SLEEP REGULATION IN THE STOP-NULL MOUSE MODEL OF SCHIZOPHRENIA

by

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DEDICATION PAGE

I dedicate this thesis to my loving mother, father and two brothers Joshua and Luke. You 've been there from the start and will be until the end. We are forever bound, forever friends.

Joshua 1:9
" Have I not commanded you? Be strong and courageous. Do not be afraid; do not be discouraged, for the LORD your God will be with you wherever you go."

Luke 11:9
"..Seek and you will find..."

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ABSTRACT

Sleep disturbances are common in patients with schizophrenia and may correlate with symptoms of cognitive and affective abnormalities. Mice lacking the cytoskeletal-associated protein Stable Tubule Only Polypeptide (STOP) display cognitive, behavioural and neurobiological deficits that mimic those seen in patients with schizophrenia. Using polysomnography and EEG power spectral analyses in conjunction with sleep deprivation, this study for the first time, provides a thorough assessment of the degree and nature of sleep wake patterns exhibited by STOP-null mice. Adult male STOP-null (KO; n=7) and wild-type (WT; n=8) mice were implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes. EEG and EMG were recorded during a 24 h baseline period (in a 12:12 light-dark cycle), followed by 6 h of sleep deprivation (via 'gentle handling' in the second half of the light phase) and a 24 h recovery period. During the 24 h baseline period, the KO mice spent more time awake and less time in non-rapid eye movement (NREM) and REM sleep than the WT mice. The KO mice had more wake and NREM sleep episodes, and shorter NREM and REM sleep episodes compared to WT mice, particularly during the 12 h dark phase. In the recovery dark phase immediately following sleep deprivation, both groups exhibited similar increases in NREM and REM sleep amounts and NREM EEG delta power relative to corresponding baseline periods. These findings indicate that the STOP-null mice sleep less and their sleep is more fragmented compared to WT mice. Furthermore, the ability of STOP-null mice to recover lost sleep is intact as evidenced by similar sleep and NREM EEG rebounds. These results are consistent with the sleep patterns observed in individuals with schizophrenia, including reduced sleep time and fragmented sleep.

LIST OF ABBREVIATIONS USED

ANOVA	Analysis of variance
Bdr	Blind drunk
CNS	Central nervous system
CT	Computed tomography
DA	Dopamine
DISC-1	Disrupted in schizophrenia
DSM	Diagnostic and Statistical Manual of Mental Disorders
E17	Embryonic day 17
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
FFT	Fast-fourier transform
Glu	Glutamate
5-HT	5-hydroxytryptamine
Hz	Hertz
i.p.	Intraperitoneal
KO	Knock out
LFP	Local field potential
MAM	Methylazoxymethanol
MAP	Microtubule associated protein
ME	Main effect
mGluR	Metabotropic glutamate receptors
MRI	Magnetic resonance imaging
NA	Noradrenaline
NRG-1	Neuregulin-1
NMDA	N-methyl-D-aspartate
NREM	Non-REM
NREMS	NREM sleep
NT	Neurotransmitter
PET	Positron emission tomography
PV	Parvalbumin
REM	Rapid eye movement
REMS	REM sleep
SCN	Suprachiasmatic nucleus
SD	Sleep deprivation
SNARE	Soluble NSF attachment protein receptor
SNAP	Synaptosomal associated protein
STOP	Stable tubule only polypeptide
SWS	Slow wave sleep
SZ	Schizophrenia
WT	Wild type
ZT	Zeitgeber time

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CHAPTER 1– INTRODUCTION

1.1 SCHIZOPHRENIA

1.1.1 What is Schizophrenia?

Schizophrenia (SZ) is a devastating neuropsychiatric disorder that is characterized by disturbances in emotions, thoughts, language, and behaviour, as well as withdrawal from reality. Approximately 1 percent of individuals are affected worldwide, regardless of race, age or gender. The age of onset is typically between 15 and 25 years old. While men tend to develop SZ earlier than women, both genders are affected at equal rates (Schultz et al., 2007). The clinical manifestations, course and severity of this disease are varied and diverse, and though SZ is largely hypothesized to stem from a combination of genetic and environmental factors, its etiology remains to be determined (Lewis & Levitt, 2002).

1.1.2 History, Symptoms and Diagnosis

The definition of SZ has changed considerably from the initial identification. Psychiatrists Karl Ludwig Kahlbaum and Ewald Hecker categorized forms of 'madness' in the mid-19th century (Ban, 2004). In 1908, Eugen Bleuler was the first to introduce the term SZ (Berrios et al., 2003). Both he and psychiatrist Emil Kraeplin subdivided the illness into groups based on prominent symptoms and prognoses, which ultimately formed the basis of diagnostic criteria for SZ that would be included in recent editions of the Diagnostic and Statistical Manual of Mental Disorders (DSM) (Palha & Esteves 1997). Since the establishment of the first DSM, the diagnostic description of SZ has evolved considerably, as researchers aim to refine diagnostic criteria and improve the assessment of mental disorders.

Current criteria for the diagnosis of SZ are described in the DSM, 5th edition (5th ed.; *DSM–5;* Suppl.Table 1). Diagnosis is made based on specific symptoms (Suppl.Table 1; Criterion A) and deterioration in occupational and social functioning (Suppl.Table 1; Criterion B). Furthermore, symptoms must be present for a specific duration (see Suppl.Table 1, Criterion C for details). This classification system, in which clinical findings are segregated into positive, negative and cognitive symptoms, has been empirically validated in various studies (Bilder et al., 1985; Liddle, 1987). Positive symptoms refer to the presence of abnormal features, and are generally recognized as hallucinations, delusions and disorganized speech and behaviour, and agitated body movements. Negative symptoms refer to the absence of normal behaviour, and are generally recognized as deficits in affective and social domains. Lastly, cognitive symptoms include disruptions to attention, working memory and executive functioning (Andreasen, 1995).

Sleep disruption also occurs in SZ (see below for a more detailed description), but sleep abnormalities lack diagnostic specificity, as they do not reliably distinguish SZ from other neuropsychiatric disorders. As a result, sleep disturbances are not included as primary symptoms of SZ. However, sleep experts could help in discriminating sleep disorders in SZ from sleep disorders like narcolepsy, which can include hypnagogic hallucinations (Benson, 2008).

1.1.3 Clinical Time Course

An individual can experience full blown psychotic symptoms such as hallucinations and delusions without any preceding warning signs, i.e., acute onset SZ, or the disease can begin with a ‘prodromal phase’. The prodromal phase is marked by seemingly mild behavioural changes including abnormal sleep patterns, mild attentional and cognitive deficits, and social withdrawal.

Following the prodromal phase, the 'active phase' is characterized by psychosis, i.e., the inability to distinguish reality from non-reality, manifesting in delusional and hallucinatory symptoms. The severity of delusions and hallucinations can increase and decrease at any given time, and a patient is said to be in the 'residual phase' when psychotic symptoms decline. Furthermore, psychosis and disorganized behaviours in SZ may recur episodically as a patient relapses or exhibits acute exacerbation (Ram et al., 1992).

1.1.4 Etiology and Pathophysiology

Over the past several decades there have been significant efforts to understand the neurobiological basis of SZ. There is a plethora of research now that supports the notion that SZ is primarily associated with abnormal brain functioning (Atluri et al., 2013; Guo et al., 2013; Lewis & Lieberman, 2000). However, many questions remain and need to be answered to fully unveil the various neurobiological mechanisms and environmental influences that underpin the disease. Generally speaking, SZ is thought to be a neurodevelopmental disorder (dysfunction in neuron and cell growth and neural pathways) involving the interplay of susceptibility genes and environmental factors (Lewis & Levitt, 2002).

Etiology:

Genetic factors strongly contribute to the susceptibility to SZ. For instance, twin and adoption studies show that SZ is highly heritable (up to 80%). Different combinations of mutations within certain genes that act synergistically increase risk for developing SZ (Gejman et al., 2011). It has been suggested that multiple, interacting susceptibility genes, as well as highly penetrant, single rare mutations may exist to predispose an individual to SZ (Fabi et al., 2013). Furthermore, these various genetic mutations differ among individuals with SZ, and are

thought to play a role in neural development (O'Tuathaigh et al., 2007). Specific candidate genes that have been implicated in predisposing an individual to SZ include: neuregulin 1 (Stefansson et al., 2002), which plays a role in axon development and neural transmission, and dystrobrevin-binding protein 1 (DTNBP1) i.e. dysbindin (Straub et al., 2002), which regulates synaptic structure and function.

Nevertheless, genetics cannot entirely explain SZ, as the concordance rates for monozygotic twins ranges from 50-80 percent, and sporadic types of the illness exist. This indicates that epigenetic and environmental factors also contribute to susceptibility to SZ (Gejman et al., 2011). Environmental factors that are thought to be heavily involved in the development of SZ include prenatal complications, such as exposure to various teratogens, especially during critical periods of embryonic brain formation. Other risk factors include complications related to child birth, including premature birth, low birth weight, and poor oxygenation (Cannon et al., 2002). It has also been postulated that SZ is an autoimmune response activated by antibodies produced by the mother in response to viral infection during pregnancy (Lukkari et al., 2012).

Pathophysiology:

A range of neurological abnormalities have been found in patients with SZ (Cahn et al., 2002). Magnetic resonance imaging (MRI) and X-ray computed tomography (CT) studies have found neuroanatomical abnormalities in the brain of post mortem and living subjects with SZ, including enlarged lateral and third ventricles, gray matter atrophy in the frontal and temporal lobes, as well as a decrease in total brain volume (Benson 2008). Functional MRI and positron emission tomography (PET) studies have found reduced metabolism in the right frontal cortex (hypofrontality) and left temporal regions of the brain in patients with SZ (Riehemann et al.,

2001; Ehlis et al., 2012). Although these structural abnormalities are apparent at the onset of illness and thus are not considered to be a result of continual degeneration, these changes do not seem to be specific to SZ as they appear in other mental illnesses such as bipolar disorder (Ehlis et al., 2012).

In addition to structural and functional brain changes, altered levels of neurotransmitters have long been implicated in the etiology and pathophysiology of SZ (Carlsson & Lindqvist, 1963). The 'dopamine hypothesis' has been central to SZ research and has been extensively investigated. This hypothesis states that hallucinations and delusions correlate with hyperactivity of the mesolimbic dopamine system. Overactive and hypersensitive mesolimbic D2 receptors are thought to underlie the chemical synaptic dysregulation responsible for inducing psychosis in SZ (Carlsson & Lindqvist, 1963). This has been confirmed by studies that have found a positive correlation between therapeutic dose of antipsychotic medications and amount of D2 receptor blockage (Creese, et al., 1996.). Also, amphetamines, which increase levels of extracellular dopamine, have been shown to induce psychosis in patients without SZ, mimicking paranoid symptoms or worsening positive schizophrenic symptoms. There are limitations to the dopamine hypothesis, however. It cannot explain negative symptoms, as dopamine receptor antagonists have been ineffective in treating negative symptoms, and dopamine receptor agonists cannot induce negative symptoms. Furthermore, atypical antipsychotics act on more than just the dopamine system. Conversely, hypo-activity of mesocortical dopamine neurons has been thought to underlie negative symptoms of SZ (Dworkin & Opler, 1992).

Other neurotransmitters that play a role in SZ include serotonin and noradrenaline (Fournet et al., 2012). The potency of second generation antipsychotic medications correlates

with serotonin and α-adrenergic receptor blockade (i.e., have antagonistic effects at these receptors). Another neurotransmitter highly implicated in SZ is the excitatory neurotransmitter glutamate, based on the recent findings of SZ susceptibility genes that play a role in glutamatergic transmission (Bagot et al., 2012). Hypoglutamatergic signaling via NMDA receptors that causes chemical synaptic dysregulation as well as electrical synaptic irregularities have been found in SZ. Further proof of glutamate dysfunction in SZ comes from the findings that drugs that block NMDA receptors mimic positive and negative symptoms of SZ, while mGluR2/3 agonists have been shown to reduce positive and negative symptoms, and are prospective treatments for SZ (Feinberg et al., 2002).

1.2 SLEEP DISTURBANCES IN SCHIZOPHRENIA

In addition to disturbances in perception, emotion, language and thought, sleep disturbances are prevalent in about 80 percent of patients with SZ (Cohrs, 2008). Abnormal sleep patterns have been shown to be an early predictor and a trigger for psychosis, relapse and a sign of deteriorating course of the illness. It is unsurprising then that the quality of sleep correlates with quality of life in SZ patients (Zarcone et al., 1987; Hofstetter et al., 2005). Despite the high prevalence of sleep disturbances in SZ (Haffmans, et al., 1994), research has yielded inconsistent findings and no specific sleep pattern has been associated with SZ (Waters & Manoach, 2012).

1.2.1 Basics of Sleep

Studies using polysomnography (PSG) and EEG spectral analysis have shed light on the relationship between schizophrenia and sleep regulatory mechanisms, but before I describe sleep patterns associated with SZ, I will briefly introduce and define relevant sleep terminology.

1.2.1.1 How is Sleep Assessed?

Sleep and wake states can be clearly identified by specific neurophysiological criteria using PSG. For PSG, a subject is fitted with EEG electrodes to measure brain wave activity, EMG electrodes to measure skeletal muscle activity, and EOG electrodes to measure eye movements. The electrodes of the EEG can monitor very small voltage fluctuations caused by synchronized activity in very large numbers of neurons in cerebral cortex (Brown et al., 2012)

EEG waveforms are classified according to their amplitude and frequencies, and allow us to distinguish between non-rapid eye movement sleep (NREMS), rapid eye movement sleep (REMS) and wake as follows: NREMS is characterized by high voltage and low frequency EEG activity in the delta range (0.5-4.5 Hz) and low EMG activity. NREMS can be further broken down into 3 stages, N1, N2, and N3 (slow wave sleep). Delta waves are most prominent in N3. REMS is characterized by low voltage and high frequency EEG activity predominantly in the theta range (5-9Hz), and very low EMG activity reflecting muscle atonia, with occasional twitching. Wake is characterized by a low voltage and fast frequency EEG, and high EMG activity (Benson & Feinberg, 2011).

PSG provides an objective assessment of sleep variables such as total sleep time, sleep latency, the number of awakenings during sleep, and sleep efficiency. These parameters, in addition to the wave characteristics in the EEG, EMG and EOG, allow us to determine and assess sleep architecture (Swihart et al., 2012) .

1.2.1.2 How is Sleep Regulated?

The sleep-wake cycle in humans and animals is regulated by two processes that interact with each other: homeostatic and circadian (about a day) processes (Borbély, 1982). The homeostatic process regulates sleep intensity, which is measured by EEG slow-wave (delta) activity, the spectral power of the EEG in the frequency range of 0.5-4.5 Hz, during NREMS. This internal homeostatic drive increases during wakefulness, and dissipates during sleep, enabling organisms to compensate for the loss of sleep (which could be induced spontaneously or by sleep deprivation, for example) or the surplus of sleep (i.e., prolonged sleep in the morning or daytime naps). In short, sleep pressure builds with accumulating sleep loss, and subsequent to sleep deprivation there is a rebound in the intensity and amount of sleep, particularly slow wave sleep (Pace-Schott & Hobson, 2002).

The circadian process uses an endogenous clock housed in the suprachiasmatic nucleus (SCN) of the hypothalamus. The clock in the SCN is modulated by periodic environmental cues, called zeitgeibers, such as the light/dark cycle, and generates circadian rhythms in sleep/wake cycles. In diurnal animals, the circadian process facilitates wakefulness during the day, allowing consolidation of sleep at night. Sleep research in rodents has provided useful information on the SCN's sleep-wake regulatory role. Lesions to the rat SCN, for instance, fragment sleep, with sleep occurring at random with respect to time of day, but do not significantly affect the total amount of sleep or the amount of recovery sleep subsequent to sleep deprivation (Stenberg , 2007; Schwartz & Roth, 2008).

1.2.2 Sleep in Schizophrenia

On average, about 80 percent of patients with schizophrenia report disturbances in sleep, such as severe insomnia, sleep-wake reversal, and fragmented sleep (Benson & Feinberg, 2011). While insomnia prevails during the prodromal phase and is considered a predictor of psychotic relapse, medicated patients who are clinically stable can also experience sleep disturbances. The type of sleep disturbances reported is dependent upon clinical course and severity of symptoms.

1.2.2.1 Subjective Sleep Quality in Schizophrenia.

Subjective reports of sleep impairment in SZ patients are positively correlated with exacerbation of symptoms. Lengthy bouts of total insomnia occur with the onset of psychotic symptoms. while during periods of less psychotic agitation, patients still complain of insomnia, with increased sleep onset latencies, diminished total sleep time, and sleep interrupted by frequent episodes of wake (Chouinard et al., 2004). However, sleep abnormalities are not restricted to individuals with active psychosis. Patients that are clinically stable and on medication also report difficulties sleeping, with an inability to fall asleep quickly and frequent awakenings in the night.

Furthermore, SZ patients may report circadian rhythm disruption, including sleep-wake reversal (i.e., the individual sleeps during the day and stays up during the night) and circadian phase delay or advance (i.e., delayed or early sleep onset time) (Wulff et al., 2010). When SZ patients exhibit a circadian rhythm disruption, their sleep quality is markedly reduced; these patients often report that during periods of sleep they feel fidgety and restless, and experience fearful hypnagogic hallucinations and nightmares (Kyung Lee & Douglass, 2010). Not surprisingly, poor sleep quality is a predictor of impaired quality of life and poor coping skills

(i.e., substance abuse) in SZ patients which in turn can cause a patient to relapse (Hofstetter et al., 2005).

1.2.2.2 Objective sleep characteristics based on PSG in Schizophrenia

Many studies have described sleep disturbances in schizophrenia using objective measures of sleep with PSG. The findings tend to be consistent with reported subjective complaints as described above (Monti et al., 2013). However, PSG studies have also reported inconsistent findings due to variations in experimental design, control groups, insufficient sample size, inclusion criteria such as age, clinical features and clinical history, and medication status of the patient (Cohrs, 2008). A meta-analysis of 219 studies involving sleep recordings in patients with schizophrenia evaluated these confounding variables (Chouinard et al., 2004). The following 3 variables were found to be a primary effect of SZ, and not an indirect effect of medication: increase in sleep latency; decreased total sleep time; and decreased sleep efficiency (Chouniard et al., 2004). Another commonly reported finding in both unmedicated and medicated patients is the trouble in reaching a state of uninterrupted sleep, or sleep interrupted by many bouts of wake (Lauer et al., 1997; Poulin et al., 2003). Slow wave sleep (SWS) deficits and reduced REMS latency onset have also been identified in SZ; however, neither has been consistently reported (Lauer et al., 1997). Again, the discrepancies may be related to differences in the medication status of the patient, or, in the case of SWS, in differences in the criteria used to score SWS (Waters & Manoach, 2012).

1.2.2.3 Sleep-Related EEG Activity

As already mentioned, the deepest stage of NREMS (N3 or SWS) is defined by the predominance of slow waves in the EEG. Slow wave activity can be quantified using power spectral analysis of the EEG as delta power which corresponds to a 0.1-4.5 Hz component in the power spectrum. Many studies have shown that EEG delta power during NREMS is reduced in patients with schizophrenia, and that this reduction correlates with negative symptoms and cognitive deficits (Sarkar et al., 2010). Another characteristic of NREMS EEG is spindle activities (12-15 Hz) that are observed mainly during the intermediate state of NREMS (N2). The number and amplitude of NREMS EEG spindles are diminished in both unmedicated and medicated SZ patients (Wilson & Argyropoulos, 2012; Ferrarelli et al., 2007). This finding may indicate abnormality in the reticular thalamic nucleus and thalamocortical mechanisms of sleep spindle generation in SZ (Ferrarelli et al., 2007). In contrast to the reduction in delta power and spindles, power in the beta (20-35 Hz) and gamma (35-45 Hz) bands of the EEG across all sleep stages (including both NREMS and REMS) was increased in un-medicated schizophrenic patients, compared to healthy subjects (Tekell et al., 2005).

1.2.2.4 Slow-Wave Sleep Homeostasis

The SWS impairments bring up questions regarding the mechanisms that govern homeostatic drive and regulation. To reiterate, the widely held view holds that sleep pressure accumulates during wakefulness, and dissipates during SWS, which becomes shorter in duration across continuous bouts of NREM sleep cycles in the night in humans (Feinberg et al., 1969). In healthy individuals that are sleep deprived or subjected to long durations of wake, SWS constitutes a greater portion of NREM sleep following sleep loss, suggesting that the homeostatic

response serves restorative functions in the CNS. This can be seen as an increase or rebound in SWS or EEG delta power in NREMS following sleep deprivation, and this rebound increase may be impaired in SZ (Benson, 2006; Benson & Feinberg, 2011). In 1967, Luby & Caldwell examined the potential dysregulation of sleep homeostasis in SZ in a small sample of un-medicated patients, and found that there was minimal recovery of SWS (stage 4 NREMS; note that stages 3 and 4 are not distinguished according to the most recent criteria) activity following 85 hours of total sleep deprivation. SZ subjects exhibited a minimal 2 percent increase in stage 4 of NREMS, markedly less than the 28 percent increase exhibited by nonpsychotic controls. Interestingly, one SZ subject within the study exhibited a paradoxical decline in NREMS stage 4 delta activity following sleep deprivation (this decrement was compensated for by an increase in NREMS stage 1).

Another study examined EEG delta activity in 14 male unmedicated patients who were subjected to one full night of SD. Interestingly, these patients lacked stage 4 (SWS) during baseline as well as recovery nights; however, analyses revealed modest increases in delta power and periods of increased delta power during the recovery period following SD relative to baseline. This led the authors to conclude that the homeostatic drive in schizophrenia is functional but perhaps slightly attenuated compared to healthy controls (Benson & Feinberg, 2011).

Lastly, Hoffman et al. (2000) reported diminished EEG delta activity over continuous cycles of NREM sleep in unmedicated SZ patients, further supporting a diminished homeostatic drive in SZ.

1.3 ANIMAL MODELS OF SCHIZOPHRENIA

Reliable and predictive animal models of schizophrenia would be useful to explain underlying genetic and neurological processes, as well as to develop effective drug treatments to treat symptoms, and to aid prevention of the illness (Jones et al., 2011). There is no single animal model in the literature that fully replicates all the symptoms of schizophrenia. The majority of rodent models of SZ exhibit behavioural phenotypes that mimic the positive symptoms (e.g. behavioural hyperactivity and impaired sensori-motor gating), while fewer models recapitulate the negative and cognitive symptoms (e.g., social withdrawal and memory deficits) (Marcotte et al., 2001). Many rodent models replicate the changes in mesolimbic dopamine function observed in patients with schizophrenia, and these models fall into four broad categories: neurodevelopmental, pharmacological, lesion and genetic (Lipska & Weinberger, 2000).

In the following section, I will briefly discuss genetic knock out mouse models of schizophrenia, and then review the sleep patterns in three well characterized rodent models of schizophrenia that have been published to date, which leads to my study.

