The effects of a combined $GABA_B$-/GHB receptor stimulation on social cognition, neuroendocrine function, sleep, and memory in healthy male subjects

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1. Summary

Gamma-aminobutyric acid (GABA) is the central inhibiting neurotransmitter in the human nervous system. Two GABA receptors are known to date: $GABA_A$ and $GABA_B$. The stimulation of both receptors leads to a wide variety of effects on cognition, mood, vigilance, sleep and other neuropsychological functions. $GABA_A$ and $GABA_B$ receptor dysfunction seems to play a key role in stress, anxiety and depression (Cryan & Slattery, 2010). γ-Hydroxybutyrate (GHB) – a GABA metabolite - is an endogenous compound found in the human brain (Bessman and Fishbein, 1963). Central GHB releasing neurons and specific GHB receptors form the GHBergic neurotransmitter system, which function is still widely unknown (Maitre, 1997). Despite its high affinity to GHB receptors, most of its neuronal and behavioral effects are mediated by an agonist action at $GABA_B$ receptors (Engberg and Nissbrandt, 1993). It was reported that GHB increases dopamine and norepinephrine levels in a biphasic mode (Maitre, 1997). GHB is approved for the treatment of narcolepsy in the USA and Europe and for alcohol withdrawal syndrome in Italy and Austria. However, GHB is also misused by a small part of the population: the most frequently reported reasons are recreation purposes, to enhance sex, to be sociable and to achieve altered states of consciousness (Sumnall et al., 2008). These effects seem to be associated with neuroendocrine alterations, which are mostly unknown in detail. An increase of growth hormone release is the most consistent finding (Van Cauter et al., 1997). In addition, plasma levels of cortisol and ACTH after GHB challenge indicated an activation of the HPA axis in the resting state, compared to an HPA axis inhibition under stress conditions (Nava et al., 2007). Neurosteroids and oxytocin are candidates of primary interest for the endocrine mediation of prosocial and prosexual behavior as suggested by animal studies (Baskerville et al., 2009, Frye et al., 2000). Thus, a release of these hormones might be an explanation for the contact stimulating effects of the drug. Interestingly, GHB is also an enhancer of slow wave sleep (SWS) in humans, which is a remarkable pharmacological property, given that most of the psychopharmacological drugs rather decrease SWS (Van Cauter et al., 1997). Recent studies have demonstrated that SWS is involved in declarative memory consolidation but the role of a combined stimulation of $GABA_B$ and GHB receptors in SWS-mediated memory consolidation is widely unknown so far (Born and Wagner, 2009). Early studies also reported anxiolytic and antidepressant effects of GHB in psychiatric patients (Danon-Boileau et al., 1962, Rinaldi et al., 1967). Neuroimaging, especially functional magnetic resonance imaging (fMRI) and MR spectroscopy (MRS) have revolutionized psychiatric biomarker research. As such, assessing brain network connectivity patterns during resting state in healthy subjects and major depressive disorder (MDD) patients and modulating them with pharmacologic or other interventions, has proven to be one of the most promising strategies in the field (Phillips et al., 2015, Hasler and Northoff, 2011). In terms of molecular imaging, MRS has been established as a valid method to study pathological and therapeutic neurotransmitter alterations in mood disorders (Caverzasi et al., 2012), and growing evidence indicates alterations of brain GABA and Glutamate (Glu) concentrations in MDD (Jun et al., 2014, Chang et al., 2003, Hasler et al., 2007). The combination of assessing brain network connectivity patterns using resting state fMRI/EEG and brain GABA and Glu concentrations using MRS seems a most promising approach in current neuroimaging biomarker research and are implemented in the study presented here.

Moreover, the effects of GHB on putative plasma biomarkers for depression and sleep physiology, such IL-beta, IL-6, IL-10, TNF-alpha, CRP, IL-4, IL-18 und IFN-gamma, tryptophan, kynurenin, kynureninic acid, neopterine, malondialdehyd, qinolinsäure, IDO (indolamine-2,3-deoxygenase) and substance P are investigated in our study. The unique spectrum of effects of a combined $GABA_B$-/GHB receptor stimulation by GHB on neuropsychiatric functions such as sexual and prosocial behavior, neuroendocrine systems and sleep makes it a pharmacological research tool and model substance of high interest. Since there are no such systematic studies of a combined $GABA_B$-/GHB receptor stimulation in healthy human subjects, we aim to investigate the effects of GHB on sleep, pharmacokinetics, putative depression and sleep biomarkers, mood, cerebral GHB-, GABA- and Glu –concentrations, resting state fMRI, as well as cognitive and social functions in healthy volunteers. The study consists of two separate experiments (PUK-GHB01 and PUK-GHB02), including 32 participants in PUK-GHB01 and 20 participants in PUK-GHB02. Social cognition, brain activity and neuroendocrinology will be examined in the first, sleep architecture, pharmacokinetics, putative depression and sleep biomarkers, cognition, memory consolidation, as well as GHB-, GABA-, and Glu-MRS and resting state fMRI/EEG in the second experiment.

2. Background

2.1 Study substance

Sodium oxybate is the international drug name for the sodium salt of gamma-hydroxybutyric acid (GHB). In research the terms are used synonymously. GHB is merchandized as Xyrem® in the U.S., Canada and Europe (incl. Switzerland) by Jazz Pharmaceuticals, Valeant Pharmaceuticals International and UCB-Pharma. In these countries it is the first line treatment for excessive daytime sleepiness and cataplexy in patients with narcolepsy. GHB is approved in Germany as an anesthetic, Somsanit® (Dr. F. Köhler Chemie) and is approved in Austria and Italy for the treatment of opioid and alcohol withdrawal as Alcover® (Laboratorio Farmaceutico). In Switzerland, GHB is a controlled substance (category A+). In this study Xyrem® oral solution will serve as source for GHB, which is used as a model substance for a combined $GABA_B$-/GHB receptor stimulation.

2.2 Dosing

The dosing used in our two studies will be lower than the common therapeutic dosing of GHB. The usual starting dose for the treatment of narcolepsy is about 4.5 g/night (about 65 mg/kg), split into 2 equal doses: one immediately before going to bed, the second 2.5-4 hours later. The dose can be increased up to a maximum of 9 g/night (about 130 mg/kg). The proper dose has to be found individually under supervision of an experienced physician. Once established, the individual dose is taken nightly as long as the disorder persists (Compendium, 2010). In the treatment of alcohol withdrawal syndrome (AWS) and prevention of relapses, GHB is given during daytime. The usual application is a daily total of 3.5-7 g (about 50-100 mg/kg) in 3 divided doses (Boscolo-Berto et al., 2012).

2.2.1 PUK-GHB01: social cognition and hormones

In the first two sessions of the first trial, we will give either 20 mg/kg (corresponding to 1.4 g in a 70 kg subject, n=16) and 35 mg/kg (corresponding to 2.45 g in a 70 kg subject, n=16) compared to placebo, to test dose-dependent effects of GHB on social cognition and neuroendocrine parameters. In the third an fourth session of the first trial, we will give 35 mg/kg (n=32) compared to placebo. These doses are low and medium, respectively, compared to the therapeutic doses used in narcolepsy. In other studies concerning subjective or cognitive effects of GHB, doses such as 10 mg/kg (Grove-White and Kelman, 1971), 21 mg/kg (Delay et al., 1965), 15 mg/kg vs. 30 mg/kg (Rosen et al., 1997), 16-64 mg/kg (Carter et al., 2009a) up to 40-72 mg/kg (Abanades et al., 2006) were used. The dose selected for our first trial is promising in mediating

significant subjective and cognitive alterations, without causing too many unwanted side effects such as dizziness, nausea and somnolence.

2.2.2 PUK-GHB02: sleep and memory

In the second trial, we will give 50 mg/kg GHB compared to placebo, to test the effects of the drug on sleep architecture, the electroencephalogram (EEG) in sleep and wakefulness, neuroendocrine parameters, pharmacokinetics, depression and sleep biomarkers, cognition, and memory consolidation during sessions 1 and 2. We will give the same dose vs. placebo to assess the concentrations of GHB, GABA and Glu in sleep- and depression-related brain areas using MRS/EEG and resting state connectivity using fMRI/EEG during sessions 3 and 4 (see figure 1). This dosage is used commonly in the treatment of narcolepsy. In comparable studies concerning GHB-mediated effects on sleep, doses such as 1 x 35-50 mg/kg (Van Cauter et al., 1997), 1 and 2 x 64 mg/kg (Rosen et al., 1997), 2 x 42 mg/kg (Scharf et al., 2003) and 2 x 42-64 mg/kg (Ondo et al., 2008) were used. The dose selected for our second trial is common both in therapeutic trials and scientific studies and is promising in mediating significant effects on sleep architecture, the EEG, pharmacokinetics, depression and sleep biomarkers, cognition, neuroendocrine parameters and memory consolidation. Before inclusion in the study, all subjects will be screened regarding the presence of specific sleep disorders (e.g. sleep-related breathing disorders) by using polysomnography.

