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Exploring the relationships of gamma-hydroxybutyrate and sleep on metabolism, physiology, and behavior in humans

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PART I

Neurobiological and Clinical Effects of *gamma*-Hydroxybutyrate / Sodium Oxybate

CHAPTER 2

Introduction to *gamma*-Hydroxybutyrate / Sodium Oxybate: Neurobiology and Clinical Effects

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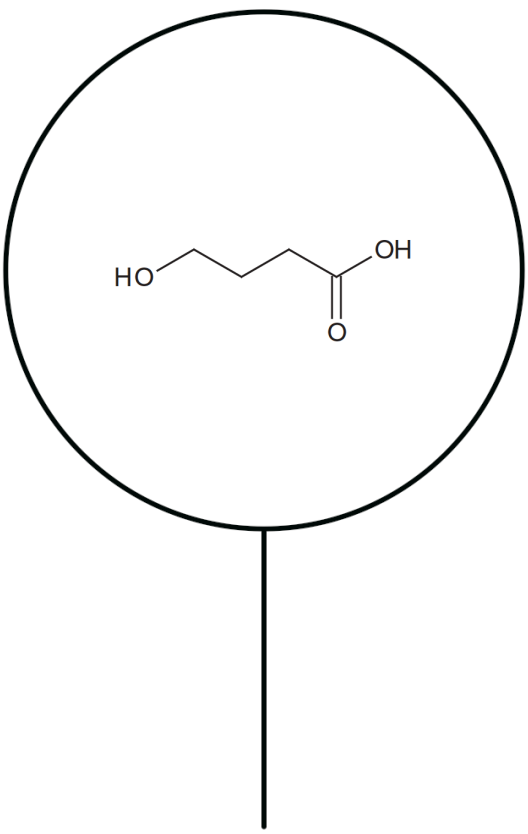
Authors: Daniel Pardi and Jed Black.



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1. Introduction to GHB / SXB

1.1 Background

Gamma-Hydroxybutyrate (GHB) is an endogenous short-chain fatty acid that is synthesized within the central nervous system (CNS)[8]. It is an active metabolite of the inhibitory neurotransmitter gamma-Aminobutyric acid (GABA)[9–11] (Figure 1) and, to a much lesser extent, of 1,4-butanediol (1,4-BD)[12] and gamma-butyrolactone (GBL)[8]. The latter two substances are also commercially produced synthetically for a wide range of industrial purposes. Sodium oxybate (SXB) is the sodium salt of GHB and is the form of GHB that is used therapeutically in humans. In this review, the term 'illicit GHB' is used to refer to illegally manufactured GHB and/or congener substances, such as industrial 1,4-BD and GBL, substances manufactured as unfit for human consumption.

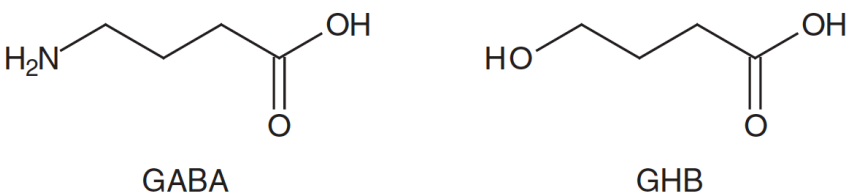


FIGURE 1. Chemical structure of gamma-hydroxybutyrate (GHB) and gamma-aminobutyric acid (GABA).

GHB was first synthesized in the early 1960s (by Henry Laborit) and was found to readily cross the blood-brain barrier into the CNS[13], where it imparts distinct pharmacological effects. Soon thereafter, it was discovered that GHB is an endogenous substance[8]. Further evidence suggested a role for GHB as a neuromodulator/neurotransmitter, as GHB is synthesized in neurons heterogeneously distributed throughout the CNS, stored in vesicles, released via potassium-dependent depolarization into the synaptic cleft, and undergoes reuptake into the nerve terminal (see section 2). Additionally, specific GHB binding sites have been identified in the CNS (see section 2). The GHB binding sites appear to share functional similarity with $GABA_B$ receptors, with both being G protein-coupled receptors.

Henry Laborit synthesized GHB in an attempt to create a GABA analogue that replicated the CNS function of GABA, but that was more slowly metabolized and that would readily cross the blood brain barrier[13]. Early work in humans demonstrated a correlation between increased blood concentrations of exogenously administered GHB and decreased levels of consciousness[14]. In 1962, the first human study of GHB was reported, in which GHB was used as a surgical anesthetic in 26 patients[15]; indeed, anesthesia became its first clinical use[16]. A remarkable feature of GHB appears to be its unique ability to induce CNS

depression with minimal effects on the respiratory and circulatory system, and without significant adverse effects[17,18]. Early research with GHB also examined its ability to induce a sleep-like state [19,20]. In 1964, Helrich *et al.*[14] induced sleep in 16 healthy study participants with intravenous administration of GHB at doses ranging from 5.9 to 9 g.

While initial evaluation of GHB in animals and humans explored its sleep-promoting or anesthesia-inducing capacity, most of the more recent human therapeutic research has focused on its use in narcolepsy. Nevertheless, other potential therapeutic effects of GHB have been explored, including: reduction of intracranial pressure[21,22]; tissue-sparing and neuroprotective effects in ischemia-induced challenges[23,24]; relief of pain, anxiety and tension during childbirth[25]; impact on anxiety conditions[26]; treatment of the alcohol withdrawal syndrome[27–34]; treatment of heroin (diamorphine) dependence[35,36]; efficacy in improving the pain, fatigue and sleep fragmentation of fibromyalgia syndrome[37,38]; antidepressant effects[39]; reduction of the negative effects of cocaine self-administration[40,41]; treatment for hyperkinetic movement disorders[42–44]; treatment of infection[45]; and hypnotic potential in healthy individuals[46] and those with insomnia[47].

As mentioned above, there has been extensive recent investigation into the usefulness of GHB (in the form of SXB) in the treatment of excessive daytime sleepiness, nocturnal sleep fragmentation and cataplexy in patients with narcolepsy. This research has established SXB as an important first-line agent in the treatment of narcolepsy. SXB (Xyrem®) was approved in July 2002 by the United States' Food and Drug Administration (FDA) as a drug for the treatment of cataplexy in patients with narcolepsy[48]. Furthermore, in November 2005, the FDA approved a label extension to include SXB as a treatment for excessive daytime sleepiness in patients with narcolepsy. These data, together with other preliminary data on the impact of SXB on sleep and wakefulness, are addressed in detail in this review, as is the neurobiology of GHB. (For a further review describing the effects of SXB in the treatment of cataplexy in narcolepsy, readers are referred to the Xyrem® International Study Group[49].)

1.2 Abuse Liability

Illicit GHB is a term used to describe multiple substances, including substances with structural similarity to GHB, which have been reported to have abuse potential when consumed in a certain fashion and, since March 2000, have been considered Schedule I controlled substances by the United States' Drug Enforcement Agency (DEA)[50]. Some of these substances have been manufactured for a variety of valid industrial and research purposes; their method of use makes them legal or illicit. It should be noted that most of these substances are not produced under good manufacturing practices necessary for human consumption, as they are intended for manufacturing purposes; thus, these substances can contain toxic impurities that may contribute to some of the negative effects imputed to illicit GHB. These substances include GHB analogues such as GBL and 1,4 BD, gamma-

hydroxyvalerate (GHV) and gamma-valerolactone (GVL). Furthermore, the various GHB analogues can have distinct toxicity and pharmacokinetic profiles from pure GHB; for example, GBL crosses the blood-brain barrier more easily and, therefore, smaller oral doses can cause much higher CNS exposure[12]. The main category of abuse of these agents relates mostly to intoxicant usage; however, their usage in sexual assault (the 'date rape drug') has received notoriety.

Physical dependence has been described with the usage of these substances. Most frequently, dependence is reported after long periods of abuse and repetitive high doses throughout the day and night[51]; some abusers report escalating their daily GHB intake to 150 g/day[52]. High frequency administration, across the day and night, appears to be a common feature to those who present with withdrawal symptoms[29]. When serious withdrawal has been induced, symptoms may be severe and take up to 12 days to resolve, usually without sequelae, but can result in death[29,51].

To examine the abuse liability of GHB in rhesus monkeys, Woolverton *et al.*[53] evaluated GHB in procedures predictive of abuse and dependence. After being trained in drug discrimination paradigms to discriminate D-amphetamine, pentobarbital or triazolam from saline, monkeys given GHB (0.01–10 mg/kg per intravenous injection) maintained self-administration marginally above saline levels. These researchers concluded that GHB has low potential for abuse. McMahon *et al.*[54] also used rhesus monkeys to evaluate the effects of 1,4 BD and GBL on various behavioral procedures that measure positive reinforcing effects and discriminative stimulus effects versus $GABA_A$ receptor modulators. These GHB analogues did not substitute for pentobarbital, midazolam or flumazenil, indicating qualitatively different effects from the abuse-related, positive reinforcing effects of $GABA_A$ receptor modulators.

Most recently, the work of Carter *et al.*[55] evaluated the relative abuse liability of GHB in 14 volunteers with a history of drug abuse. Psychomotor, subjective and cognitive effects of a broad range of GHB doses (2–18g/70kg) were compared to placebo and two abused sedative/hypnotic drugs, triazolam (0.5mg/70kg and 1mg/70kg) and pentobarbital (200mg/70kg and 400mg/70kg). This study was conducted at a residential research facility under double-blind, double-dummy conditions. Generally, on most measures of likelihood of abuse (e.g., ratings of liking the reinforcing effects), the effects of pentobarbital were significantly greater than those of triazolam, and the effects of GHB were between two reference compounds. Furthermore, GHB produced relatively less memory impairment and more negative effects such as nausea and sedation, occasionally leading to accidental overdose (defined as greater sedation than intended). In total, the authors noted that the negative effects limited the desirability of GHB for the participants.

To monitor the trends in abuse of illicit GHB (including analogues), Anderson *et al.*[56] identified cases from the California Poison Control System computerized database between 1999 and 2003. Illicit GHB exposures decreased precipitously by

76% from baseline (1999; n=426) to the final study year (2003; n=101). These data were then compared to the American Association of Poison Control Centers and Drug Abuse Warning Network (DAWN) data and drug use prevalence from the National Institute for Drug Abuse survey data. These organizations reported a parallel decreased trend to those of the California Poison Control System. These authors concluded that, nationally, a clear decrease in illicit GHB abuse case incidence is likely[56]. For comparative purposes, the absolute and relative abuse incidence of illicit GHB is further discussed. All proceeding comparisons in this paragraph are based on the DAWN quarter 3 (Q3) and quarter 4 (Q4) 2003 report. For Q3-Q4 2003, DAWN estimates 305,731 drug-related emergency department (ED) visits that involved a major substance of abuse. For this time period, drug-related ED visits were alcohol associated in 141,343 visits (46% of the total), cocaine associated in 125,921 visits (41% of the total) and marijuana associated in 79,663 visits (26% of the total), while illicit GHB (including analogues) accounted for 990 drug-related ED visits (0.3% of the total)[57].

Like many CNS depressants, illicit GHB has been implicated in a number of sexual assault cases. Over 60 chemicals have been associated with sexual assault[57] and any drug that has CNS depressant properties can be used as such. In response to this social problem, the Violence Intervention Program, Women's Hospital, University of Southern California Medical Center, Los Angeles, CA, USA, performed a study to obtain information about the relationship of alcohol and drug usage in these victims[58]. In this study, a total of 2003 women who presented to rape treatment centers throughout the U.S. allowed for their urine specimens to be analyzed via gas chromatography combined with mass spectrometry. Nearly two-thirds of the samples contained alcohol and/or drugs; a substantial subset of the specimens contained more than one substance. The predominant substances found were alcohol and marijuana, present in 63% and 30% of samples, respectively. Other substances that appeared in the collected samples included cocaine, diazepam, amphetamines, methamphetamines and others. Illicit GHB was found less frequently than the above-mentioned drugs; <3% of the positive samples contained GHB. Additionally, over the 2-year study period, illicit GHB, as a drug of sexual assault, appeared to be declining in frequency of appearance in tested samples. The author of the study concluded that the attention currently being focused on 'date-rape drugs', such as illicit GHB, is misleading the public about the drugs most commonly related to sex crimes, namely alcohol and marijuana[58].

2. Biology and Pharmacology of GHB / SXB

2.1 Distribution

In mammals, GHB is present in micromolar concentrations in all brain regions investigated, although the concentration differs by region[59,60]. The highest relative concentration is found in the striatum of humans and monkeys, reaching concentrations between ~11 and 25 µmol/L[11]. Localization of GHB within cytosolic and synaptosomal fractions[61] suggests a mechanism for presynaptic accumulation. The concentration of GHB is higher in the developing brain than in the adult brain[62].