1.3.1 Genetic Rodent Models

As I previously mentioned, the etiology of schizophrenia appears to involve a large genetic component, and although no single gene fully explains the cause of this disorder, many candidate genes are linked to increased risk of developing SZ (Harrison & Weinberger, 2005; Gogos & Gerber, 2006; Allen et al., 2008). Genome wide studies have located various linkage regions that contain alleged susceptibility genes which typically segregate to proteins that play roles in neuronal plasticity, and glutamatergic and dopaminergic synapse formation and function (Harrison & Weinberger 2005). Most genetic animal models have been created to reproduce

changes in mRNA and proteins that are associated with SZ pathology, and aim to replicate specific SZ endophenotypes that are stable, and measurable features of the disease (Gottesman & Gould, 2003; Braff et al., 2007; O'Tuathaigh & Waddington, 2010). Certain susceptibility genes that encode proteins involved in neuron morphology and proliferation include: disrupted-in-schizophrenia 1 (DISC1), dysbindin-1, STOP (also known as MAP6), neuregulin-1 (NRG-1), and SNAP (synaptosomal associated protein)-25. These genes have been proposed as biomarkers for early detection of schizophrenia and have contributed to the understanding of abnormal dopamine and glutamate function which underlies the pathophysiology of SZ. The loss of these genes in KO mice have been shown to mimic many features of the disease (for a review see (Jones et al., 2011; Volle et al., 2012). The human STOP gene can be found in the 11q14 chromosomal region, which has been associated with schizoid disorders (Brzustowicz et al., 2000). More details on the STOP null mouse model are discussed below, in section **1.4.3**

DISC 1 KO mice replicate a number of neurological features of schizophrenia including: enlarged lateral ventricles, and diminished cortical thickness and brain volume (Jaaro-Peled et al., 2010). Behavioural deficits include locomotor hyperactivity (Clapcote et al., 2007), and reduced social ability (Li et al., 2007), while cognitive abilities such as working memory and executive function also seem to be impaired (O'Tuathaigh et al., 2007). Mice heterozygous and null for dysbindin-1 exhibit behavioural and cognitive alterations, such as reduced social interaction and impaired working memory (Bhardwaj et al., 2009). Dysbindin-1 mutants have also been shown to display morphological changes such as alterations in dendritic spines at excitatory asymmetric synapses in the hippocampus (Feng et al., 2008). Also, the down regulation and overexpression of Neuregulin-1 lead to heightened aggression, impaired social

novelty (i.e. avoid novel social interactions), as well as other cognitive deficits reminiscent of negative symptoms in schizophrenia (O'Tuathaigh et al., 2007).

A number of studies have assessed circadian rhythm and sleep disruption in transgenic SZ animal models (see Pritchett et al., 2012, specifically section titled "SCRD in schizophrenia-relevant mouse models: existing evidence", for an in-depth review). These studies, however, did not use PSG recordings and thus for brevity will not be reviewed here, with the exception of the Bdr mutant mouse model (described in depth below), for which the most recent and well characterized sleep analysis exists (out of SZ *transgenic mouse* models).

1.4 SLEEP IN ANIMAL MODELS OF SCHIZOPHRENIA

Few studies have provided in-depth analyses on the rest-activity or sleep-wake patterns in rodent models of schizophrenia. Two recent and comprehensive sleep studies exist for the neurodevelopmental MAM/E17 in rats (Phillips et al., 2012) and the transgenic blind-drunk (Bdr) mouse [a model of synaptosomal-associated protein (Snap)-25] (Oliver et al., 2012). A third study (Andrieux et al., 2002) provided limited information on sleep in the STOP (Stable Tubule Only Polypeptide) null mice, a transgenic knock out model. In the next sections, I will briefly review the sleep patterns in these three rodent models of SZ.

1.4.1 MAM-E17 Rat

The MAM-E17 model derives from previous studies looking at the effect of methylazoxymethanol (MAM), an anti-mitotic and nucleic acid alkylating agent that alters neuroblast proliferation in the central nervous system (CNS) and affects the development of the brain. Administration of MAM to pregnant rat dams during critical periods of embryonic brain development caused dramatic changes in the brain structure and behaviour of offspring,

including microencephaly, hyperactivity and learning deficits (Jones et al., 2011). Furthermore, rats injected with MAM at gestational day (GD) 17 (or E17) exhibited neuroanatomical deficits, including diminished cortical thickness in frontal brain regions, and reduction of parvalbumin-expressing (PV+) interneurons in the prefrontal cortex and ventral hippocampus (Lodge & Grace, 2009). MAM-E17-induced rats also presented cognitive abnormalities including impaired spatial working memory, attention and learning (Gourevitch et al., 2004, Featherstone et al., 2007, Moore et al., 2006). These histopathological patterns and behavioural abnormalities in MAM are similar to those seen in humans with SZ (Phillips et al., 2012).

1.4.1.1 Sleep patterns in MAM/E17 rat

Using EEG, local field potential (LFP) and unit recordings, Phillips et al. (2012) reported disruptions in the structure and amount of NREM sleep in MAM injected rats, i.e., fragmented NREM sleep, reduced amount of NREM sleep and impaired slow-wave propagation, as well as deficient ripple-spindle coordination. No changes in REM sleep were found. The fragmented sleep architecture seen in MAM/E17 rats is similar to what has been found in patients with SZ (Wulff et al., 2010). Furthermore, loss of cortical PV+ interneurons (which impairs circuits underlying slow wave propagation) found in MAM/E17 has also been seen in post-mortem brain slices from patients with SZ (Lewis et al., 2005). Lastly, the reduced sleep spindle density, and thalamic abnormalities seen in MAM-E17 have also been found in patients with SZ (Adriano et al., 2010; Ferrarelli et al., 2010). Thus, MAM-E17 appears to be an adequate model to study the links between neuropathology, sleep architecture and neurophysiology underlying SZ.

1.4.2 Bdr ('blind drunk') Snap-25 Mouse

The blind drunk (Bdr) Snap-25 missense mutation leads to enhanced binding affinity to the soluble NSF attachment protein receptor (SNARE) complex (Jeans et al., 2007). The SNARE complex mediates docking of synaptic vesicles with the presynaptic membrane; therefore, this mutation causes abnormalities in exocytosis and vesicle recycling, leading to synaptic dysfunction and abnormal neurotransmitter release. Genetic, pathological and functional studies have highlighted various links between the Snap-25 gene and SZ, which include altered Snap-25 expression in human patients (Corradini et al., 2009) and mouse models (Pletnikov et al., 2008). In humans with SZ, altered SNAP-25 gene expression was associated with cognitive deficits including impaired verbal working memory and attention (Golimbet et al., 2010). Furthermore, human subjects with SZ exhibited increased SNAP-25 binding at the striatal synapse (Barakauskas et al., 2010). Panda et al. (2002) found that disruption of this gene in mice (Bdr mutants) coupled with prenatal complications resulted in impaired sensorimotor gating (i.e., prepulse inhibition deficit) that was corrected by antipsychotics.

1.4.2.1 Rest-activity rhythm in Bdr Snap-25

Oliver et al. (2012) examined the circadian phenotype of blind-drunk (Bdr) mutant mice, which were heterozygous for the Snap-25 mutation, using video tracking of wheel running activity. The authors found that activity rhythms in Bdr mice were phase advanced and also fragmented under a 12:12 light:dark cycle, compared to WT mice, similar to what is seen in some patients with SZ (Pritchett et al., 2012). Retinal inputs to the suprachiasmatic nucleus did not differ between Bdr mice and WT mice, and both in vivo and in vitro analyses found that clock gene rhythms within the SCN were normally phased in the Bdr mouse. Consistent with phase advance in circadian rhythms, arginine-vasopressin mRNA expression within the SCN (the

best defined output peptide of the SCN), as well as corticosterone blood levels, was phase advanced in mutants. The authors concluded that disruption in synaptic connectivity within the SCN (which alters its output signals) resulted in the abnormal circadian phenotype of Bdr mouse mutants. Abnormal neurotransmitter systems like NMDAR hypofunction are thought to underlie the intrinsic desynchrony and circadian misalignment that have previously been implicated in neuropsychiatric disorders (Lisman et al., 2008).

In sum, Oliver et al. (2012) document a possible connection between disrupted circadian rhythms and synaptic dysfunction in a murine model of SZ.

1.4.3 STOP KO Mouse

Neuropathological studies indicate that changes to synaptic structure and function in SZ are linked to abnormal cytoskeletal functioning (Benitez-King et al., 2004). Complete loss of the cytoskeletal-associated protein STOP induces marked impairment in microtubule cold stability, as well as synaptic abnormalities such as depleted synaptic vesicle pools, and both short and long term plasticity abnormalities (Andrieux et al., 2002). Thus, the STOP protein appears to be involved in synaptic plasticity, neurogenesis, neurotransmission, and neuron architecture.

Mice lacking STOP exhibit severe pathologies that resemble numerous SZ-like symptoms and characteristics (Andrieux et al., 2002; Powell et al., 2007a; Fournet et al., 2012). These include brain pathologies such as enlarged ventricles and abnormally small thalamic nuclei compared to wild type mice (Powell et al. 2007), hypoglutamatergic functioning (Brenner et al., 2007), increased dopaminergic transmission in the nucleus accumbens (Brun et al., 2005), marked accumulation of 5-hydroxytryptamine (5-HT) in the raphe nuclei and depleted 5-HT amounts in target regions of serotonergic projections (Fournet et al., 2012).

Behaviourally, STOP KO mice showed increased sensitivity to mild stress and locomotor effects of psychostimulants (Brun et al. 2005; Bouvrais-Veret et al. 2007), deficits in pre-pulse inhibition (i.e., sensorimotor gating) (Fradley, et al., 2005), social withdrawal, depressed 'mood' (Fournet et al., 2012), impaired learning and memory (as reflected by deficits in performing object recognition tests and Morris water maze task; Powell et al., 2007), and maternal care defects (Andrieux et al., 2002).

1.4.3.1 Sleep in STOP KO Mouse

Andrieux et al. (2002) examined sleep and various wake behaviours including grooming, feeding, and activity in STOP KO and WT mice. Based only on visual observation for 3 hours at an unspecified time during the evening, the authors reported that STOP KO shifted behaviours more often, particularly showing increased transitions between 'walk' and 'still' phases, compared to WT mice. The total 'sleep' amount in STOP KO mice was less than in WT counterparts, but the number of 'sleep' episodes was similar in both genotypes, indicating that single 'sleep' episodes were shorter in the STOP mice. These findings suggest that loss of the STOP peptide disrupts the ability to maintain sleep, while the ability to initiate sleep remains intact.

However, a solid conclusion cannot be drawn based on these findings because the reliability of assessing sleep by visual observation alone is subject to error, and without PSG recordings sleep architecture cannot be evaluated. Furthermore, because the mice were sampled at only one relatively short time period (3 hours) during the light phase, the observed differences in sleep patterns may not be applicable to the sleep patterns across the 24 h period. The abnormality also could be not due to the genetic manipulation but instead could be attributed to differences in circadian organization between strains of mice (Huber et al., 2000).

Finally, other aspects of sleep regulation were not assessed in the Andrieux et al. (2002) study. Notably, it is unknown whether the homeostatic response to sleep loss is intact in STOP KO mice. Furthermore, given the role of sleep on memory consolidation, it is possible that sleep deficits could contribute to cognitive impairments in STOP mice.

1.5 STUDY OBJECTIVES

1.5.1 Goals

The main goals of this thesis project are to characterize the sleep pattern of STOP KO mice and its regulation by circadian and homeostatic mechanisms, and to determine whether STOP mice show sleep disturbances that resemble those seen in SZ patients. The second goal will aid in assessing the validity of this model of SZ in terms of sleep regulation, and thus its usefulness for future SZ research. PSG and EEG analyses will be used. My specific aims are:

1) To determine spontaneous sleep-wake patterns of STOP KO and WT mice during a 24 h baseline period; and

2) To evaluate sleep recovery following 6 h of sleep deprivation in STOP KO and WT mice.

1.5.2 Hypotheses

Based on the study by Andrieux et al. (2002) as well as the sleep deficits observed in patients with SZ (see section **1.2.2**), I hypothesize the following:

1) Under the baseline condition, STOP KO mice will have a shorter duration of individual sleep episodes, and therefore a smaller total amount of sleep as compared to WT mice; and

2) During recovery from sleep deprivation, STOP KO mice will show smaller rebounds in sleep amount and NREMS EEG delta activity, possibly due to their difficulties in maintaining sleep.

CHAPTER 2– MATERIALS AND METHODS

2.1 ANIMALS & BREEDING

Adult male STOP KO (homozygous recessive) mice (n=7) and wild type littermates (n=8), between the ages of 3 and 4 months, weighing 22-34 grams at the time of surgery, were used in the current study. The animal handling protocol followed the regulations of the Canadian Council on Animal Care, and the experimental protocol was approved by the Dalhousie University Committee on Laboratory Animals. Mice were raised and housed in groups of 1-5 in ventilated cages under a standard 12 hour light: 12 hour dark photoperiod (lights on [Zeitgeber Time (ZT) 0] at 07:00h) at 23 ± 1ºC, with ad libitum access to food and water.

The mice used in this experiment originated from a colony maintained by Dr. Annie Andrieux in Grenoble, France (Andrieux et al., 2002). Animals were bred in-house from heterozygous STOP KO mouse breeding trios (pure C57Bl/6 background) at Dalhousie University's Carleton Care Facility. Two females and 1 male per cage produced litters yielding an average of 4-9 pups. At 21-25 days post-natal, pups were weaned and separated by sex into new cages. Litters were expected to generate the standard Mendelian ratios, i.e., 25 percent WT, 50 percent heterozygous, and 25 percent STOP KO. However, due to issues regarding male fertility, maternal defects, and pups' abilities to retrieve mother's milk that are associated with STOP-KO mice (Andrieux et al., 2002), their survival rate was lower than expected with litters yielding less than 10 percent homozygous recessive animals (including females).

Females were not used in this study due to the variable influences estrogen and progesterone exert on the sleep wake cycle. By using males only we eliminated any confounds that the different hormone profiles between males and females may present.

2.2. GENOTYPING:

Mouse progeny were coded by ear punching. The ear clip biopsies were used for extraction of genomic DNA and polymerase chain reaction (PCR) (see Kajitani et al., 2010). Isolation of genomic DNA was performed using Taq DNA polymerase (with RED Extract-N-Amp™ Tissue PCR Kit, Sigma Aldrich Co., LLC) and customized primers (Invitrogen, Life Technologies) (for protocol details refer to **figure A2**). For PCR, DNA products were amplified using a thermal cycler under the following conditions: Denaturation at 94 ° C for 5 min, 31 cycles of denaturation at 94° C for 45 sec, annealing at 62° C for 45 sec and extension at 72°C for 1 min; followed by extension at 72° C for 10 min. PCR amplified DNA was then electrophoretically separated on a 2% agarose gel and DNA was visualized by ethidium bromide and UV-trans illumination. GCAGATGCCCTCAACAGGCAAATCCGG (forward) and CTGGGAAAACCAGTGTGGAAACTGTTA (reverse) primers generated a 300 kbp band if the WT allele was present, and GGCGAATGGGCTGACCGCTTCCTCGTG (forward) and AGTCTCACCTGTAAGAGCTGCTTACTG (reverse) primers generated a 600 kbp band if the STOP KO allele was present. Heterozygous animals were identified upon presentation of bands in both 300 kbp and 600 kbp regions. See figure A3 for an agarose gel representing the DNA bands.

2.3 SURGERY:

For surgery, mice were anesthetized with the KRAS anesthetic (ketamine (100mg/mL) – xylazine (20mg/mL)-acepromazine (10mg/mL) mixture- 200mg/mL dose; each mouse was injected intra peritoneally with 2mL KRAS per 10 grams of body weight). Depth of surgical

anesthesia was verified by an absence of response to a light toe pinch. The mouse's head was then shaved and the scalp cleaned with 70 % ethanol and providone iodine antiseptic, and their eyes were lubricated with sterile lubricant gel.

Subsequently, the mouse was placed in a stereotaxic frame using standard ear bars. An incision approximately 0.7 cm in length was made to the scalp, revealing the skull and neck muscles. Three small holes were gently punctured (less than 0.5 mm deep) with an 18- gauge syringe needle through the skull over the frontal and parietal cortices (for bipolar EEG recording) and the cerebellum (for the ground electrode). The specific placements of EEG electrodes were as follows: Frontal cortex: 1 mm rostral to bregma and 1 mm right of the midline; Parietal cortex: 2.5 mm caudal to bregma and 1 mm left of the midline; Cerebellar ground: 1 mm caudal to lambda and 1mm right of the midline (see figure A1).

Each cortical electrode consisting of a miniature stainless steel screw was implanted so that the tip of the screw gently made contact with the surface of dura matter, while EMG wires were embedded into the nuchal muscles of the mouse. The opposite end of all electrodes consisted of gold pins which were bonded to a small plastic connector (Plastics One Inc., Roanoke, VA) using dental acrylic.

Following surgery, mice were closely monitored while recovering from anesthesia before being transferred into clean, individual cages and immediately placed on a heat pad. At this time, animals were given subcutaneous injections of 0.1 mL Anafen analgesic, 0.1mL Baytril (5mg/mL) antibiotic, and 1 mL of warm sterile lactated Ringer's. 2mL of Baytril was also mixed in mouse water bottles (50mg/mL), and Baytril-containing water was given to the mice for about 5 days post-surgery. Animals were returned to animal care for 1 week of recovery and were monitored daily with body weight checks, and given mashed mouse chow, treats (i.e. cereal,

nuts) and a nutrient fortified water gel for every day during recovery and for the remainder of the experiment. There were no significant differences in percent change in body weight from surgery to sacrifice between genotypes (see Suppl. Table 1 for body weights).

2.4 EXPERIMENTAL DESIGN AND DATA ACQUISITION

After one week following surgery, mice were individually transferred from the animal colony room to a designated recording room for the rest of the experiment. The recording room housed two wooden recording chambers (23 x 18 x 19 cubic inches) each fitted with an electric fan for ventilation and an incandescent light timed to maintain the same Light:Dark 12:12 lighting cycle with lights on at 7am. Mice were placed individually inside a clear Plexiglas cage (15 x 12 x 12 cubic inches) placed within an individual recording chamber. 2 mice, usually a STOP KO and a WT, were recorded at the same time. Mouse mash, nutrient fortified water gel and water were available ad libitum. The next day, each mouse was tethered to a flexible recording cable within the chamber. To secure the head stage connector on the mouse's head to the recording cable, the mice were lightly anesthetized with isofluorane (isofluorane mixed with oxygen at 4% for induction and 2% for maintenance). The other end of the cable was then connected to a swivel attached to the top of the recording chamber. The swivel allowed the mouse to freely roam within its cage, which contained bedding made of wooden chips, paper nesting material, and mouse hay. Each animal remained connected to the cable for a habituation period of one week prior to the start of EEG/EMG recording. The entire recording lasted for 54 hours (see Figure A1), beginning with a 24 hour baseline period starting at 1 pm (ZT6), of which animals were left undisturbed to go through spontaneous cycles of sleep and wake. Mice were monitored periodically throughout the day to ensure that the EEG/EMG recording ran normally.

Following the baseline recording, sleep deprivation was conducted via 'gentle handling' for 6 h during the second half of the light phase (1pm to 7pm). This was done by introducing novel objects, gentle cage tapping, or slightly moving the bedding tray, when signs of sleep were evident in animal's behaviour or EEG (i.e. large amplitude slow waves).

Mice were never directly touched during sleep deprivation, and left undisturbed while eating, drinking, or grooming. The number of interventions required to keep the animal awake was recorded continuously across the 6 hours of sleep deprivation (see Figure A5). At the end of sleep deprivation (7pm/ZT 12; at lights off), EEG/EMG recording continued for an additional 24 hours of undisturbed sleep to assess recovery sleep.

Animals were euthanized immediately following recovery recording and their body weights were measured.

EEG and EMG signals were amplified and band-pass filtered using an amplification system (Grass Telefactor, West Warwick, RI). EEG signals were amplified x5000 and band-pass filtered between 0.1 and 100 Hz. EMG signals were amplified x5000 and band-pass filtered between 0.3 and 100 Hz. The signals were digitized at 256 Hz, and stored on a computer. For data acquisition, Vital Recorder software (Kissei Comtec, Irvine, CA, USA) was used, while off line data analyses were conducted with SleepSign software (Kissei Comtec).

2.5 SLEEP-WAKE SCORING AND ANALYSES

The SleepSign software automatically scored EEG/EMG recordings into 10 seconds epochs of wakefulness (characterized by low voltage, high frequency EEG, and high voltage, fast frequency EMG), non-rapid eye movement (NREM) sleep (characterized by high voltage, low frequency EEG [dominated by delta activity in the 0.5-4.5 Hz range], and low voltage EMG),

rapid eye movement (REM) sleep (characterized by low-voltage, high frequency EEG [dominated by theta activity in the 5-9 Hz range] and very low voltage EMG with sporadic muscle twitches). For details on how epochs were scored see Figure A4.A. Automatically scored recordings were visually inspected, and manual corrections were made when there was a discrepancy between autoscoring and visual inspection. The inspector was blind to genotype. The variables analyzed were: amount (minutes), episode number and mean duration of episodes (seconds) of wakefulness, NREM sleep, and REM sleep. Other variables analyzed include: number of episodes per nine individual and ascending time blocks in each behavioral state and number of transitions between sleep and wake states. All variables were analyzed at various time points (i.e., baseline versus recovery; light versus dark phase).

EEG power spectra during wakefulness, NREM sleep, and REM sleep were computed in 0.5-Hz bins (range 0.5-50 Hz) and analyzed using Fast Fourier transform (FFT; Hanning Window) in 2-second windows in the following frequency bands: delta [0.5-4.5 Hz], theta [5-9Hz], alpha [9.5-12 Hz], beta [12.5-30 Hz], and gamma [30.5-50 Hz] (Mochizuki, 2004; Fisher, 2013). For the 5 frequency bands and the power spectrum, relative (normalized) EEG power values were calculated for each behavioral state by expressing the absolute EEG power at each frequency bin (0.5-25Hz) as a percentage of the total EEG power (0.5-50 Hz) during the 12 hour baseline light and the 12 hour baseline dark, in each individual animal. For the NREM sleep delta power time course, relative power values were calculated by expressing absolute NREM delta power values (in 2 hour intervals) as a percentage of delta power values over the 24 hour baseline recording in each individual animal. Normalizing to total power serves as a means to control variability between animals (Hines et al., 2013; Morairty et al., 2013). Epochs with EEG artifacts were included in the sleep-wake analyses but not in the FFT analysis (the acceptable

upper limit of epochs with EEG artifact was 80 percent of total number of epochs). One WT mouse (out of 8) was excluded from the FFT analysis of the EEG because the recording had a large amount (greater than 80 percent) of EEG artifacts.

2.6 STATISTICAL ANALYSES

Statistical analysis was carried out using Statview 5.0 (SAS Institute Inc., Cary, NC, USA) and SPSS version 20.0 (Chicago, IL, USA). Unpaired sample t-tests and two-way repeated measures ANOVA were used to assess the sleep/wake variables in STOP KO and WT mice at various time points (i.e., baseline versus recovery; light versus dark phase). Post hoc multiple comparison t-tests (Bonferroni) were used when ANOVAs revealed significant interactions. Probabilities of 0.05 or less were considered significant. To ensure that assumptions of the ANOVA analysis were met, normality (Shapiro-Wilk test), equality of variances (Levene's test) and sphericity (Mauchly's test) were assessed in SPSS. If these assumptions were violated (as indicated by a p value greater than 0.05 (failure to reject the alternative hypothesis) the data were transformed by taking the log10 of values.

CHAPTER 3– RESULTS

3.1 BASELINE SLEEP/WAKE STATES

3.1.1. Baseline Wake

3.1.1.1. Total Amount

During the 24 h baseline period STOP KO mice spent significantly more time awake (+65 min) compared to WT mice (t_{13} = -2.58, P = 0.023; Figure 1A). However, the amounts of wake were not significantly different between STOP KOs and WTs in either the 12 h light phase (t_{13} = -1.13, P = 0.28) or 12 h dark phase (t_{13} = -1.60, P = 0.13; Figure 1A).