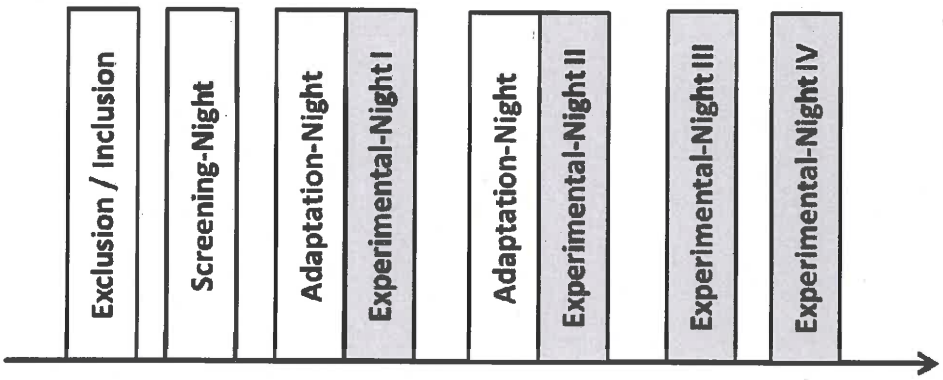

Figure 1: Basic Flow Chart of PUK-GHB02

2.3 Side effects

Typical side effects (10-20% of the treated patients) of GHB are dizziness, nausea and headaches. Common side effects (≥1/100 to <1/10) are anorexia, abnormal dreaming, drowsiness, disorientation, somnambulism, depression, sleep disturbances, cataplexy, fear, insomnia, nervousness, somnolence, tremor, imbalance, falls, attention deficits, hypesthesia, paresthesia, sedation, blurred vision, arterial hypertonia, dyspnea, snoring, emesis, gastric pain, diarrhea, sweating, muscle cramps, arthralgia, enuresis, asthenia, sleepiness, peripheral edema. Occasional side effects (≥1/1000 to <1/100) are weight loss, psychosis, paranoia, abnormal thinking, hallucinations, agitation, suicide attempt, sleep disorder, myoklonus, amnesia, restless leg syndrome, eczema (Compendium, 2010).

2.4 Toxicity

The toxic effects of GHB are dose-dependent: low doses (10 mg/kg) induce euphoria, increased libido, and disinhibition, while doses of 30-50 mg/kg lead to sleep and general anesthesia (Wong et al., 2004). The median lethal dose (LD_{50}) of GHB in rats is 1.7 g/kg (Snead, 2002). In humans, massive overdoses and the combination of GHB with CNS depressants such as alcohol are associated with serious coma necessitating

intubation and intensive care, the number of reported fatal cases associated with the drug appears very limited. Post-marketing surveillance indicates GHB has an acceptable safety profile for controlled clinical application (Wedin et al., 2006).

3 Aims of the project

3.1.1 PUK-GHB01: social cognition and hormones

The GABAergic system is the principal mediator of neuronal inhibition in the brain. Dysfunction of GABA neurotransmission has been purposed to play a key role in psychiatric disorders, including anxiety and depression. While the link between GABA and anxiety is clearly established, the importance of GABAergic dysfunction in the etiology and course of depression remains unclear. Animal studies showed that alterations of $GABA_B$ receptor function cause antidepressant-like behavior (Cryan and Slattery, 2010). Some human studies indicate a beneficial effect of the $GABA_B$ agonist baclofen on depression symptoms (Krupitsky et al., 1993). Baclofen is further known to impair cognition by its sedative effect and to increase concentrations of both growth hormone (Cavagnini et al., 1977) and cortisol (Morosini et al., 1985). GHB is an endogenous short-fatty acid and GABA metabolite found in the human brain (Bessman and Fishbein, 1963). Despite its high affinity to GHB receptors, most of its neuronal and behavioral effects are mediated by an agonist action at $GABA_B$ receptors (Engberg and Nissbrandt, 1993). Presumably, exogenous administered supraphysiological concentrations of GHB produce qualitatively different neuronal actions than those produced by endogenous GHB concentrations. In high concentrations GHB might act on the high affinity GHB receptor and on the $GABA_B$ receptor, as the neuronal and behavioral effects of GHB and the specific $GABA_B$ agonist baclofen can be differentiated experimentally (Wong et al., 2004). GHB was reported to increase dopamine and norepinephrine levels in a biphasic mode (Maitre, 1997). A certain concern regarding the use of GHB for research purposes is its abuse and addictive potential. The drug is abused by a small percentage of the population (<1%) as a recreational drug in a home setting or as a "club drug" (Sumnall et al., 2008). The development of addiction in patients treated with GHB is rare (0.015 %) compared to illicit use (4-21 %) (Carter et al., 2009b). The subjective effects of GHB constitute a continuum between relaxation, prosocial and prosexual stimulation in lower doses (10 mg/kg) up to profound sedation and sleep-induction in higher doses (50 mg/kg) (Bosch et al., 2012). GHB-mediated effects seem to be associated with neuroendocrine alterations, which are mostly unknown in detail. An increase of growth hormone release is the most consistent finding (Van Cauter et al., 1997). The effects of GHB on the hypothalamic-pituitary-adrenal (HPA) axis were examined in several trials, but the results remain controversial. Plasma levels of cortisol and ACTH after GHB challenge indicated an activation of the HPA axis in the resting state, compared to an HPA axis inhibition under stress conditions (Nava et al., 2007). Neurosteroids and oxytocin are candidates of primary interest for the endocrine mediation of prosocial and prosexual behavior as suggested by animal studies (Baskerville et al., 2009, Frye et al., 2000). In humans, the prosocial effects of methylenedioxymethamphetamine (MDMA, "ecstasy"), which phenomenological resemble to GHB-effects, correlated with a rise in plasma oxytocin levels (Dumont et al., 2009). Therefore, we propose to investigate the effects of a combined $GABA_B$-/GHB receptor stimulation on the relations of social cognition and neuroendocrine parameters compared to placebo in 32 healthy subjects. GHB will serve as a model substance for the combined $GABA_B$-/GHB receptor stimulation. The first experiment will focus on the acute effects of GHB in waking participants, assessing correlating changes in cognitive capacities, brain activity and endocrine blood parameters. We hope to gain new insights into the neurobiological underpinnings of prosocial and prosexual experience and behavior by these means. As anhedonia, reduced social interaction and loss of libido are key symptoms of depression, we aim to investigate the potential relevance of a combined $GABA_B$-/GHB receptor stimulation for these psychobiological targets.

3.1.2 PUK-GHB02: sleep and memory

The GABAergic system is strongly involved in the regulation, maintenance and termination of sleep. Benzodiazepines, which mainly exert their effects via $GABA_A$ receptor activation, have sedating and sleep-inducing effects, but clearly interfere with physiological sleep architecture. Although the restorative function of sleep seems intuitively obvious, some researchers follow the hypothesis that a primary function of sleep pertains to the consolidation of memory (Born and Wagner, 2009). Encoding and retention of information are understood as complementary processes which need distinct neuronal activation pattern for their realization. Memory consolidation serves the strengthening of newly encoded, preliminary memory traces. Recent studies could demonstrate that sleep after learning enhances retention not only of declarative memory (Gais and Born, 2004), but also of emotional (Wagner et al., 2001), and procedural memories (Stickgold, 2005). Moreover, the type of memory consolidation is dependent on specific sleep stages. It was shown that retention of hippocampus-dependent declarative memory benefits mainly from SWS-dominated early nocturnal sleep, whereas amygdala-dependent emotional memories and also procedural memories improve from REM sleep during the late night in the first place (Gais and Born, 2004). Brain stimulation that enhanced SWS oscillations was found to enhance selectively the retention of declarative memories in humans (Marshall et al., 2006). A previous study has shown that GHB is a robust enhancer of slow wave sleep (SWS) in patients with sleep disorders and in healthy subjects (Van Cauter et al., 1997). A deteriorative effect of GHB on memory encoding was shown in humans (Carter et al., 2009a, Grove-White and Kelman, 1971). Given, that encoding and consolidation of information are functional counterparts and that GHB reliably induces SWS, an enhancement of declarative memory consolidation under the influence of GHB seems convincing. Impaired sleep is a key symptom of depression, and brain resting state network alterations offer plausible mechanisms for antidepressant treatment effects related to sleep (Bosch et al., 2013). Moreover, alterations of GABA and glutamate concentrations can be found in patients with MDD (Jun et al., 2014, Chang et al., 2003, Hasler et al., 2007), potentially serving as diagnostic and therapeutic biomarkers. GHB has distinct neurobiological and clinical effects, which make it an interesting tool for the pharmacological modification of depression biomarkers (Bosch et al., 2012). This attempt is absolutely novel, as it has never been performed in humans to date. Therefore, we want to apply MRS/EEG of the anterior cingulate cortex (ACC), Nucleus accumbens (Nacc), and amygdala and assess resting state connectivity using fMRI/EEG directly after GHB-induced sleep (50 mg/kg GHB p.o. vs. placebo) and on the following morning in sessions 3+4 of experiment PUK-GHB02 (see figure 1).
Hence, we propose to investigate the effects of GHB on sleep architecture, pharmacokinetics, and putative depression- and sleep biomarkers, cognition, declarative memory, as well as GHB-, GABA-, and Glu-MRS/EEG and resting state fMRI/EEG compared to placebo in 20 healthy subjects. The proposed study will provide the unique opportunity to investigate the neurobiology of a combined $GABA_B$-/GHB receptor stimulation on the regulation of sleep and memory. Given that the interest in the neurobiology of memory and sleep and their possible correlation to plasma, saliva and cerebral changes, is constantly increasing, a better understanding of the possible neurochemical consequences of GHB application is an appealing research approach. Memory deficits and sleep disturbances are characteristic symptoms of major depressive disorder. In this study, GHB serves as a model compound for a combined $GABA_B$-/GHB receptor stimulation, which could help to develop new treatments targeting specific neurobiological alterations in affective disorders.

