task**
t – 30 min	Urine test
t – 25 min	IV-catheter
t – 20 min	Blood sample 1 / Vital signs*
t – 17 min	GSQ 1
t – 15 min	VAS 1
t – 13 min	POMS 1*
t – 10 min	SADI 1*
t_0	**GHB / Placebo application**
t + 06 min	Pause
t + 25 min	VLMT 1
t + 28 min	CANTAB/ DMS
t + 35 min	Blood sample 2 / Vital signs*
t + 38 min	GSQ 2
t + 40 min	VAS 2
t + 41 min	Sexual Arousal Task*
t + 50 min	SADI 2*
t + 52 min	CANTAB/ RTI
t + 57 min	VLMT 2
t + 60 min	Blood sample 3 / Vital signs*
t + 63 min	VAS 3
t + 66 min	GSQ 3
t + 68 min	POMS 2*
t + 70 min	Charity Donation
t + 75 min	MET
t + 90 min	Social Value Orientation
t + 100 min	Blood sample 4 / Vital signs*
t + 102 min	SADI 3*
t + 104 min	GSQ 4
t + 106 min	VAS 4
t + 109 min	MASC
t + 120 min	VAS 5
t + 135 min	Blood sample 5 / Vital signs*
t + 138 min	GSQ 5
t + 141 min	POMS 3
t + 150 min	SADI 4
t + 163 min	Reciprocity task
t + 182 min	VAS 6
t + 183 min	POMS 4*
t + 190 min	Blood sample 6 / Vital signs*
t + 198 min	GSQ 6
t + 225 min	End of Session

Supplementary Figure 2: GSQ Scores GHB vs. placebo A) silliness, B) happiness, and C) sexual arousal, and D) sensitivity to sound.



Supplementary Figure 3: Correlations of feeling of pleasure when being prosocial under placebo (r=-.30, p=.29) A), and under GHB (r=-.65, p=.008) B) in the reciprocity task.

A)



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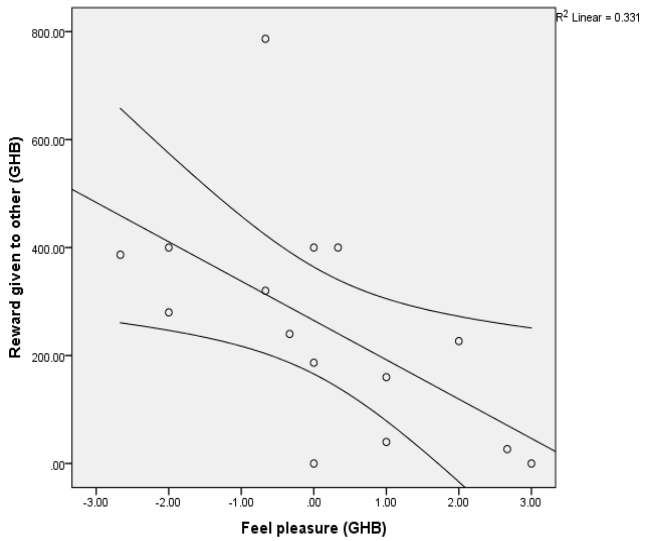
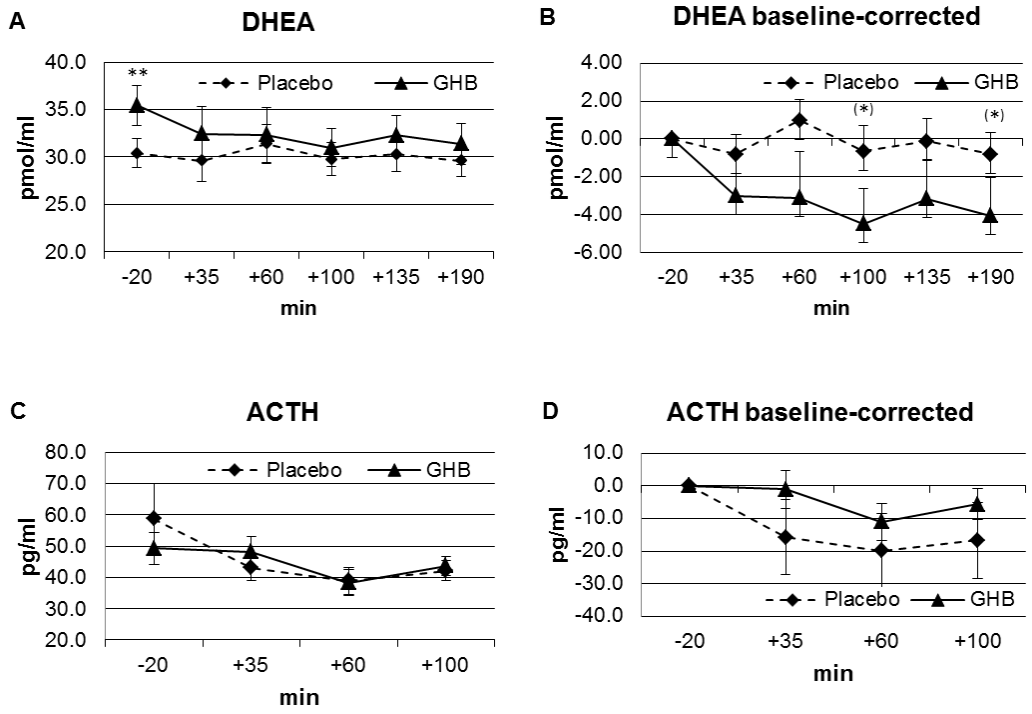