The time course of wake amount was analyzed in 2 h intervals during the 24 h baseline recording (Figure 2A). STOP KO mice displayed a lower amount of wake at ZT12-14 (start of the dark phase). During the dark phase, while WT mice showed a steady decline in amount of wake, STOP KO mice had relatively unchanging wake amounts throughout the dark phase. Thus, during the second half of the dark phase (ZT18-20 and ZT22-24), STOP KO mice exhibited higher amounts of wake (Main Effect of Time: $F_{1, 13}$=40.21, P<0.001; Main Effect of Group: $F_{1, 13}$=5.54, P=0.035; Group x Time interaction: $F_{1, 13}$= 3.21, P<0.001).

To quantify the variation in the distribution of wake amount across the dark phase, the ratio of the second half of the dark phase versus the first half of the dark phase was calculated for each group (Figure 2B). This ratio was significantly smaller in WTs (mean ± SEM, 0.67 ± 0.04) compared to STOP KOs (0.95 ± 0.073; t_{13}= -3.531, P=0.004): in the WT mice, wake amount was higher during the first half compared to the second half of the dark phase, while in the STOP KOs wake amount was similar in both halves of the dark phase, resulting in a ratio closer to 1.

Furthermore, there was no significant difference between genotypes in the ratio of the amount of wake in the light phase versus the amount of wake over 24 h (t_{13}=-0.075, P=0.94; Figure 3A): approximately 30% of total wake over the 24 h period occurred during the light phase for both STOP KO and WT mice. There were also no significant group differences in the absolute difference between the maximum and minimum values of wake amount over 24 h (t_{13}=0.40, P=0.70 Figure 3B).

3.1.1. 2. Episodes: Frequency and Duration

Although STOP KO mice exhibited a greater amount of wake during the 24 h baseline recording compared to WT, there were no significant differences in the number (t_{13}=-0.11, P=0.91; Figure 4A) or mean duration of wake episodes (t_{13}=0.46, P=0.65; Figure 5A) between genotypes during this 24 h period. However, in the 12 h baseline light phase, STOP KO mice tended to exhibit a lower number of episodes (t_{13}=1.85, P= 0.088; Figure 4A), with a longer mean duration of episodes, compared to WT mice (t_{13}=-2.00, P=0.067; Figure 5A). In the 12 h baseline dark phase, STOP KO mice had a significantly greater number of episodes (t_{13}=-2.21, P=0.045; Figure 4A) and showed a non-significant trend of a shorter mean duration of episodes (t_{13}=1.87, P=0.084; Figure 5A).

The number of wake episodes as a function of episode duration was calculated for both the 12 h baseline light phase (data not shown) and baseline dark phase (Figure 6). There were very few significant differences between genotypes during the light phase; thus, we focused on the dark phase for the episodes per duration analysis. In the 12 h baseline dark phase, STOP KO mice displayed a significantly greater number of wake episodes in 'mid-duration' time bins (i.e., 80-150 s, 160-310 s, 320-630 s, and 640-1270 s; $P < 0.05$ vs. WT for each comparison), along with a significantly lower number of wake episodes in 'long-duration' time bins (>2560 s),

compared to WT mice (Main Effect of time bin: $F_{1,13}$=35.60, P<0.001; Main Effect of group: $F_{1,13}$=4.73, P=0.049; Interaction: $F_{1,13=}$ 2.84, P=0.007; Figure 6A). Additionally, both genotypes had the highest number of wake episodes in the 10 s duration bin compared to the other 8 duration bins. These 10 s wake bouts or 'brief awakenings' are a measure of sleep fragmentation, and thus tend to affect sleep consolidation. Although the number of brief awakenings was not different between genotypes, taking into account the significantly greater number of short duration NREMS sleep episodes in STOP KOs compared to WTs (see Figure 6B and the sections on NREMS for more detail), it appeared that the frequent brief awakenings that both genotypes experienced, might have been more disruptive in STOP KO mice.

3.1.2. Baseline NREMS

3.1.2.1 Total Amount

In contrast to wake, STOP KO mice had less amount of NREMS (−53 min) compared to WT mice during the 24 h baseline period (t_{13} = 2.20, P = 0.044; Figure 1B). As was the case for wake, there were no significant differences in the amount of NREMS between STOP KO and WT mice in the 12 h light phase (t_{13} = 1.00, P = 0.3340: Figure 1B), or 12 h dark phase (t_{13} = 1.47, P = 0.16; Figure 1B). Also, the ratio between NREMS amount in the second half of the dark phase versus the first half of the dark phase during baseline was significantly lower in STOP KO mice compared to WT mice (t_{13}=2.30, P=0.039; Figure 2D), reflecting the increasing NREMS amounts in WTs, and a flat distribution in STOP KOs, across the 12 h baseline dark phase.

The 2 h time plot of the 24 h baseline recording (Figure 2C) illustrated significant differences in the data between STOP KO and WT mice that were selective to the dark phase

(ZT12-14, 18-20 and 22-24; Main Effect of time: $F_{1,13}$=35.1, P<0.001; Main Effect of group: $F_{1,13}$=4.74, P=0.049; and interaction: $F_{1,13}$=2.6, P=0.005; post hoc tests P<0.05).

As shown in Figure 3A, approximately 70% of NREMS over the 24 h baseline period occurred during the light phase for both STOP KO and WT mice, and there were no significant differences between genotypes in the ratio of the amount of NREMS in the light phase versus the amount of NREMS over 24 h (t_{13}=-1.07, P=0.30; Figure 3A). There was also no significant difference in the absolute difference between the maximum and minimum values of NREMS amount over 24 h (t_{13}=0.58, P=0.57 Figure 3B).

3.1.2.2. Episodes: Frequency and Duration

Similar to wake, in the 24 h baseline period, there were no significant differences in the number (t_{13}=0.10, P=0.92: Figure 4B) or mean duration of NREMS episodes (t_{13}=-1.0, P=0.34: Figure 5B). Also, like wake, in the 12 h light phase, there were no significant differences in the number of episodes (t_{13} = 1.83, P = 0.090: Figure 4B) or mean duration of episodes of NREMS (t_{13} = 1.29, P = 0.22: Figure 5B). Yet, similar to the pattern observed in wake, in the 12 h dark phase, STOP KO mice had a significantly greater number of episodes (t_{13}= 2.14, P=0.032: Figure 4B) and significantly shorter duration of episodes (t_{13}=-4.51, P=0.001: Figure 5B) compared to WT mice.

As shown in Figure 6B, STOP KO mice had more ‘short duration’ NREMS episodes lasting 10 and 20-30 sec compared to WT mice, and less ‘mid-range duration’ episodes lasting 160-310 and 320-630 sec (Main Effect of time bin: $F_{1,13}$=28.07, P<0.001; Main Effect of group: $F_{1,13}$=4.547, P=0.053; Group x Bin interaction: $F_{1,13}$=6.715, P<0.001; post hoc tests P<0.05 vs. WT for each comparison).

3.1.3. Baseline REMS

3.1.3.1. Total Amount:

In the 24 h baseline period, there was no significant group difference in the total amount of REMS (t_{13}=1.84, P=0.089: Figure 1C). Similarly, there were no significant differences between genotypes in the 12 h light (t_{13}=0.48, P=0.64:) or 12 h dark (t_{13}=2.0, P=0.066) periods. The ratio of the second 6 h of the baseline dark phase versus the first 6 h of the baseline dark phase was not significantly different between genotypes, indicating no genotype difference in the daily rhythms of REMS (t_{13}=1.45, P=0.17, Figure 2F).

Statistical evaluation of the 2 h REMS time plot of the 24 h baseline revealed no significant interaction, and thus differences between genotypes at specific time points could not be assessed statistically (Main Effect of time: $F_{1,13}$=32.5, P<0.001; Main Effect of group: $F_{1,13}$=3.38, P=0.089; interaction: $F_{1,13}$=1.5, P=0.13; Figure 2E).

Lastly, like wake and NREMS, there were no significant differences between genotypes in the ratio of the amount of REMS in the light phase compared to the dark phase (~80% of REMS occurred in the light phase; t_{13}=-1.70, P=0.11; Figure 3A), or the absolute difference in maximum and minimum REMS amounts (t_{13}=1.33, P=0.21, Figure 3B).

3.1.3.2. Episodes: Frequency and Duration

There were no significant differences in either the number or mean duration of REMS episodes during the 24 h baseline period (t_{13}=1.2, P=0.23: Figure 4C; and t_{13}=0.23, P=0.82, respectively; Figure 5C) or 12 h light phase (t_{13}=-0.88, P=0.39: Figure 4C; t_{13}=-1.07, P=0.30; Figure 5C, respectively). However, during the 12 h dark phase, the mean duration of individual REMS episodes was shorter compared to WT (t_{13}=2.3, P=0.039: Figure 5C), while the number

of REMS episodes between genotypes was not significantly different (t_{13}=-0.97, P=0.35: Figure 4C).

There were no significant differences in the frequency per duration distribution of REMS episodes (Figure 6A).

3.1.4. Baseline Sleep/Wake State Transitions

To examine sleep/wake architecture in greater detail, a transitions analysis assessing the dynamics between wake, NREMS and REMS was employed (Figure 7).

The average number of transitions between sleep/wake states was assessed in the 12 h light phase (top of the figure) and the 12 h dark phase (bottom of the figure). In the 12 h dark phase, STOP KO mice transitioned more frequently from wake to NREMS (+ 55.5%; t_{13}=-2.16, P=0.049) and from NREMS to wake (+74.5 %; t_{13}=-2.67, P=0.018,) compared to WT mice. The opposite patterns were observed in the light phase, although these differences were not significant (wake to NREMS: -20%; t_{13}=3.1, P=0.088 ; NREMS to wake: -22.5%; t_{13}=3.3, P=0.11).

NREMS to REMS transitions occurred less frequently compared to NREMS to wake or wake to NREMS transitions; they also did not significantly differ between genotypes in either the dark phase or light phase. The results were similar for REMS to wake transitions. Also, neither STOP KO or WT mice made transitions from REMS to NREMS, or wake to REMS, which would indicate abnormal sleep-wake cyclicity and do not typically occur under normal circumstances or healthy individuals.

Overall, these data provide further evidence for increased sleep and wake fragmentation in STOP KO mice during the dark phase.

3.1.5. Baseline EEG power spectra

EEG power spectra were normalized to the total power (0.5-50 Hz) for wake, NREMS and REMS. Figures 8 (5 frequency bands) and 9 (0.5-25 Hz power spectra) illustrate a typical increase in delta power (0.5-4.5 Hz) in NREMS, and a peak in theta power (5-9.5 Hz) in REMS, observed in both STOP KO mice and WT mice.

3.1.5.1. Wake Power Spectra

There were no significant differences during wake in any of the five EEG frequency bands, including delta, theta, alpha, beta and gamma between STOP KO and WT mice (light phase: Figure 8A; dark phase: Figure 8B). EEG power spectra curves also did not differ between genotypes (light phase: Figure 9A; dark phase: Figure 9B).

3.1.5.2. NREMS Power Spectra

In the 12 h light phase during NREMS, STOP KOs exhibited a non-significant increase in EEG delta power compared to WTs (t_{12}=-1.90, P=0.082: Figure 8C). This difference was also observed in the power spectra plotted in 0.5 Hz frequency bins, where the peak in the delta range appeared to be higher in STOP KOs compared to WTs (Figure 9C). Also, during the light phase, STOP KO mice had significantly lower alpha power (9.5-12 Hz; corresponding to low-frequency spindles) compared to WT mice (t_{12}=2.61, P=0.023).

In the dark phase, there were no trends or significant differences between STOP KO and WT mice in any of the five frequency bands, including delta, theta, alpha, beta and gamma (Figure 8D); nor did EEG power spectra curves differ between genotypes in the dark phase (Figure 9D).

3.1.5.2. REMS Power Spectra

In REMS during the light phase, alpha power was lower in STOP KOs compared to WTs, while gamma power (30-50 Hz) was higher in STOP KOs compared to WTs. (t_{12}=3.99, P=0.0018: t_{12}=-2.66, P=0.021: Figure 8E). Additionally, theta peak appeared to be lower in the power spectra in STOP KOs compared to WTs during the baseline light phase (Figure 9E); this difference was not detected in the frequency band analysis (Figure 8E), because the lower peak was still in the theta range.

During the baseline dark phase, the only significant differences observed in the frequency bands was in REMS: similar to the light phase, alpha power was lower in STOP KOs compared to WTs (t_{12}=3.17, P=0.0081: Figure 8F), while gamma power was higher in STOP KOs compared to WTs (t_{13}=-3.07, P=0.0097: Figure 8F). Furthermore, there was a leftward shift in the EEG power spectra curve of STOP KO mice, revealing a slower theta peak (~ 8.5 Hz in WT mice to ~7 Hz in STOP KO mice) compared to WT mice during the baseline dark phase (Figure 9F). As in the baseline light phase, theta peak also appeared to be lower in power spectra in STOP KOs compared to WTs during the dark phase (Figure 9F); this difference was not detected when assessed in frequency bands (Figures 8F).

3.1.6. NREMS EEG Delta Power

Figure 10 illustrates a 4 h time plot of normalized NREMS EEG delta power (a measure of sleep intensity) across the 24 h baseline recording. The time plot analysis reveals a significant Main Effect of time ($F_{1,12}$=5.89, P<0.001), Main Effect of group ($F_{1,12}$=4.93, P=0.048) and significant Group x Time interaction ($F_{1,12}$=5.14, P=0.001). Post hoc tests revealed that throughout the dark phase, KO mice displayed lower EEG power in NREMS, and this difference

was significant for the first and last 4 h of the dark phase (ZT 12-16 and ZT 20-24, respectively; $P<0.05$). Contrary to this pattern, during the light phase, KO mice had generally higher EEG delta power values, and this difference was significant in the middle of the light phase (ZT4-8).

WT mice exhibited a gradual decline in NREMS EEG delta power across the 24 h baseline, with peak at the beginning of the dark phase, reaching a minimum at the end of the light phase (post hoc tests, $P<0.05$). Hence, while WT mice exhibited a pronounced 24 h rhythm, the distribution of NREMS EEG delta power in the STOP KO across the 24 h baseline period appeared relatively flat, indicating that sleep intensity in STOP KOs remained relatively constant, and was unaffected by time of day.

3.1.6 Summary of the Results (baseline)

1. STOP KO mice showed more wake and less sleep, particularly NREMS, compared to WT mice over a 24 h period.
2. In the 12 h dark phase, STOP KO mice had more frequent wake episodes, shorter but more frequent NREMS episodes, and shorter REMS episodes, which resulted in more frequent transitions between wake and NREMS.
3. STOP KO mice had lower EEG alpha power (9.5-12 Hz; corresponding to low-frequency spindles) in NREMS in the light phase only, and lower EEG alpha and higher EEG gamma power (30-50 Hz) in REMS in both the light and dark phase, compared to WT mice.

3.2 SLEEP DEPRIVATION

In order to assess recovery sleep patterns following sleep loss, animals were deprived of sleep via gentle handling during the second 6 h of the light phase. In light of the nocturnal nature and polyphasic sleep patterns in rodents, 6 h of sleep deprivation during the light phase would constitute large sleep loss for mice. All animals were successfully sleep deprived to lose approximately 80 percent of their sleep observed during the corresponding baseline time period. The number of interventions required to keep STOP KO and WT animals awake increased over the 6 h period of sleep deprivation in both groups (Suppl. figure A5.A), demonstrating an accumulation of sleep pressure. STOP KO animals appeared to require more interventions than WT mice, although these differences were not significant at any time intervals during sleep deprivation, in either 1 h intervals (Suppl. figure A5.A) or when totalled across the 6 h period (t_{13}=0.86, P=0.40; Suppl. figure A5. B).

3.3 RECOVERY RECORDING:

3.3.1 Recovery NREMS

3.3.1.1 First 12 h after Sleep Deprivation (dark phase)

3.3.1.1.1 Total Amount

In the first 12 h immediately following sleep deprivation (corresponding to the 12 h dark phase), both STOP KO and WT mice had increased amounts of NREMS compared to the time-matched 12 h baseline period (Main Effect of condition $F_{1,13}$=37.00, P=0.00: Figure 11A). A non-significant interaction revealed that this rebound was similar in the STOP KO and WT mice ($F_{1,13}$=0.50, P=0.49). The percent increase in NREMS amount from the baseline dark phase was +75% in STOP KO mice and +28% in WT mice, which were not significantly different (Figure 11C).

3.3.1.1.2 Episodes: Frequency and Duration

The number of NREMS episodes in the first 12 h following SD increased relative to the time-matched baseline period in both groups (+36% in STOP KO; +40 percent in WT; Main Effect condition : $F_{1,13}$ =16.14, P=0.001: Figure 12A). However, there was no significant interaction and, thus, no differences between genotypes in the magnitude of this rebound ($F_{1,13}$ =1.06, P=0.32)

In contrast to the number of NREMS episodes, there was no change in the mean duration of NREMS episodes in the first 12 h of recovery sleep following SD compared to the time-matched baseline period in both groups (no Main Effect condition : $F_{1,13}$=1.78, P=0.20: no significant interaction: $F_{1,13}$ =1.99, P=0.18; Figure 13A).

In sum, both STOP KO and WT animals had a greater number of NREMS episodes that were similar in length (i.e. no change in mean duration) during the first 12 h of the recovery period as compared to baseline conditions.

3.3.1.2. Second 12 h after Sleep Deprivation (light Phase)

3.3.1.2.1 Total Amount

In the second 12 h following SD (light phase), STOP KO still maintained a slight rebound in NREMS amount, while in WT mice NREMS amount returned approximately to baseline levels (Group x Condition Interaction: $F_{1,13}$ =4.70, P=0.049; post hoc tests: STOP KO: P<0.05 WT: P>0.05: Figure 14A). The percent change relative to baseline during the light phase for NREMS amount was significantly higher for KO mice compared to WT mice (+7.5% for STOP KO mice and -1.25% for WT mice; t_{13}=-2.20, P=0.047; Figure 14 C)

3.3.1.2.2 Episodes: Frequency and Duration

There were no significant differences in the number or mean duration of NREMS episodes in the 12 h recovery light phase compared to baseline levels (see Suppl. figure A6A,B). For the number of NREMS episodes (Suppl. figure A6A), this lack of difference indicates that the rebound increase that occurred initially following SD diminished over time. For the mean duration of NREMS episodes (Suppl. figure A6B), there was no initial rebound immediately following sleep deprivation (dark phase), and this trend was maintained in the light phase.

3.3.1.3. Two h Time Course across 54 h recording: Relative and Absolute Changes

To examine the time course of recovery sleep, an analysis of NREMS amount in 2 h bins was conducted for the entire 54 h experiment (Figure 15A). A significant effect of time ($F_{1,13}$ =35.71, P<0.001) and significant interaction was found ($F_{1,13}$ =3.27, P<0.001). Subsequent post hoc analysis revealed that the NREMS rebound in both STOP KO and WT mice was most robust in the first 2 h (ZT12-14) following SD. Within subjects post-hoc tests (P<0.05) showed that during this period, STOP KO had 29.1 min more of NREMS amount relative to baseline, while WT had 17.3 min more. Between subjects post-hoc tests revealed that STOP KO and WT mice differed from each other at the following time points during recovery (P<0.05): In the first 2 h following sleep deprivation, which corresponded to the beginning of the dark phase (ZT12-14), STOP KO had more NREMS, while at the end of the dark phase (ZT 22-24), STOP KO had less NREMS. Towards the end of the light phase (ZT8-12), STOP KO had more NREMS than WT mice. Also, similar to the baseline time course, there appeared to be a relatively flat distribution of NREMS amount in the STOP KO mice particularly during the 12 h dark phase, in contrast to the more robust change over time seen in WT mice (i.e., the NREMS amounts in WT mice increased linearly from the start of the

recovery dark phase to the end of recording, while the NREMS amounts decreased linearly from the start of the recovery light phase to the end).

In order to assess the magnitude of the rebound in NREMS amount, percent change of NREMS amount relative to baseline values was calculated in 4 h intervals in each group (Figure 15B). Because some animals did not sleep during certain 2 h time intervals during the baseline dark phase, we could not compute the percent change in 2 h bins. There were no significant differences between genotypes at any time point; however, this figure clearly illustrates that the NREMS rebound peaked immediately following SD and dissipated over time in both groups (Main Effect time: $F_{1,13}$ =3.14, P=0.014). From ZT12 to ZT16, percent change was reduced by about half to one-third in the two groups and, as expected, continued to decline towards the end of the dark phase, indicating that increased sleep need induced by 6 h of SD continued to dissipate as animals recovered some of lost sleep at the same rate in both genotypes.

As an alternative way to assess the change in NREMS amount from baseline, the absolute difference in NREMS amount (in min) during recovery relative to baseline was computed in 2 h bins (Figure 15C). The absolute difference in NREMS amount in the first 2 h following SD (ZT12-14) in STOP KO mice (29.1 min) was greater than in WT mice (17.2 min), although it barely reached statistical significance (t_{13}= 1.20, P=0.051) An analysis taking into account all the time points throughout the 24 h recovery period (and time-matched baseline period) revealed a significant Main Effect of time ($F_{1,13}$ =3.00, P=0.001), but no significant interaction ($F_{1,13}$=0. 86, P=0.58), which supported that both genotypes showed similar rebounds in NREMS amount.

3.3.2 Recovery REMS

3.3.2.1 First 12 h after Sleep Deprivation (dark phase)

3.3.2.1.1 Total Amount

During the recovery dark phase, similar to NREMS, both STOP KO and WT mice had increased amount of REMS compared to the time-matched baseline dark phase (Main Effect of condition $F_{1,13}$=33.28, P<0.001: Figure 11B). A non-significant interaction indicated that this rebound was similar between STOP KO and WT mice (F=0.16, P=0.17). The percent increase in REMS amount from baseline during the dark phase in STOP KO mice was +245%, and +130% in WT mice; there was no significant difference between genotypes (Figure 11C).

3.3.2.1.2. Episodes: Frequency and Duration

The number of REMS episodes during the recovery dark phase increased relative to the time-matched baseline period in both groups ($F_{1,13}$ =37.73, P<0.001: Figure 12B); however, there were no differences between genotypes in the magnitude of this increase (no Group x Condition interaction: $F_{1,13}$=1.541, P=0.236). Likewise, there was a significant increase in the mean duration of REMS episodes during the recovery dark phase compared to the time-matched baseline period in both groups ($F_{1,13}$=6.324, P=0.026: Figure 13B), but this increase was similar in STOP KO and WT mice (no Group x Condition interaction: $F_{1,13}$=0.066, P=0.80).

3.3.2.2 Second 12 h after SD (light Phase)

3.3.2.2.1 Total Amount

In the second 12 h following SD (light phase), the amount of REMS returned to baseline values in both STOP KO and WT mice (Main Effect condition: $F_{1,13}$=0.48, P=0.50; interaction: $F_{1,13}$=0.23, P=0.64: Figure 14B). The percent change in REMS amount from baseline during the light

phase was not significantly different between STOP KO mice (+1.0%) and WT mice (+6.5%) (Figure 14C).

3.3.2.2.2 Episodes: Frequency & Duration

As was the case for NREMS, there were no significant differences in the number or mean duration of REMS episodes between genotypes, or within genotypes in the second 12 h of recovery following SD (light phase) compared to the time-matched baseline (see Suppl. figure A6C,D). The number and mean duration of REMS episodes in the recovery light phase were back to baseline levels in both STOP KO and WT mice.

3.3.2.3 Two h Time Course across 54 h recording: Percent and Absolute Changes

Like NREMS, an analysis was conducted on REMS amount in 2 h bins across the entire 54 h experiment (Main Effect of time: $F_{1,13}$=31.0, P<0.001 and interaction: $F_{1,13}$=1.86, P=0.008; Figure 16A). In the first 2 h following sleep deprivation, STOP KO mice had 4.5 minutes more REMS amount compared to WT mice (post hoc tests: P<0.05; 5.5 min in STOP KO, and 1.0 min in WT). Furthermore, STOP KO mice showed the same flat distribution as in the baseline period, i.e., relatively unchanging REMS amounts over the course of the 12 h dark phase, while in WT mice REMS amounts increased linearly. The flat distribution of data points in STOP KOs and more defined change in WTs was not as prominent in the light phase, however. Other significant differences between genotypes were seen at recovery ZT18-22, during which STOP KO mice had less amounts of REMS compared to WTs (post hoc tests: P<0.05; Figure 16A).