3.2 Open questions

3.2.1 PUK-GHB01: social cognition and hormones

Since the discovery of GHB in the year 1960, an extensive body of research has been performed concerning its neurobiological effects in animals and humans. Its potency to induce pleasurable feelings, affiliative behavior and sexual arousal led to illicit use of GHB for recreational purposes. In spite of these facts, neither the prosocial and prosexual effects of GHB, nor the underlying neuroendocrine alterations were examined scientifically so far. Various hormones were shown to be involved in the processing of social cognition,

appetitive behavior and binding. It is most likely that the neuropeptide oxytocin plays a central role for the subjective and behavioral effects of GHB, as the secretion of this hormone was shown to correlate with the prosocial effects of the phenomenologically comparable club drug MDMA (Dumont et al., 2009). Other hormones of interest are centrally synthesized progesterone analogs such as alloprenanolon (3α, 5α-THP) and allotetrahydrodeoxy-corticosterone (THDOC). These neurosteroids are released after GHB application in rats and induce prosexual behavior (Barbaccia, 2004). Additionally, the results regarding the GHB-modulation of the HPA axis remain contradictory and need further investigation. The relations of GHB-mediated subjective and behavioral changes to alterations of cerebral activity and neuroendocrine function remain unstudied.

3.2.2 PUK-GHB02: sleep and memory

As mentioned above, a complementary effect of memory encoding and consolidation and a link of these processes to the wake-sleep cycle has been postulated. Declarative memory consolidation was related to early night SWS. If GHB enhances declarative memory consolidation possibly due to an enhancement of SWS remains unknown. GABA and glutamate neurotransmission are key players both in MDD (Phillips et al., 2015, Hasler and Northoff, 2011), and sleep physiology (Ackermann and Rasch, 2014). GHB was proposed as an experimental therapeutic in depression and interesting tool for the pharmacological modulation of depression biomarkers (Bosch et al., 2012). Here, we want to add data about cerebral GABA, Glu, and GHB concentrations in the ACC, Nacc, and amygdala and resting state connectivity in depression-related networks, with regard to a potential use of GHB as biomarker modulator in depression. In summary, the complex relationships of the GHB and $GABA_B$ receptor mediated GHB effects on sleep architecture, EEG, pharmacokinetics, depression- and sleep biomarkers, cognition, memory, as well as GHB-, GABA-, and Glu-MRS/EEG and resting state fMRI/EEG pose various questions, which are addressed in the proposed study.

3.3 Working hypotheses

We expect to find significant alterations of sexual arousal, social cognition, pharmacokinetics, depression and sleep biomarkers, cerebral GHB-, GABA-, and Glu-concentrations, resting state connectivity, cognition, and declarative memory functions after GHB administration compared to placebo. Moreover, we predict that these effects are correlated with specific changes in serum biomarker levels, brain activation patterns and sleep architecture, respectively.

4 Study design

4.1 Primary outcome measures

In the first part of the study (PUK-GHB01), plasma hormone levels, subjective effects assessed by questionnaires, and social cognition parameters assessed by specific computed test batteries, as well as brain activity assessed by electroencephalography (EEG) and functional magnet resonance imaging (fMRI) will serve as primary outcome measures. The results of the neuropsychological test battery performance will serve as longitudinal safety parameters to detect unexpected cognitive impairment after GHB intake. In the second part (PUK-GHB02), plasma hormone levels, GHB levels in plasma, urine, hair, and nails, sleep biomarkers such as brain-derived neurotrophic factor (BDNF), fragile x mental retardation protein (FMRP), IL-beta, IL-6, IL-10, TNF-alpha, CRP, IL-4, IL-18 und IFN-gamma, tryptophan, kynurenin, kynureninic acid, neopterine, malondialdehyd, qinolinsäure, IDO (indolamine-2,3-deoxygenase), substance P, and genetic markers, cognition, memory consolidation assessed by procedural and declarative memory tests, polysomnographic parameters, cerebral GHB-, GABA-, and Glu-concentrations in the ACC, Nacc, and amygdala, resting state connectivity, and measures of daytime sleepiness such as the Epworth Sleepiness

Scale (ESS), as well as subjective mood measures such as the Visual Analog Mood Scale (VAMS), and the Positive and Negative Affect Scale (PANAS) will serve as primary outcome measures.

4.2 Design of the Experiments PUK-GHB01 and PUK-GHB02

4.2.1 PUK-GHB01: social cognition and hormones

Thirty-two male healthy subjects will be assessed with questionnaires (GHB Specific Questionnaire [GSQ], Sexual Arousal and Desire Questionnaire [SADI], Profile of Mood States [POMS], Visual Analog Scale [VAS]), tests of social cognition (Sexual Arousal Task [SAT], Multifaceted Empathy Test [MET], Social Gaze Test [SGT], Movie for the Assessment of Social Cognition [MASC]), neuropsychology (Cambridge Neuropsychological Automated Test Battery [CANTAB], Flanker Task, Verbal Memory Test [VLMT]), neuroeconomic tests (Reciprocity Task [RT], Social Value Orientation [SVO], Charity Donation [CD]), electroencephalography (EEG), functional magnet resonance imaging (fMRI) and neuroendocrine blood tests (oxytocin, 3,5-THP, THDOC, ACTH, prolaktin, testosterone, cortisol, progesterone, N-Arachidonoylethanolamin, 2-Arachidonoylglycerol, Palmitolethanolamid, Arachidonic acid, Linoleboylethanolamid, Oleoylethanolaid, Stearoylethanolamid, prostaglandin E2 and GHB) after application of GHB (days 1+2 20 or 35 mg/kg p.o., days 3+4 35 mg/kg p.o. vs. placebo – cross over design). The neuropsychological and neuroeconomic test as well as the tests of social cognition have already been implemented in trials of the Experimental and Clinical Pharmacopsychology research group of the Psychiatric University Hospital Zurich. The neurocognitive test will be performed on a study computer of the experimental and clinical pharmacopsychology research group of the Psychiatric University Hospital Zurich. The fMRI experiments will be performed at the MR Center of the University Hospital of Psychiatry Zurich. The assessment will follow the Good Clinical Practice (GCP) guidelines. Both neurocognitive and neuroendocrine tests will be performed paralleled, under two different conditions (20 or 35 mg/kg GHB p.o. vs. placebo; t0= GHB application) on two days spaced at least 1 week apart. Neurocognitive testing will be performed during the peak of subjective GHB effects (t+ 30 until t + 90). Blood tests for neuroendocrinologic measures will be taken six times (t-30 min, t0, t + 20 min, t + 35 min, t + 65 min, t +95 min, t + 124 min and t + 180 min). A total amount of blood of 150 ml will be taken during each experiment. The complete assessment will last about five hours. The two fMRI sessions will start at least one week after the two first experimental days, and succeed with at least one week in between. Each session takes a time of 2.5 hours. Furthermore, one pre- and one post-test session will be performed for medical check-up.
All experiments are monitored by a medical doctor. In case of any unexpected incidence threatening the subject's health or if the subject wishes to abandon the study, the procedure will be stopped immediately. All participants will provide urine for a drug screening to control for current drug use. Any drug use pattern will be assessed using the Interview for Psychotropic Drug Consumption (Quednow et al., 2004). The subjects will receive 225 CHF for participating in the first to experimental days and the pre- and post-tests. An additional amount of 75 CHF can be earned through the neuroeconomical tests. The participation in the two fMRI sessions will be compensated with additional 100 CHF.