GHB is also found in micromolar concentrations in peripheral tissues such as heart, kidney, liver and muscle, with the highest concentration found in brown adipose tissue (37 µmol/L)[63].

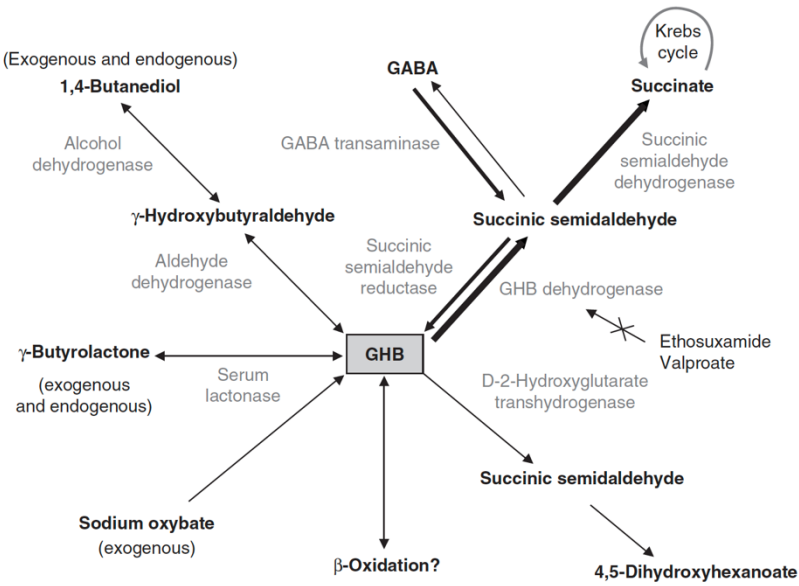


FIGURE 2. Synthesis and metabolism of gamma-hydroxybutyrate (GHB). The major pathways are shown by the thick arrows. GABA = gamma-aminobutyric acid.

2.2 Synthesis

Radiolabel studies have shown that GABA is the major precursor of GHB in the brain[9,64]. GHB formation occurs in GABAergic neurons[65] as approximately 0.05% (*in vitro*)[66] to 0.16% (*in vivo*)[64] of GABA is metabolized into GHB under normal conditions. Thus, the concentration of GHB is roughly 0.1% of the concentration of GABA[67] in the central nervous system.

The synthesis of GHB from GABA occurs mainly via the enzyme GABA transaminase, which converts GABA to succinic semialdehyde (SSA)[12,68] (see Figure 2). SSA is reduced to form GHB via the enzyme SSA reductase (SSR), an aldehyde reductase enzyme with high affinity and specificity for SSA. This enzyme is mostly present in the cytosol and, to a lesser extent, in the synaptosomal fraction[69,70]; a second catabolic pathway through a mitochondrial SSR has also been described[71], although evidence for the existence of this pathway is limited[72].

Another postulated precursor of endogenous GHB is 1,4-BD[73]. Snead *et al.*[12] showed that, in the brain, 1,4-BD is metabolized to gamma-hydroxybutyraldehyde by the enzyme alcohol dehydrogenase. Subsequently, gamma-

hydroxybutyraldehyde is metabolized via aldehyde dehydrogenase to produce GHB. It has also been observed that GBL is metabolized to GHB via serum lactonases[74]. Minor pathways for the production of GHB, including conversion from 1,4-BD and GBL, are shown in Figure 2.

2.3 Metabolism

The major route of GHB metabolism is via conversion to SSA and succinate, as shown in Figure 2. Radio-isotope studies show that intraventricularly administered GHB is metabolized rapidly into SSA[75,76]. Studies using [^{3}H]-GABA showed total brain turnover time of 26 minutes for GHB[64]. GHB is catabolized by the SSR enzyme (aldehyde reductase 1) in the presence of nicotinamide adenine dinucleotide phosphate ($NADP^+$)[77–79]; this enzyme is now known as NADP-dependent GHB dehydrogenase (GHB-DH)[80]. SSA and the co-substrate NADPH[65] are the products formed by GHB-DH from GHB and NADP+[79]. This process is also coupled to the reduction of D-glucoronate, which releases NADPH[81,82] and activates the pentose phosphate pathway (potentially more prevalent during metabolism of exogenously administration GHB than seen with endogenous levels)[83]. After GHB is converted into SSA, SSA is converted to succinate, which is eventually metabolized to CO_2 and H_2O via the tricarboxylic acid cycle (Krebs cycle).

The enzyme GHB-DH is inhibited by various antiepileptic drugs (valproate, ethosuximide, barbiturates) and certain short-chain fatty acids[84]. These compounds induce increased brain GHB concentrations by inhibiting GHB metabolism[66,84].

SSA dehydrogenase (SSADH) deficiency is a rare autosomal recessive disorder, linked to the ALDH5A1 gene on the short arm of chromosome 6. People with this condition have elevated CNS concentrations of GABA and GHB due to an inability to break down these substances. This condition has a serious impact and can result in delayed development, ataxia, hypotonia, hyporelexia, seizures, mental retardation, hyperkinesis, psychosis, myopathy, ocular abnormalities and other neurological manifestations[85].

Direct transport of GHB to the mitochondria may also be possible. A mitochondrial enzyme, GHB-oxoacid-transhydrogenase (D-2-hydroxyglutarate transhydrogenase), is capable of reducing GHB to SSA, suggesting that the mitochondria is a location of GHB metabolism[82,86,87].

In vivo experiments suggest that it is unlikely that a significant amount of GHB converts back to GABA[75,88,89]; however, *in vitro* research has shown that it is possible[89–91]. Other possible routes for GHB degradation include β-oxidation, and conversion to GBL and 1,4-BD[92] (Figure 2).

2.4 GHB as a Neurotransmitter

Several lines of evidence suggest that GHB acts as a neurotransmitter. First, studies using [^{3}H]-GHB have shown that neuronal and synaptosomal GHB is released via potassium-induced neuronal depolarization; this release is calcium dependent[93–95]. Interestingly, there is suggestion of heterogeneous GHB release within the CNS, with greater extracellular release occurring within the cortex, hippocampus and striatum, and lesser exocytotic release in caudal regions[67]. Second, there appears to be an active transport mechanism, as the existence of a high-affinity sodium- and potassium-dependent GHB uptake system has been observed in the synaptosomal membrane[96,97].

2.5 GHB Binding Site(s) in the Brain

Two GHB binding sites have been discovered, a high-affinity binding site (30–580 nmol/L, Kd1) and a low-affinity binding site (2.3–16 µmol/L, Kd2)[98,99]. Both sites are G protein linked and are of the G_i or G_0 family[100]. In the CNS, the highest density of high- and low-affinity GHB binding sites is found at the neuronal synapse[98], and [^{3}H]-GHB binding is displaced only by GHB and analogues — findings that support the existence of discrete GHB binding site(s) and the role of GHB as a neurotransmitter[96,99]. The GHB high-affinity binding site is absent from peripheral tissues such as kidney, liver, muscle or heart, although GHB is present in significant amounts in these organs[99]. GHB might function as a metabolic intermediate in the periphery[101].

Studies analyzing the location of GHB binding sites show heterogeneous CNS distribution. Maximal high-affinity binding occurs in the hippocampus, dentate gyrus, olfactory system, nucleus accumbens, septum, caudate putamen, substantia nigra, ventral tegmental area, and in the cortex (prefrontal, frontal, parietal, temporal, cingulate and entorhinal)[99,102,103]. Intermediate concentrations of GHB high-affinity binding are found in the amygdala and the thalamus. Snead and Liu[99] also showed significant binding in the pons and hypothalamus.

The distribution of GHB high-affinity binding sites in rat brain appears specific, as it does not match the distribution of $GABA_A$ or $GABA_B$ receptors[104]. Some GHB binding sites, however, have been shown to be located on cholinergic interneurons and on a population of GABAergic interneurons containing enkephalin immunoreactivity[105].

It has been demonstrated that some antipsychotics bind to GHB binding site(s). Among them is (–)-sulpiride, which, at therapeutic dosages, may have an influence on psychotic symptoms partly via an interaction with GHB binding sites[106].

2.5.1 Stimulation of GHB Binding Site(s)

Discrete activity has been associated with GHB binding site(s), as the selective GHB binding site antagonist NCS-382 has been shown to induce inhibition of calcium

conductance[107] and a decrease in nitric oxide synthase (NOS) activity[108]. Stimulation of presynaptic GHB binding sites, via exogenously administered GHB, induces neuronal cell membrane hyperpolarization via potassium extrusion or entry of chloride ions[109,110]. However, much remains unclear regarding the role of the GHB binding site(s), including the effect of endogenous GHB at its binding site(s) and the contribution of this response to the behavioral effects associated with supraphysiological brain concentrations of GHB induced by exogenous GHB administration. Furthermore, these effects are not antagonized by $GABA_B$ receptor antagonists, suggesting these effects are mediated at the GHB binding site[107].

2.5.2 Stimulation of $GABA_B$ Receptors by GHB

GHB has been shown to have selective but weak affinity for $GABA_B$ receptors, with an IC50 of 150 µmol/L[111]. This suggests that supraphysiological brain GHB concentrations, achieved by exogenous administration of GHB[95,112], are necessary for GHB to bind to $GABA_B$ receptors[99,113].

Binding of GHB to postsynaptic $GABA_B$ receptors augments potassium conductance via inwardly rectifying potassium [GIRK or Kir3] currents[111]; Kir3 channels are of particular importance in anti-nociception in animal models[114]. In Xenopus oocytes, GHB activated $GABA_B$ R1/R2 receptors, with a maximal stimulation of 69% compared with the $GABA_B$ receptor agonist (–)-baclofen. A combination of GHB and (–)-baclofen did not amplify the effect of each agent alone and did not stimulate the $GABA_B$ receptor in a linearly additive manner[115]. Cellular recordings of hippocampal or thalamocortical neurons after local application of GHB also demonstrated neuronal hyperpolarization. $GABA_B$ receptor antagonists block this GHB-induced response[116–118]. Furthermore, in the prefrontal cortex, the GHB binding site antagonist NCS-382 did not suppress these hyperpolarizations[119]. Kaupmann *et al.*[104] studied the effects of GHB in $GABA_B$ R1 –/– mice, which lack functional $GABA_B$ receptors. After GHB or GBL application, $GABA_B$ R1 –/– mice did not show the hypolocomotion, hypothermia, increase in striatal dopamine synthesis or the electroencephalogram (EEG) delta wave induction seen in wild-type mice. These authors concluded that all the effects of GHB studied were $GABA_B$ receptor dependent.

2.6 Intracellular Response to GHB

Several cellular effects in various brain regions have been observed after GHB has been applied in experimental conditions, including alterations in intracellular calcium[107], cyclic guanosine monophosphate (cGMP)[120], cyclic adenosine monophosphate (cAMP)[120], NOS[108] and increases of inositol phosphate concentrations[121]. Stimulation of GHB binding site(s) has been shown to cause an increase of up to 123% in the concentration of cGMP in the hippocampus 45 minutes after intraperitoneal administration of GHB 500 mg/kg[121]. The stimulation of GHB binding site(s) by GHB induces a progressive decrease in NOS activity (this reduced NOS activity presumably explains the increase in cGMP concentrations previously reported); other agonists of the GHB binding site(s) reproduce this

effect. The effect on these substances is blocked by $GABA_B$ and GHB antagonists, antiepileptic drugs and opioid receptor antagonists[108,122,123].

GHB has also been shown to induce a G protein-mediated decrease in adenylyl cyclase activity via the presynaptic GHB binding site(s); this effect is blocked by a GHB antagonist but not by a $GABA_B$ receptor antagonist[120]. The effect of GHB on intracellular response depends on the type of receptor stimulated and manifests regional variability within the brain.

2.7 Effects on Neurotransmitter Systems

2.7.1 *Dopaminergic System*

Exogenous administration of GHB raises concentrations of GHB to many times higher than endogenous concentrations in dopaminergic regions of the brain[124,125]. It appears possible that the GHBergic system participates physiologically in the control of the dopaminergic system in the nigrostriatal and in the mesocorticolimbic pathways[126,127]. Research has shown that GHB has multiple effects on dopaminergic neurons. Initially, at certain concentrations, less dopamine is released under the influence of GHB, as GHB reduces impulse flow and inhibits firing of dopaminergic terminals[128,129]. Decreased dopamine release is also seen with administration of baclofen, a $GABA_B$ receptor agonist[116,126,130–133]. It is possible that the effects of GHB on dopaminergic neurons are mediated via the GHB binding site(s) and/or $GABA_B$ receptors. With GHB, the decrease in dopamine release is followed by tissue accumulation of dopamine in the neuron[134–140]. By inhibiting impulse flow, administration of GHB stimulates the kinetic properties of tyrosine hydroxylase, the rate-limiting enzyme controlling the synthesis of dopamine from tyrosine[141–146]. Histofluorescent analysis of different catecholamine systems has shown that dopamine fibers appear swollen and abnormal following administration of GHB, suggesting that GHB alters metabolism within the neurons[147]. In summary, it appears that pharmacologic GHB affects dopaminergic neurons by inhibiting dopamine release, increasing dopamine synthesis and inhibiting end-product inhibition of the enzyme tyrosine hydroxylase. Both latter mechanisms increase the amount of available dopamine in the neurons prior to GHB washout[148].