The REMS rebound can be clearly seen in the plot of percent change from baseline in 4 h bins (Figure 16B), which shows, as for NREMS, that the compensatory increase in sleep following SD was the greatest immediately following SD, and dissipated over time during the dark phase (Main Effect time: $F_{1,13}$=4.297, P=0.003; interaction: $F_{1,13}$=1.370, P=0.256).

The plot of absolute difference in 2 h bins (Figure 16C) shows that at ZT12-16, STOP KO mice had 2.5 minutes more REMS compared to baseline while WT mice had less than a minute more REMS compared to baseline. (t_{13}= -1.4, P=0.18), further illustrating that the magnitude of REMS rebound did not significantly differ between genotypes.

3.3.3 Recovery NREMS EEG Delta Power

EEG delta power during NREMS is an index of sleep intensity. To analyze the time course of NREMS EEG delta power, normalized NREMS delta power (% of 24 h baseline) was calculated in 4 h bins across the entire 54 h recording (Figure 17A). An ANOVA of the values for the first 4 hours of recovery (ZT12) and the time-matched baseline period revealed that STOP KOs and WTs exhibited similar increases in NREMS delta power (Main Effect of condition: $F_{1,12}$=13.2, P=0.004; no Group x Condition interaction: $F_{1,12}$=0.001, P=0.97; Figure 17B). At the start of the dark phase in recovery, relative change in delta power was at its maximum in WT, and continued to decline over time, dipping below baseline values (i.e. negative rebound) subsequent to the acute positive rebound for 4 h. This negative rebound in WT mice was prominent throughout the recovery light phase. Conversely, relative change in delta power in STOP KO mice remained fairly constant from the start of the dark phase to the end of the light phase, with no signs of negative rebound.

An ANOVA that took into account the entire 54 h recording for the 4 h time plot delta power revealed a significant Main Effect of time ($F_{1,12}$=9.64, P<0.001) and a significant interaction ($F_{1,12}$=5.41, P<0.001). Post hoc tests showed that STOP KOs had higher delta power values compared to WTs throughout the entire light phase (P<0.05). These differences are indicative of the negative rebound in delta power that occurred in WT mice, compared to the lack of rebound in STOP KO during the light phase. To more clearly show the changes in delta power that occurred during recovery from sleep deprivation, the percent change relative to 4 h time matched

baseline intervals was calculated (Figure 16B). This clearly illustrates the 'negative' rebound in the WT that persisted into the light phase (Main Effect time: $F_{1,12}$=7.754, P<0.001, interaction: $F_{1,12}$=0.52, P=0.76).

1.3.4 Summary of the Recovery Results

1. STOP KO and WT mice showed similar rebounds in NREMS and REMS amounts during the first 12 h (dark phase) following sleep deprivation.

2. The amount of NREM sleep and REM sleep increased during the first 2 h immediately following sleep deprivation in both STOP KO and WT mice; however, changes in absolute amounts of NREM and REM sleep relative to baseline levels were greater in STOP KO than in WT mice. Over the 24 h of recovery period after SD, percent changes relative to baseline levels were not significantly different between STOP KO and WT mice at any 4 h intervals for either NREMS or REMS.

3. STOP KO and WT mice had similar rebounds in NREMS EEG delta power during the first 4 h of the recovery dark phase, although STOP KO mice did not show a subsequent trend of negative rebound, as seen in WT mice.

CHAPTER 4– DISCUSSION

The present study revealed that STOP KO mice exhibit alterations in sleep patterns compared to WT mice during both baseline periods and recovery periods following 6 h of sleep deprivation. Specifically, KO mice spent more time awake and less time in NREMS over a 24 h baseline period compared to WT mice. These features were particularly prominent in the 12 h dark (active) phase, when KO mice exhibited more wake episodes and shorter, but more frequent, NREMS episodes, as well as shorter REMS episodes, resulting in more frequent transitions between wake and NREMS. These results imply that KO mice have no difficulty initiating sleep and wake states, but have difficulty in maintaining consolidated states of wake and sleep, especially during the dark phase. Furthermore, both KO and WT mice exhibited pronounced daily rhythms in wake, NREMS and REMS amounts during the baseline period. However, specific to the dark phase, KO mice exhibited atypically flat distributions of sleep-wake state amounts while WT mice exhibited the expected decreases and increases in wake and sleep states, respectively.

In addition, KO mice exhibited lower EEG alpha power, corresponding to low frequency spindles (9.5-12 Hz), in both NREMS and REMS, as well as higher EEG gamma power (30-50 Hz) in REMS, the latter probably reflecting increased arousal.

Following 6 h of sleep deprivation in the second half of the light phase, a 24 h assessment of recovery sleep revealed that both genotypes had similar rebounds in NREMS and REMS amounts as well as increased NREMS EEG delta power relative to baseline, indicating that the ability to compensate for sleep loss following sleep deprivation remained intact in KO mice. Subsequent to the initial increase in NREMS delta power after sleep deprivation, WT mice exhibited a gradual decline in delta power during the dark phase, with values eventually

dropping and remaining below baseline levels for the remainder of the 24 h recovery period. This negative rebound was most prominent in the recovery light phase, and was not exhibited by KO mice whose delta power values reverted to and maintained at baseline levels.

4.1 BASELINE SLEEP-WAKE AND EEG PATTERNS

4.1.1 Sleep Initiation is Intact, while Sleep Maintenance is Impaired in STOP KO Mice

During the 24 h baseline recording of uninterrupted sleep and wake, KO animals exhibited significantly greater amounts of wake, accompanied by significantly less NREMS amount and a non-significant trend for reduced REMS amount as compared to WT animals (see Figure 1). Amounts of wake, NREMS and REMS calculated in 2 h time bins revealed that the majority of these differences occured in the second 6 h of the dark phase (see Figure 2), where sleep amount peaked during the late night, compared with the early night, in WT mice, while in KO mice sleep amount remained stable or 'flat' throughout the entire course of the night (dark phase). This flat distribution in KO mice may be reflective of altered circadian rhythms in these mice.

Despite these differences in the amount and distribution of sleep and wake states, animals maintained their normal diurnal variation (i.e. the ratio of wake and sleep amount observed in the dark compared with the light period was similar between genotypes). Specifically, the light-dark ratio (see Figure 3) revealed that both KO and WT mice are clearly nocturnal sleepers, with only approximately 30% of wake (and 70% of NREMS and REMS) occurring during the light (rest) phase. Consistent with these results, Brun et al. (2005) and Begou et al. (2007) showed that STOP KO had more locomotor activity during the dark phase as compared to the light phase. In these studies, however, STOP KO mice had more locomotor activity during the first half of the dark phase as compared to the second half, which is in contrast to the flat distribution of wake

amount during the dark phase in the current study. This apparent discrepancy may be reconciled by assuming that the KO animals in the present study spent more time in active wakefulness in the first half of the dark phase, and more in quiet wakefulness during the second half of dark phase. The level of motor activity could not be determined by the EMG in the current study.

The decrease in NREMS amount in KO animals was due to a reduction in the mean duration of episodes, i.e., shortening of NREMS episodes (see Figure 5), while the number of NREMS episodes increased during the dark phase (see Figure 4). Wake episodes were also shorter and more frequent in KO animals compared to WT animals. This reduction in the mean duration and increase in the number of wake and NREMS episodes indicates a highly fragmented sleep–wake behaviour in KO mice. As for REMS, the mean duration of single episodes was reduced in KOs compared to WT in the dark phase, while the number of episodes did not significantly differ between the two groups.

To better understand the inability of KO mice to sustain sleep during the dark phase, the frequency of wake, NREMS and REMS episodes as a function of episode duration was analyzed (i.e. an assessment of the number of episodes per consecutive time bins). During the dark (active) period, wakefulness in mice usually occurred in very long bouts (Li et al., 2013). It is interesting that KO mice had less number of long wake bouts (>2560 s) as compared to WT animals (Figure 6A). KO animals also exhibited significantly more short episodes (10 and 20-30 sec bins) of NREMS, which were accompanied by significantly fewer 'long duration' time bins of NREMS (Figure 6B). Additionally, an analysis of the numbers of transitions between sleep and wake states (Figure 7) reveals that KO mice had significantly more transitions from wake to NREMS, and from NREMS to wake compared to WT. Collectively, these data indicate that KO mice have trouble maintaining long periods of wake and NREMS, exhibiting fragmented wake and

NREMS.

In summary, these results suggest that KO mice have no difficulty initiating sleep and wake states but rather had difficulty maintaining consolidated states of wake and sleep particularly during the dark phase.

4.1.2 Baseline EEG Power Spectra Changes in STOP KO mice

In addition to sleep wake architecture abnormalities based on standard polysomnographic scoring, spectral analyses of the sleep-wake EEG revealed that KO mice exhibit alterations in alpha and gamma power, as well as NREMS delta power. In particular, KO mice had lower EEG alpha power (9.5-12 Hz) in NREMS (light phase only) and REMS, while EEG gamma power (30-50 Hz) in REMS was greater compared to WT mice (see Figure 8). These EEG differences may reflect a disruption of the neural networks underlying alpha and gamma oscillations in KO mice.

As for EEG slow wave activity (delta power) during NREMS, there were no significant differences during the 12 h light or 12 h dark phase; however, KO animals did exhibit a trend of greater delta power during the light phase and lower delta during the dark phase compared to WT animals (see Figure 8). The distribution of NREMS delta computed in 4 h bins (see Figure 10) shows that while WT animals exhibited robust diurnal rhythm across the 24 h baseline, and a gradual decline in the levels of NREMS delta, KO animals exhibited no evident increase or decrease, i.e., 'flatness', in the distribution of values over the 24 h baseline period. One possibility is that this flatness may reflect impaired sleep homeostasis in STOP mice, and this issue is discussed in the next section.

4.2 HOMEOSTATIC RESPONSE TO SLEEP DEPRIVATION

It was of interest to assess whether STOP KO mice would show deficits in compensatory responses to sleep loss because of their apparent difficulty in sustaining sleep under baseline conditions. Sleep deprivation typically results in compensatory increases, or rebounds, in sleep amount and sleep intensity, and is a standard method to examine sleep homeostasis (Franken et al., 2009). All mice were successfully sleep deprived via gentle handling, losing approximately 80% of sleep over the 6 hour period. KO and WT did not differ in the number of interventions required to keep them awake, which gradually increased with time awake, indicating similar accumulations of sleep pressure between the two groups.

KO and WT mice showed similar rebounds in NREMS and REMS amounts during the first 12 h (dark phase) following sleep deprivation (see Figure 11). These rebounds occurred immediately (first 2 h) following sleep deprivation (see Figures 15 and 16). These rebounds in NREMS and REMS amounts were due to an increase in the numbers of NREMS and REMS episodes (Figure 12) in both genotypes, as compared to the time-matched baseline period, while mean duration of single episodes (Figure 13) did not change. These findings indicate that KO mice have the ability to compensate homeostatically for sleep loss following sleep deprivation.

On a closer examination, however, the increase that occurred in NREMS and REMS amounts during the first 2 h of recovery (the dark phase), as indexed by a greater absolute change in amount from baseline levels, was more rubust in STOP mice than in WT mice (see Figures 15C and 16C). The greater immediate rebound may reflect an altered homeostatic response.

The magnitude of sleep rebounds dissipated with increasing recovery time, as reflected by a reduction in NREMS and REMS amounts and frequencies during the second 12 h of recovery (light phase). All sleep measures returned to baseline levels, except for slightly elevated levels of

NREMS amount in late light phase in KO mice. Thus, despite impaired sleep maintenance and fragmentation under baseline conditions, STOP-KO mice seem to exhibit similar overall compensatory responses to sleep loss as those of WT mice, which indicates largely intact sleep homeostasis in KO mice, excpet the immediate (within 2 h after SD) response which seems to be more pronounced in KO mice.

As was the case for NREMS amount and episode frequency, KO and WT mice exhibited similar rebounds in NREMS delta power immediately following SD (recovery dark phase) (Figure 17). The rebound in NREMS delta lasted only 4 h following SD, decaying rapidly in WT mice. Delta power reached baseline levels in the next 4 h of the recovery period, but continued to decline throughout the rest of the recovery recording, resulting in a negative rebound (i.e., fell below baseline values) during the recovery light phase. This negative rebound was not observed in KO mice, which maintained the baseline levels of delta power following the initial rebound during the first 4 h period after sleep deprivation. This absence of negative rebound in KO mice may support sleep recovery, and may be due to greater NREMS propensity (as indicated by higher EEG delta levels; see Figure 17A) during the light phase as compared to WT. Furthermore, it appears to correlate with the slightly sustained rebound in NREMS amount in the second 12 h of recovery (light phase) exhibited only by KO mice (Figure 14C), suggesting a greater need to replenish sleep even after a significant time lapse following SD. An explanation for sustained recovery in KO mice may be related to their inability to maintain NREMS sleep under baseline conditions. However, these are fairly minor differences and therefore overall, sleep homeostasis is largely intact in KO mice.

4.3 COMPARISONS OF SLEEP PATTERNS IN STOP KO MICE AND OTHER RODENT MODELS OF SCHIZOPHRENIA

The present findings of sleep patterns in STOP KO mice show various degrees of similarities to, and differences from, previous studies that have assessed sleep-wake and activity patterns in different rodent models of SZ (Andrieux et al., 2002, Oliver et al., 2012; Phillips et al., 2012). These studies employed a variety of techniques including visual observation (Andrieux et al., 2002), video tracking of circadian wheel running behaviour (Oliver et al., 2012), EEG, as well as local field potential (LFP) and unit recordings (Phillips et al., 2012). The depth of sleep analysis varies among the different studies, but none assessed the homeostatic response to sleep deprivation. The current study in STOP KO mice is the first to assess homeostatic sleep patterns in response to sleep deprivation in an animal model of SZ.

The current findings of shorter sleep bouts and increased transitions between sleep and wake in STOP KO mice are consistent with the findings of Andrieux et al. (2002) based on behavioural observations without polygraphic recordings. The study reported that STOP KO mice shifted more frequently between wake and sleep states, and slept less during an unspecified 3 h period during the dark phase. Whether this finding was selective to the dark phase as in the present study is unclear because mouse behaviours were not recorded during any other time period.

The sleep fragmentation in STOP KO mice is also in accord with the findings by Oliver et al. (2012) for another mouse model of SZ, the Bdr-Snap-25 mouse, which exhibited highly fragmented rest and activity patterns. However, although the study did not assess sleep architecture with PSG recordings, it was reported that total sleep time over a 24 h period was

similar between Bdr mutants and WT mice, contrary to the current finding of decreased sleep amount in STOP KO mice and previous studies using STOP KO mice and MAM/E17 rats (Andrieux et al., 2002; Phillips et al., 2012). However, it is important to note that in the Oliver (2012) study, sleep time was not directly measured but estimated as a correlate of total immobility within running wheels, which may not accurately represent total sleep time as animals may be immobile while polygraphically awake.

More recently, a very comprehensive sleep study by Phillips et al. (2012) recorded cortical EEG and circadian behaviour of MAM/E17 rats, a neurodevelopmental model of SZ. To recapitulate, the model utilizes offspring of rat dams that are injected with the mitotoxin MAM (methylazoxymethanol-acetate) on day 17 of gestation, which disrupts limbic-cortical circuitry.

Despite methodological disparities between the current study and the study by Phillips et al., a number of sleep-wake characteristics of STOP KO mice are very similar to those exhibited by MAM/E17 rats. These include: increased wake amount and decreased NREMS amount, reduced NREMS duration, and intact diurnal rhythms of NREM and REM sleep. However, there are also notable differences between the two studies. In particular, it appears that the NREMS bout duration is shorter in MAM mice in both the light and dark phases, while this difference is restricted to the dark phase in STOP KO mice (we also found a reduction in REMS episode duration, but Phillips et al. did not). Additionally, spectral analysis of NREMS EEG in MAM/E17 revealed a clear reduction in delta power during both the light and dark phases (most robust in the first 6 h of the light phase) as compared to control animals, while STOP KO mice exhibited non-significant trends of increased delta power in the light phase and reduced delta power during the dark phase. Whether these differences can be attributed to a difference in species, animal model, or the methodologies and analyses employed is unclear. For instance, in

MAM/E17 rats EEG sleep and wake parameters were analyzed over 48 h and averaged in 6 h time bins, while in STOP KO mice, sleep and wake parameters were analyzed over 24 h and averaged in 2, 4 and 12 h time bins.

In addition to assessing sleep-wake architecture and EEG spectral analyses, Phillips et al. (2012) studied the neurophysiological properties of NREMS using cortical depth recordings with tetrodes. It is interesting to note that a reduction in the density and amplitude of sleep spindles (10-15 Hz) generated during NREMS were observed in MAM/E17 rats. Sleep spindles are thought to play a role in memory consolidation during sleep, and impairments in spindle properties are thought to contribute to impairments in memory and attention in SZ (Waters and Manoach, 2012). Although we did not assess sleep spindles per se, STOP KO mice did exhibit a slight reduction in alpha power (9.5-13 Hz), which corresponds to low-frequency spindles, during NREMS. Interestingly, STOP KO mice also exhibit attention, memory and learning deficits which may be associated with the reduction in alpha power exhibited by the animals (Powell et al., 2007). Further analysis is required to see if the reduction in alpha power relates to changes in spindles in STOP KO mice.

Sleep is homeostatically regulated, and the deficits in SWS in MAM/E17 and in schizophrenia patients pose questions regarding the nature of homeostatic regulatory mechanisms in SZ. Furthermore, given that SWS is important for attention and memory processes, and its quality may predict disease outcome in SZ (Luby & Caldwell, 1967; Benson et al., 1996; Hofstetter et al., 2005) it is important to better understand the mechanisms that govern sleep homeostasis in animal models of SZ. Thus, there is a need to assess the ability to restore sleep following sleep deprivation, and the present study was the first to do so in an animal model of SZ. Despite the lack of a 24 h rhythm in NREMS delta activity, and slight reduction

during the 12 h baseline dark phase in STOP KO mice, there were no differences between the KO mice and WT mice in homeostatic sleep response to sleep deprivation, indicating that STOP KO mice have a normal ability to restore SWS. It remains to be seen if other animal models exhibit impairments in SWS restoration after sleep loss and, if so, how these might relate to their deficits in SWS.

To conclude, while homeostatic regulation of sleep following sleep deprivation has yet to be assessed in other SZ animal models besides STOP, fragmented sleep architecture, poor consolidation of nocturnal activity and reduced sleep amount seem to prevail in the animal literature. Thus, in addition to providing confirmatory evidence for these results, the current study adds new information to the literature by demonstrating that sleep homeostasis is intact in the STOP KO mouse model of SZ.

4.4 COMPARISONS OF SLEEP PATTERNS IN STOP KO MICE AND HUMAN STUDIES OF SCHIZOPHRENIA

The current findings are in accord with previously published studies which reported patients with schizophrenia exhibited reduced sleep time, fragmented sleep-wake behaviour, and abnormal alpha and gamma EEG oscillations (Chouinard,et al., 2004; Cohrs, 2008; Klimesch, 1999; Waters & Manoach, 2012; Wilson & Argyropoulos, 2012).

Actigraphy monitoring showed that reduced sleep time, sleep interruption and difficulties maintaining sleep are stable characteristics of the disorder, independent of medication status (Waters & Manoach, 2012), all of which were seen in STOP mice. Circadian rhythm disruptions, such as phase advances and delays, are also a common feature of SZ (Wulff et al., 2010).

Contrary to this, the present study found that STOP mice maintained a normal diurnal rhythms (i.e., the proportion of wake and sleep amount observed in the dark compared with the light period confirmed these animals were nocturnal, and the ratio was similar to that in WT animals). Despite the intact L/D ratio, however, STOP mice presented flat distributions of wake and NREMS across their dark phase. One possibility for this may be that STOP KO mice have altered circadian rhythms. Further investigation on circadian rhythm chronobiologic parameters is needed to address this possibility (see the Future Studies section below).

There are limited data on the spectral features of the sleep EEG in SZ patients (Waters & Manoach, 2012). Most studies have reported a decrease in EEG slow wave activity during slow wave sleep, i.e., NREMS delta, although there are also negative reports (Goder et al., 2006). In line with the latter, the current study in STOP mice found no significant differences compared to WT controls, in NREMS delta during the 12 h dark phase or 12 h light phase, suggesting that slow wave sleep intensity is intact in KO mice. Nonetheless, the distribution of NREMS delta during baseline sleep seemingly lacked a diurnal variation in KO mice (see Figure 10). Furthermore, there does appear to be a trend of reduced NREMS delta during the dark phase and increased NREMS delta during the light. These changes in NREMS delta-particularly the flat 24 h rhythm in KO mice may reflect an altered ability to accumulate sleep pressure, which in turn may explain why KO mice exhibit impairment in their ability to maintain consolidated sleep. In contradiction to this however, the number of sleep deprivation interventions required to keep the animals awake did not significantly differ between genotypes. Furthermore, the recovery patterns following sleep deprivation (previously described in section 4.2 of the discussion) reveal KO and WT mice do not differ in their ability to restore lost sleep**,** and hence these animals do exhibit the ability to accumulate sleep pressure.

A more well-established spectral characteristic of the sleep EEG in SZ is reduced sleep spindles (Wilson & Argyopoulos, 2012), which was observed in STOP mice during NREMS in the current study. Low-frequency sleep spindles correspond to alpha power, which is thought to play a role in memory consolidation and attention (Klimesch et al., 1998). Reductions in alpha power are often seen in SZ patients and are commonly associated with cognitive symptoms (Phillips et al., 2012). Interestingly, these cognitive impairments, which are hallmark symptoms of SZ, have also been reported in STOP mice (Eastwood et al., 2007; Powell et al., 2007; Bégou et al., 2008; Kajitani et al., 2010; Volle et al., 2012). Gamma oscillations are also related to cognitive processes such as perceptual learning and selective attention (Fell et al., 2001); thus, abnormalities in gamma generation may be linked to perceptual and cognitive distortions in SZ (Sperling et al., 1996). Interestingly, patients with SZ exhibit increased gamma power both during REMS and bouts of psychosis (Hermann & Demiralp, 2005).

Lastly, the homeostatic drive in STOP KO mice appears to be largely intact as demonstrated by similar numbers of interventions required in the two groups, and similar rebounds in NREMS amount and NREMS EEG delta power immediately following SD in STOP mice as in WT mice. This suggests that the accumulation and release of sleep pressure are for the most part intact in STOP mice. This is also consistent with one study in humans that found modest increases in NREMS delta power during the recovery period following 1 day of SD relative to baseline levels in SZ patients compared to controls (Benson & Feinberg, 2011). However, discrepancies do exist in the literature on this point as well, and no recovery of SWS amount following 85 hours of total sleep deprivation has also been observed in a small sample of unmedicated patients (Luby & Caldwell, 1967).

It is uncertain whether the sleep abnormalities found in STOP mice and in SZ originate from a deficit in the mechanisms that control the timing of sleep and wake, i.e., the SCN, the neurons that initiate sleep (e.g., the ventro-lateral pre-optic area) and wake (e.g., the tubero-mammillary nucleus and locus coeruleus) states, or the incapacity of these neurons to maintain consolidated states of sleep and wake despite intact timekeeping mechanisms. See section 4.5 (below) for speculations on the underlying mechanisms that may be responsible for the sleep problems in STOP KO mice.