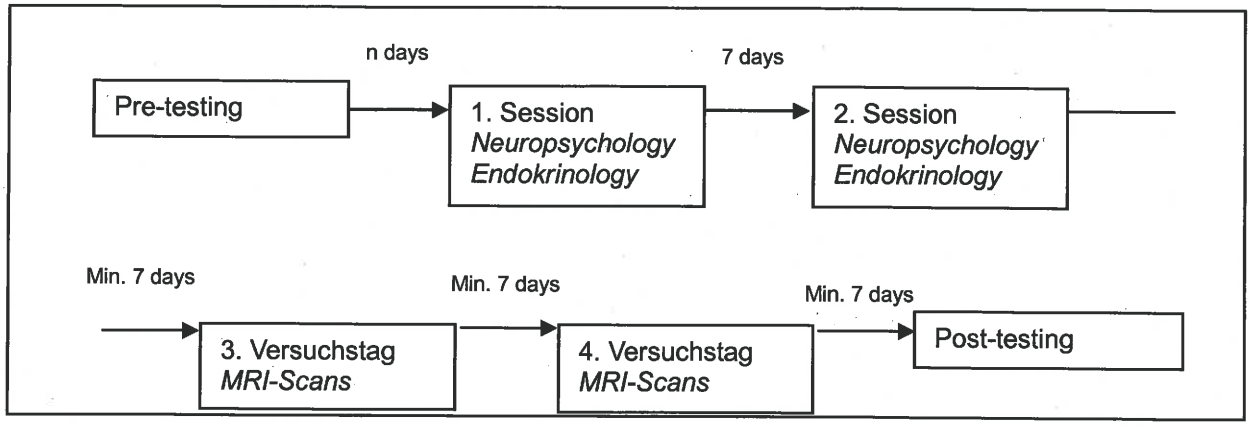

***Chart 1** Order of study sessions*

4.2.2 PUK-GHB02: sleep and memory

Following screening procedures prior to enrollment into the experiment, 20 male healthy subjects will be assessed with an actiwatch (for two weeks), questionnaires (Eigenschaftswörterliste [EWL-60], Epworth Sleepiness Scale [ESS], Alcohol Use Identification test [AUDIT], Pittsburgh Sleep Quality Index, Morning Questionnaire [MQ], Positive and Negative Affect Schedule [PANAS], Visual Analog Mood Scale [VAMS], Evening Questionnaire [EQ], Karolinska Sleepiness Scale [KSS], Beck Depression Inventory [BDI], Persönlichkeitsfragebogen, State-Trait Anxiety Inventory [STAI], Mehrfach-Wortschatz-Intelligenztest [MWT-B], Symptomcheckliste 90 [SCL-90]), cognitive tests (Random Number Generation Task [RNG], IOWA Gambling Task [IGT], Psychomotor Vigilance Task [PVT], Delay Discounting Task [DDT]), tests of procedural and declarative memory consolidation (Learning of Texts Task [LTT], Two-Dimensional Object-Location Memory Task [2D-OLMT], Walker Finger Sequence-Tapping Task [FSTT], Paired Associate Word List [PAWL]), polysomnography (electroencephalography [EEG], electromyography [EMG] and pulse oxymetry), magnetic resonance spectroscopy with electroencephalography (MRS/EEG), functional magnetic resonance imaging with electroencephalography (fMRI-EEG), saliva cortisol, and blood tests (IL-beta, IL-6, IL-10, TNF-alpha, CRP, IL-4, IL-18 und IFN-gamma, tryptophan, kynurenin, kynureninic acid, neopterine, malondialdehyd, qinolinsäure, IDO (indolamine-2,3-deoxygenase), substance P , cortisol, progesterone, BDNF, FMRP, genetic markers, prolaktin) after two applications of GHB (50 mg/kg p.o. vs. placebo – cross over design). Each subject will pass through 7 study nights: one screening night, two adaptation nights and four experimental nights (GHB vs. placebo) (see chart 1). The screening night will take place before the first experimental session. The two first experimental nights will be preceded by one adaptation night. The experimental nights 1+2 will take place in the sleep laboratory of the University of Zurich in collaboration with Prof. Landolt. The experimental nights 3+4 will take place in the magnetic resonance imaging center (MRZ) of the Psychiatric Hospital of the University of Zurich. The assessment will follow the Good Clinical Practice (GCP) guidelines. Memory testing consists of two steps. First, learning tasks are performed prior to sleep. Second, recalling tasks are performed one hour after awakening. Blood tests for neuroendocrinologic, pharmacokinetic, and depression and sleep biomarker measures will be taken 18 times in total (adaptation nights 6x, experimental nights 6x, post-experimental-night evenings 2x, MRS-fMRI/EEG-nights 4x). Further pharmacokinetic samples will be taken as follows: five hair samples (T0: before testing, T1: days post-experimental night I, T2: seven days post-experimental night I, T3: two days post-experimental night II, T4: six weeks post-experimental night I, T5: before experimental night III, T6: the day after experimental night

III, T7: the day after experimental night IV), seven sweat samples (one sweat patch per night), four urine samples (after every adaptation and experimental night), nine nail samples (weekly nail clipping). A total amount of blood of 250 ml will be taken during the whole experiment GHB02. The experiment is monitored and all blood drawings are conducted by a medical professional or study nurse. In case of any unexpected incidence threatening the subject's health or if the subject wishes to abandon the study, the procedure will be stopped immediately. All participants will provide urine for a drug screening and absolve exhalative alcohol testing to control for current alcohol and drug use. Any drug use pattern will be assessed using the Interview for Psychotropic Drug Consumption (Quednow et al., 2004). Screening of all subjects includes a daytime screening examination and screening-night in the sleep laboratory, to exclude the inability to sleep in an unknown environment and the presence of specific sleep disorders (e.g. sleep-related breathing disorders, leg movements during sleep). Subjects will receive 800 CHF for participating in the study, plus additional 100 CHF for the hair samples on T4, and another optional 50 CHF for the nail samples.

4.3 Neurocognition, Multiple Sleep Latency and Brain Activity Assessments

4.3.1 Neuropsychological testing

The Cambridge Neuropsychological Test Automated Battery (CANTAB) is a classical neuropsychological test battery measuring visual memory, attention, working memory, executive function and planning. The test batteries begin with simple tests, progressing to more complex tests with repeated test elements. The tests are administered using a computer with a touch-sensitive screen and a tool for reaction timing. CANTAB has been proposed for the assessment of neurotoxic effects (Fray and Robbins, 1996). These tasks are already available in our laboratory and will be applied according to standard procedures.

4.3.2 Tests of sexual arousal and social cognition

The Sexual Arousal Task (SAT), the Multifaceted Empathy Test (MET), the Movie for the Assessment of Social Cognition (MASC) and the Social Gaze Test (SGT) will be used to measure sexual arousal, emotion perception, empathy, and joined attention.
The Sexual Arousal Task (SAT) was designed to investigate the sexual arousal state of the participants. It is a based on 15 images from the International Affective Picture System (IAPS), comprising neutral objects, neutral female portraits, neutral couple pictures and explicitly erotic single and couple pictures. In the first part of the task, subjects are shown these pictures in a randomized order, whereas the short presentation time (1 s per image) can be elongated by mouse clicking in a high frequency, if the participants like the picture. This "effort condition", allows measuring difference in the effort of the subjects to see pictures with different degree of arousal content. In the second part of the task, the subjects are asked to rate how "pleasant", "erotic", "attractive", "likeable" and "exciting" they felt about the picture.
The Multifaceted Empathy Test is a photo-based measure to assess empathy in a multidimensional way. It is designed to measure cognitive and emotional empathy simultaneously. The MET consists of a series of photographs, showing people in emotionally charged situations. To assess cognitive empathy, subjects are asked to determine the mental states of the individuals depicted in the photographs. Emotional empathy is assessed by measuring the emotional reactions in response to the pictures. In the Social Gaze Test (SGT), participants are gazed by virtual characters or observe them looking at someone else. In dynamic animations, virtual characters then exhibit socially relevant facial expressions or arbitrary facial movements. Joined attention can be measured by duration of eye contact and interaction with the virtual character. It was shown that the perception of socially relevant facial expressions selectively activates the ventral MPFC. These tasks are already available in our laboratory and will be applied according to standard procedures.

4.3.3 Neuroeconomical testing

We will perform the Social Value Orientation (SVO), Charity Donation (CD) and Reciprocity Task (RT) to assess changes in neuroeconomical reactions under the influence of the drug.
The Reciprocity Task is a monetary distribution task, which is used to investigate the social behavior between two participants. Out of time and infrastructural reason, it is not possible to conduct an interaction between various subjects. That's why a cover story had to be used to create a reliable social interaction condition. So, the subjects are told that they were interacting with other study participants, whereas they interacted just with virtual participants.
The task consists of five interactions with five virtual participants. In each interaction the subject is asked to distribute a value between 0 - 8.- CHF between himself and the opposite (virtual) participant. This task allows to a certain extent to measure the degree of altruism in a monetary distribution situation.
In the Charity Donation Task, participants are given the possibility to donate a value between 0-40.- CHF of their own monetary participant compensation to one of ten listed charity organizations. After that the subjects are asked to rate how pleased they felt about their donation. The participants are further informed that only one of both donations of the two main testing sessions would randomly be chosen to be paid out.
The Social Value Orientation (SVO) is another monetary distribution test, which is used to characterize the social behavior of a subject. It allows assigning the subjects to either an altruistic, prosocial, individualistic or competitive category.
We use a SVO version composed of 15 different decision scales. The subjects are instructed to choose for each scale the preferred monetary distribution. They are further told that their decision would directly affect their one final monetary participant compensation (You receive) and the one of the next participant of the study (other receives).