Subsequent to the GHB-mediated decrease in dopamine release and increase in intercellular dopamine synthesis, a transient increase in dopamine release is observed during washout[149,150]. Animals pretreated with a GHB receptor antagonist showed no increase in extracellular dopamine induced by GHB. Therefore, in a similar way, GHB may be affecting dopaminergic neurons via GHB binding site(s) and $GABA_B$ receptors[126,130,131,151]. These neurons could be enkephalinergic[105] and partly controlled via GHB binding site(s), as naloxone (an opioid receptor antagonist) has been reported to block the GHB-induced increase in dopamine synthesis in the striatum[152]; it should be noted that naloxone can antagonize GABA receptors[153].

Furthermore, 6 hours after GHB administration (500 mg/kg intraperitoneally), an increase in dopamine D1 and D2 receptor mRNA expression has been observed. Repeated exposure to GHB (500 mg/kg intraperitoneally twice daily for 10 days), followed by a 14-hour withdrawal period, induced increased mRNA expression for the D1 and D2 receptors. The author cautions that this study analyzed concentrations of dopamine receptor mRNA, not actual receptor numbers or activity[154].

2.7.2 *Serotonergic System*

GHB can induce an increase in serotonin turnover in the striatum and in the mesolimbic areas[133,141,155,156] without significantly changing absolute concentrations of serotonin. This effect can be seen as an accumulation of 5-hydroxy-indoleacetic acid (5-HIAA), the main metabolite of serotonin, together with no significant change in potassium-induced increase in extracellular serotonin concentrations after administration of GHB[128,129]. Furthermore, GHB may affect transport of tryptophan, the precursor of serotonin, through the blood-brain barrier and/or through the neuronal membrane, as evidenced by tryptophan accumulation after *in vivo* administration of GHB[157].

Baclofen-induced activation of $GABA_B$ receptors mimics some aspects of the serotonergic activity of GHB[133], suggesting the effect of GHB on serotonergic neurons may be in part mediated by GHB-induced activation of $GABA_B$ receptors; the role of GHB binding site(s) in the effects of GHB on serotonergic activity is unclear.

This action on serotonin may account for the ability of GHB to stimulate growth hormone (GH) release (see section 3.1.1), as co-administration with the serotonin receptor antagonist metergoline significantly reduces this increase[158]. Metergoline has also been shown to lower GH concentrations in patients with acromegaly[159].

2.7.3 *Opioidergic System*

GHB has been found to increase brain concentrations of the endogenous opioids dynorphin and encephalin[160,161]. After a single intraperitoneal dose of GHB 500 mg/kg, an increase in proenkephalin mRNA concentrations in the whole dorsal striatum (+60%), but not in other areas such as the nucleus accumbens, was seen between 15 and 90 minutes after injection. An increase in prodynorphin mRNA expression was observed in the frontal cortex (+90%) and hippocampus (+55%) 6 hours after GHB administration[123]. Chronic exposure to GHB (500 mg/kg intraperitoneally twice daily for 10 days) induced significant increases in both proenkephalin and prodynorphin mRNA concentrations in various brain regions[161,162].

GHB appears not to act as an agonist at the μ-, δ-, and κ-opioid receptors[162]. The opioid receptor antagonist naloxone (10 mg/kg) blocks the GHB-induced increase in cGMP and inositol phosphate turnover in response to GHB[121] and decrease in

glucose utilization[163]; however, these effects may be mediated via GABA receptors as naloxone has no effect on GHB-induced sleep or dopamine metabolism[152,164,165]. Thus, GHB could act on opioid interneurons via GABAergic and/or GHBergic agonism. Again, it should be noted that naloxone can antagonize GABA receptors[153], and GHB binding site(s) antagonists block the GHB-induced accumulation of met-enkephalin and inhibition of release of met-enkephalin, which participates in the presynaptic regulation of dopamine release[165]. Thus, GHB could act on opioid interneurons via GABAergic and/or GHBergic agonism.

2.7.4 Cholinergic System

The effects of GHB administration on the cholinergic system are unclear, although GHB binding site(s) have been found on cholinergic interneurons[105]. A study by Sethy *et al.*[166], using anesthetic doses of GBL (750 mg/kg intraperitoneally), produced a time-dependent increase in acetylcholine in the striatum and hippocampus, with maximal increase occurring 15 minutes after administration. However, a correlation between the increase in acetylcholine and the depth of anesthesia produced by GBL was not observed[166]. In contrast, a microdialysis study in freely moving rats showed a dose- dependent reduction in extracellular hippocampal acetylcholine concentrations induced by both GHB (200 and 500 mg/kg intraperitoneally) and baclofen (10 and 20 mg/kg intraperitoneally). Furthermore, a $GABA_B$ receptor antagonist prevented this effect, while a GHB receptor antagonist did not block this effect. These findings indicate that the GHB-induced reduction of hippocampal acetylcholine release is mediated by $GABA_B$ receptors[167]. Of note, the disparity in responses between the two studies may be mediated by the different dose levels utilized but may also highlight neuropharmacological differences between GHB and GBL.

2.7.5 Noradrenergic System

Anatomical, neurochemical and electrophysiological studies have provided evidence that $GABA_B$ receptors are involved in the regulation of noradrenergic neurons emanating from the locus coeruleus (LC). Activation of $GABA_B$ receptors by baclofen inhibits spontaneous firing of these neurons and causes membrane hyperpolarization due to an increase in potassium conductance[168]. In reports as early as 1980, GHB was shown to affect noradrenergic transmission in the CNS. Intraperitoneal administration of GHB increases brain noradrenaline (norepinephrine) synthesis and utilization, particularly in the neocortex[169]. Recently, Szabo *et al.*[170] used *in vivo* extracellular unitary recordings to monitor the effect of sustained administration of GHB (40 mg/kg/day) on the burst firing of LC noradrenergic neurons. Two days and 10 days of continuous 24-hour GHB administration decreased the firing activity of LC neurons by ~50% when compared with controls. In contrast, withdrawal of GHB administration after 10 days of continuous 24-hour treatment resulted in a 33% augmentation in LC activity for 36 hours compared with controls. Thus, chronic GHB treatment inhibits the burst firing of LC noradrenergic neurons while the drug is present in meaningful

concentrations and enhances LC noradrenergic firing during and beyond drug washout.

Speculatively, as noradrenergic activity is implicated in arousal[171], inhibition of LC noradrenergic activity by GHB, as seen in animal models[170], may contribute to the observed sleep enhancements noted in the literature[47,172–174]. Conversely, the augmentation of LC noradrenergic neurons on washout, as seen in animal models[170], might help explain the observations of improved wakefulness and reduced cataplexy seen during the daytime after chronic nighttime administration of GHB in patients with narcolepsy[49,175,176].

Lastly, withdrawal effects have not been seen in clinical trials[177], but have been reported in the literature with regard to frequent, regular dosing of illicit GHB[51,178]. These data on noradrenergic neurons might partially or fully explain any GHB withdrawal phenomenon. Further research is necessary to assess these hypotheses.

2.7.6 Glutamatergic System

GHB has been shown to affect glutamate transmission via GHB site(s) and/or $GABA_B$ receptors[179]. The ability of GHB to depress the amplitude of the first and the second evoked alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate excitatory postsynaptic potentials suggests that GHB inhibits excitatory synaptic transmission by decreasing the probability of release of glutamate[180]. Further research has shown that this effect of GHB on glutamate levels is concentration dependent, whereby a direct GHB binding site-mediated facilitation is observed at nanomolar concentrations and an indirect $GABA_B$ receptor-mediated inhibition predominates at millimolar concentrations[179,181]. This effect is partially reversed by GHB binding site(s) antagonists. Of note, $GABA_B$ agonists also produce a concentration-dependent decrease in basal and potassium-evoked concentrations of glutamate[182].

3. Hormonal and Metabolic Effects of GHB / SXB

3.1 Hormonal Effects of GHB / SXB

3.1.1 Growth Hormone

The effects of GHB on GH had been described as early as 1970[183]. Van Cauter *et al*.[184] investigated the GH response to GHB in healthy young men. Eight such men each participated in four experiments involving bedtime oral administration of placebo, GHB 2.5 g, GHB 3.0 g or GHB 3.5 g. Polysomnography (PSG) sleep recordings were performed every night, and blood samples were obtained at 15-minute intervals from 20:00–08:00 hours. There was a doubling of GH secretion in all GHB-treated groups, resulting from an increase in the amplitude and the duration of the first GH pulse after sleep onset. This stimulation of GH secretion was significantly correlated with a simultaneous increase in the amount of stage IV non-rapid eye movement (NREM) sleep.

It is uncertain whether the ability of GHB to stimulate GH secretion is exclusively tied to its ability to stimulate slow-wave sleep (SWS) during NREM sleep, which is the phase of sleep during a human's 24-hour diurnal cycle when most GH is released, particularly in men[184]. There appears to be a linear relationship between SWS and GH secretion, so that during aging, SWS and GH secretion decrease with the same chronology[185].

Interestingly, many of the studies investigating the ability of GHB to stimulate GH release were performed during the daytime without allowing the study participant to sleep. This suggests that the ability of GHB to stimulate GH secretion is not dependent on sleep induction. Some studies that evaluated the effect of GHB on GH in the absence of sleep are notable. Gerra *et al.*[186] investigated GH responses to GHB with or without the benzodiazepine receptor antagonist flumazenil. Nine male healthy volunteers (aged 23.2 ± 2.5 years) were submitted to three tests in random order: (i) oral GHB administration; (ii) oral GHB and intravenous flumazenil administered 15 minutes prior to GHB; and (iii) oral placebo and intravenous saline administration. Blood samples for GH assays were collected during the three tests 15 minutes prior to drug administration, at the time of administration, and 15, 30, 45, 60 and 90 minutes afterward. GHB induced a significant increase in plasma GH concentrations; however, intravenous flumazenil 15 minutes prior to GHB administration antagonized the action of GHB on GH secretion. No changes in GH concentrations were obtained with placebo and saline. This study suggests that some of the effects of GHB on GH are mediated at $GABA_A$ receptors.

The observation that baclofen stimulates GH secretion in healthy men but not in patients with Parkinson's disease[187] prompted research to test the effect of GHB on GH secretion in these patients. GHB, like baclofen, is a $GABA_B$ receptor agonist. $GABA_B$ receptor activation has been shown to have a positive effect on GH releasing hormone (GHRH), which in turn has a positive effect on the release of GH[188]. Volpi *et al.*[187] studied ten healthy men and ten patients with *de novo* parkinsonism who were administered sodium valproate (800 mg orally), GHB (25 mg/kg/body weight orally) or baclofen (10 mg orally). All drugs induced a significant increase in serum GH concentrations in the healthy controls. Growth hormone secretion in parkinsonian patients did not change after baclofen or sodium valproate administration, whereas normal responsiveness to GHB was observed. This suggests a different mechanism underlying the GH response to GHB compared with the other drugs. Volpi *et al.*[189] administered GHB to healthy and parkinsonian patients to investigate whether muscarinic cholinergic receptors mediate the GH secretion induced by GHB; both study groups were tested in the absence and the presence of the anticholinergic agent pirenzepine. Both healthy controls and parkinsonian patients showed a significant rise in serum GH concentration in response to GHB (25 mg/kg/ bodyweight orally), although a slightly, but significantly, lower response was observed in parkinsonian patients. Pretreatment with pirenzepine (100 mg orally 2 hours before GHB) completely suppressed the GHB-induced GH release in both controls and parkinsonian patients. These data indicate that a cholinergic mechanism mediates the GH

response to GHB in healthy men and that this is preserved in the parkinsonian brain.