4.5 MECHANISMS UNDERLYING THE SLEEP PROBLEMS IN STOP KO MICE

In addition to various cognitive, mood, social and sleep deficits, STOP mice exhibit a number of neurochemical irregularities (Brun et al., 2005; Hanaya et al., 2008; Fournet et al., 2012) that are also found in SZ humans (Desbonnet et al., 2009). Of particular interest are the various abnormalities in DA signalling and increases in basal cerebral glucose metabolism, which seem to lend support for the present findings of increased wake amount, decreased NREMS amount, and shortened REMS duration.

4.5.1 Dopamine

STOP deletion has been shown to affect dopamine levels in the nucleus accumbens. Specifically, Brun et al. (2005) found that dopamine efflux elicited by electrical stimulation was increased specifically in the nucleus accumbens of STOP mice, as compared to WT controls, recapitulating the mesolimbic hyperdopaminergic activity seen in SZ humans. Although the link between dopaminergic activity and specific sleep/wake pathways has never been directly assessed in STOP null mice and the involvement of dopamine in sleep regulation is not fully

understood, psychostimulants increase dopamine levels and can affect circadian rhythms, and exacerbate positive symptoms in schizophrenia (Honma & Honma, 2009). Thus, it is tempting to speculate on the underpinnings of increased wake and decreased sleep in relation to dopamine, specifically, the possibility that increased dopamine levels might be responsible for increased wake and sleep fragmentation in STOP KO mice. It has been previously hypothesized that the insomnia of SZ patients may be linked to an over-active dopamine system, based on pharmacological experiments showing that D2 receptor agonists enhanced wake and reduced sleep. Conversely, drugs that block D2 receptors have been shown to increase sleep (Monti et al., 1988).

4.5.2 Cerebral Glucose Metabolism

Hanaya et al., (2008) found that STOP null mice exhibited increased basal rates of cerebral glucose metabolism in the anterolateral and ventromedial hypothalamic nuclei. To explain their findings, these authors cited previous studies (Szymusiak et al., 1998) that showed that activation of the ventromedial hypothalamic nucleus led to excitation of the midbrain reticular formation and subsequent increases in wake and alertness. Conversely, stimulation of the anterior hypothalamic nuclei led to increased sleepiness and somnolence, which may also be linked to blunted emotions seen in SZ (Ticho and Radulovacki, 1991). Also, lesions to anterior hypothalamic regions results in increased wake, and these regions have been associated with insomnia (Szymusiak and Satinoff, 1984; Sallanon et al., 1989). Thus, heightened metabolic activity of the anterior hypothalamic nuclei in STOP KO mice may disrupt normal sleep-wake regulation in these animals.

To conclude, it remains to be seen whether the neurochemical abnormalities that have been observed in STOP mice are a predominant cause of their sleep pathologies, or secondary to the neurochemical mechanisms that underpin other symptoms in SZ.

4.6 METHODOLOGICAL CONSIDERATIONS

Transgenic animal models like STOP KO mice are highly valuable for studying the role of genetics on SZ phenotypic expression. Animal models also enable highly controlled genetic, anatomical, physiological and behavioural studies that cannot be conducted in humans. However, they are not without methodological concerns (Chen et al., 2006). For instance, it is not possible to model all features of such a heterogeneous disorder as SZ in one animal model. This especially applies to conventional knock out models like STOP KO mice that manipulate only one gene. Psychiatric disorders involve the interaction of multiple genes that induce the disease phenotype, and are modulated by environmental factors (Desbonnet et al., 2009). Also, because KO models like the STOP null mouse lack the gene from the time of conception, there is a high chance that compensatory mechanisms may act to counter the deficits induced by the gene manipulation, thus producing animals with less robust phenotypes. This compensation also interferes with studying the role of the target gene. Employing a method like RNA interference (RNAi), which permits selective knock down of genes with better spatial control and reversibility, may be a better alternative when attempting to model a heterogeneous disease like SZ with symptoms that vary with age and time (Kelly & Bianchi, 2012).

Another issue particularly with animal models of psychiatric or mood disturbances is the effects of the mother's care on pups. The question arises whether KO pups are treated differently

as compared to WT pups by heterozygous mothers or WT (foster) mothers. Studying the impact of maternal defects on pup viability, Andreiux et al (2002) found that KO and WT pups died at similar rates when cared for by STOP KO mother. Despite reporting that offspring from heterozygous mothers developed normally, the study did not clarify to what extent or whether KO animals were treated differentially compared to WT offspring by heterozygous mothers. For instance, if KO mice receive different treatment as compared to their WT and heterozygous littermates prior to weaning, this may add to the effect of the gene deletion. Thus, whether phenotypes are a direct result of the gene deletion or are modulated by environmental factors like maternal care remains unknown. Interestingly, Volle et al. (2012) reported that KO pups were more sensitive to maternal deprivation compared to WT pups, in that KO animals but not WT exhibited locomotor activity impairments in adulthood that were exacerbated by maternal deprivation in childhood. This suggests that KO mice are more sensitive possibly due to the gene deletion, but whether mothers treat KO pups differently compared to WT still remains to be determined, and thus whether behavioural defects in these animals are solely a direct result of the gene deletion remains unclear.

4.7 FUTURE DIRECTIONS

4.7.1 Studying Circadian Rhythms in STOP KO mice using constant conditions

To better understand the flat distribution of wake, NREMS and REMS across the dark phase in STOP mice, it would be useful to examine circadian behaviours under constant conditions (DD). It seems as though these animals may not respond to the dark with increased alertness like WT mice. By using running wheels we can assess the circadian rhythm of voluntary locomotor activity. Video tracking circadian wheel-running behavior under constant conditions (DD) combined with EEG and

EMG recordings would clarify how robust the internal clock in STOP mice is and to what extent it is able to persist in the absence of external time cues. This would allow for a thorough and accurate quantification of circadian rhythm regulation in these animals.

4.7.2 Assessing Neurotransmitter Systems that are commonly altered in Sleep-wake and SZ in STOP KO Mouse Model

A number of neurotransmitters play a role in sleep-wake circuitry (for reviews see Brown et al., 2011; Espana & Scammel, 2012; and Schwartz & Roth 2008). Wake is maintained and regulated by neurons within the brainstem and the posterior hypothalamus that utilize acetylcholine, serotonin, noradrenaline, dopamine, histamine and hypocretin (also known as orexin). Projections from the cell bodies containing these transmitters within these regions support behavioural and cortical arousal by activating the cortex and forebrain areas. Conversely, NREMS is generated when these ascending arousal mechanisms are suppressed by neurons in the preoptic area of the hypothalamus (Espana & Scammel, 2012).

In order to elucidate the function of MAP6 and its relation to the neuropathalogic mechanisms associated with schizophrenia and its possible involvement in sleep disruption, future research should aim to employ neurobiological techniques that measure levels of neurotransmitters involved in sleep wake circuitry in STOP mice (see above for potential candidates). Concentrations of endogenous neurotransmitters can be assessed from samples obtained from in vivo microdialysis, and then via HLPC and electrochemical detection (See Fournet et al., 2012 for method). Prospective experiments should aim to target specific brain regions specifically associated with sleep promotion and maintenance (Espana et al., 2012; Schwartz & Roth 2008). Of specific interest is the 'sleep-wake' switch mechanism governed by

the hypothalamus, whereby the ventrolateral preoptic nucleus (VLPO) is sleep promoting and has reciprocal interactions with the wake promoting monoaminergic pathways (Schwartz & Roth, 2008). During wake, the monoamine nuclei actively fire, to inhibit the VLPO from firing. Conversely, during sleep VLPO neurons inhibit neurons that release monoamine neurotransmitters like serotonin from firing. This back and forth interaction results in sleep-wake transitions, and the rapid discharge from the VLPO during sleep results in transition to sleep and its maintenance. Furthermore, animals with lesions to the VLPO exhibit decreased sleep time and highly fragmented sleep with a greater number of awakenings (Lu et al., 2000). The fact that STOP mice exhibit the pattern of reduced sleep time and fragmented NREMS may suggest an alteration to the VLPO system, and the neurotransmitters regulating its activity. It would be interesting to assess concentrations of endogenous neurotransmitters such as GABA and galanin within the VLPO of STOP mice, as well as serotonergic inputs from the raphe nuclei, noradrenergic inputs from the locus coerelus to the VLPO, and VLPO outputs to monoaminergic cell body regions.

Finally, the loss of orexin has been shown to induce behavioural states instability, as in the case of orexin KO mice which are unable to maintain wake and have fragmented NREMS (Mochizuki et al., 2004). Given the wake promoting effect of orexinergic neurons that project from the lateral hypothalamus to monamine nuclei, i.e., the locus coerelus and dorsal raphe nucleus, it may be useful to assess orexin levels in STOP null mice within these brain regions to better understand the mechanisms underlying reduction in sleep time and fragmented sleep patterns in these animals, as well as in schizophrenia (Benson, 2006; Stenberg, 2007).

4.7.3 Therapeutic interventions

Another avenue of future research should consider therapeutic intervention of sleep in STOP KO mice. The marked impairment in microtubule stability, as well as synaptic abnormalities induced by the gene deletion (Andrieux et al., 2002; Volle et al., 2012), has been associated with behavioural disturbances, such as maternal defects and hyperactivity, and it has been shown that these behavioural deficits can be ameliorated with typical and atypical antipsychotic medications (Brun et al., 2005; Delotterie et al., 2010). It remains to be seen whether antipsychotics could correct STOP sleep-wake abnormalities. Interestingly, Fradley et al. (2005) treated STOP mice with clonazepam (used to treat mania and anxiety disorders), which was shown to reduce their hyperlocomotion. Perhaps then, treating STOP mice with clonazepam may be effective in consolidating their sleep.

Also, it would be interesting to treat pups from birth with antipsychotics and compare to pups treated at different time points in their life span to determine when therapeutic intervention is most effective. Finally, other therapeutic interventions, to test on these mice could include melatonin administration, and even non-pharmacologic approaches such as changing the timing of the light/dark cycle, and scheduled exercise (Pritchett et al., 2012).

4.8 CONCLUSIONS

This study has provided a thorough assessment of the nature of sleep/wake patterns exhibited by STOP-null mice. Furthermore, this is the first experiment to assess sleep-wake patterns in response to sleep deprivation in an animal model of schizophrenia. The primary goal for doing so was to establish whether STOP-null mice are a valid model of SZ symptomology in terms of sleep patterns. My initial predictions that STOP mice would exhibit shorter durations of

individual sleep episodes and therefore total amounts of sleep as compared to WT were supported by the data, while the initial hypothesis of smaller rebounds in sleep amount and delta activity after sleep deprivation in STOP mice (due to difficulty maintaining sleep under baseline conditions) was not supported, as both genotypes exhibited similar increases in NREMS and EEG delta power after sleep deprivation.

Strikingly, these aspects of sleep architecture observed in STOP mice bear a close resemblance to those found in other animal models of SZ and in humans with SZ. This provides support that this model is an appropriate model to study SZ, and to further explore the link between neuronal abnormalities, and sleep-wake regulatory mechanisms in the neuropsychiatric disease.

Future research should aim to understanding how cognitive deficits relate to sleep impairments, and whether medications used to correct sleep abnormalities also correct other symptoms as well. Furthermore, treating sleep via pharmacologic and nonpharmacologic interventions may help to reduce symptoms and improve patients' quality of life.

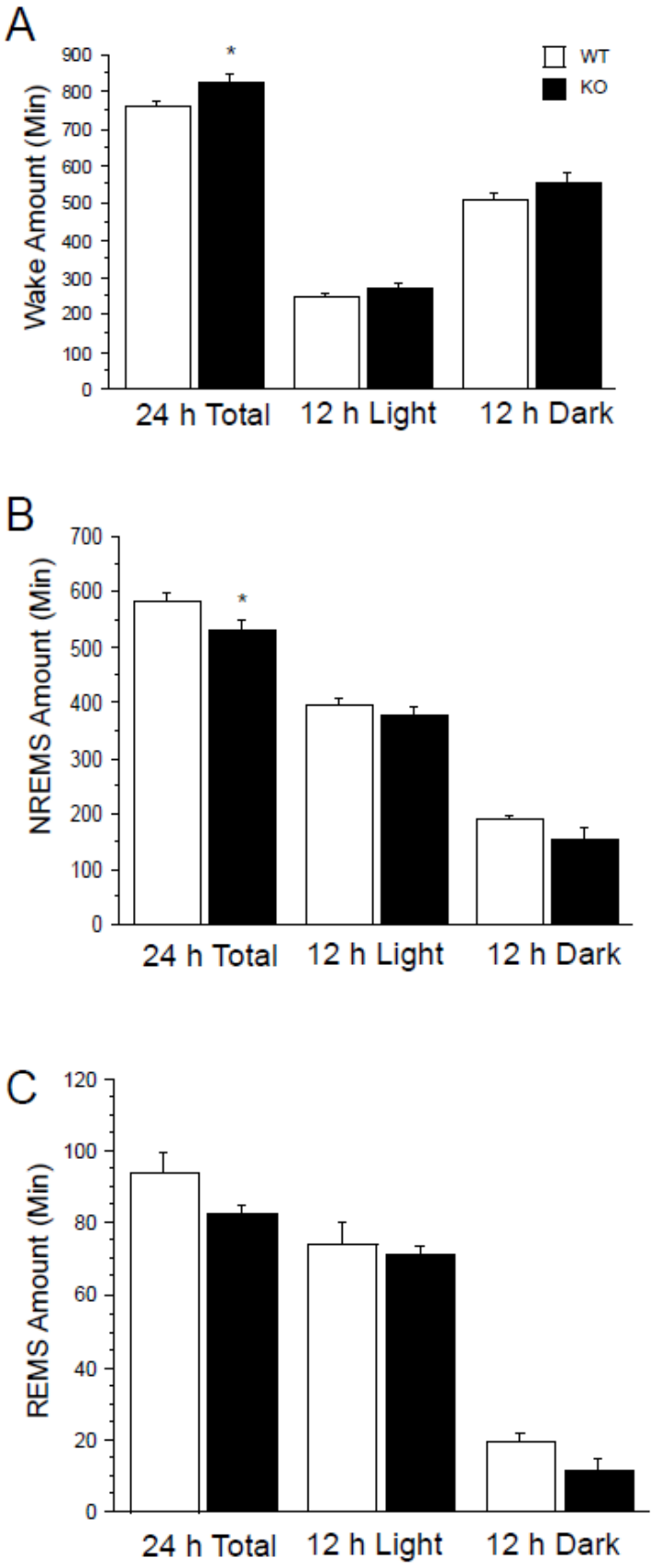

A
Wake Amount (Min)
900
800
700
600
500
400
300
200
100
0
*
WT
KO
24 h Total
12 h Light
12 h Dark
B
NREMS Amount (Min)
700
600
500
400
300
200
100
0
*
24 h Total
12 h Light
12 h Dark
C
REMS Amount (Min)
120
100
80
60
40
20
0
24 h Total
12 h Light
12 h Dark

Figure 1. Amounts (in min) of wake (A), NREMS (B), and REMS (C) during the baseline recording in STOP KO and WT mice. Values (means + SEM) are shown for the entire 24 h period, 12 h light phase, and 12 h dark phase. Compared to the WT mice, the STOP KO mice showed more wake (A) and less NREMS (B) during the 24 h baseline period, although there was no significant group difference in either the light or the dark phase. There was a trend of a decrease in REMS amounts, especially during the dark phase, in the KO compared to the WT mice (C). N=8 WT, N=7 KO. * Different from WT mice; $P<0.05$.

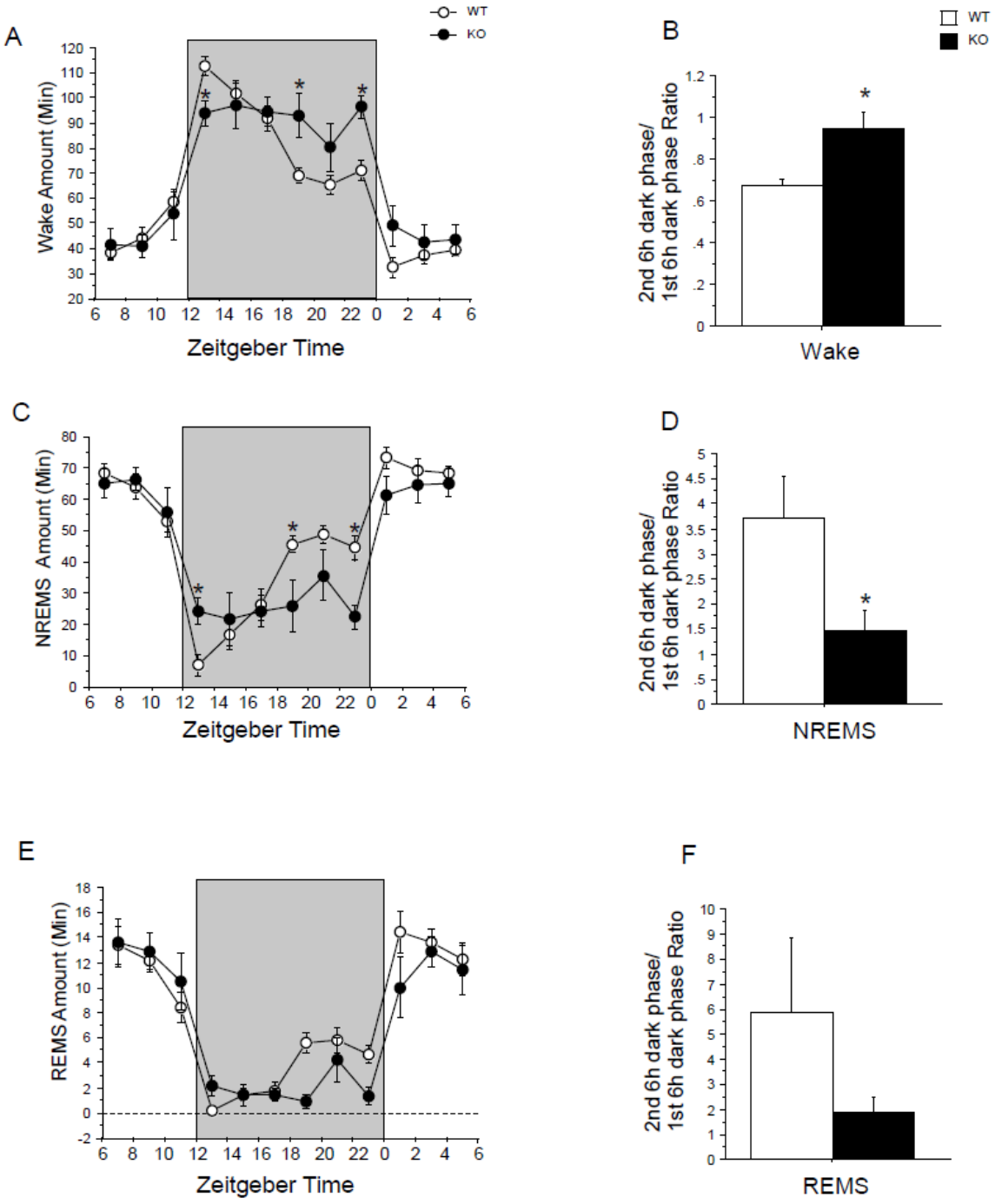

A
WT
KO
Wake Amount (Min)
Zeitgeber Time
B
2nd 6h dark phase/
1st 6h dark phase Ratio
Wake
C
NREMS Amount (Min)
Zeitgeber Time
D
2nd 6h dark phase/
1st 6h dark phase Ratio
NREMS
E
REMS Amount (Min)
Zeitgeber Time
F
2nd 6h dark phase/
1st 6h dark phase Ratio
REMS

Figure 2. Time course in 2 h intervals (left) of wake (A), NREMS (C), and REMS (E) amounts across the 24 h baseline recording in the STOP KO and WT mice, and the ratio of the second half to the first half of the baseline dark phase (right) for wake (B), NREMS (D), and REMS amounts (F). All values are displayed as means + SEM. Left: The STOP KO mice showed less wake (A) and more NREMS (B) during the first 2 h of the dark phase (ZT12-14), and more wake (A) and less NREMS (B) during the second half of the dark phase (ZT18-20 and 22-24), compared to the WT mice. Shaded areas represent the dark phase. Right: The ratio of the second half of the dark phase to the first half of the dark phase was significantly higher for wake (B) and lower for NREMS (D) in the STOP KO mice than in the WT mice. The ratio for wake, NREMS, and REMS (F) was close to 1 in the STOP KO mice, indicating a flat distribution over the course of the dark phase. N=8 WT, N=7 KO. * Different from WT mice; $P<0.05$ (Bonferroni post-hoc comparisons in A and C).

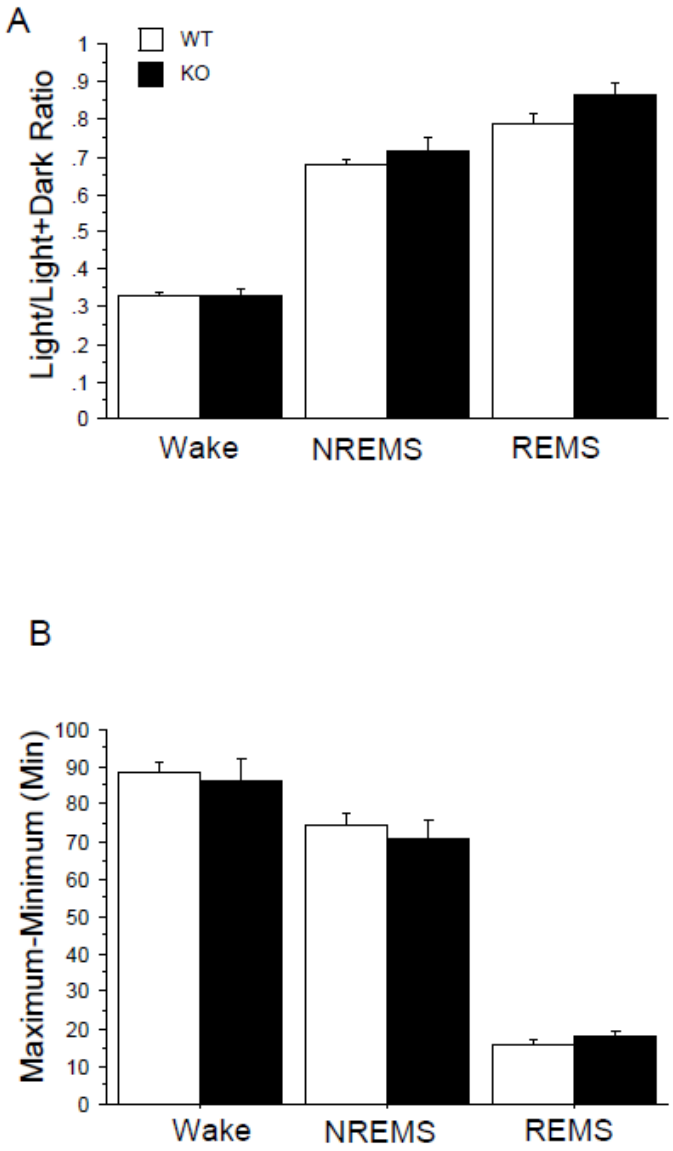
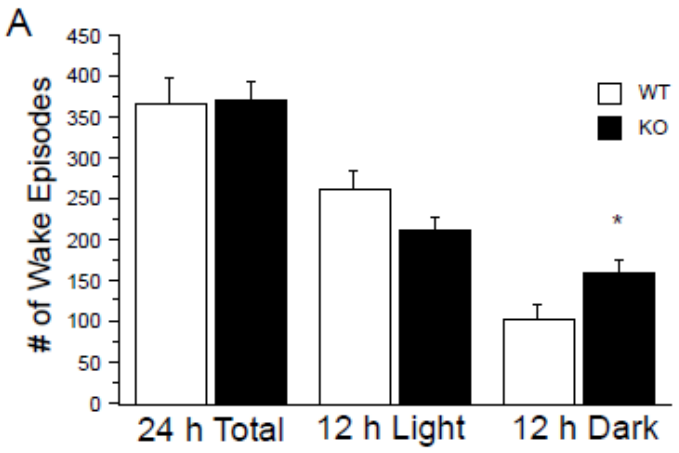
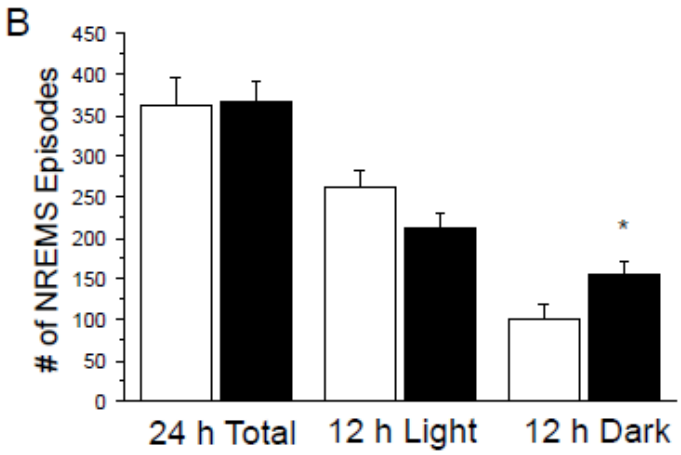


Figure 3. The ratio of the amount in the 12 h light phase to the amount in the full 24 h period (L/[L+D] ratio; A), and the amplitude of the daily variation (Max-Min; B) for wake, NREMS, and REMS in the STOP KO and WT mice at baseline. All values are displayed as means + SEM. The max-min amplitude for each behavioral state was calculated across the 24 h baseline recording. There was no significant difference between the STOP KO and WT mice in either parameter for any behavioural state.