4.3.4 Cognitive and memory testing

Six tasks are used in our study for the assessment of the effects of GHB on nocturnal memory consolidation. The Paired-Associate Word List (PAWL) consists of 46 pairs of nouns, which are presented sequentially on a computer screen. Testing occurs through a cued-recall procedure one time immediately after learning and one time after nocturnal sleep. Memory function is determined by the percentage of recalled matching words (Marshall et al., 2006). The Walker Finger Sequence-Tapping Task (FSTT) is a test of procedural memory, requiring to repeatedly finger-tap with the non-dominant left hand a 5-element sequence presented on a computer monitor. A training period with twelve 30-s intervals is performed anterior to the sleep period. Retrieval testing after sleep consists of a practice run followed by three 30-s test intervals. Performance is determined by the number of correctly completed sequences/30-s Interval (Walker et al., 2002). Moreover, we will implement the following tasks: the Random Generation Task (RNG) assesses executive functions such as inhibition, updating, and monitoring by requiring the participant to randomly name numbers between 1-10 over a defined period of time (Towse and Neil, 1998). In the Iowa Gambling Task (IGT), participants make a series of choices from four decks of cards and receive a reward and/or a loss. "Bad" decks have large rewards but periodically large losses while "good" decks have small but steady rewards and lead to higher payouts overall. Participants must learn the magnitude and probability of rewards from accumulating experience over trials, and over time many healthy participants learn to favor the good decks. Poor performance on this task, on the other hand, is commonly linked to a failure to properly weigh the long-term consequences of choices, such that large losses are neglected in favor of chasing large rewards (Bechara et al., 1994). The Psychomotor Vigilance Task (PVT) is a 10-min lasting test, where subjects have to response to the appearance of a stimulus on a computer screen with pressing a button as fast as possible. It is widely used to assess neurocognitive consequences of sleep loss and circadian misalignment (Basner et al., 2015). The Delay Discounting Task is a paper and pencil test which assesses if subjects tend to choose less reward immediately or higher

reward after a time delay, and it is used to assess impulsive behavior and is sensitive towards sleep deprivation (Kirby et al., 1999).

4.3.5 Electroencephalography (EEG) and Flanker Task

Electroencephalogram (EEG) recordings are performed using Bio-Semi (Amsterdam, The Netherlands) ActiveTwo electrode system with 64 scalp electrodes. Additional single active electrodes are attached on the outer canthus of each eye to record the horizontal electrooculogram (EOG) and infraorbitally and supraorbitally to the left eye to record the vertical EOG. Electrophysiologic signals are bandpass filtered at .01 to 67.0 Hz and digitized at 512 Hz.
In a modified version of the Erikson Flanker task subjects are required to respond as fast and accurate as possible according to the direction pointed by a centrally presented arrow with either the index finger of the left hand (<) or the right hand (>). Two additional arrows flanking the target arrow on the left as well as the right side either favored the target response (congruent trials, <<<<< or >>>>>) or primed the other response (incongruent trials, >><>> or >>>>>). The stimuli are presented on a black background for 650 ms. In order to assure a constant error rate throughout participants, the difficulty level during the task was programmed in a dynamical way. If an error is committed, the presentation time of the following stimulus will be prolonged by 20 ms. If there is a correct trial, presentation time will be shortened by 20 ms for the next trial. This should assure sufficient error trials and therefore ERNs for further evaluation. Furthermore, a feedback about the correctness of the response is given 3 seconds after response. In the case of a correct response a smiling face appears on the screen, if response is incorrect a sad face appears and if response is to slow, a question mark appears. In total 240 trials are presented randomized during 20 min. To counterbalance sequential effects each of the four stimuli follows every other stimulus with equal probability. To increase time-pressure, subjects are informed, that the absence of response will count as error and that they should response as fast and accurate as possible.

4.3.6 Functional Magnetic Resonance Imaging (fMRI)

Functional Magnetic Resonance Imaging (fMRI) will be performed using the Philips Achieva 3.0 TX MR-System in the MR-Center of the University Hospital of Psychiatry Zurich. For functional imaging (fMRI), a T2*-weghted single-shot echoplanar sequence will be used in combination with an 8-channel head array (TE = 35 ms, TR = 3000 ms (θ = 82°), FOV = 220 mm, matrix = 80x80 reconstructed on 128 x128, voxel size: 2.75 x 2.75 x 4 mm, SENSE acceleration factor R = 2.0). Intrinsic functional connectivity at rest (Resting State [rs] –fMRI, 10 min), brain perfusion (Arterial Spin Labeling [ASL], 5 min) and brain anatomy (3D T1-weighted scan, 5 min) will be performed at baseline, followed by a post-Challenge Scan (rsfMRI/ASL: 12-15 min), an fMRI-Scan with the Sexual Arousal Task using the program Presentation® (Neurobehavioral Systems Neurobehavioral Systems, Inc. Albany, USA; 10-20 min) and a post-Task Scan (rsfMRI/ASL: 12-15 min). In the the experimental nights 3+4 of PUK-GHB02, we will perform resting state fMRI/EEG scans.

4.3.7 Magnetic Resonance Spectroscopy (MRS)

A 2D J-resolved point-resolved MRS (JPRESS) will be used to investigate GHB induced metabolite alterations in the ACC. The method allows direct assessment of up to 18 brain metabolites (Fuchs et al., 2014) including myo-inositol, Glu, glutamine as well as Glu and GABA, as we and our associates have previously shown (Hulka et al., 2014, Walter et al., 2009, Northoff et al., 2007). Additionally, we are establishing a method to assess local GHB concentrations, which will be correlated with GHB plasma levels. Because of the smaller extension of the NAcc and the amygdala non-water suppressed MRS via the metabolite cycling technique (Hock et al., 2013) will be used. This method is optimally applicable for the metabolic profiling of the neurocircuitry of mood as shown for measurements of small brain volumes including NAcc and amygdala

(Hock et al., 2014b). In addition, 1D navigator acquisitions interleaved with MRS measurements will be used for real-time subject-motion detection, which has been shown feasible in MRS of human spinal cord (Hock et al., 2013), liver (Hock et al., 2014a) and heart muscle (Hock et al., 2015). Moreover, additional water scans will be acquired allowing absolute quantification by means of methods like ERETIC (Heinzer-Schweizer et al., 2010) which has been recently implemented for absolute metabolite determination using small MRS voxels (Zoelch et al., 2014).
MRS data will be acquired on our 3 T MR scanner equipped with related hardware as described above. Following a high resolution structural T1 weighted scan (acquisition time ca. 5 min) JPRESS measurements with acquisition parameters similar to (Hulka et al., 2014), of the ACC and non-water suppressed MRS of the NAcc and the amygdala similar to (Hock et al., 2014b) will be performed including navigator subject motion detection and absolute quantification. For MRS data quantification, Profit2 (Fuchs et al., 2014) JPRESS and LCModel, Version 6.3-1J [(Provencher, 1993), non-water suppressed MRS) will be used. After visual inspection of the acquired MRS data sets and evaluation of the navigator acquisitions to exclude data sets distorted by subject motion, Cramér-Rao lower bounds will be used to identify reliably quantified metabolites. In addition, signal to noise and line shape of the unsuppressed water peak will be used as quality criteria. All demographic, clinical, neuropsychological and MRS data, as well as correlations will be analyzed using SPSS® 22. We will use a combined MRS/EEG set up.

4.4 Anonymization

Anonymization will occur by means of a study code (see table 1, e.g. 112103: subject 11, experiment 2, experimental day 1, blood sample 3). Codes can be decoded through a list, which will be kept inaccessible for any other person than the principal- and the co-investigators.

Number	**Significance**
1 and 2	Study subject 01-32
3	Experiment 1 to 2
4	Experimental day 1 to 4
5 and 6	for all samples: chronological sample number 01-xx for all others: 00

Table 1 *Description of the study code*

4.5 Blood and other biosamples

4.5.1 Procedure

Experiment I: Every study subject takes part in at least two experimental sessions (GHB vs. placebo). An amount of blood of 150 ml (6 samples) will be taken by the principal investigator through an intravenous catheter during each session. For both sessions, a total amount of blood of 300 ml will be taken.

Experiment II: Every study subject takes part in four experimental sessions (2 x GHB vs. placebo), plus one preceding screening-night and one adaptation night anterior to each experimental night. A total amount of blood of 250 ml (18 samples, see 6.2) will be taken by a medical professional or a study nurse through an intravenous catheter during each session. Each sample will be tested for the quantities of the plasma markers IL-beta, IL-6, IL-10, TNF-alpha, CRP, IL-4, IL-18 und IFN-gamma, tryptophan, kynurenin, kynureninic

acid, neopterine, malondialdehyd, qinolinsäure, IDO (indolamine-2,3-deoxygenase) and substance P cortisol, progesterone, GHB, BDNF, FMRP, genetic markers, and prolactin. Moreover, other biosamples will be taken as follows: five hair samples (T0: before testing, T1: 2 days post-experimental night I, T2: seven days post-experimental night I, T3: two days post-experimental night II, T4: six weeks post-experimental night I, T5: before experimental night III, T6: the day after experimental night III, T7: the day after experimental night III), seven sweat sweat samples (one sweat patch per night), four urine samples (after every adaptation and experimental night), nine nail samples (weekly nail clipping).