In narcolepsy, Overeem *et al.*[190] observed that GH secretion does still occurs during SWS. Since sleep in narcoleptics is distributed across a 24-hour period, however, so are the GH pulses leading to a more dispersed 24-hour GH profile and greater daytime secretion. To follow up on this work characterizing GH secretion patterns in narcoleptics, Donjacour *et al.,*[191] evaluated the influence of nighttime SXB administration (two times 3 g/night for 5 consecutive nights) on GH and sleep in eight male hypocretin-deficient patients with narcolepsy and cataplexy and eight controls matched for sex, age, body-mas index (BMI), waist-to-hip ratio, and fat percentage. On the fifth day of SXB administration, both groups underwent 24 hours of blood sampling at 10-minute intervals. Administration of SXB caused a significant increase in total 24-hour GH secretion in narcolepsy patients, but not in controls. However, SWS, and the cross-correlation between GH levels and SWS, more than doubled in both groups, strengthening the temporal relation between SWS and GH secretion.

3.1.2 Neurosteroids

GHB has been shown to increase the concentrations of the neurosteroids allopregnanolone and allotetrahydrodeoxycorticosterone (allo-THDOC)[192]. These neurosteroids are positive allosteric modulators of $GABA_A$ receptors and could therefore contribute to the hypnotic properties of GHB.

In rats, the effect of GHB on the concentrations of allopregnanolone and allo-THDOC and on righting reflex was assessed. GHB induced a loss of righting reflex that lasted up to 90 minutes and declined 180 minutes after GHB administration. An increase in the concentrations of the neurosteroids was also seen after administration of GHB, with a time course that matched that of the loss of righting reflex. The authors of the study concluded that the GHB-induced increase in neurosteriod concentrations was implicated in the sedative/hypnotic properties of GHB[192].

3.1.3 Prolactin

Prolactin is a pituitary-derived protein that plays an important role in metabolism and the regulation of the immune system. Its secretion is regulated by inhibitory control of dopaminergic tuberoinfundibulum neurons on D2 receptors of the arcuate nucleus and stimulated by thyrotropin-releasing factor[193,194]. At sufficient concentrations, GHB can bind to $GABA_B$ receptors and influence the release of dopamine and serotonin.[195] Accordingly, Donjacour *et al.*, designed an open-label intervention study to examine the influence of five nights of SXB administration (3 g given twice per night.) on prolactin levels in eight hypocretin-deficient patients and eight controls matched for sex, age, BMI, waist-to-hip ratio and fat percentage. Administration of SXB markedly increased prolactin secretion and enhanced the association between prolactin release and SWS in both groups. The authors

speculated that changes in the tuberoinfundibular output of dopamine could be the cause of the effect of SXB on prolactin release, and that the hypocretin system is not involved in this mechanism since SXB treatment-stimulated prolactin release did not significantly differ between narcolepsy patients and controls[196].

3.1.4 *Melatonin*

Melatonin is a pineal gland-derived hormone involved in endocrine timing, the timing of behaviors, and communication of night length in seasonal-breeding mammals[197]. It is also involved in regulation of the sleep-wake cycle, and as such, it can modulate sleep and even induce sleepiness at sufficient concentrations[198].

The secretion of melatonin is regulated by the release of norepinephrine (NE) from local sympathetic nerve fibers. This NE release happens exclusively under darkness conditions. Once released, NE binds to β-adrenergic receptors on the pinealocyte to activate cAMP-dependent protein kinase A (PKA), which in turn regulates the rate limiting enzyme in melatonin synthesis: it phosphorylates arylalkylamine N-acetyltransferase (AANAT). Under conditions of light exposure to the eye, there is an absence of local NE stimulation, and AANAT is degraded by proteasomal proteolysis. Production of melatonin is started again, typically in the evening, under conditions of dim light.

As described earlier in the article, GHB has been shown to affect noradrenergic transmission in the CNS, likely via binding to $GABA_B$ receptors when at sufficient concentrations. Given that GHB can influence NE transmission, the key neurotransmitter responsible for regulating melatonin, and including the fact that therapeutic SXB is administered chronically in certain populations, the investigation of SXB on the melatonin level is warranted.

Interestingly, the pineal gland is innervated by hypocretinergic neurons, therefore making hypocretin activity a possible modulator of diurnal melatonin synthesis and secretion[199]. Therefore, Donjacour and colleagues[200] measured plasma melatonin after 5 days of SXB administration (3 g, 2x per night) in both hypocretin-deficient narcoleptic patients and matched controls. Mean melatonin concentrations were similar between patients and controls; however, the percentage of 24-hour melatonin secreted during the daytime was significantly higher in narcoleptic patients – possibly secondary to light exposure during wake periods during nighttime fragmented sleep – and melatonin secretion exhibited a weaker coupling to sleep. SXB did not affect melatonin secretion.

3.1.5 *Ghrelin*

Ghrelin is a peptide hormone mainly produced by endocrine cells in the stomach and gastrointestinal tract, and is an important endogenous regulator of energy balance and GH secretion[201]. Ghrelin has an excitatory influence on hypocretin neurons and these systems have been shown to interact in ingestive behavior[202]. Like ghrelin, SXB administration stimulates GH release[184]. Research detailed below

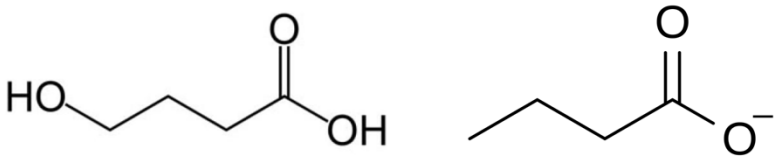
in this thesis describes the effects of SXB on the ghrelin level in narcoleptics and controls.

3.2 Metabolic Effects of GHB / SXB

3.2.1 *Insulin Sensitivity*

Butyrate

The short chain fatty acid butyrate is structurally similar to (Figure 3) and can be produced from β-oxidation of GHB. Supplemental butyrate has been shown to improve insulin sensitivity in mice[203]. Likewise, a meta-analysis evaluating human butter consumption—which contains a significant amount of butyrate per serving—showed a modest inverse association with the risk for type 2 diabetes[204]. Furthermore, inducing an increase in butyrate-producing gut bacteria, such as *Roseburia* in the feces and *Eubacterium halii* in the small intestine, associates with increased peripheral insulin sensitivity in males with metabolic syndrome who underwent a fecal transplant[205].



GHB Butyrate

FIGURE 3. Chemical structure of gamma-hydroxybutyrate (GHB) and butyrate.

Butyrate may induce beneficial metabolic effects through enhancement of mitochondrial activity, prevention of metabolic endotoxemia by strengthening the gut lining, and activation of intestinal gluconeogenesis via different routes of gene expression and hormone regulation[206]. The anti-diabetic properties of butyrate may also in part come through its influence as a histone deacetylace (HDAC) inhibitor, which is a property it shares with GHB. Some HDAC inhibitors have been shown to promote β-cell reprogramming, differentiation, proliferation and functioning, and to improve insulin resistance[207].

HDAC Inhibition

Multiple physiological and functional roles are attributed to the modification of histone acetylation and its consequences at the level of gene expression. Pharmacological concentrations of GHB significantly induce brain histone H3 acetylation and reduce *in vitro* HDAC activity[208]. It is therefore possible that GHB, at sufficient levels, could directly participate in the epigenetic modification of gene expression related to insulin-sensitizing mechanisms.

Also of interest is SXB's influence in increasing GH release[184]. While GH administration can promote reductions in body fat, which itself promotes an insulin-sensitizing effect, long-term elevations in its level have been associated with both increases and decrements in insulin sensitivity[209,210].

Given that the use of this medication in narcoleptic patients is chronic, and that multiple insulin-sensitizing mechanisms are plausible, evaluating the long-term impact of SXB therapy on insulin sensitivity is important. As such, Donjacour and colleagues studied the effects of nightly SXB treatment on β-cell functioning and insulin sensitivity in seven hypocretin-deficient patients over three months. Compared to baseline, SXB treatment increased hepatic, but decreased whole-body insulin sensitivity[211]. It should be noted that the sample of narcoleptic patients studied demonstrated increased peripheral insulin sensitivity, but normal hepatic insulin sensitivity and β-cell function, compared to matched healthy controls at baseline. Therefore, future studies should further evaluate the effect of SXB on insulin sensitivity and β-cell functioning in this population, and in populations other than hypocretin-deficient narcoleptics.

3.2.2 *Thermoregulation*

The hypothalamus integrates sensory information to help control basic life functions, including the regulation of temperature and sleep/wake states. To regulate body temperature, the median preoptic nucleus (MePOA) of the hypothalamus sends descending output to two key sites. The first is to the dorsomedial nucleus (DMH), in which activation of glutamatergic neurons elevates body temperature. The MePOA, as well as the DMH, also innervates the raphe pallidus nucleus, which in turn innervate sympathetic preganglionic neurons that cause an increase in body temperature by activating brown adipose tissue to cause thermogenesis, and by promoting vasoconstriction of superficial vascular beds, thus reducing heat loss through the skin[212].

In relation to wake and sleep states, changes in skin temperature may moderate the efficacy by which the clock and homeostat manage to initiate or maintain sleep or wakefulness[213]. In healthy individuals, body temperature, both core and skin, closely associate with sleep and arousal[214]. Relatively speaking, during wakefulness, core temperature is high while skin temperature is low. When transitioning between wake and sleep, temperature changes can be observed reliably. Just prior to the onset of sleep, skin temperature increases, allowing convective heat loss and a drop in core temperature. During sleep, core temperature stays depressed while skin temperature remains elevated[215].

Aligned with the above findings, recent work from Siegel and colleagues evaluated the association of sleep and wake to skin and environmental temperature in three natural-living, hunter-gatherer communities in their ecological niche. They found that sleep onset, in both the winter and summer, occurred during falling ambient temperature. Morning awakenings were accompanied by strong vasoconstriction in the hands and in the feet. The authors proposed that the physiological purpose

of this vasoconstriction could be to increase the temperature of the core and brain to evoke awakening[216].

Interestingly, hypocretin-deficient narcoleptic patients are characterized by skin temperature dynamics that deviate from normal controls: during wake, their skin temperature resembles that of the healthy at sleep onset[217]. Daytime sleepiness is a cardinal symptom of narcolepsy, and it has been proposed that changes in skin and core body temperature modulate neuronal activity of thermosensitive neurons in brain areas that regulate vigilance and sleepiness[218].

GHB / SXB and Body Temperature

The effects of GHB on body temperature appear to vary by dose. In rats, an intraperitoneal dose of 10 mg/kg raises core body temperature slightly, while doses of 200 mg/kg or greater lower it[219]. A similar temperature-lowering effect with higher doses of GHB has also been observed in gerbils and rabbits[220,221], and in humans under conditions of GHB abuse[222,223].

The main temperature-lowering effect of GHB seems to be dependent on $GABA_B$ receptor binding [104,224]. However, GHB may also affect body temperature through decreased heat production, via reductions in metabolism and energy utilization and increases in cutaneous circulation[101,225].

Given the impact of GHB on temperature regulation, the altered pattern of skin temperature dynamics in narcolepsy, and the positive effects of SXB on both sleep and sleepiness in narcolepsy patients, van der Heide *et al.* investigated whether the beneficial effect of SXB occurs, at least partially, by restoring physiological temperature regulation. The effects of five nights of SXB (3g, twice per night) treatment on temperature and sleep-wakefulness was tested in eight male hypocretin-deficient narcoleptic patients and matched controls[226]. At baseline, all participants underwent measurement of core, as well as proximal and distal skin temperatures, and 24-hour PSG to assess sleep/wake measures. For narcoleptic individuals, core and proximal skin temperatures were significantly lower than controls during daytime. Immediately after the fifth night of SXB treatment, the narcoleptic patients underwent a second round of temperature and PSG over a 24-hour period. The nightly administration of SXB increased the daytime proximal skin temperature in narcoleptic patients to a level similar to that of controls and normalized the predictive value of distal skin temperature and distal–proximal temperature gradient for the onset of daytime naps. This treatment protocol, however, did not affect core body temperature, distal temperature, or distal–proximal temperature gradient. Further studies should explore the role of SXB in temperature regulation and sleep in narcolepsy, as well as in other populations.

3.2.3 *Weight and Energy Balance*

When GHB was legal to purchase as a dietary supplement in the U.S., it was advertised as an agent to help reduce body fat. The effects of SXB on weight loss have been reported on in some clinical settings and there are several plausible

mechanisms for how GHB might influence adiposity. However, SXB remains to be tested rigorously as a clinical intervention for weight loss.

Clinical Reports

Weight Loss in Narcolepsy

The first clinical report found on this subject was from Dr. Ruzica Ristanovic, published in abstract form at the 2003 Associated Professional Sleep Society (APSS) conference. She analyzed the change in BMI in narcolepsy patients on SXB from baseline (n=184; median BMI=28) to 2 (n=156), 6 (n=175), and 12 months (n=97) of treatment. At each time point, there was a statistically significant reduction in BMI from baseline (2 mo = -0.20; 6 mo = -0.425; and 12 mo = -1.103).