Supplementary Figure 4: Plasma levels under GHB vs. placebo of A) DHEA uncorrected (drug: p=.1, time: p=.3, drug*time: p=.2) , B) DHEA baseline-corrected (drug: p=.1, time: p=.3, drug*time: p=.2), C) ACTH uncorrected (drug: p=.79, time: p=.07, drug*time: p=.46), D) ACTH baseline-corrected (drug: p=.36, time: p=.07, drug*time: p=.46), E) Oxytocin uncorrected (drug: p=.25, time: p=.54, drug*time: p=.89), F) Oxytocin baseline-corrected (drug: p=.81, time: p=.62, drug*time: p=.85), G) Testosterone baseline uncorrected (drug: p=.39, time: p<.01, drug*time: p=.16), H) Testosterone baseline-corrected (drug: p=.64, time: p<.001, drug*time: p=.17), I) Aldosterone uncorrected (drug: p=.60, time: p<.001, drug*time: p=.73), J) Aldosterone baseline-corrected (drug: p=.47, time: p<.001, drug*time: p=.73), K) Cortisol uncorrected (drug: p=.62, time: p<.001, drug*time: p=.60), L) Cortisol baseline-corrected (drug: p=.31, time: p<.001, drug*time: p=.60). Paired t-tests: $^{(*)}$p<.10, **p<.01.



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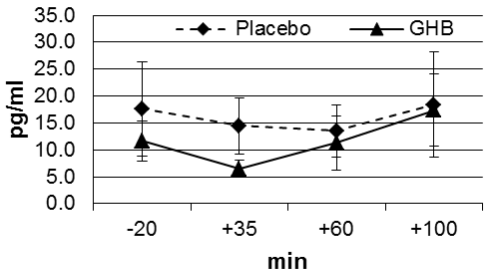
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pg/ml
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25.0
20.0
15.0
10.0
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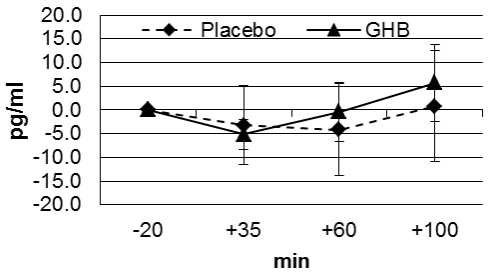
-20
+35
+60
+100
min

F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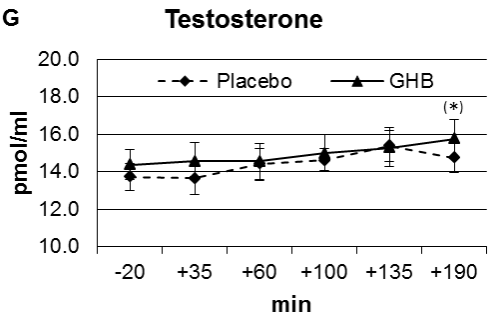
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min

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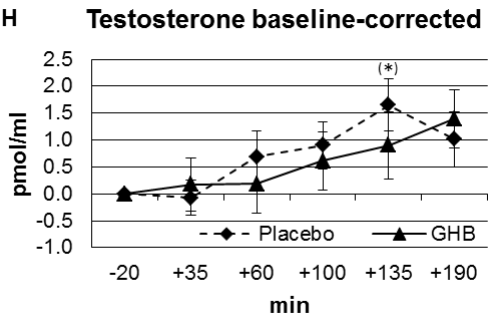
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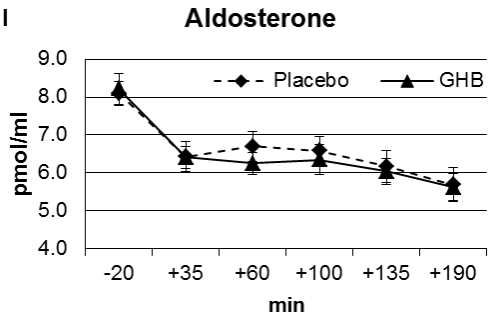
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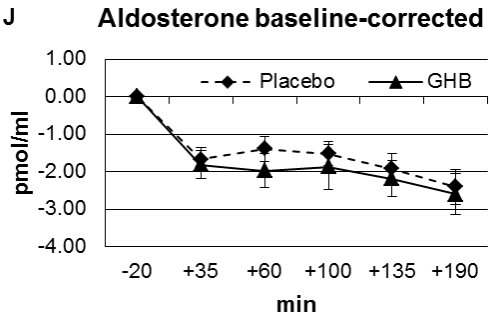
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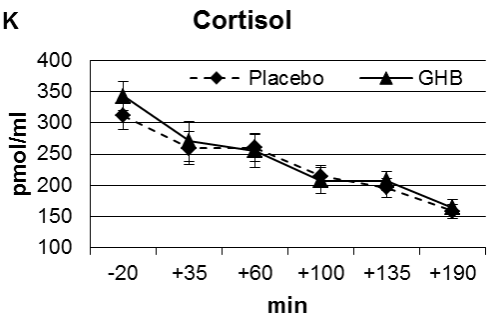
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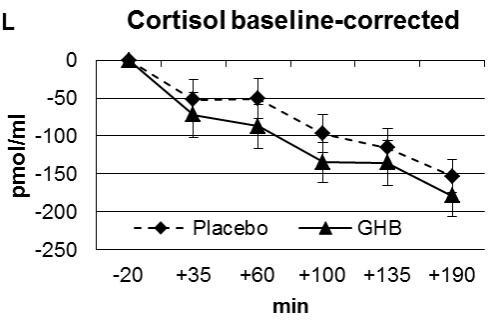
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+135
+190
min