4.5.2 Anonymization of biosamples

Anonymization of the all samples will occur as described in 4.4. The two last numbers of the code signify the chronological sample number (01-xx).

4.5.3. Blood and other biosample analyses and conservation

The quantitative analyses of the samples for oxytocin, and ACTH will be made in the Laboratory for Biological and Personality Psychology of the Department of Psychology of the University of Freiburg (Stefan-Meier-Straße 8 - 79104 Freiburg i. Br. - Germany). This happens in cooperation with and under supervision of Prof. M. Heinrichs, PhD.

The quantitative analyses of the samples for GHB, testosterone, prolactin, cortisol, progesterone, 3,5-THP und THDOC, the endocannabinoids and GHB will be made at the Institute for Biochemistry and Molecular medicine of the University of Bern (Bühlstrasse 28 - CH-3012 Bern - Switzerland). This happens in cooperation with and under supervision of Prof. J. Gertsch, PhD.

The analyses of BDNF, FMRP, IL-beta, IL-6, IL-10, TNF-alpha, CRP, IL-4, IL-18 und IFN-gamma, tryptophan, kynurenin, kynureninic acid, neopterine, malondialdehyd, qinolinsäure, IDO (indolamine-2,3-deoxygenase), substance P and genetic markers will be made at the Institute for Pharmacology and Toxicology of the University of Zurich (Winterthurerstrasse 190, CH-8057 Zürich). This happens in cooperation with and under supervision of Prof. H. Landolt, PhD.

The analyses of urine, sweat, hair, and nail samples will be made at the Institute for Forensic Medicine of the University of Zurich (Winterthurerstrasse 190, CH-8057 Zürich). This happens in cooperation with and under supervision of Prof. T. Krämer.

The samples will be conserved in the locations of the analyses until the end of the study and then destroyed.

4.6 Drug accountability

4.6.1 Documentation of substance and application information's

Drug accountability is recorded via a separate document (Anlage 8o Drug Accountability). In this document, detailed information about the study substance (incl. charge number and expiry date) as well as the abstracted, applicated and potentially retracted or lost doses per patient. Every application is signed by both the blinded person who abstracts the drug or placebo and puts it into the application-cup and the principal investigator. Presuming an average subject weight of 70 kg, a total amount of 116 900 mg GHB (= 233 ml Xyrem oral solution) will be needed for the first, and 140000 mg GHB (= 140 ml Xyrem oral solution) for the second experiment. Four bottles of 180 ml Xyrem oral solution (= 90000 mg GHB each) will be purchased for

the study.

4.6.2 Drug labels

Every charge is labeled by the manufacturer with the trade name (Xyrem) and substance name (Sodium oxybate), content in mg and ml (90000 mg in 180 ml) and the expiry date, as requested by the Swissmedic. Additionally, we will label every charge with the study charge number, the project name and the name and telephone number of the principal investigator (see Table 2).

"Nur für klinischen Versuch!"	
Chargennummer	PUK-GHB01 / PUK-GHB02
Projekt	PUK-GHB01 / PUK-GHB02
Hauptprüfer / Ansprechpartner	Prof. E. Seifritz (044 384 23 32) / Dr. O. Bosch (044 384 28 26)

Table 2 *Example for the additional study drug labels*

4.7 Bias reduction

4.7.1 Placebo

We will use a double-blind, randomized crossover design for bias reduction. The investigator, monitor, the data analysts and the study subjects will be kept unaware of the treatment assignment. A placebo will serve as comparator.

4.7.2 Blinding procedure

Blinding will occur through a clinical staff member not involved otherwise in the study. This person will randomly abstract GHB (Xyrem) or placebo (0.9% saline solution) for each subject and put it into a plastic cup with 150 ml standard orange juice using a ml-syringe. In the first experiment (SocCog) sixteen subjects will receive 20 mg/kg and sixteen subjects 35 mg/kg GHB on one occasion and placebo on the other. For the MR-sessions, the same thirty-two subjects will receive 35 mg/kg GHB in one, placebo in the other session in a double-blind, randomized manner. In the second experiment 20 subjects will receive 50 mg/kg GHB on two occasions and placebo on two others. The information about the weight of the subject is transmitted anterior to this procedure. The cup is labeled with the study-code and will be kept back until the end of the study. When the cup is filled, it will be given to the principal investigator, who hands it out to the subject. The subject drinks the whole content and returns the empty cup to the principal investigator.

4.7.3 Compliance

The compliance of the study subjects will be supervised be the investigator. As the study substance will be dosed by a third person without any contact to the investigational team, the investigator will give the study substance (or placebo) to the study subject, who will swallow it under sight. The used cup and potential residues will be collected and stored by the investigator.

5 Study populations

5.1 Subject recruitment

Subjects will be recruited by addressing psychology and medical students of the University of Zurich by

public appointments. Subject eligibility will be confirmed through a brief telephone screening interview, which will be held by the principal investigator. Inclusion and exclusion criteria will be screened briefly during this interview. After a confirmation of eligibility, potential study subjects will have a personal meeting with the principal investigator and undergo medical examination and questionnaire testing.

5.2 Inclusion criteria

We will include healthy men between 20 and 40 years for PUK-GHB01 and healthy men between 20 and 30 years for PUK-GHB02. The subjects' capability to give informed consent will be approved by a psychologist involved in the study. An informed consent form must be signed by the subject before the start of the test.

5.3 Exclusion criteria

We will exclude female subjects, persons with intake of illegal psychotropic substances more than 5 times per substance or any psychiatric axis I disorder according to DSM-V or existence of a serious somatic or neurological disease. Screening will be performed using DIA-X. To exclude current illegal substance use drug urine test will be done at the beginning of each test session.

	Inclusion	**Exclusion**
General	- the subjects capability to give informed consent must be approved by a psychologist or resident physician involved in the study - an informed consent form must be signed by the subject before the start of the test	- women - lifetime intake of illegal psychotropic substances on more than 5 occasions per substance - psychiatric axis I disorder according to DSM-IV - existence of a serious somatic or neurological disease -homosexual - smoking - medication
additional for PUK-GHB01	- men between 20 and 40 years - heterosexual	- metal implants and big tattoos
additional for PUK-GHB02	- men between 20 and 30 years	- sleep abnormalities (e.g. sleep apnea syndrome) (wakefulness after sleep onset > 20 %, apnea/hypopnea index > 5/hour of sleep, number of periodic leg movements > 5/hour of sleep) - metal implants and big tattoos

Table 3 *Inclusion and exclusion criteria of subjects*

6 Assessments of Effects

6.1 Outcome measures GHB01

Experiment	Method	Time (related to GHB application)
PUK-GHB01		
days	Cambridge neuropsychological automated test	t –40 min

1+2	battery (CANTAB) 1	
	GHB specific questionnaire (GSQ)	t –17, +38, +66, +106, +138 min, t+ 180 min
	Profile of Mood States (POMS)	t -13, +63, +141, +183 min
	Sexual arousal and desire questionnaire (SADI)	t -10, +51, +103, +160 min
	Visual Analog Scale (VAS)	t – 15 min, + 40, + 68, + 108, + 140, + 182 min
	Sexual Arousal Task	t + 41 min
	Movie for the Assessment of Social Cognition (MASC)	t +109 min
	Multifaceted empathy test (MET)	t +74 min
	Social Value Orientation (SVO)	t +90 min
	Charity Donation (CD)	t +69 min
	Reciprocity Task (RT)	t +163 min
	Social gaze test (SGT)	t +143 min
	Blood tests for endocrinology (BS-endo)	t-30 min, t0, t + 20 min, t + 35 min, t + 65 min, t +95 min, t + 124 min and t + 180 min
	CANTAB 2	t >24 h
days 3+4	Baseline-Scan	t – 30 min
	Post-Challenge Scan	t + 40-55 min
	SAT Task-related Scan	t + 60-80 min
	Post-Task Scan	t + 85-100 min

Table 4 *Outcome measures GHB01: methods and time of testing*

6.2 Outcome measures/Flow Chart GHB02

	Prescreening	
Time point	**Task**	**Time**
	Telefoninterview	
	Zusenden des Fragebogenlinks und der Probandeninformation	
	Persönlichkeitsfragebogen	30-45 min
	Epworth Sleepiness Scale	
	Pittsburgh Sleep Quality Index	
	STAI-X_2 (Trait)	
	BDI	
	SCL-90	
	MWT-B	
	Fragebogen ausgefüllt?	
	Probandenliste ergänzen	