In 2005, Husain *et al.*, published an abstract (APSS) on a chart review of 17 narcolepsy patients (11 males, 6 females; mean age 43 ± 16.6 years) in their clinic who had been on SXB (7.0 ±1.8 g/n avg.) for a mean duration of 10.7 (±7.2) months. The average weight loss was 10.4 pounds (pre-SXB weight = 182.1 ± 33.3, BMI = 27.6 ±4.0; post-treatment weight = 171.7 ± 31.2, BMI = 26.0 ± 3.5), with a maximum loss of 41 pounds.

Similarly, Turner *et al*., published an abstract at the 2006 APSS meeting on a chart review of 86 patients (22 males, 20-76 years old; 64 females, 16-76 years old) being treated with SXB therapy (3-10.8 g/night). Chart audits were conducted at three time points: baseline, first, and second office visit. At the first office visit (mean 1.7 months), 56% of patients experienced weight loss ranging from 1 to 16 lbs., with 10 patients experiencing weight loss of 10 lbs. or more. This finding was not statistically significant. At the second visit, three months after the first, 60% of the patients experienced some degree of weight loss, yet still, across the group, the amount of weight loss was not significant. Comparatively, however, from baseline to the second visit, women lost significantly more weight than men did (14 lbs ± 10 lbs., 8.3 lbs ± 4 lbs., respectively).

More recently, Donjacour *et al.*, conducted a study looking at the effects of SXB on glucose and fat metabolism in narcoleptic patients (as reported above)[211]. Compared to baseline, three months of SXB treatment significantly reduced body weight (99.2 ± 6.0 vs. 94.0 ± 5.4 kg) and increased lipolysis (4.9 ± 0.4 vs. 6.5 ± 0.6 µmol/kg FM/min), possibly accounting for the observed weight loss from treatment. This change in body weight strongly associated with alterations in glucose disposal rate per unit serum insulin (r=-0.93, p=0.003), but not with changes in endogenous glucose production per unit serum insulin (r = 0.29, p=0.535). While SXB showed a tendency to decrease systemic insulin sensitivity, it increased hepatic insulin sensitivity, suggesting tissue-specific effects[211].

Weight Change in Fibromyalgia

Fibromyalgia is a complex musculoskeletal disorder clinically characterized by widespread pain, usually accompanied by fatigue, sleep disturbance, and dyscognition[227]. A 14-week, phase 3, double-blind, randomized, controlled trial evaluated SXB (4.5 g, n = 182; 6 g, n = 183) versus placebo (n = 183) in patients with fibromyalgia. At study endpoint, compared to placebo (1.2%), a greater proportion of patients treated with SXB (4.5 g = 7.6%; 6 g = 14.3%) had a decrease in weight of ≥7%. Furthermore, a decrease in weight was reported as an adverse event in five patients in each SXB group, while an increase in weight was reported in one patient in each SXB group. Additionally, a decreased appetite was reported as an adverse event in five patients in the SXB 4.5 g group, and in three in the SXB 6 g group[228].

In a separate 14-week phase 3 study evaluating SXB in fibromyalgia, 573 patients that were enrolled at 108 centers in eight countries were randomly assigned to placebo, SXB 4.5 g/night or SXB 6 g/night. In this study, weight loss was observed in the SXB-treated groups, with a mean (standard error) weight change from baseline of −1.19 (0.22) kg in the SXB 6 g/night group and −0.43 (0.20) kg in the SXB 4.5 g/night group compared with a mean weight gain of 0.43 (0.16) kg in the placebo group[229].

The weight loss in both the narcoleptic and fibromyalgia patients is impressive as these reductions in weight took place in populations not enriched for weight loss.

Potential Mechanisms for SXB and Weight Loss

Sleep

Both feeding and sleeping behaviors in mammals are thought to result from the integration of homeostatic and circadian influences. Drug-induced alterations of one behavior (sleepiness-sleep) may hypothetically lead to changes in the other behavior (hunger-food consumption), or both behaviors can be altered via drug effect on common mechanism(s) they share.

Sleep deprivation contributes to a number of molecular, immune, and neural changes with broad effects on body systems (endocrine, metabolic, immune, and cognitive)[230], all of which can play a role in increasing adiposity. The connection between sleep and weight will be discussed in greater detail in the second half of this thesis.

The ability of SXB to reduce sleep disruption in some populations and increase SWS in all populations tested, is well-established. It is plausible that SXB-induced sleep enhancement can influence adiposity in susceptible individuals.

Arousal

The effects of SXB on wakefulness in narcolepsy, Parkinson's disease, and healthy sleep deprived adults are well documented[1,231]. This increased wakefulness may lead to increased physical activity and energy expenditure, which are key elements in the energy balance equation.

The daytime wake-enhancing effects from nighttime SXB treatment may be a result of several interacting mechanisms, including increased SWS during nocturnal sleep and/or direct pharmacological effects on wake-associated systems. For instance, after acclimation to a week or more of nightly SXB use, and subsequent down regulation of $GABA_B$ receptors, SXB may promote daytime wakefulness by desensitizing adenosine receptors (A_{1A}) on thalamic relay neurons to reduce the drowsiness-inducing tonic adenosine-mediated inhibition in the thalamus. Additionally, acclimation to SXB may promote arousal by disinhibiting excitatory glutamatergic transmission within thalamic relay neurons circuits, excitatory dopaminergic of the VTA, and excitatory noradrenergic circuits emanating from the locus coeruleus.

As per the second half of my thesis, impairments in arousal, and thus subjective and objective alertness, may lead to altered food decisions that could promote a defection from personal health standards[7].

Anorexigenic Effects

SXB may also promote an anorexigenic effect via an influence on neuromodulators like cocaine- and amphetamine-regulated transcript (CART) that are involved in feeding behavior and energy expenditure. Furthermore, the closely-related ketone β-hydroxybutyrate has been shown to induce proliferative remodeling of neurons in the energy-regulation arcuate nucleus of the hypothalamus, which may lower the body fat set point[232]. Given the clinical weight loss that has been observed, and the impressive weight loss effects from β-hydroxybutyrate, the effects of chronic exposure to SXB on body fat set point neurons should be evaluated directly. Interestingly, Fisler *et al.*[233] showed that obese rats have lower brain levels of GHB than their lean counterparts, despite an identical diet. This suggests endogenous GHB could play a role in the regulation of bodyweight.

Hormones

As discussed previously, SXB administration promotes the release of GH, which promotes lipolysis by upregulating hormone sensitive lipase (HSL) to mobilize free fatty acids from triglycerides. Additionally, three months of SXB treatment in narcoleptic patients significantly increased lipolysis and lowered body weight, with tissue-specific effects on insulin sensitivity. This increased lipolysis may have accounted for the weight loss observed in this same study[211].

Temperature

A study in rats showed a remarkable increase in body temperature after administration of very low doses of the GHB precursor, GBL. Following intraperitoneal injection of 5 mg/kg, body temperature rose rapidly to 1.8°F above normal at 15 minutes, reached a peak of almost 2.2°F at the hour mark, and was still elevated by 2°F at the 75-minute mark, dropping gradually thereafter. This thermogenic effect could contribute to increased energy expenditure and reduced adiposity over time[234].

In summary, a potent connection between sleep and energy regulation is known, as well as a unique positive effect of SXB on sleep in clinical and non-clinical populations. Given the connection between sleep and weight, and SXB's effects on sleep — in addition to its other pharmacological effects on neuronal and hormonal systems of the body — as well as the impressive clinical reports that have been published on weight loss after SXB therapy, a more thorough, direct investigation into SXB as a therapy for weight loss is warranted.

4. Clinical Effects of GHB/SXB

The effects of GHB, in the form of SXB, on sleep in humans and animals have been examined since the early 1960s[19,20,235,236]. Much of the recent research has continued to focus on the somnopharmacological properties of this compound. Animal studies included the exploration of intravenous or intraperitoneal doses as low as 10 mg/kg to as high as 1200 mg/kg. The sample sizes, doses and other aspects of the methodologies varied greatly across studies, resulting in a variety of responses ranging from sedation to torpor[235,237,238].

Much more meaningful analysis of GHB on sleep and wakefulness has been conducted in humans during the past 30 years. The clinical findings are fairly consistent and are discussed in the following sections. In humans, administration of GHB affects many aspects of sleep; additionally, a growing data pool demonstrates that it also promotes next-day wakefulness (i.e., ameliorates excessive daytime sleepiness), at least in certain clinical populations. The following sections review research on sleep and wakefulness in healthy humans and across various clinical populations. It should be noted that in the sections on fibromyalgia and narcolepsy (sections 4.2.2 and 4.2.5), all doses of GHB discussed represent a total nightly dose. In all the clinical research trials in these two indications, the total nightly dose has been split into two equal halves, with the first dose administered at bedtime and the second dose administered somewhere between 2.5 and 4 hours later. For example, a total nightly dose of 6 g would equate to a bedtime dose of 3 g and a night-time dose of 3 g, 2.5-4 hours later.

4.1 Healthy Subjects

4.1.1 *Sleep*

Early work with GHB in a healthy human population examined the ability of the drug to induce a sleep-like state[19,20]. In this population, GHB was shown to decrease sleep onset latency, promote delta activity and enhance sleep maintenance; these effects were dependent on dose and timing of administration[239]. In 1964, Helrich *et al.*[14] induced sleep in 16 healthy study participants with intravenous administration of GHB at doses ranging between 5.9 g and 9 g. Lapierre *et al.*[46] documented an effect of GHB on sleep organization in 12 healthy individuals (six male, six female; age range 23–63 years). All subjects were free from a history or current symptoms of psychopathology, as well as of any medical conditions know to influence sleep. Each participant received a single oral dose of GHB 2.25 g or placebo, in double-blind fashion, 15 minutes prior to undergoing nocturnal PSG recording. In this study, GHB had no significant effect on total sleep time (TST), and sleep latency decreased, although not significantly; stage 1 sleep decreased from a mean of 10.1% to 8.1% of TST; the percentage of TST spent in stage 2 remained unchanged; SWS increased significantly from a mean of 10.5% to 13.6% of TST and SWS latency shortened significantly. No difference was found in REM sleep latency or the percentage of time spent in REM sleep; however, REM sleep efficiency increased significantly. As expected, these effects, from single bedtime doses of GHB, were seen only during the first third of the night.

Research by Van Cauter *et al.*[184] has supported this earlier work, showing that, in addition to increasing GH secretion (see section 3.1.1), GHB increases the time spent in deep SWS in healthy human subjects. These researchers evaluated eight healthy young men who participated in each of four experiments involving bedtime oral administration of placebo, GHB 2.5 g, GHB 3.0 g and GHB 3.5 g; PSG sleep recordings were performed every night. Subjects showed reduced sleep latency at all doses of GHB compared with placebo (24 ± 5 minutes); these sleep latency scores were significantly reduced in the GHB 2.5 g (13 ± 3 minutes) and 3.5g (14 ± 2 minutes) dose groups. Subjects showed increases in deep SWS time with the 3.0 g (105 ± 9 minutes) and 3.5 g (99 ± 18 minutes) doses and decreases in deep SWS at the 2.5 g (88 ± 8 minutes) dose, which were not significant when compared with placebo (91 ± 9 minutes). Again, as expected with a single nightly dose, these effects occurred mainly during the first third of the night after sleep onset. None of these studies were designed to evaluate next-day wakefulness or cognition parameters.

4.1.2 *Mood, Prosocial, and Prosexual Effects*

In humans, GHB has a wide spectrum of properties ranging from stimulation and euphoria in lower doses, to sedation, deep sleep, and coma after ingestion of high doses. Reports from abuse cases suggest that the ingestion of certain doses of GHB elicit euphoric, prosocial, prosexual, libido-enhancing and empathogenic effects in users. Until recently, these commonly-reported effects had not been tested

directly. In 2015, Bosch et al., gave GHB (20 mg/kg) to 16 healthy males, using a randomized, placebo-controlled, cross-over design. They observed that GHB showed both stimulating and sedating effects, and elicited euphoria, disinhibition, and enhanced vitality [240]. In 2017, Bosch et al., reported on two experiments of the putative prosexual effects of GHB [241]**.** In the first experiment, the research group gave 20 and 35mg/kg GHB vs. placebo in 32 participants using a randomized, placebo-controlled, double-blind, balanced, and cross-over study designs to measure subject prosexual effects assessed using the Sexual Arousal and Desire Inventory (SADI). The prosexual effects of GHB were shown by increased SADI ratings regarding physiological, evaluative, and motivational aspects of sexual arousal. In the second experiment, brain reactivity towards erotic vs. neutral pictures was investigated in 15 participants using fMRI after 35mg/kg GHB vs. placebo. Under the placebo condition, erotic visual stimuli activated the bilateral insula, NAcc, fusiform gyrus, thalamus, and left occipital pole. Post administration of GHB, even sexually neutral pictures of persons induced subjective sexual arousal and increased activation of the bilateral NAcc and right anterior cingulate cortex, which significantly correlated (left NAcc by trend). GHB also increased connectivity between NAcc and ventromedial prefrontal cortex during processing of visual erotic cues, i.e., in the condition in which subjective sexual arousal was highest.