A
# of Wake Episodes
450
400
350
300
250
200
150
100
50
0
WT
KO
*
24 h Total
12 h Light
12 h Dark


B
# of NREMS Episodes
450
400
350
300
250
200
150
100
50
0
*
24 h Total
12 h Light
12 h Dark

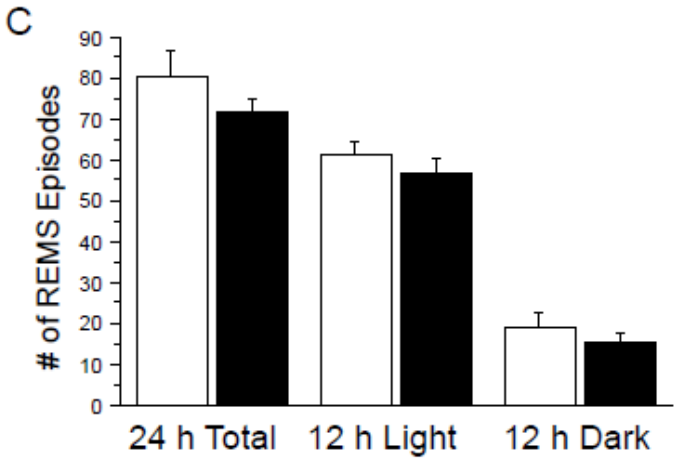

C
# of REMS Episodes
90
80
70
60
50
40
30
20
10
0
24 h Total
12 h Light
12 h Dark

Figure 4. Number of episodes of wake (A), NREMS (B), and REMS (C) during the baseline recording in the STOP KO and WT mice. Values (means + SEM) are shown for the entire 24 h period, 12 h light phase, and 12 h dark phase. The STOP KO mice had more wake (A) and NREMS episodes (B) during the 12 h dark phase, compared to the WT mice. There were no significant differences between groups in either the light phase or the entire 24 h period. N=8 WT, N=7 KO. *Different from WT mice; $P<0.05$

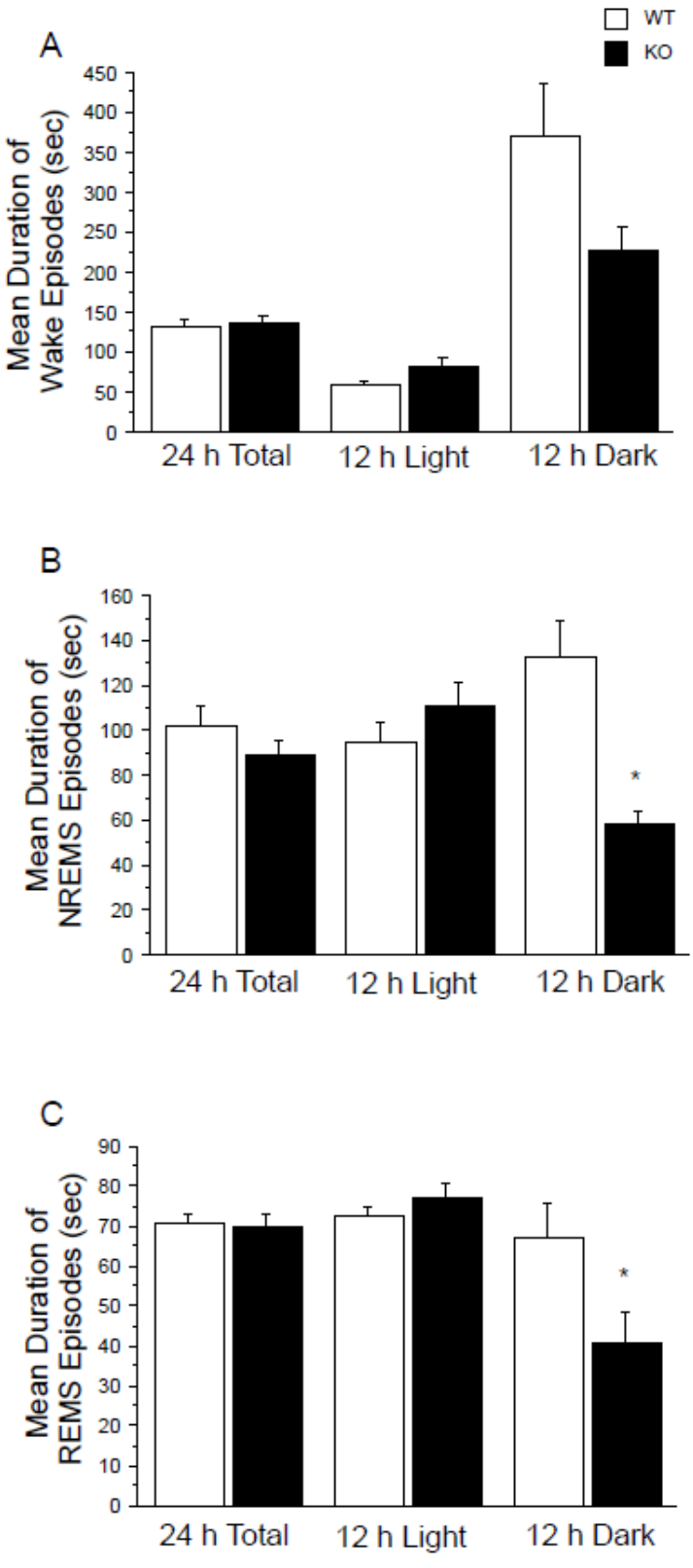

A
WT
KO
Mean Duration of Wake Episodes (sec)
450
400
350
300
250
200
150
100
50
0
24 h Total
12 h Light
12 h Dark
B
Mean Duration of NREMS Episodes (sec)
160
140
120
100
80
60
40
20
0
*
24 h Total
12 h Light
12 h Dark
C
Mean Duration of REMS Episodes (sec)
90
80
70
60
50
40
30
20
10
0
*
24 h Total
12 h Light
12 h Dark

Figure 5. Mean duration of episodes (in seconds) of wake (A), NREMS (B), and REMS (C) during the baseline recording in the STOP KO and WT mice. Data (means + SEM) are shown for the entire 24 h period, 12 h light phase, and 12 h dark phase. Compared to the WT mice, the STOP KO mice had shorter NREMS (B) and REMS episodes (C), as well as a trend of shorter wake episodes (A), during the 12 h dark phase. There were no significant differences between groups in either the light phase or the entire 24 h period. N=8 WT, N=7 KO.* Different from WT mice; $P<0.05$.

Baseline Dark Phase

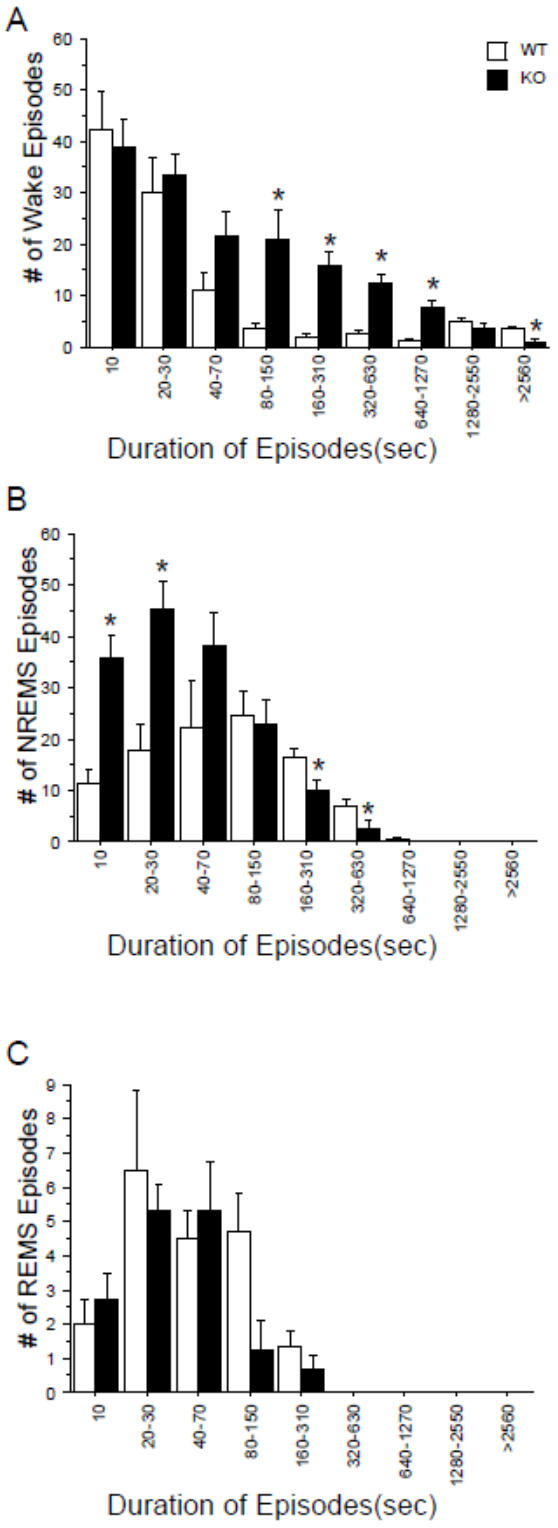


Figure 6. Number of episodes of wake (A), NREMS (B), and REMS C) as a function of episode duration during the baseline dark phase in the STOP KO and WT mice. All values are displayed as means + SEM. All episodes of wake, NREMS, and REMS were divided into 9 consecutive bins on a logarithmic scale. The STOP KO mice had more wake episodes lasting 80-1270 s but fewer episodes lasting >2560 s, compared to the WT mice (A). The STOP KO mice had more short (<30 s) episodes and fewer long episodes (>160 s) of NREMS (B). There were no significant group differences in REMS episode duration (C). N=8 WT, N=7 KO. *Different from WT mice; $P<0.05$ (Bonferroni post hoc comparisons).

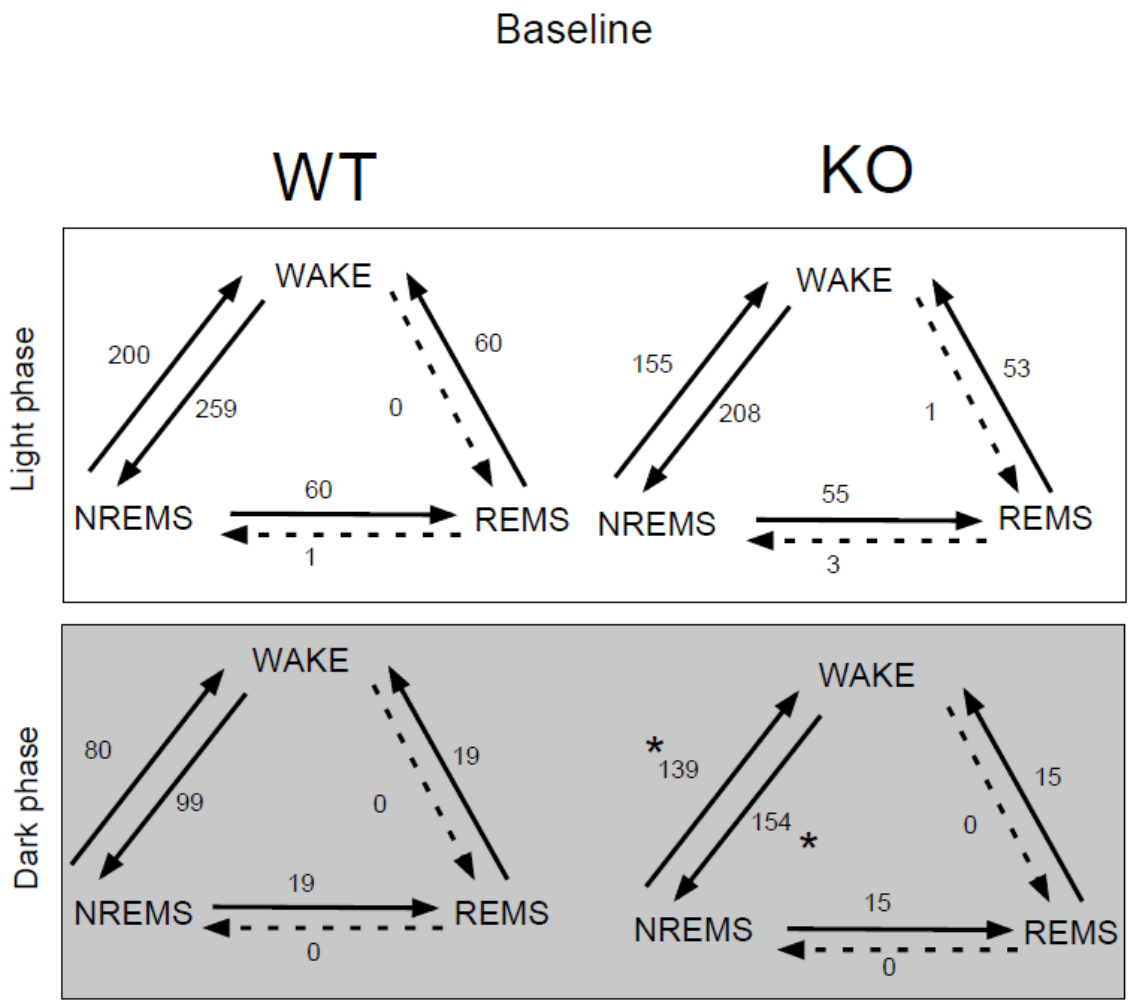


Figure 7. Number of transitions between wake, NREMS, and REMS during the baseline light (top) and dark (bottom) phases in the STOP KO and WT mice. The mean number of transitions between behavioral states is indicated along the arrows between states (the dashed arrows represent rarely occurring transitions). The STOP KO mice made significantly more transitions from wake to NREMS and from NREMS to wake during the 12 h dark phase (bottom). There were no significant differences between groups in the light phase (top). N=8 WT, N=7 KO. *Different from WT mice; $P<0.05$.

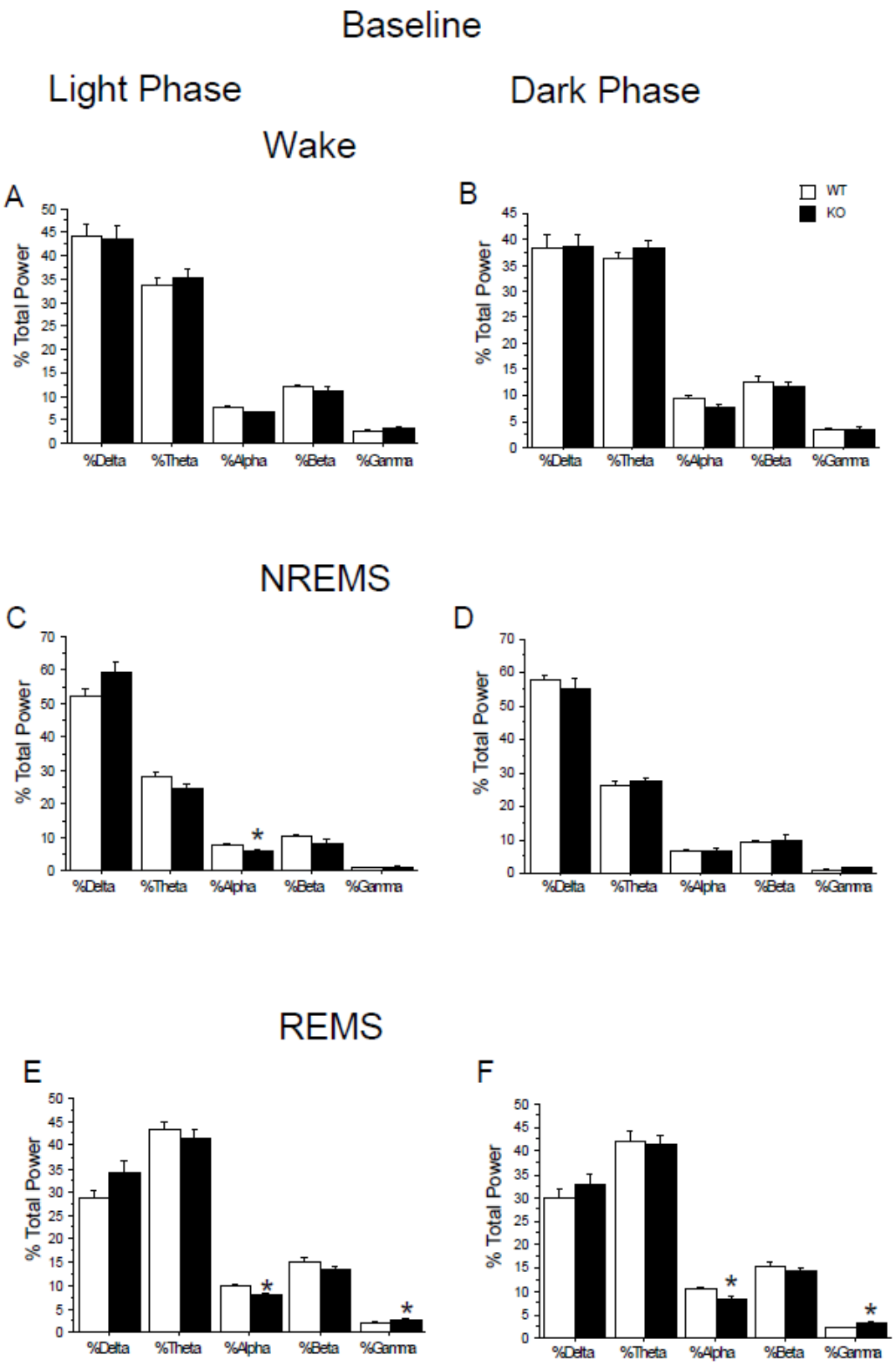

Baseline
Light Phase
Dark Phase
Wake
A
B
WT
KO
% Total Power
%Delta
%Theta
%Alpha
%Beta
%Gamma
NREMS
C
D
REMS
E
F
*

Figure 8. EEG power values in 5 frequency bands (delta, theta, alpha, beta, and gamma) in the baseline light (left) and dark (right) phases during wake (A, B), NREMS (C, D), and REMS (E, F) in the STOP KO and WT mice. Values (means + SEM) are shown for each frequency band. EEG spectral power in each frequency band was normalized to total EEG power (0.5-50 Hz) in each behavioral state. Compared to the WT mice, the STOP KO mice displayed significantly lower alpha power (9-12.5 Hz) in NREMS (C) in the light phase only, and significantly lower alpha power and higher gamma power in REMS during both the light (E) and dark phases (F). There was a trend of an increase in delta power (0.5-4.5 Hz) both in NREMS (C) and REMS (E), especially during the light phase. There were no significant group differences in the wake EEG during either the light or the dark phase, and in the NREMS EEG during the dark phase. N=7 WT, N=7 KO. *Different from WT mice; $P<0.05$

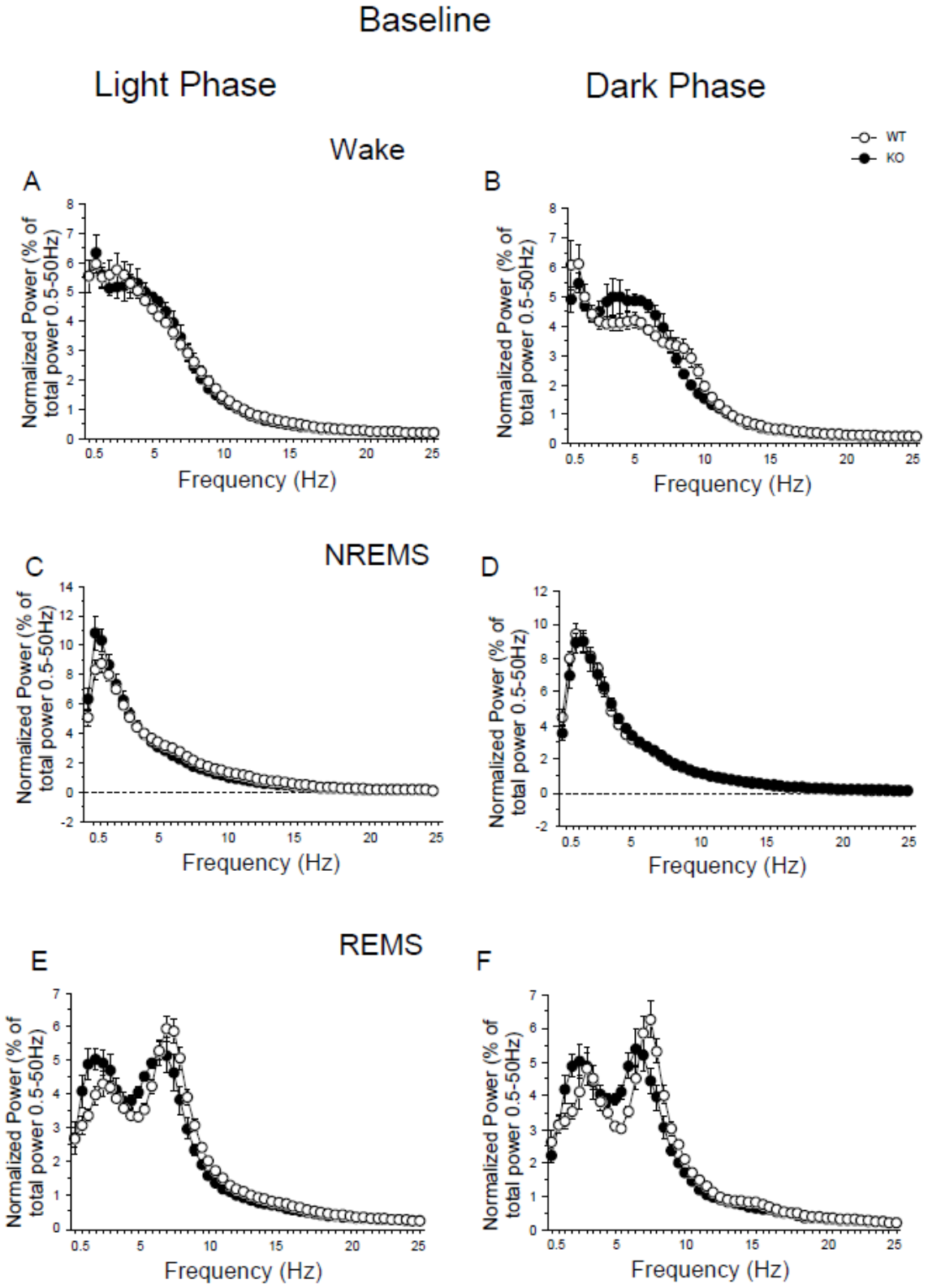

Baseline
Light Phase
Dark Phase
Wake
NREMS
REMS
A
B
C
D
E
F
WT
KO
Normalized Power (% of total power 0.5-50Hz)
Frequency (Hz)

Figure 9. Power spectra of the EEG (0.5-25 Hz) during wake (A, B), NREMS (C, D), and REMS (E, F) in the baseline light (left) and dark (right) phases in the STOP KO and WT mice. All values are displayed as means + SEM. EEG power was normalized to the total EEG power (0.5-50 Hz) for each 0.5 Hz bin in each animal. The STOP KO mice exhibited a slower peak in theta power (4.5-9 Hz) in REMS during the dark phase (F). N=7 WT, N=7 KO.