	Screening Night	

Time point	Task	Time
20:00	Empfang	15 min
	Einverständniserklärung	5 min
	Urine drug test	5 min
	Physical Examination	15 min
	Psychiatr. Interview (SKID-I Screening)	10 min
	Alcohol use identification test (AUDIT)	5 min
	Quednow Drug Interview	20 min
22:00	EEG/PSG installation	60 min
	Nail sample 1	
	Saliva_scree	
	Abend-Toilette	20 min
23:00	EEG/PSG connection (IMP)	
23:40	Subject in bed	
23:45	Karolinska Drowsiness Test	5 min
23:50	Waking EEG	10 min
00:00	**SLEEP**	420 min
08:00	CAR Saliva 1	
08:10	Karolinska Drowsiness Test	5 min
08:15	CAR Saliva 2	
08:15	Morning questionnaire (MQ)	5 min
08:20	Waking EEG	10 min
08:30	CAR Saliva 3	
08:30	EEG/PSG deconnection	10 min
	Morgen-Toilette (ohne Duschen)	10 min
08:45	CAR Saliva 4	
08:50	STAI-X1	5 min
08:55	EWL-60	15 min
09:10	Duschen etc.	
09:30	CAR Saliva 5	
	End of Session	

	Adaptation Night 1	
Time point	**Task**	**Time**
21:30	Empfang	15 min
21:45	Blood sample 1 (Venflon; EDTA)	10 min
21:55	Drug/Alcohol test	5 min
22:00	EEG/PSG installation	60 min
	Saliva_adap1	
22:00	Light meal (optional)	
	Sweatpatch installation	
	Hair Sample 1	
	Abend-Toilette	
23:15	EEG/PSG connection (IMP)	20 min
23:35	Subject in bed	
23:45	Blood sample 2 (Venflon; EDTA)	2 min

	Karolinska Drowsiness Test	2 min
23:50	Waking EEG	10 min
0:00	**SLEEP**	420 min
08:00	Blood sample 3 (Venflon; EDTA)	5 min
08:10	Karolinska Drowsiness Test	5 min
08:15	Morning questionnaire (MQ)	5 min
08:20	Waking EEG	10 min
08:30	EEG/PSG deconnection	10 min
	Morgen-Toilette/Urine sample 1	10 min
8:40	Random Number Generation Task (RNG)	10 min
8:50	Psychomotor Vigilance Task incl. SSS	10 min
9:00	STAI-X1	5 min
9:05	EWL-60	15 min
9:20	Duschen etc.	
	End of Session	

	Experimental Night 1	
Time point	**Task**	**Time**
19:30	**Empfang**	**15 min**
20:00	Blood sample 1 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	15 min
20:40	Drug/Alcohol test	5 min
20:45	EEG/PSG installation	60 min
	Saliva_exp1	
	Light meal (optional)	
	Sweatpatch installation	
21:45	STAI-X1, PANAS, EWL-60, EQ, VAMS	20 min
22:05	Paired Word List Task 1	40 min
22:45	Walker 1	10 min
	Abend-Toilette	
23:10	EEG/PSG connection	20 min
	Subject in bed	
23:30	***** GHB or Placebo *****	5 min
23:47	Karolinska Drowsiness Test	2 min
23:50	Waking EEG	10 min
23:55	Blood sample 2 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	5 min
00:00	SLEEP	420 min
08:00	CAR Saliva 1	
	Blood sample 3 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	10 min
08:10	Karolinska Drowsiness Test	5 min
08:15	CAR Saliva 2	
	Morning questionnaire (MQ)	5 min
08:20	Waking EEG	10 min
08:30	CAR Saliva 3	
	EEG/PSG deconnection	10 min

08:40	Morgen-Toilette/Urine sample 2	10 min
08:45	CAR Saliva 4	
08:50	Paired Word List Task 2	40 min
09:30	CAR Saliva 5	
09:35	Walker 2	10 min
09:45	IOWA Gambling Task (IGT)	20 min
10:05	Random Number Generation Task (RNG)	10 min
10:15	Psychomotor Vigilance Task incl. SSS	10 min
10:25	Delay discounting task	5 min
10:30	STAI-X1, PANAS, EWL-60, EQ, VAMS	20 min
10:50	Duschen etc.	
	End of Session	

	Post Acute Measurement 1	
Time point	**Task**	**Time**
12:00 (t12h)	PANAS, EWL-60, VAMS	
16:00 (t16h)	PANAS, EWL-60, VAMS	
19:45	Empfang	
20:00 (t24h)	PANAS, EWL-60, VAMS	
	Blood sample 4 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	
	Nail sample 2	

	Adaptation Night 2	
Time point	**Task**	**Time**
21:30	Empfang	15 min
21:45	Blood sample 1 (Venflon; EDTA)	10 min
21:55	Drug/Alcohol test	5 min
22:00	EEG/PSG installation	60 min
	Saliva_adap2	
22:00	Light meal (optional)	
	Sweatpatch installation	
	Hair Sample 1	
	Abend-Toilette	
23:15	EEG/PSG connection (IMP)	20 min
23:35	Subject in bed	
23:45	Blood sample 2 (Venflon; EDTA)	2 min
	Karolinska Drowsiness Test	2 min
23:50	Waking EEG	10 min
0:00	**SLEEP**	420 min
08:00	Blood sample 3 (Venflon; EDTA)	5 min
08:10	Karolinska Drowsiness Test	5 min
08:15	Morning questionnaire (MQ)	5 min
08:20	Waking EEG	10 min
08:30	EEG/PSG deconnection	10 min

	Morgen-Toilette/Urine sample 1	10 min
8:40	Random Number Generation Task (RNG)	10 min
8:50	Psychomotor Vigilance Task incl. SSS	10 min
9:00	STAI-X1	5 min
9:05	EWL-60	15 min
9:20	Duschen etc.	
	End of Session	

	Experimental Night 2	
Time point	**Task**	**Time**
19:30	**Empfang**	**15 min**
20:00	Blood sample 1 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	15 min
20:40	Drug/Alcohol test	5 min
20:45	EEG/PSG installation	60 min
	Saliva_exp2	
	Light meal (optional)	
	Sweatpatch installation	
21:45	STAI-X1, PANAS, EWL-60, EQ, VAMS	20 min
22:05	Paired Word List Task 1	40 min
22:45	Walker 1	10 min
	Abend-Toilette	
23:10	EEG/PSG connection	20 min
	Subject in bed	
23:30	***** GHB or Placebo *****	5 min
23:47	Karolinska Drowsiness Test	2 min
23:50	Waking EEG	10 min
23:55	Blood sample 2 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	5 min
00:00	SLEEP	420 min
08:00	CAR Saliva 1	
	Blood sample 3 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	10 min
08:10	Karolinska Drowsiness Test	5 min
08:15	CAR Saliva 2	
	Morning questionnaire (MQ)	5 min
08:20	Waking EEG	10 min
08:30	CAR Saliva 3	
	EEG/PSG deconnection	10 min
08:40	Urine sample 2	10 min
08:45	CAR Saliva 4	
08:50	Paired Word List Task 2	40 min
09:30	CAR Saliva 5	
09:35	Walker 2	10 min
09:45	IOWA Gambling Task (IGT)	20 min
10:05	Random Number Generation Task (RNG)	10 min
10:15	Psychomotor Vigilance Task incl. SSS	10 min

10:25	Delay discounting task	5 min
10:30	STAI-X1, PANAS, EWL-60, EQ, VAMS	20 min
10:50	Duschen etc.	
	End of Session	

	Post Acute Measurement 2	
Time point	**Task**	**Time**
12:00 (t12h)	PANAS, EWL-60, VAMS	
16:00 (t16h)	PANAS, EWL-60, VAMS	
19:45	Empfang	
20:00 (t24h)	PANAS, EWL-60, VAMS	
	Blood sample 4 (Venflon; 2 EDTA, Protein, Kalium-Fluorid, Heparin-Plasma, 2 PAX)	
	Nail sample 2	

Time point	**Sample**	**Time**
Screening night	Nail sample 1	
Adapt night 1	Hair sample 1	
Post akut 1	Nail sample 2	
Adapt night 2	Hair sample 2	
Post akut 2	Nail sample 3	
2 days post EN 2	Hair sample 3	
2 weeks post EN 2	Nail sample 4 (at home)	
3 weeks post EN 2	Nail sample 5 (at home)	
4 weeks post EN 2	Nail sample 6 (at home)	
5 weeks post EN 2	Hair sample 4	
	Nail sample 7	
6 weeks post EN 2	Nail sample 8 (at home)	
7 weeks post	Nail sample 9 (at home)	

EN 2		
8 weeks post EN 2	Nail sample 10 (at home)	
9 weeks post EN 2	Nail sample 11 (at home)	
10 weeks post EN 2	Nail sample 12 (at home)	
11 weeks post EN 2	Nail sample 13 (at home)	
12 weeks post EN 2	Nail sample 14 (at home)	
13 weeks post EN 2	Nail sample 15 (at home)	
14 weeks post EN 2	Nail sample 16 (at home)	