Together, these findings align with the subject reports by demonstrating that GHB between doses of 20-35mg/kg can increase physiological, evaluative, and motivational ratings of sexual arousal, likely by stimulating hedonic sexual functioning and lowering the threshold for erotic perception, which is related to increased susceptibility of mesolimbic reward pathways.

4.2 Clinical Populations

4.2.1 *Insomnia*

The early work of Laborit *et al.*[13,16,242–244], Helrich *et al.*[14] and Jouany *et al.*[245] characterizing the sleep-promoting effects of GHB led to speculation by Mamelak that this substance may improve the sleep of patients with insomnia or fragmented sleep associated with a history of psychiatric illness. In their 1973 report, Mamelak *et al.*[47] describe the placebo-controlled, crossover administration of low-dose GHB to five patients (three males and two females; age range 35–60 years) with all-night PSG monitoring. In all subjects, an increase in delta sleep was observed after GHB compared with placebo, along with a subjective improvement in sleep quality and the restorative nature of sleep; no rebound insomnia or withdrawal effects were seen. In 1995, Reder *et al.*[246] explored the use of GHB to induce SWS in a patient with fatal familial insomnia. In this patient, who had been devoid of SWS, GHB induced 3 hours of SWS and had no effect on REM sleep. Furthermore, this patient showed enhanced daytime alertness, as evidenced by his greatly improved ability to answer simple questions.

4.2.2 *Fibromyalgia*

Fibromyalgia is a perplexing disorder where disrupted sleep is believed to result from its primary pathophysiology or to perhaps even contribute to or exacerbate symptoms. Patients with fibromyalgia often exhibit an electroencephalogram (EEG) pattern of excess alpha activity during NREM sleep, especially during SWS, and also evidence of reduced SWS, which may correlate with musculoskeletal pain and mood disturbance[247,248]. Of interest, work by Lentz *et al*.[249] reproduced results previously reported by Moldofsky *et al.*[248] that disrupted SWS correlated with the rapid appearance of discomfort/pain in healthy controls. This research showed that disrupting deep SWS, without reducing total sleep or sleep efficiency, for three consecutive nights in 12 healthy, middle-aged, sedentary women without muscle discomfort was associated with the appearance of fibromyalgia symptoms, including a decreased pain threshold, increased discomfort, fatigue and the inflammatory flare response in skin.

Two trials conducted by Scharf *et al.*[37,38] evaluated the effects of GHB in the treatment of fibromyalgia. The first 11 patients underwent a 4-week open-label pilot trial to evaluate the effect of GHB on nighttime sleep and daytime fatigue, among other endpoints. PSG recordings were used to evaluate sleep stages, sleep efficiency and the presence of the alpha anomaly in NREM sleep. Daily diaries were kept to asses fatigue levels. After administration of GHB, there was a significant increase in SWS, a decrease in the severity of the alpha anomaly, and a significant improvement in subjective fatigue by more than 110% compared with baseline[38]. Moreover, EEG registrations of sleep showed that GHB induced an increase in delta sleep by ~60% and decreases in NREM sleep periods with alpha intrusion by ~70%.

This preliminary finding led Scharf *et al.*[37] to conduct a second, more rigorous study to evaluate the effect of GHB on fibromyalgia. This 24-patient (all female), double-blind, randomized, placebo-controlled, crossover trial evaluated the effects of GHB on subjective sleep quality; objective PSG sleep variables such as alpha intrusion, SWS (stage 3/4) and sleep efficiency; and subjective daytime fatigue and alertness, among other endpoints. During GHB treatment, subjective sleep quality significantly improved by 33%, compared with only 10% during placebo treatment. SWS (stage 3/4) was significantly increased from 14.9 ± 6.5 minutes at baseline to 21.5 ± 7.1 minutes at endpoint, with a change of 6.6 ± 8.6 minutes. Additionally, alpha intrusion, measured as a percentage of NREM sleep, significantly decreased changed from a baseline of 36.9 ± 10.7 minutes to 25.8 ± 11.8 minutes, which represented a –11.1 ± 7.1-minute change, and sleep latency and REM sleep were significantly decreased compared with placebo. Subjective fatigue scores were evaluated by assessing morning fatigue, end-of-day fatigue and overall fatigue. Morning alertness significantly improved by 18% for GHB compared with 2% for placebo. The authors concluded that GHB effectively addressed nighttime sleep and excessive daytime sleepiness in this study group.

A double-blind, placebo-controlled study by Moldofsky *et al.*[250] assessed the effects of SXB 4.5 g and 6 g/night administered to fibromyalgia patients for 8 weeks on sleep physiology and sleep/wake-related symptoms; 151 patients completed the study (54

placebo, 51 SXB 4.5 g, and 46 SXB 6 g). Data showed that SXB treatment improved EEG sleep physiology and sleep-related fibromyalgia symptoms. Compared with placebo, both doses of SXB achieved statistically significant improvements in daytime sleepiness, morning fatigue, sleep, daytime functioning, vitality, and in general and morning tiredness; both doses also demonstrated decreased REM sleep. SXB 6 g/night improved afternoon, evening and overall fatigue, reduced wakefulness after sleep onset, and increased stage 2, slow-wave, and total NREM sleep versus placebo. Moderate correlations were noted between changes in subjective sleep and pain measures.

A phase 3 double-blind, randomized, placebo-controlled trial conducted by Russel *et al.*[228] evaluated the effect of SXB administered at doses of 4.5 and 6 g per night during 14 weeks in patients with fibromyalgia (a previous prospective, randomized clinical trial to evaluate the potential role of SXB in the management of fibromyalgia had already been carried out by the same group[251]); 334 patients completed the study (111 placebo, 119 SXB 4.5 g, and 104 SXB 6 g). In general, significant effects of SXB were observed as early as 1 week after initiating therapy and maintained throughout the 14-week trial duration. Significant reductions in pain, fatigue and in the Fibromyalgia Impact Questionnaire total score, an indicator of improvement in fibromyalgia patient functioning, were observed with SXB relative to placebo. There was a statistically significant reduction in patient-reported sleep disturbance with SXB relative to placebo, observed in both SXB groups at the first evaluation (week 4) and maintained throughout the study; a significantly reduced frequency of waking up several times per night was also observed for both SXB doses. The proportion of patients who reported a global improvement of ‘‘much’’ or ‘‘very much’’ better was 48.3% in the SXB 4.5 g group and 45.4% in the SXB 6 g group, significantly greater than the 27.2% for placebo[228].

Another phase 3 double-blind, randomized, placebo-controlled 14-week trial conducted by Spaeth *et al.*[229] (376 patients completed the study; 131 placebo, 129 SXB 4.5 g, and 116 SXB 6 g) also showed significant improvements in function, fatigue, tenderness, health-related quality of life, and subject's impression of change in overall wellbeing. A significant improvement in sleep quality was observed for both SXB 4.5 g/night and SXB 6 g/night versus placebo. Improvements in functionality related to sleep were also significantly greater for SXB 4.5 g/night and SXB 6 g/night versus placebo. There were strong correlations between a subjective measure of restorative sleep and pain ($r=0.68$; $p<0.001$), fatigue ($r=0.78$; $p<0.001$), global assessments ($r=0.59$; $p<0.001$) and sleep quality ($r=0.56$; $p<0.001$). Post-hoc analyses demonstrated statistically significant associations of moderate strength between the changes in the Jenkins Sleep Scale (JSS) and in the Functional Outcomes of Sleep Questionnaire (FOSQ), and the changes in other clinical outcomes (pain, multidimensional function, fatigue). Significantly greater proportions of subjects reported feeling ‘much better’ or ‘very much better’ at week 14 and had their condition rated as ‘very much improved’ or ‘much improved’ by investigators with SXB 4.5 g/night and SXB 6 g/night versus placebo.

Overall, multiple large, robust and well-designed clinical trials have shown that SXB has therapeutic effects not only on sleep, but also on other important clinical fibromyalgia symptoms, such as fatigue and pain. Since the induction of alpha activity during SWS (often observed in fibromyalgia patients) has been shown to produce fibromyalgia-like symptoms in healthy individuals[248,249], the increased pattern of alpha-delta sleep may underlie the exacerbated pain experienced by fibromyalgia patients[252]. Given that SXB was shown to reduce the incidence of alpha-delta sleep in fibromyalgia patients[37,250], and that SXB was able to restore normal delta sleep in an experimental model of alpha-delta sleep[253], it is possible that the therapeutic effects of SXB on fibromyalgia-associated pain may be linked to its action on alpha activity during SWS. But further research is needed to fully understand SXB's effects on fibromyalgia pathophysiology.

4.2.3 Parkinson's Disease

Parkinson's Disease (PD) is strongly associated with excessive daytime sleepiness (EDS) and nocturnal sleep dysfunction. In fact, both PD and dopaminergic treatments for it can cause EDS. Intrinsic changes in sleep architecture include reduced SWS and reduced sleep spindles[254,255].

To evaluate SXB in PD, Ondo *et al.*[256] evaluated 27 patients receiving a mean SXB dose of 7.8 g per night. Patients were titrated over the first 6 weeks, then maintained on a steady dose for another 12 weeks. From baseline to endpoint, mean overnight SWS time increased almost 90% (from 41.3 ± 33.2 to 78.0 ± 61.2 minutes) and subjective sleep quality improved by about 30% (Pittsburgh Sleep Quality Inventory score significantly lowered from 10.9 ± 4.0 to 6.6 ± 3.9). In response, subjective sleepiness scores improved by nearly 28% (Epworth Sleepiness Scale score dropped from 15.6 ± 4.2 to 9.0 ± 5.0) and fatigue improved by about 18% (Fatigue Severity Scale score dropped from 42.9 ± 13.2 to 36.3 ± 14.3). However, there were no significant changes in Unified Parkinson Disease Rating Scale scores. It was concluded that, given the robust efficacy and good tolerability of SXB in this patient sample, and the lack of effective treatment for EDS in patients with PD, controlled trials with SXB in PD using objective measures of daytime sleepiness are justified[256].

4.2.4 Alzheimer's Disease

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder that comprises between 60-80% of all age-related dementias[257]. The disease has three primary groups of symptoms: cognitive symptoms, non-cognitive symptoms, and instrumental symptoms. The cognitive symptoms can include memory loss, language issues, and diminished executive functioning. The non-cognitive symptoms can include behavioral disturbances, depression, hallucinations, delusions, and agitation. The instrumental symptoms refer to issues that impair the ability to perform activities of daily life (e.g., driving, shopping, dressing, and eating unaided)[258].

Pathogenesis of Alzheimer's Disease

The neurological processes that give way to AD can take decades to occur[259]. It is believed that the accumulation and aggregation of amyloid beta (Aβ) peptides is the initial step in the complex series of biochemical and physiological processes, termed the 'amyloid cascade.' Aβ accumulation is thought to result from an imbalance between its production via the amyloidogenic processing of the amyloid peptide precursor and its removal from the brain through various clearance pathways and enzyme-mediated degradation. Impaired clearance of Aβ has been proposed to play a key role in the development of sporadic forms of AD[260,261]. Therefore, lowering Aβ concentrations in the brain constitutes a potential therapeutic approach to protect against amyloidopathy and cognitive decline and/or delay the onset of AD[262]. During this preclinical period, the activity of several critical proteins, regulated by multiple inherited or environmental factors, participates in homeostasis of Aβ.

Because the process by which AD develops can take years-to-decades, identifying factors that can even slightly alter the pathogenic process could lead to delayed onset and reduced disease severity.

Neurobiological Effects of GHB on AD Pathogenesis

Pharmacologic doses of SXB have been shown to induce multiple effects that could have a beneficial impact on limiting AD pathophysiology, including HDACs inhibition, gene expression of Aβ-degrading brain protease, neprilysin, and SWS induction[263].

Research by Klein *et al.*[264] evaluated the effects of auto-administration of SXB to APPSWE mice who express high concentrations of the mutant Aβ, develop significant amyloid plaques, and display memory deficits. SXB induced the overexpression of brain neprilysin, reduced cerebral Aβ contents, counteracted phosphoramidon-induced brain neprilysin inhibition and Aβ accumulation, and prevented cognitive deficits. Because SXB doses used in this study were clinically relevant, data suggested that chronic oral administration of GHB or its analogs may be considered for strategies against presymptomatic or established AD.