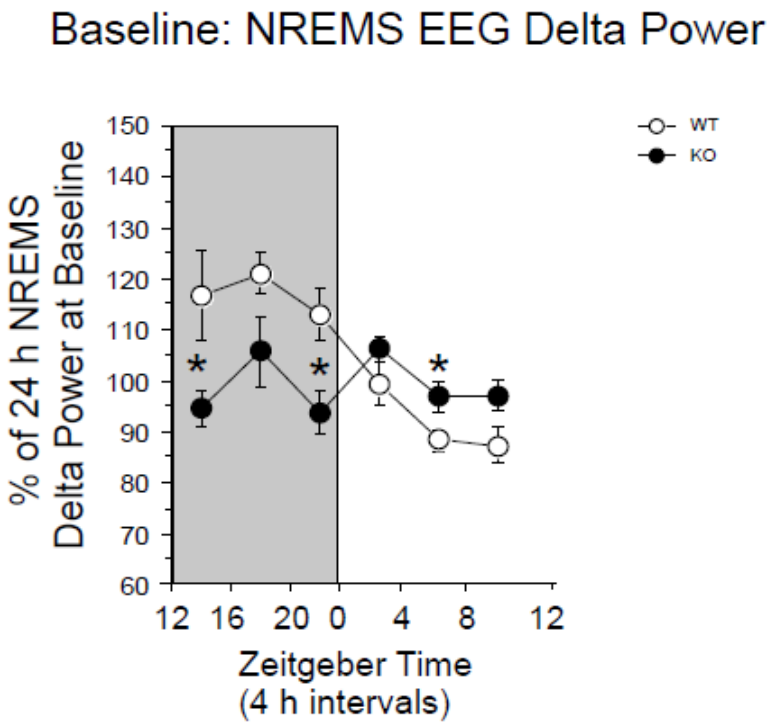


Figure 10. Time course of normalized EEG delta power (0.5-4 Hz) during NREMS in 4 h intervals throughout the 24 h baseline recording in the STOP KO and WT mice.

The time points for the second half of the light phase are plotted after the first half of the light phase. All values are displayed as means + SEM. Throughout the dark phase (shaded gray), STOP KO mice displayed lower NREMS EEG delta power than WT mice, reaching statistical significance for the first and last 4 hours of the dark phase (ZT12-16 and ZT20-24, respectively). Contrary to the dark phase, during light phase, STOP KO mice showed a trend of higher EEG delta power values, with a significant group difference in the middle of the light phase at ZT4-8. The distribution of EEG delta power across the entire baseline appeared flat in KO mice, while WT mice appeared to have a pronounced daily rhythms with delta values that peaked at the beginning of the dark phase, and were lowest at the end of the light phase. Relative (normalized) power values were calculated by expressing absolute NREM delta power values (in 2 hour

intervals) as a percentage of delta power values over the 24 hour baseline recording in each individual animal. N=7 WT, N=7 KO. *Different from WT mice; $P<0.05$ (Bonferroni post hoc comparisons).

First 12 h after SD
(Dark phase)

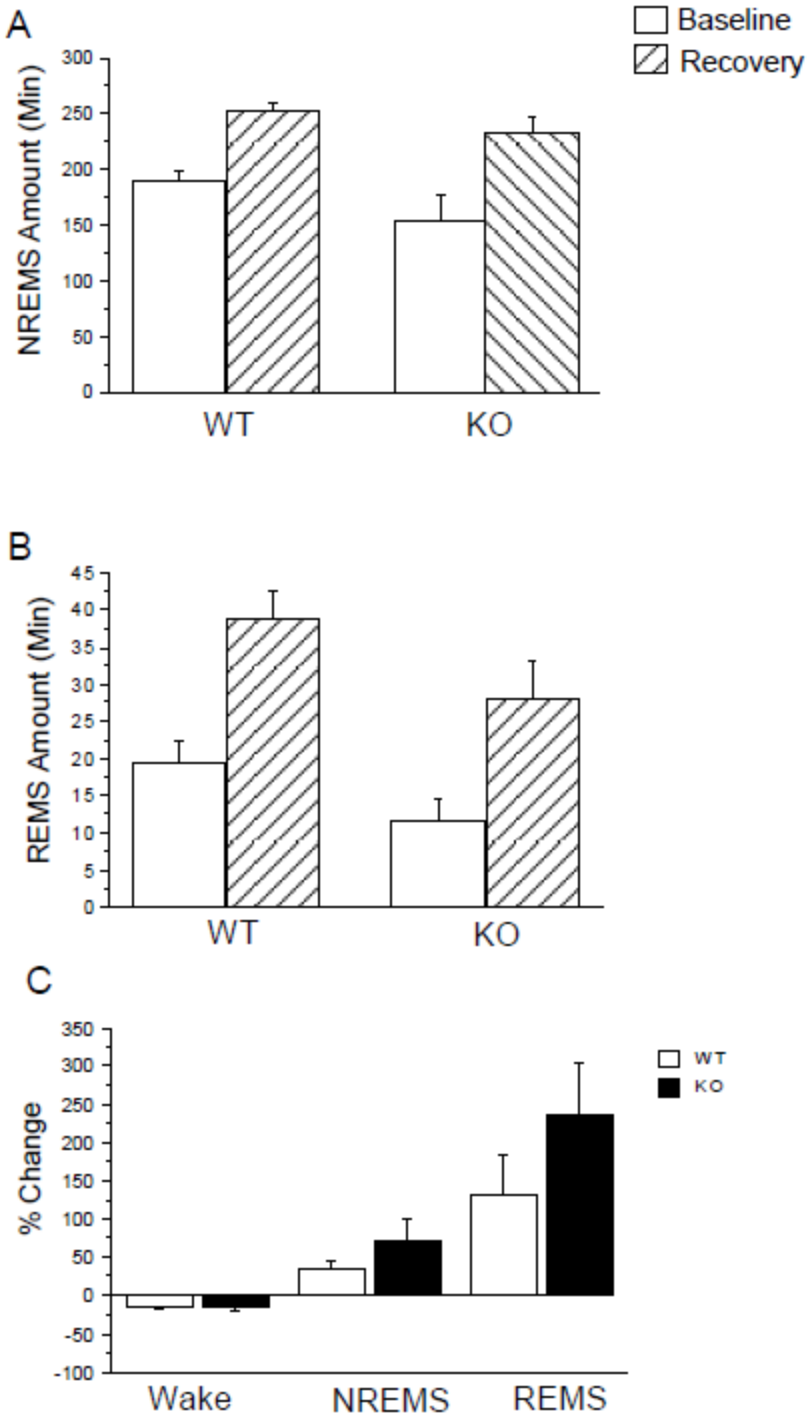


Figure 11. Amount (in min) of NREMS (A), and REMS (B) during the first 12 h period (dark phase) of recovery (hatched bars) following sleep deprivation and the time-matched baseline period (white bars) in the STOP KO and WT mice, and the percent change from baseline for each sleep-wake state (C). All values are displayed as means + SEM. The STOP KO mice and WT mice exhibited similar increases in amounts of NREMS (A) and REMS (B) during the first 12 h following SD, as indicated by a significant Main Effect of condition and a non-significant Group x Condition interaction for both sleep states. N=8 WT, N=7 KO.

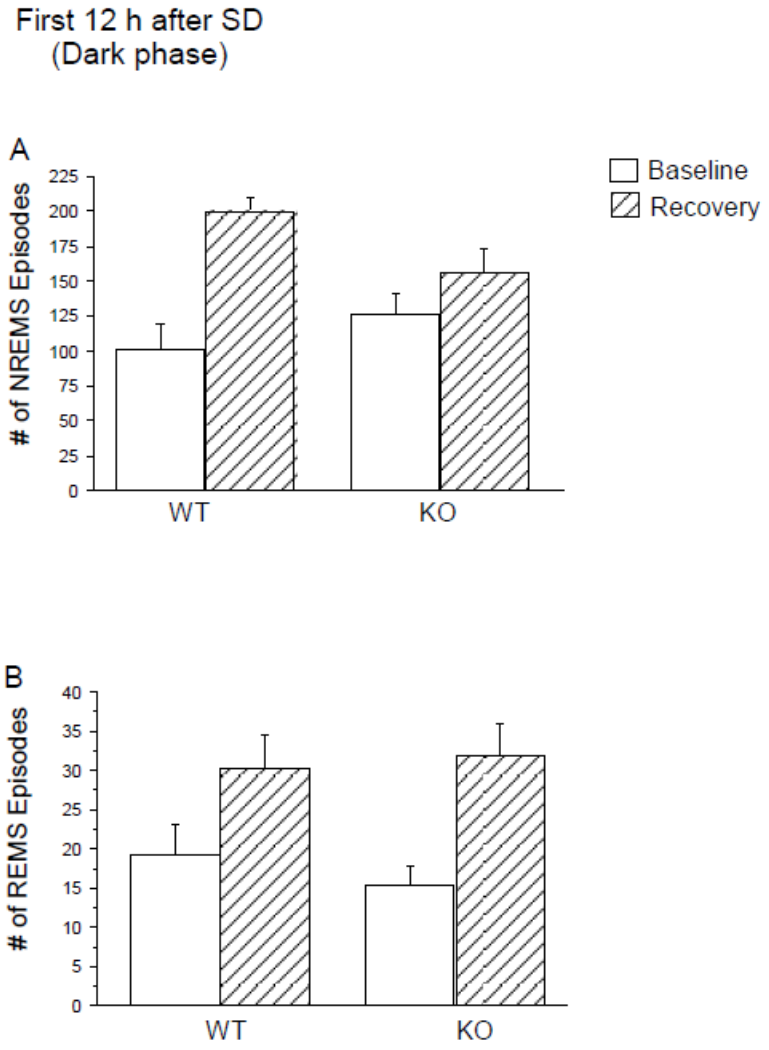


Figure 12. Number of episodes of NREMS (A), and REMS (B) in the first 12 h period (dark phase) of recovery (hatched bars) following sleep deprivation and the time-matched baseline period (white bars) in the STOP KO and WT mice. All values are displayed as means + SEM. The STOP KO mice and WT mice showed similar increases in the number of episodes of NREMS (A) and REMS (B) during the first 12 h following SD. There was a significant Main Effect of condition but no Group x Condition interaction. N=8 WT, N=7 KO.

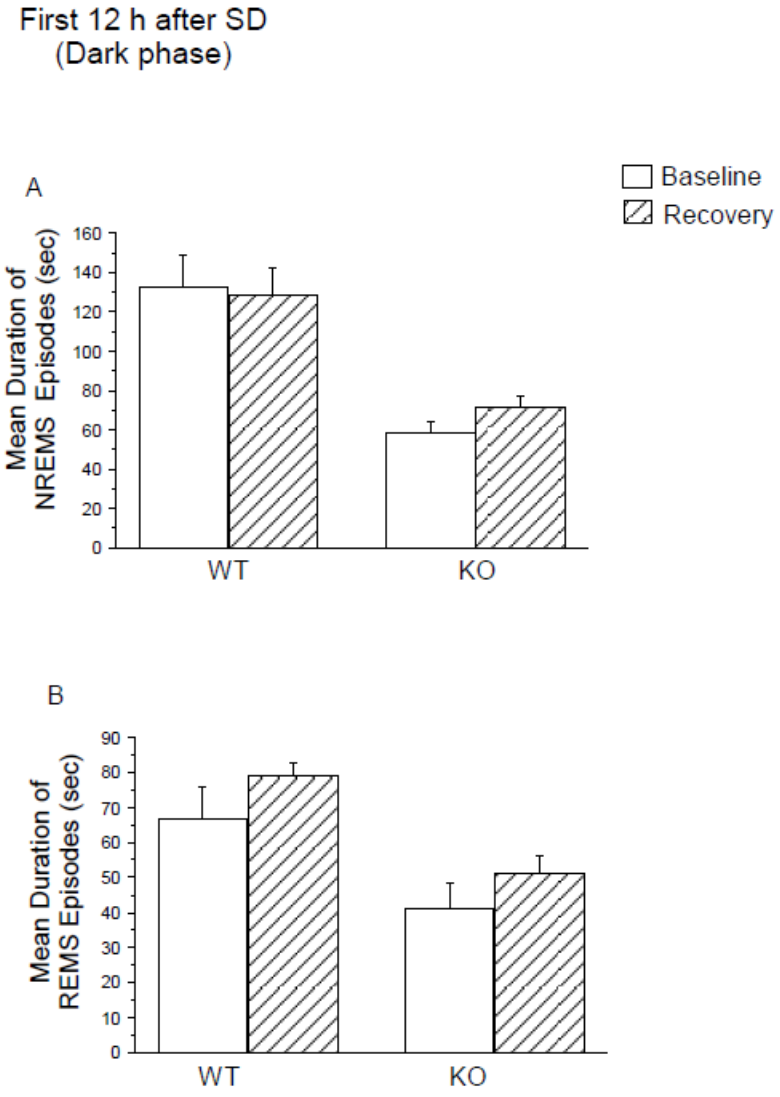


Figure 13. Mean duration of episodes (in seconds) of NREMS (A), and REMS (B) in the first 12 h period (dark phase) of recovery (hatched bars) following sleep deprivation and the time-matched baseline period (white bars). All values are displayed as means + SEM. The STOP KO mice and WT mice exhibited similar increases in the mean duration of REMS episodes (B) during the first 12 h following SD. There was no rebound increase in the mean duration of NREMS episodes (A). N=8 WT, N=7 KO.

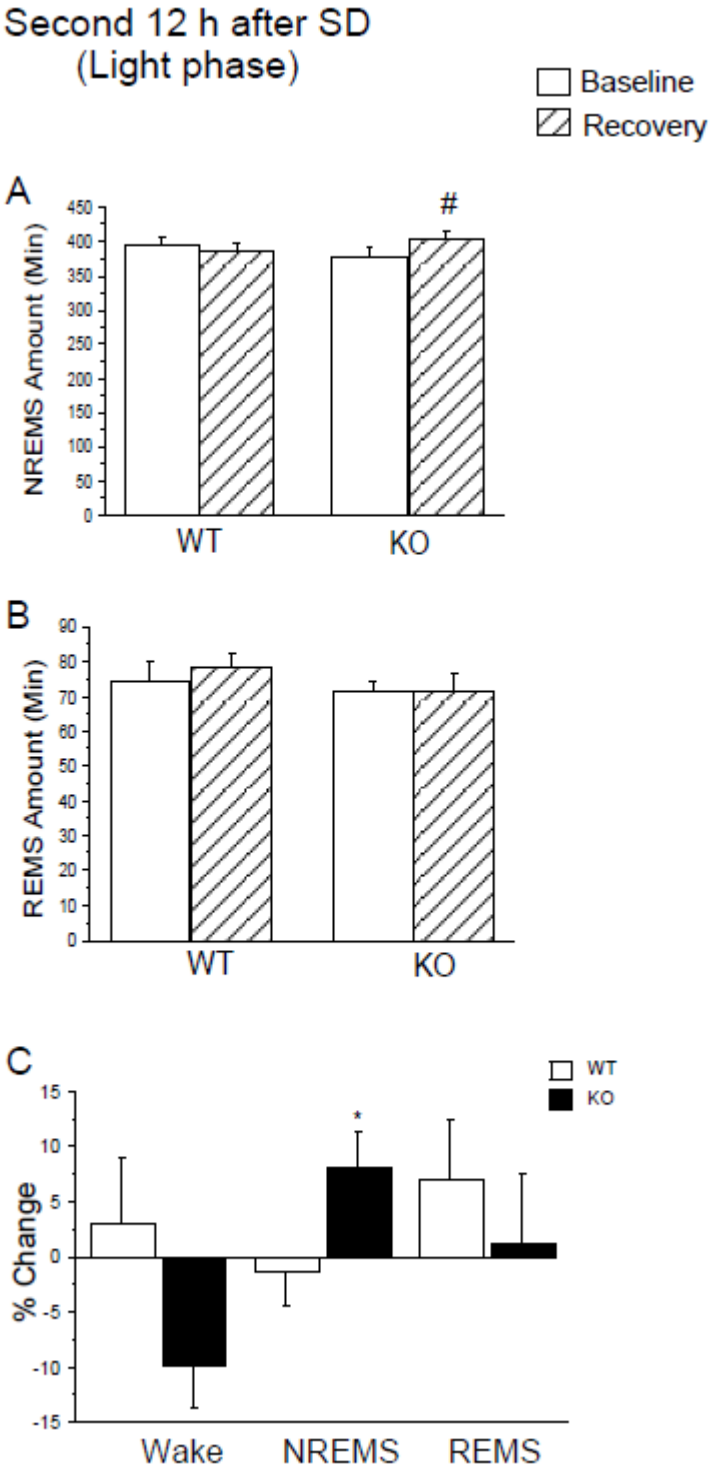

Second 12 h after SD
(Light phase)
Baseline
Recovery
A
NREMS Amount (Min)
450
400
350
300
250
200
150
100
50
0
#
WT
KO
B
REMS Amount (Min)
90
80
70
60
50
40
30
20
10
0
WT
KO
C
WT
KO
% Change
15
10
5
0
-5
-10
-15
*
Wake
NREMS
REMS

Figure 14. Amount (in min) of NREMS (A), and REMS (B) in the second 12 h period (light phase) of recovery (hatched bars) following sleep deprivation and during the time-matched baseline period (white bars) in the STOP KO and WT mice, and the percent change from baseline for each sleep-wake state (C). All values are displayed as means + SEM. There was a significant main effect of condition in NREMS amount (A). STOP KO mice exhibited an increase in the amount of NREMS during recovery compared to baseline in the second 12 h following sleep deprivation, while there was no significant difference between baseline and recovery for WT mice. Also, STOP KO mice exhibited a greater percent change in NREMS amount between baseline and recovery relative to WT mice (C). There was no significant main effect of condition in REMS amount (B). WT, n=8 KO, n=7. * Different from WT group, # different from corresponding baseline; $P < 0.05$ (Bonferroni post hoc comparisons).

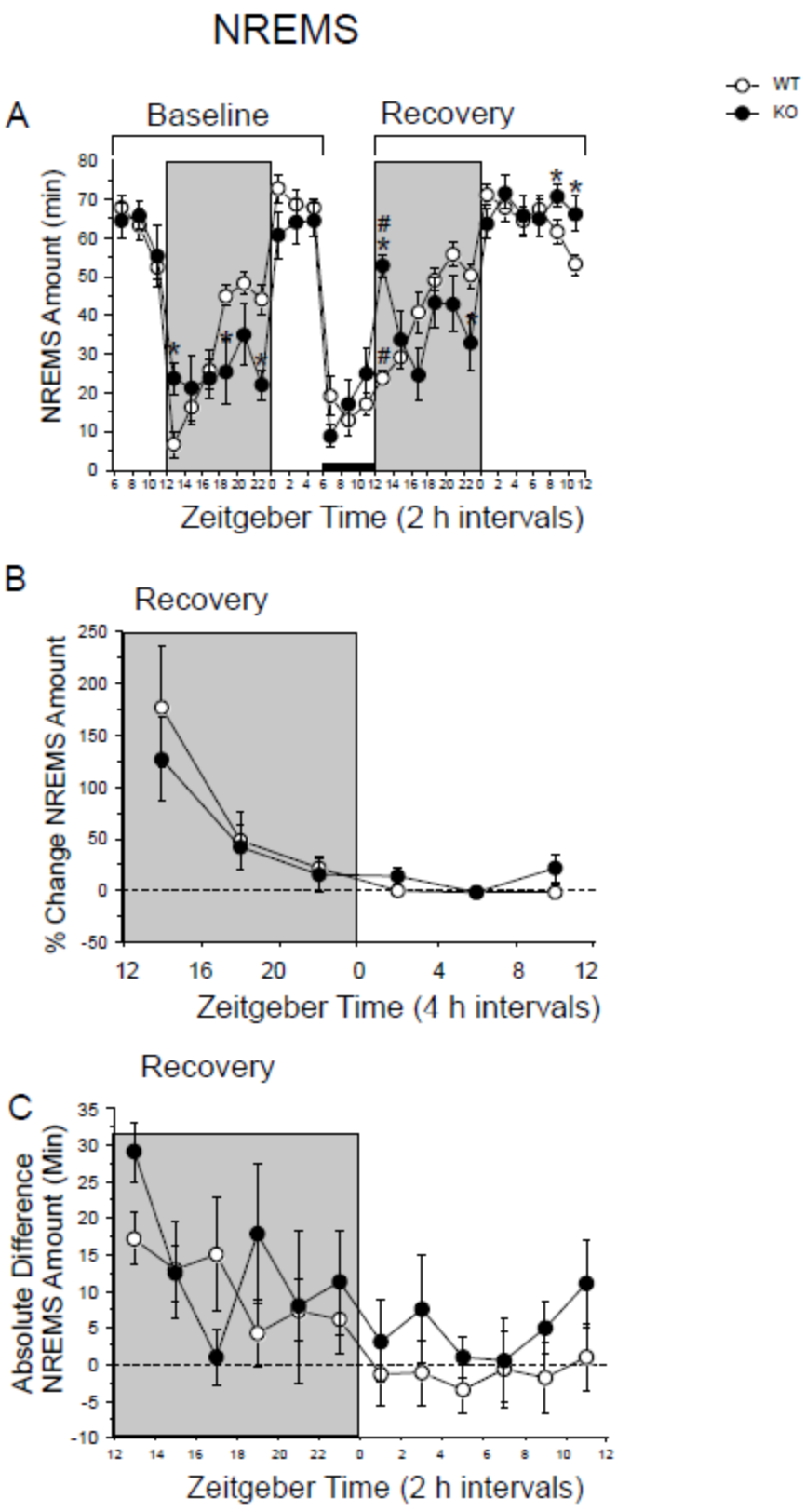

NREMS
WT
KO
A
Baseline
Recovery
NREMS Amount (min)
Zeitgeber Time (2 h intervals)
B
Recovery
% Change NREMS Amount
Zeitgeber Time (4 h intervals)
C
Recovery
Absolute Difference NREMS Amount (Min)
Zeitgeber Time (2 h intervals)

Figure 15. Time course of amount (in min) of NREMS in 2 h intervals across the 24 h baseline recording, 6 h of sleep deprivation (black bar) and 24 h recovery period (A) in the STOP KO and WT mice, and percent change from time-matched baseline in 4 h intervals (B) and absolute difference from time-matched baseline in 2 h intervals (C).