	Experimental night 3	
Zeit (geplant)	**Task**	**Time**
21:15	**Empfang**	
21:30	Drug/Alcohol test	
21:55	Blood sugar measurement	
22:00	Light meal (optional)	
22:15	MR-Fragebogen, VAS, PANAS, VAMS, EQ	
22:20	EEG/PSG installation	
	Sweatpatch 5	
23:00	**MRI Baseline**	
	Anatomisches MRI	
	MRS	
	Resting Sate fMRI	
23:30	***GHB or Placebo***	
00:00	Blood Sample 1 (Butterfly; 2 EDTA)	
00:05	**MRI Session 1**	
	Anatomisches MRI	
	MRS NAcc, ACC, Amygdala	
	Resting Sate fMRI	
01:40	Blood Sample 2 (Butterfly; 2 EDTA)	
01:45	VAS, PANAS, VAMS	
	Abend Toilette	
	EEG/PSG connection	
01:50	Karolinska Drowsiness Test	

02:00	Waking EEG	
	Sleep	
08:00	Karolinska Drowsiness Test	
08:05	Morning questionnaire	
08:10	Waking EEG	
08:20	VAS, PANAS, VAMS,	
	Hair sample 2	
	EEG deconnection	
	Morgen-Toilette	
	EEG connection	
08:30	**MRI Session 2**	
	Anatomisches MRI	
	MRS NAcc, ACC, Amygdala	
	Resting Sate fMRI	
10:10	EEG deconnection	
	End of session	

	Experimental night 4	
Zeit (geplant)	**Task**	**Time**
21:15	**Empfang**	
21:30	Drug/Alcohol test	
21:55	Blood sugar measurement	
22:00	Light meal (optional)	
22:15	MR-Fragebogen, VAS, PANAS, VAMS, EQ	
22:20	EEG/PSG installation	
	Sweatpatch 6	
23:00	**MRI Baseline**	
	Anatomisches MRI	
	MRS	
	Resting Sate fMRI	
23:30	***GHB or Placebo***	
00:00	Blood Sample 1 (Butterfly; 2 EDTA)	
00:05	**MRI Session 1**	
	Anatomisches MRI	
	MRS NAcc, ACC, Amygdala	
	Resting Sate fMRI	
01:40	Blood Sample 2 (Butterfly; 2 EDTA)	
01:45	VAS, PANAS, VAMS	
	Abend Toilette	
	EEG/PSG connection	
01:50	Karolinska Drowsiness Test	
02:00	Waking EEG	
	Sleep	

08:00	Karolinska Drowsiness Test	
08:05	Morning questionnaire	
08:10	Waking EEG	
08:20	VAS, PANAS, VAMS,	
	Hair sample 2	
	EEG deconnection	
	Morgen-Toilette	
	EEG connection	
08:30	**MRI Session 2**	
	Anatomisches MRI	
	MRS NAcc, ACC, Amygdala	
	Resting Sate fMRI	
10:10	EEG deconnection	
	End of session	

7 Safety

7.1 Safety measures

Experiment	Method	Time (related to GHB application)
PUK-GHB01 days1+2	Blood pressure/pulse (BP/P)	t -40, 0, +20, +40, +120 min
PUK-GHB02	Supervision, EEG-Measures	see Flow Chart 6.2

Table 5 *Safety measures: methods and time of testing*

7.2 Security management in case of unexpected adverse effects

Each series of experiments will be supervised by a clinical doctor. Special attention will be paid to the possible occurrence of side effects, such as disinhibition, dizziness, nausea, drowsiness and respiratory impairment. In case of unexpected adverse effects, immediate help and quick transfer to an emergency unit is guaranteed.

8 Statistics

8.1 Primary and secondary endpoints

The primary endpoint of PUK-GHB01/02 is the detection of alterations in brain activity, GHB-, GABA-, and Glu-concentrations, and blood parameters after the administration of GHB. The secondary endpoint of trial I is the correlation between serum hormone levels and neurocognitive as well as subjective measures. The secondary endpoint of trial II is the correlation of blood parameters with cognitive alterations as well as subjective measures and the correlation of cerebral GHB-, GABA-, and Glu-concentrations with results of the other experiments GHB01 and GHB02.

8.2 Number of subjects

Comparable studies to the effects of GHB and other challenges on neuroendocrine and cognitive parameters in awake and sleeping subjects (Carter et al., 2009a, Dumont et al., 2009, Grove-White and Kelman, 1971, Marshall et al., 2006) revealed effect sizes of d=0.7-1.2. With a conservative assumption of d=0.7, an α-error probability of 5%, and a power of 80% (two-tailed), we would need at least 19 subjects to detect significant differences in each experiment.

8.3 Statistical analyses

We will use repeated measurements ANOVA for all dependent variables in both experiments.

8.4 Significance level

The significance level for both experiments will be $p \leq 0.05$ (=5%).

8.5 Drop outs

Drop outs will be excluded from the study.

9 Study specific precautions and obligations

9.1 Specific precautions

For the study participants it is not allowed to use any illegal drugs during or seven days prior to the experiments. Participants should abstain from alcohol at least 48 h hours. Furthermore, it is not allowed to drive motor vehicles or to use machines subsequent to the experiments.

10 Investigators obligation

10.1 GCP and law

The study will be conducted according to the protocol, Good Clinical Practice (GCP) guidelines and the law.

10.2 Serious adverse events (SAE) and changes of protocol

Serious adverse events (SAE), serious adverse drug reactions (Serious ADR) as well as changes in the protocol will be communicated to Swissmedic and the ethical commission.

10.3 Insurance

In case of any unforeseen health problems the study participants are covered by insurance (Zürich Versicherungs-Gesellschaft Nr. 9.723.614), medical treatment will be provided free of charge. The legal venue is Zurich.

11 Ethical considerations

11.1 Risk-benefit analysis

We aim to perform a pharmacological challenge study with GHB under two circumscribed conditions (wake and sleep) with two single expositions to the substance compared to placebo for each subject in the first experiment and two single expositions in the second experiment. GHB is internationally approved for the

treatment of narcolepsy and was shown to have a favorable safety profile both for therapeutic and experimental use (Kantrowitz et al., 2009). However, concern has been raised regarding toxicity and possible dependency from GHB and the possibility of its misuse or abuse (Nicholson and Balster, 2001). The risk of acute toxicity using the herein proposed doses is extremely low, as multicenter studies and post marketing surveillance for GHB (Xyrem) suggested a very low incidence of unexpected side effects even in long term use (Wang et al., 2009). Residual toxicity of GHB is very small as endogenous and exogenous GHB is rapidly and completely converted into CO2 and H2O through the tricarboxylic acid cycle (Krebs cycle) (Maitre, 1997). Regarding the often cited concern about the potential addictive effect of GHB, animal studies showed a relatively low reinforcing effect compared to barbiturates and benzodiazepines. GHB and its precursors 1,4-butanediol (BDL) and gamma-butyrolactone (GBL) were not able to substitute for pentobarbital, methohexital, midazolam, diazepam or flumazenil in rhesus monkeys. Self-administration was lower than with these substances and the total maintained self-administration was marginally above saline levels (McMahon et al., 2003). In humans, a clear differentiation has to be done between illicit uncontrolled and medically supervised use of the substance. Cases of physical dependence have been reported in recreational illicit GHB users, but exact statistical analysis remains difficult as the estimated number of unreported non-dependent users is not known (Miotto et al., 2001, Degenhardt et al., 2002). Compared to this, reported rates of substance dependency as defined by DSM-IV criteria and physical dependency respectively, after the use of therapeutically applied GHB are one case for every 6500 patients treated and one case for every 3300 patients treated, or approximately 0.015% and 0.031% (Wang et al., 2009). These data apply to controlled daily use over periods up to 30 years. Thus, the cumulative post marketing and clinical experience indicates a very low risk of abuse/misuse of therapeutically applied GHB. Given that this study implies only a maximum of four exposures to the drug in doses, which are lower or equivalent to therapeutic doses, and that the expected results are important for the understanding of the complex relationships between hormonal, cerebral, cognitive and behavioral functions in humans, the risk-benefit analysis clearly suggests a benefit for conducting both trials.

12 Quality control

12.1 Data access

Access to the original data is guaranteed to the members of the ethical commission and the authorities, inspections may take place.

12.2 Privacy

The participants will remain anonymous; no personal identifying information will be published, passed on to authorities or elsewhere. Electronic data will be password protected; paper data will be stored in a key locker.

12.3 Monitoring

Trial monitoring will occur on-site before, during and after the trial. Dr. Lea Hulka (see page 2) will act as monitor and ensure that the trial is conducted and documented properly. A written report will be submitted to the sponsor after each trial-site visit by the monitor. The monitoring agreement consists of the monitoring requirements according to the Guidelines of Good Clinical Practice (GCP, 1996, Chapter 5.18 Pp.: 26-28; see Anlage 8m and 8n, "Monitoring-Checklisten"). The monitor has direct access to the complete source data, with exception of the subject identification list.

12.4 Case Report Forms

The Case Report Forms (CRF) of our study will contain a header, safety related modules and efficacy related modules. The header information includes key identifying information (Study number, site number, subject identification number). The safety module includes documentation of demography, adverse events, vital signs, medical history, physical exam and drug screening. The efficacy module consists mainly from baseline measurements (subjective state, hormones).

13 Publication of data

Data will be reported in peer-reviewed international scientific journals and presented at international and national congresses of psychiatry and neuropsychopharmacology.

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