Sleep and AD

Compared to cognitively normal older adults, sleep disruption is more pronounced in older individuals with mild cognitive impairment (MCI) or AD—with over 60% having at least one clinical sleep disorder—or in individuals at high genetic risk for developing AD (APOE4+ carrier)[265,266].

Sleep disturbance appears to be one of the earliest observable symptoms of AD, often present even before diagnosis[266]. Mounting evidence indicates that sleep disruption has a causal and bi-directional relationship with AD pathophysiology. This is substantiated by the fact that insomnia and sleep apnea are simultaneously features of, and risk factors for MCI and AD, decreasing their onset age[267,268]; conversely, superior sleep quality or successful treatment of sleep disturbances

can protect cognitive function and decrease the risk of developing MCI and AD[268–270]. Importantly, poor sleep correlates with the severity of cortical Aβ burden and with CSF measures of Aβ, both in cognitively normal older adults and in MCI and AD patients[271–273].

NREM sleep seems to be of special relevance to the pathophysiology of AD, since cognitive impairment in AD patients is most significantly associated with poor NREM sleep quality[271,272]. NREM SWS decline is accelerated in AD patients relative to age-matched controls, with the magnitude of sleep disruption and the severity of AD appearing to be correlated[271,274,275]. Also, the levels of tau and Aβ protein in the CSF of AD patients predict the degree of reduced SWS time and the decreases in sleep efficiency and REM sleep[271]. Importantly, the experimental increase in cortical Aβ was shown to lead to a fragmentation of NREM sleep[276,277], while the experimental decrease in NREM sleep and increase in wake time was shown to escalate Aβ production and its cortical deposition[276].
NREM sleep promotes the clearance of extracellular Aβ that accumulates during wakefulness[278]; disrupted NREM SWS and excess wakefulness may therefore increase Aβ accumulation, which itself impairs NREM SWS, thereby promoting its own accumulation[279]. NREM sleep disturbance can also impair cellular repair processes and the regulation of oxidative stress, enhancing the metabolic distress and oxidative damage induced by wakefulness[280,281]; oxidative stress in turn promotes Aβ accumulation[282], which further promotes oxidative stress[283], leading to an amplification of Aβ accumulation through another positive feedback loop. Aβ aggregation then triggers increased sleep disruption, further feeding this vicious cycle that accelerates AD pathogenesis. By being associated with Aβ accumulation, sleep disruption can also be linked to the AD-associated progression of cognitive decline. CSF Aβ (and tau and orexin) levels correlate with both sleep and cognitive measures, supporting the association between sleep, AD pathophysiology and cognitive decline[271].

Although the precise mechanism through which Aβ disrupts NREM sleep is unknown, it has been proposed that it may be associated with a disruption of NMDA and $GABA_A$ receptor function[284 285] leading to a reduction in the generation of low-frequency (<1 Hz) slow oscillations of NREM sleep[286].

Pharmacologically-Induced SWS to Ameliorate AD Pathogenesis

Since sleep is a modifiable factor, it can also be a target for AD therapy. The increasingly evident association between sleep disturbances and AD pathophysiology suggests that therapeutic interventions aiming at restoring NREM SWS may be beneficial by increasing Aβ clearance, decreasing metabolic distress, promoting cellular repair processes, regulating oxidative stress, and, consequently, delaying cognitive decline.

Given the growing evidence of the impact of sleep disturbances on AD pathophysiology, the ability of GHB to improve sleep quantity and quality in both healthy and clinical populations, particularly in promoting SWS and delta power[173,174], may be of interest in the context of AD therapy. In humans, NREM

sleep induced by GHB treatment seems to be beneficial for the consolidation of declarative memory[287]. Also, pharmacological studies have shown that therapeutic doses of GHB elicit a substantial GABAergic potentiation in the brain[288], which, in turn, has been shown to significantly attenuate the severe SWS changes observed in a mouse model of AD[289].

The effects of GHB in increasing NREM SWS[46] could potentially attenuate the vicious cycle of Aβ accumulation and AD progression promoted by poor NREM SWS[278,279]. However, this possibility still needs to be adequately studied so that a proper insight into the role of GHB in hampering AD pathogenesis can be obtained.

4.2.5 *Narcolepsy*

GHB has a three decade history and the most robust clinical dataset of all drugs assessed in the treatment of narcolepsy[290,291]. Broughton and Mamelak[172] and Mamelak *et al.*[174] initially hypothesized that GHB could reduce sleep fragmentation in narcolepsy through its known effects on increased sleep consolidation, SWS augmentation and REM sleep facilitation. Their seminal combined work in patients with narcolepsy led to the beginnings of an understanding of GHB as a potent treatment not only for improving sleep, but also for controlling cataplexy and enhancing daytime alertness[174,290].

Further work by Mamelak *et al.*[292], Scharf *et al.*[293], Scrima *et al.*[294], Lammers *et al.*[295], and others provided long-term, controlled evidence supporting the effectiveness of GHB in narcolepsy. Recently, extensive research, through multiple large controlled and open-label studies—including a comparative study with modafinil in the treatment of daytime sleepiness—has led to an understanding of GHB as a primary and unparalleled treatment for the complex of narcolepsy symptoms: sleepiness, cataplexy and sleep fragmentation. The following sections focus only on the sleep and wakefulness (i.e., impact on excessive daytime sleepiness) data generated from the work of multiple researchers.

4.2.5.1 *Initial Exploration*

The initial exploration of GHB by Broughton and Mamelak[290,291] in 20 subjects with narcolepsy proved promising in improving sleep continuity and reducing daytime sleepiness in narcolepsy. PSG analysis revealed statistically significant changes in sleep, including increased SWS duration and sleep efficiency, and reduced stage 1 sleep, sleep fragmentation, REM sleep latency and REM sleep density with treatment compared with baseline. Additionally, daytime sleep duration and number of sleep episodes were significantly reduced.

Scharf *et al.*[293] conducted a treatment trial of GHB, enrolling 30 patients (17 women and 13 men) in an open-label study comparing pretreatment baseline measures with those after 1 month and 6 months of treatment. By the end of the first week of treatment, a statistically significant improvement over baseline was evident in daytime sleep attacks. Sleep attacks were reduced by 70% of baseline at this time. All symptoms were found to improve still further until the end of the 6-

month observation period. PSG at 1 month versus baseline revealed significantly increased total sleep time, sleep efficiency and percentage of time spent in SWS; significant reductions in total wake time, awakenings and REM sleep latency were also noted. Changes in nocturnal sleep observed at 4 weeks continued throughout the 6 months of treatment, as demonstrated by PSG in a subset of 12 patients who underwent repeat PSG at 6 months.

Long-term open-label data were collected in a combined effort by Mamelak, Scharf and Woods and reported in the mid-1980s[292]. This report included experience of 48 patients (21 men and 27 women) treated with GHB 4.5–9 g for durations of 6 months to 9 years. Thirty-six of the 48 patients (75%) became symptom free over time, with GHB given as monotherapy in some patients and the addition of low-dose dexamphetamine or methylphenidate (<30 mg/day) in others. An additional six patients experienced an incomplete but clinically meaningful response, while the remaining six patients reported a lack of response, which resulted in discontinuing treatment. No patients discontinued treatment because of adverse effects.

4.2.5.2 Early Placebo-Controlled Trials

In 1989 and 1990, Scrima *et al.*[294,296] published the first reports of a blinded, placebo-controlled trial of low-dose GHB in narcolepsy. This U.S. government-sponsored study employed a 4-week (29-day) crossover design with a 6-day washout period. Twenty patients (ten men and ten women) were randomized to treatment with the relatively low dose of GHB (50 mg/kg) or placebo per night during the first period. After the washout period, patients were transferred to the contrasting treatment for 29 days in a double-blind fashion. By the end of week 1, subjective 'arousals from sleep' and hypnagogic hallucinations had decreased significantly in patients receiving GHB compared with in those receiving placebo (p=0.035 and p=0.008, respectively). This improvement continued for the duration of treatment. Objective measures of sleep were also significantly influenced by GHB administration. Specifically, decreased stage 1 sleep (p=0.012), increased stage 3 sleep (p=0.008), increased delta (stages 3 and 4 combined) sleep (p=0.049), fewer stage shifts (p=0.002) and fewer awakenings (p=0.006) occurred after GHB versus placebo.

Despite substantial effects on nocturnal sleep, low-dose GHB did not significantly improve measures of daytime alertness (both subjective and objective) compared with placebo in this study. These measures included the Stanford Sleepiness Scale (SSS), the number of sleep attacks per day, the number of naps per day and the multiple sleep-latency test (MSLT). However, a trend toward reduced sleepiness on the MSLT (p=0.074) as well as a significant increase in MSLT wakefulness (p=0.03) and a significant reduction in sleep-onset REM episodes (p=0.020) during MSLT on the final day (day 29) of GHB versus placebo was seen.

Lammers *et al.*[295] initiated a similar blinded, placebo-controlled, crossover study of low-dose GHB in 24 patients (13 men and 11 women) with narcolepsy and cataplexy. Nocturnal sleep-related subjective and objective (PSG) measures

showed a response generally consistent with the previously mentioned controlled and uncontrolled studies. A marked reduction in hypnagogic hallucinations (p=0.008), an increase in SWS (stages 3 and 4, p=0.053), a reduction in REM-sleep awakenings (p=0.016) and a decrease in time spent awake during REM sleep (p=0.007) was observed during GHB treatment compared with placebo. Of interest, all parameters of subjective daytime sleepiness were substantially improved, despite the low dose of GHB used. Patient-reported number of sleep attacks (p=0.001) and severity of daytime sleepiness (p=0.028) were reduced. MSLT results, however, were not significantly different from placebo, although only seven subjects had adequate MSLT data for analysis.

The FDA solicited the participation of Orphan Medical in providing the necessary research and development work required for regulatory approval of GHB for medicinal use in narcolepsy. At the time, no medication had undergone the rigorous evaluation required for approval in cataplexy treatment. The following section summarizes the substantial exploration of GHB conducted under the direction of Orphan Medical in approximately 1000 patients with narcolepsy.

4.2.5.3 Multicenter Clinical Trials

A large number of multicenter clinical trials of SXB in the treatment of narcolepsy have been sponsored by Orphan Medical in the past decade. The results of these trials provide definitive and essential information on the efficacy of this agent in excessive daytime sleepiness and nocturnal sleep fragmentation, as well as on its tolerability and safety profile. The results of the most relevant multicenter clinical trials, at the time of writing, are summarized in this section.

The assessment of the impact of SXB on subjective measures of daytime sleepiness was assessed in a 4-week placebo-controlled, multicenter trial in which 136 patients with narcolepsy were randomized to receive SXB 3, 6 or 9 g or placebo nightly for 4 weeks[175]. A clear dose-related improvement on all measures assessed, including the ESS and the number of inadvertent naps/sleep attacks, was observed. These effects reached statistical significance at the 9 g dose for both measures ($p = 0.001$ and $p = 0.012$, respectively). An open-label extension trial monitored these patients for another 12 months after the initial 4-week double-blind period. This study included bi-monthly Epworth Sleepiness Scale (ESS) measures of sleepiness[297]. Mean ESS for patients before enrolling in the preceding placebo-controlled trial was 18 and was significantly improved to 13 ($p < 0.001$) during the 1-year period, with little fluctuation over time and no evidence of tachyphylaxis.

To further explore the dose relationship of SXB-induced effects on night-time sleep and daytime sleepiness, a subsequent multicenter study subjected patients to a 'forced' dose titration of SXB[173]. Twenty-five patients (7 men and 18 women) were evaluated during a baseline period, then administered SXB 4.5 g for 4 weeks followed by 6 g for 2 weeks, then 7.5 g for 2 weeks, and then 9.0 g for 2 weeks. Measures assessed included PSG, ESS and the 20-minute Maintenance of Wakefulness Test (20-min MWT). The main findings of this trial included a robust, dose-related and statistically significant increase in SWS duration at doses of 7.5 g

and 9 g, and in delta power (a measure of the rate of occurrence of approximately 0.5–4Hz EEG activity coupled with the amplitude of the waves in this frequency range) across all doses compared with baseline. Additionally, REM sleep latency was significantly decreased, and REM sleep duration significantly increased during the first night of administration of SXB 4.5 g. In contrast, with longer administration, the shortened REM sleep latency abated and total REM sleep duration decreased in a dose-dependent manner, so that at a dose of 9 g, REM sleep duration was moderately but statistically significantly reduced relative to baseline. No consistent effects were seen on sleep latency, total sleep duration, stage 1 or 2 sleep duration, or the number of awakenings, across all doses, in this study.

Subjective sleepiness showed significant improvement, as ESS group means decreased (improved) with all doses of SXB ($p<0.001$) compared with baseline. The study participants, when receiving the 7.5 g ($p<0.001$) and 9 g ($p<0.001$) doses, showed significant improvement compared with pre-baseline assessment. Objective sleep-latency measures during the 20-min MWT also evidenced robust improvement with both 7.5 g ($p<0.01$) and 9 g ($p<0.001$) compared with baseline. MWT group means more than doubled at the 9 g dose (baseline = 4.5 minutes, 9 g = 10.6 minutes). Results of this study parallel those found by Broughton and Mamelak[290] and Mamelak *et al.*[174] almost 30 years ago.
The effects of SXB on nocturnal sleep and excessive daytime sleepiness were again tested in a multicenter, placebo-controlled, parallel-group trial. This study evaluated 228 patients for 8 weeks[298], with doses of 4.5 g, 6 g and 9 g (Figure 4).

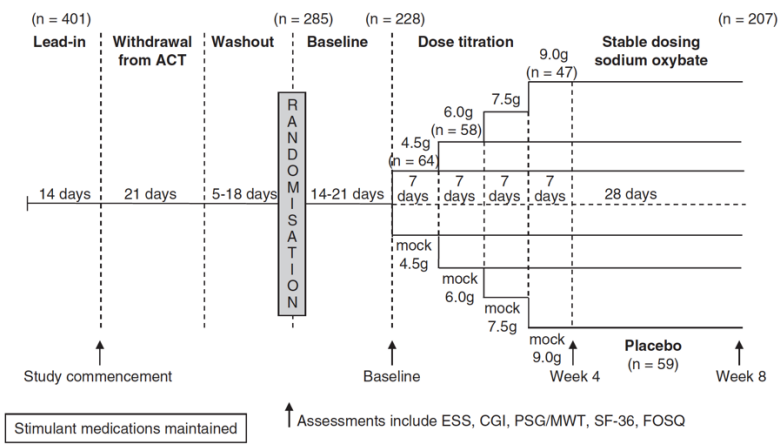


FIGURE 4. Design of an 8-week randomised, double-blind, placebo-controlled, parallel-group trial comparing the effects of three doses (4.5, 6.0 and 9.0g nightly in two evenly-divided doses 2.5–4 hours apart) of orally administered sodium oxybate with placebo in 228 patients diagnosed with narcolepsy.[298]

As expected from the results of prior studies, SXB had a significant and remarkable effect on nighttime sleep (Figure 5): SWS (4.5 g, $p<0.05$; 6 g and 9 g, $p<0.001$) and

delta power (4.5 g, p=0.006; 6 g and 9 g, p<0.001) were significantly increased at all doses of SXB compared with placebo. REM sleep duration was again moderately decreased (p<0.05) at the 9 g dose only. A significant decrease in the number of awakenings (p<0.05) and stage 1 sleep (p<0.001) occurred at the 6 g and 9 g doses and a modest increase in total sleep duration (p<0.05) was observed at the 9 g dose. Improvement in measures of sleepiness was again consistent with previous research.

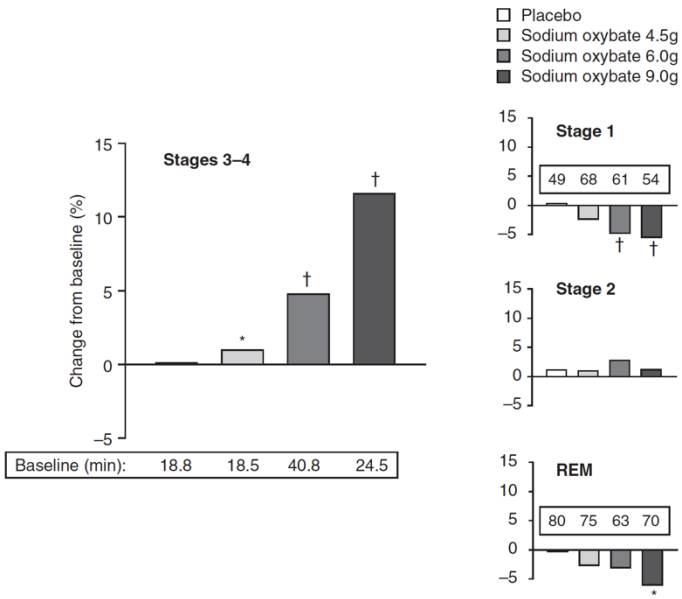


FIGURE 5. Effect of sodium oxybate on polysomnographically recorded sleep stages in 193 patients with narcolepsy.[298]

Subjective sleepiness showed significant improvement at both 6 g and 9 g via decreased (improved) ESS (Figure 6) and number of inadvertent naps/sleep attacks (p<0.001 for both measures on both doses.). Additionally, sleepiness improved, with a 10-minute MWT increase from 7.6 minutes at baseline to 17.7 minutes at 9 g (p<0.001); this magnitude of improvement for a drug taken at night on daytime sleepiness is unusual and unprecedented.

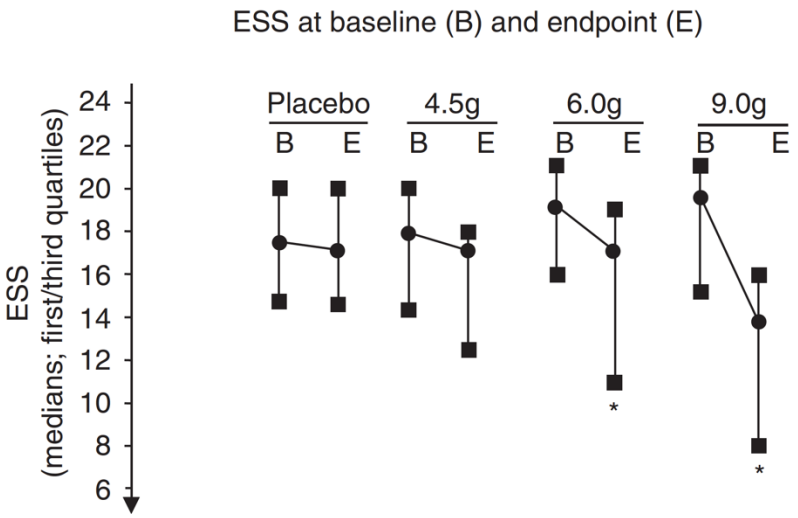


FIGURE 6. Effect of sodium oxybate on subjective sleepiness, as assessed using the Epworth Sleepiness Scale (ESS), in 228 patients with narcolepsy.[298]

Ultimately, a comparative study was designed to evaluate the effect of SXB on daytime sleepiness compared with modafinil[299]. This double-blind placebo-controlled (double-dummy) trial compared SXB alone, modafinil alone, modafinil in combination with sodium oxybate, and placebo (Figure 7) in 222 patients with narcolepsy with or without comorbid cataplexy for an 8-week treatment period.

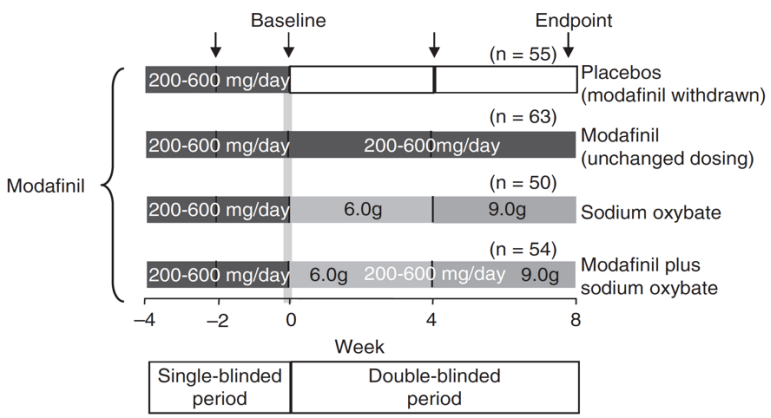


FIGURE 7. Design of an 8-week randomised, double-blind, double-dummy, placebo-controlled, parallel-group, multicentre trial (trial 4) comparing the effects of orally administered sodium oxybate and modafinil with placebo in the treatment of daytime sleepiness and sleep fragmentation in narcolepsy.[299]

Patients entering the study who had been receiving stable, effective and tolerable doses of modafinil for daytime sleepiness were randomized to one of these four

treatment groups. Statistically significant improvement in daytime sleepiness, as measured by ESS (Figure 8) and the number of inadvertent naps/sleep attacks, was found in the group of patients receiving SXB alone compared with modafinil alone. The combination therapy group had the greatest overall improvement in subjective and objective measures of sleepiness.

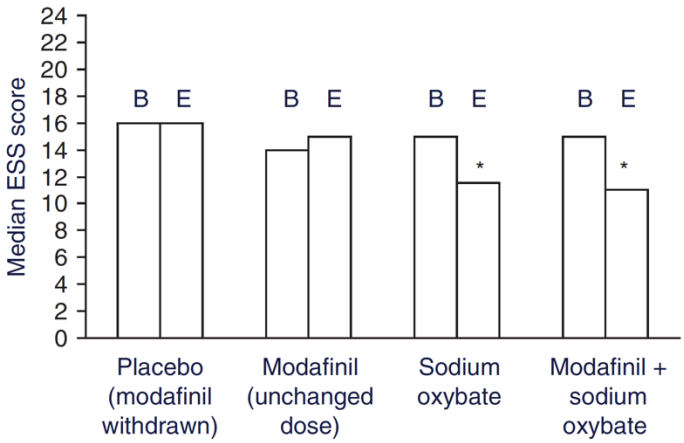


FIGURE 8. Mean change in sleep latency on the Epworth Sleepiness Scale (ESS) after 8 weeks of treatment with placebo, modafinil, sodium oxybate, or a combination of modafinil and sodium oxybate in patients with narcolepsy.[299]

Throughout these multicenter clinical trials, the most common reported adverse events in SXB recipients included dose-related headache, nausea, dizziness and somnolence.

5. Conclusions

5.1 Neurobiology

GHB is an endogenous short-chain fatty acid synthesized locally within the CNS, mostly from its parent compound GABA. Approximately 1–2% of GABA converts to GHB, which is relatively rapidly converted into CO_2 and H_2O through the Krebs cycle. GHB for exogenous administration was first synthesized in the early 1960s and found to readily cross the blood-brain barrier into the CNS, where it displays distinct pharmacological effects. Evidence suggests a role for GHB as a neuromodulator/neurotransmitter, as GHB is synthesized in neurons heterogeneously distributed throughout the CNS, stored in vesicles, released via potassium-dependent depolarization into the synaptic cleft, and undergoes reuptake into the nerve terminal. Under endogenous conditions and concentrations, and depending on the cell group affected, GHB may increase or decrease neuronal activity by inhibiting the release of the primary co-localized neurotransmitter. For example, GHB may decrease neuronal activity when inhibiting the release of the excitatory neurotransmitter dopamine and increase neuronal activity when inhibiting the release of the inhibitory neurotransmitter GABA. After exogenous administration, it is likely that GHB acts at GHB binding

site(s) and $GABA_B$ receptors, although it appears that most of the behavioral effects are mediated through the $GABA_B$ receptor. On neurons, supraphysiological concentrations of GHB have a qualitatively different effect than endogenous GHB concentrations. These elevated levels, mostly acting through $GABA_B$ modulation on various neuron groups, decrease neuronal activity. On washout from supraphysiological concentrations, increased neuronal responsiveness has been observed. This activity may underlie the sleep modulation seen when GHB is administered before nighttime sleep onset and, conversely, the wakefulness stimulating effects observed during the day following nighttime administration.

5.2 Clinical Effects

GHB, administered in the form of SXB, modulates sleep in healthy subjects, and sleep and wakefulness in clinical populations, including groups with insomnia, fibromyalgia and narcolepsy. In narcolepsy, the results of large, multicenter trials corroborate earlier work and demonstrate a consistent effect of SXB on SWS activity, yielding substantial, dose-related increases in SWS duration and delta power. Additionally, dose-related reductions in stage 1 sleep and number of awakenings are apparent in the larger studies, as well as modest increases in total sleep duration and reductions in REM sleep duration at a dose of 9 g. Multiple measures of daytime sleepiness demonstrated consistent short- and long-term improvement when SXB was administered in combination with stimulant therapy or as the only wake-promoting treatment. In addition, compared with modafinil, SXB as monotherapy appears to produce equal or greater improvement in daytime sleepiness in patients with narcolepsy with, or without, co-morbid cataplexy.