In the first 2 h of recovery immediately following SD (ZT 12) STOP KO mice displayed significantly greater amounts of NREMS compared to WT mice, while both genotypes exhibited significant and similar rebounds in NREMS amount during this 2 h interval (A). This rebound, which was most pronounced immediately following SD and dissipated throughout the dark phase (shaded gray) overtime, is also illustrated in the percent change 4 h plot between baseline and recovery (B). NREMS amounts returned to baseline values at the onset of the light phase (ZT0), as reflected by virtually a zero percent change at this time-point (B). Finally, non-significant increases between genotypes in the absolute difference in 2 h intervals of NREMS amount (C) also corroborate the finding that rebounds were similar for both STOP KO and WT mice. All values are displayed as means + SEM. N=8 WT, N=7 KO. * Different from WT mice, # different from corresponding baseline; all $P < 0.05$ (Bonferroni post hoc comparisons).

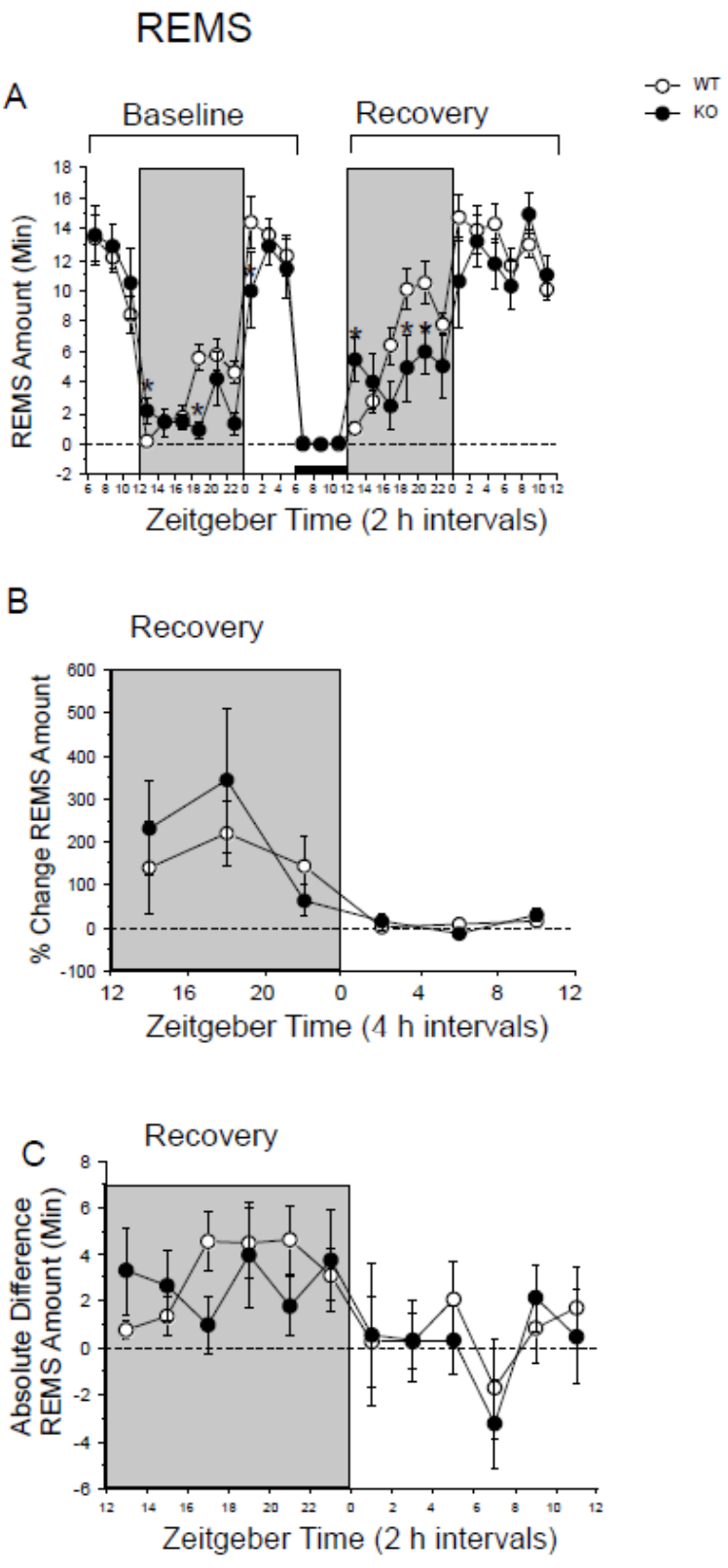

REMS
A
Baseline
Recovery
WT
KO
REMS Amount (Min)
Zeitgeber Time (2 h intervals)
B
Recovery
% Change REMS Amount
Zeitgeber Time (4 h intervals)
C
Recovery
Absolute Difference REMS Amount (Min)
Zeitgeber Time (2 h intervals)

Figure 16. Time course of amount of REMS in 2 h intervals across the 24 h baseline recording, 6 h of sleep deprivation (black bar) and 24 h recovery period (A) in the STOP KO and WT mice, and percent change from time-matched baseline values in 4 h intervals (B) and absolute difference from time-matched baseline values in 2 h intervals (C). In the first 2 h of recovery immediately following SD (ZT 12) STOP KO mice displayed significantly greater amounts of REMS compared to WT mice (A); however, unlike NREMS there was no significant rebound in either genotype for REMS amount. Yet, as is evident in the percent change between baseline and recovery in 4 h intervals of REMS amounts (B), like NREMS, both genotypes exhibited recovery values that were above baseline in the dark phase (shaded gray), and returned to baseline values at the onset of the light phase (ZT 0). There were no significant difference in the absolute difference between baseline and recovery at any timepoint between genotypes (C) All values are displayed as means + SEM. N=8 WT, N=7 KO * Different from WT mice; $P < 0.05$ (Bonferroni post hoc comparisons).

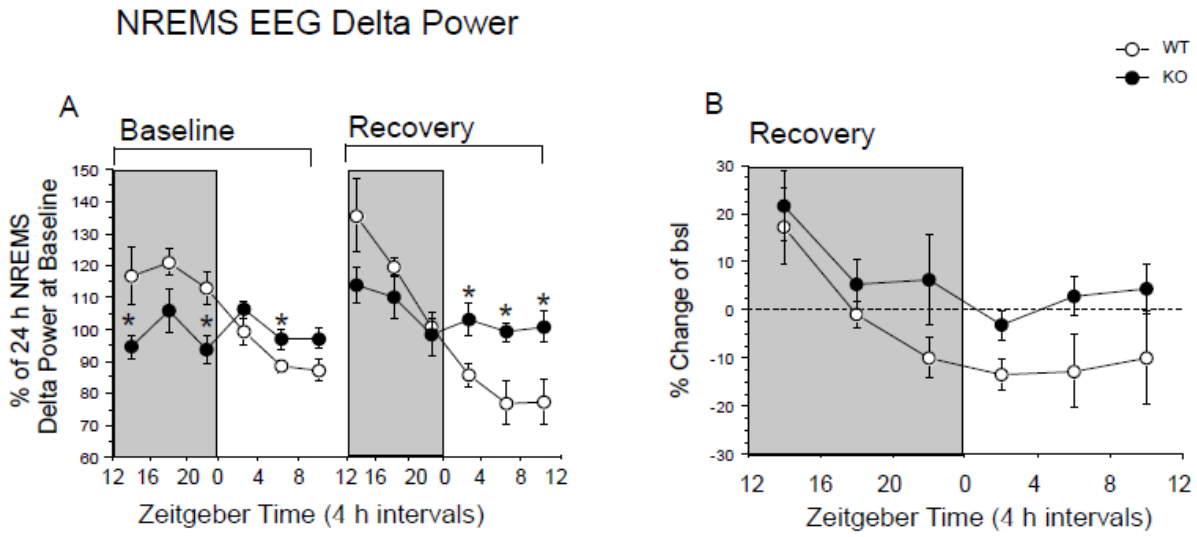


Figure 17. Time course of normalized NREMS EEG delta power (0.5-4 Hz) in 4 h intervals across the 24 h baseline recording (reproduced from Figure 10), and 24 h recovery period (A), and percent change from time-matched baseline values in 4 h intervals (B) in the STOP KO and WT mice. In A, the percent EEG delta power values were obtained by normalizing raw delta power to the average of delta power during 24 h baseline at 2 h intervals in each animal. NREMS delta power was significantly elevated in both genotypes in the first 4 h of recovery following SD (A; see the text for statistics). There were no significant differences in the percent change between baseline and recovery for NREMS EEG delta power (B). However, while STOP KO animals exhibited a simple return to baseline levels towards the end of the dark phase (shaded gray), WT mice only exhibited elevated delta power in the first 4 h of recovery relative to baseline, with values dropping below baseline values (i.e. negative rebound) towards the end of the dark phase and remaining low throughout the light phase

All values are displayed as means + SEM. N=7 WT, N=7 KO. *Different from WT mice (Bonferroni post hoc comparisons).

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APPENDIX A- SUPPLEMENTARY MATERIAL

Supplementary Table 1: DSM-5 Criteria for Schizophrenia

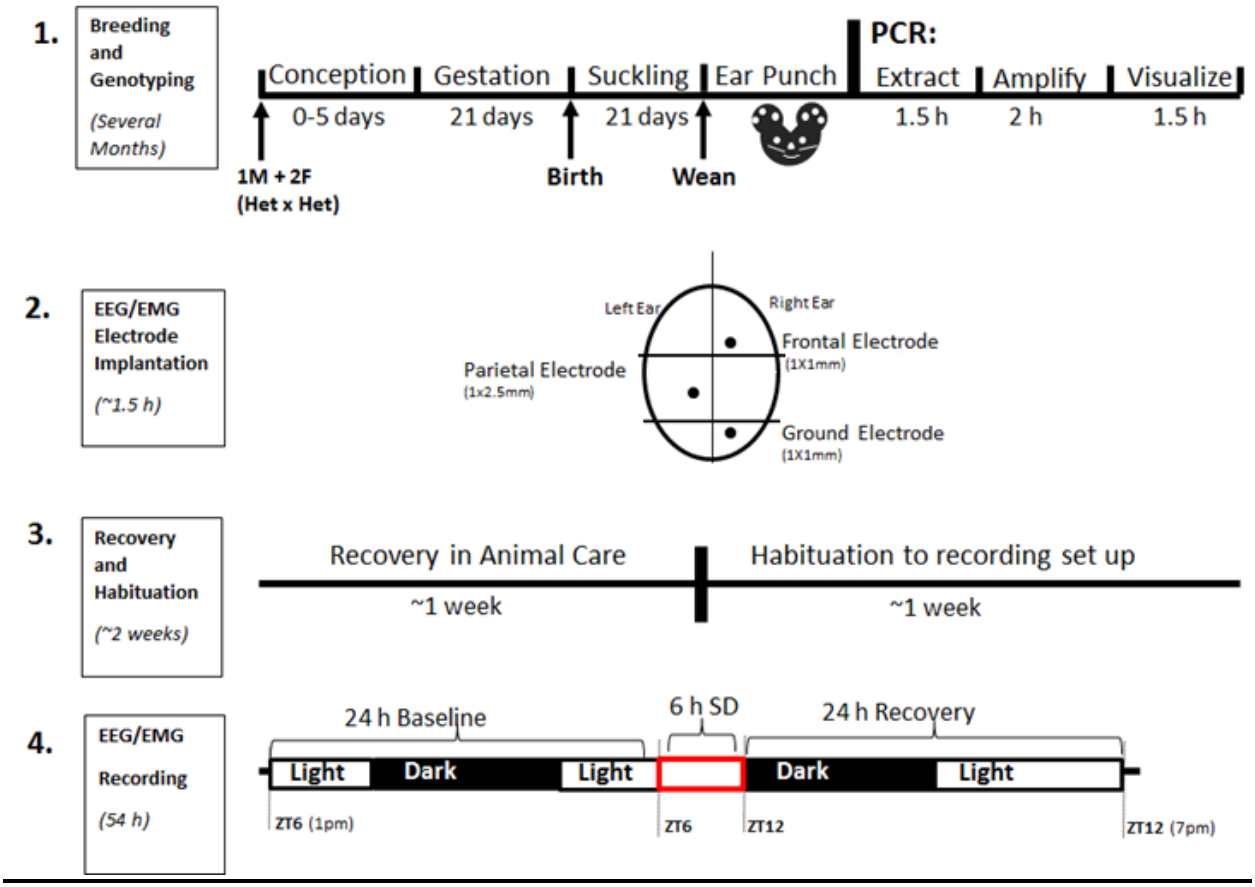
Criterion A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least two of these should include 1–3 (1) delusions (2) hallucinations (3) disorganized speech (4) grossly abnormal psychomotor behavior, including catatonia (5) negative symptoms, e.g., diminished emotional expression or avolition
Criterion B. **Social/occupational dysfunction:** For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning, such as work, interpersonal relations, or self-care, are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).
Criterion C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).
Criterion D. **Schizoaffective and major mood disorder exclusion** Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either (1) no major depressive or manic episodes have occurred concurrently with the active phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.
Criterion E. Substance/general mood condition exclusion Substance/general medical condition exclusion: The disturbance is not attributed to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.
Criterion F. **Relationship to Global Developmental Delay or Autism Spectrum Disorder:** If there is a history of autism spectrum disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least 1 month (or less if successfully treated).

DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th edition. Adapted from the American Psychiatric Association: Diagnostic and statistical manual of mental disorders, 5th ed. Washington, DC: American Psychiatric Press; 2013. Copyright 2013 American Psychiatric Association.

Supplementary Table 2. Body weights of STOP KO and WT mice at different time points during the experiment.

Body Weight	**Genotype**	
	WT(n=8)	KO(n=7)
At surgery (g)	27.8±1.6	26.3±0.8
At habituation (g)	27.8±1.5	24.9±1.2
At sacrifice (g)	27.2±1.4	25.7±1.1
% Change in Body Weight (surgery to habituation)	0	-4.3±3.0
% Change in Body Weight (surgery to sacrifice)	-0.36±2.8	-2.3±3.5

Data are displayed as means ± SEM. No significant differences between genotypes. The numbers of mice are specified in brackets. Range of Days between surgery to sacrifice for each genotype; KO: 16-22 days, WT: 15-25 days.



Supplementary Figure A1. Experimental Procedures.

This study began with breeding and genotyping STOP KO and WT mice (1); Het-heterozygous; M-male; F-female; PCR-Polymerase Chain Reaction. When the genotype of mice was established, heterozygous males and females pups were kept for ongoing breeding to maintain colony, while only the male STOP KO and male WT pups were kept for this experiment. Surgery consisted of EEG/EMG electrode implantation using specific stereotaxic placements (2). Animals were then given time to recover from surgery and habituate to the recording set up (3), followed by EEG/EMG recording for 54 h (4). Sleep deprivation (SD) was conducted via gentle handling. EEG-electroencephalogram; EMG- electromyogram; ZT - Zeitgeber time. Food and water were available ad libitum throughout the experiment. Animals were kept under a 12L:12D cycle.

DNA Extraction:
Using REDExtract-N-Amp Tissue PCR Kit:
1) Add 20ul extraction solution and 5ul tissue prep solution to each ear punch tissue sample (can make a master mix of 4:1 ratio no more than 2 h in advance)
2) Incubate tissue at 55°C for 30-60 minutes.
3) Incubate at 95°C for 3 minutes
4) Add 20ul Neutralization Solution per tube, vortex then store at 4°C
PCR Amplification:
1) To prepare PCR samples, for each animal add 2ul DNA, 3ul WT Primer mix, and 5ul REDExtract-N-Amp per PCR tube (to save time, make a master mix of 3:5 primer:RED, and load 8ul per tube)
2) Replicate for KO Primer mix (2 PCR tubes per animal)
3) Place tubes in Thermal Cycler and run "STOP" program under Main
Note: Primers are stored in SIGMA labelled tubes at -20°C
Gel Electrophoresis:
1) Run amplified DNA samples on a 1% agarose gel for 50-60 minutes @100V
2) Incubate in ethidium bromide (diluted 1:200 from stock) for 20 minutes covered on shaker.
3) Visualize under UV light

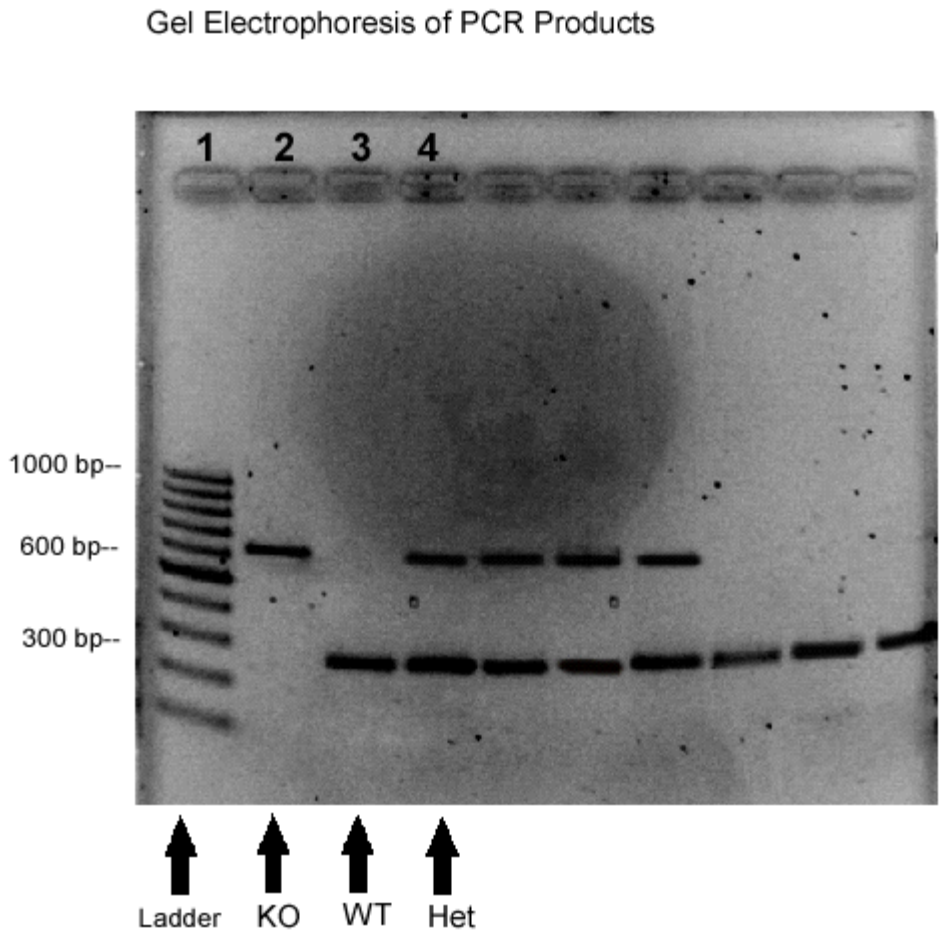
Primer Recipe:
To prepare a Master Mix for each primer set:
-add 3ul WT forward primer (fwd) and 3ul WT reverse primer (rev) to 94ul dH2O: final conc. = 1.5uM
- add 3ul MUT forward primer (fwd) and 3ul MUT reverse primer (rev) to 94ul dH2O: final conc. = 1.5uM
3ul primer mix (1.5uM) into 10ul solution = 0.45uM final concentration.

	nmol protein	ml 0.1xTE buffer	Final concentration
WT STOP (fwd)	37.3	0.746	50uM
WT STOP (rev)	29.0	0.580	50uM
MUT STOP (fwd)	42.9	0.858	50uM
MUT STOP (rev)	48.7	0.974	50uM

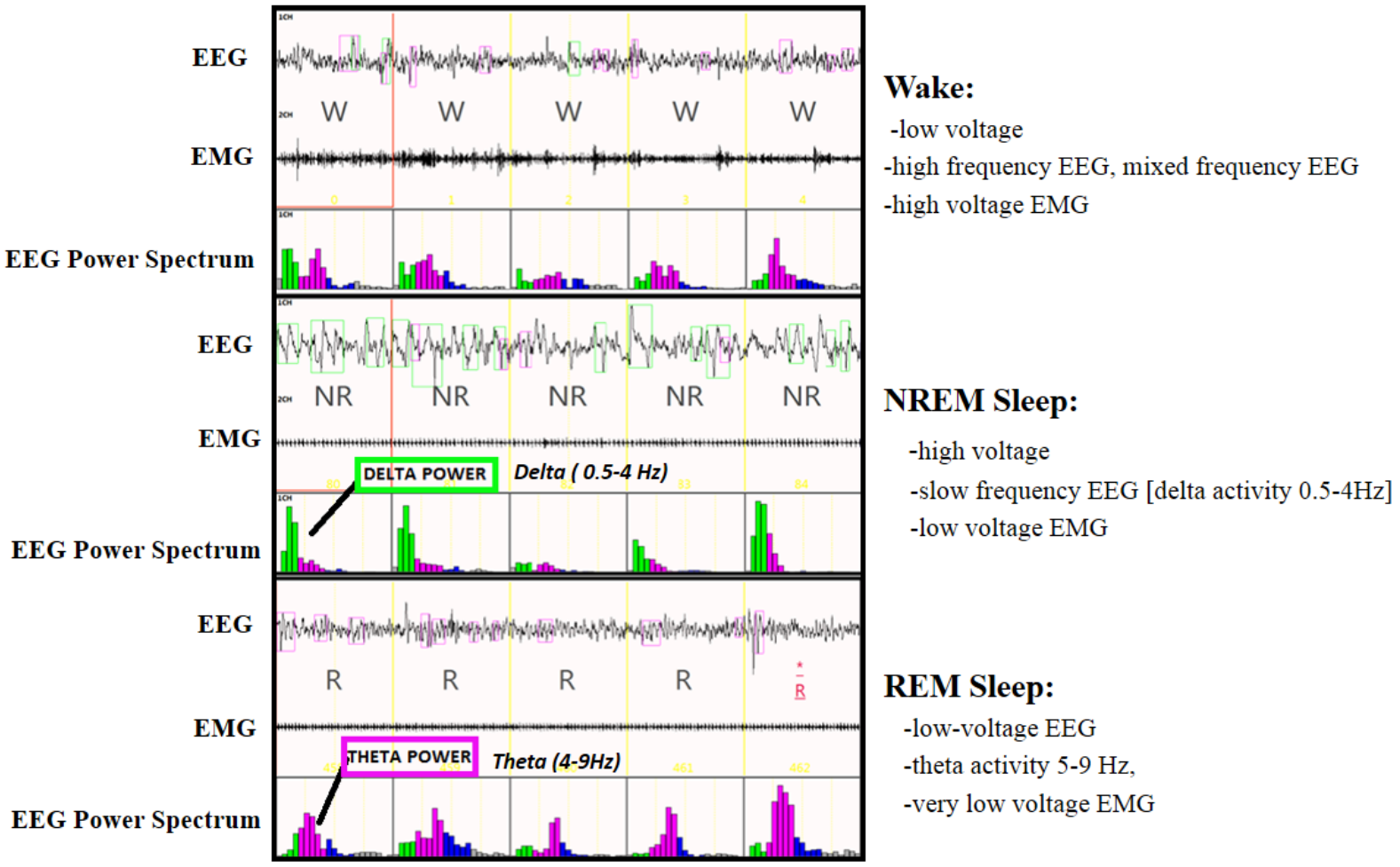
Supplementary Figure A2. Genotyping Protocol.

Genotyping was carried out using the above protocol (Kajitani et al., 2010). DNA extraction was performed on ear punches obtained from 15-40 mice of both sexes at a time. Following DNA extraction using Taq polymerase and RED Extract N amp kit, tissue samples were amplified via PCR using a thermal cycler programmed at specified conditions optimized for STOP KO mice.

PCR products were then pipetted into the wells of an agarose gel, and visualized with ethidium bromide under a UV light. See Suppl. Figure A3, for a representative gel of PCR products that denote DNA bands that correspond to genotype.

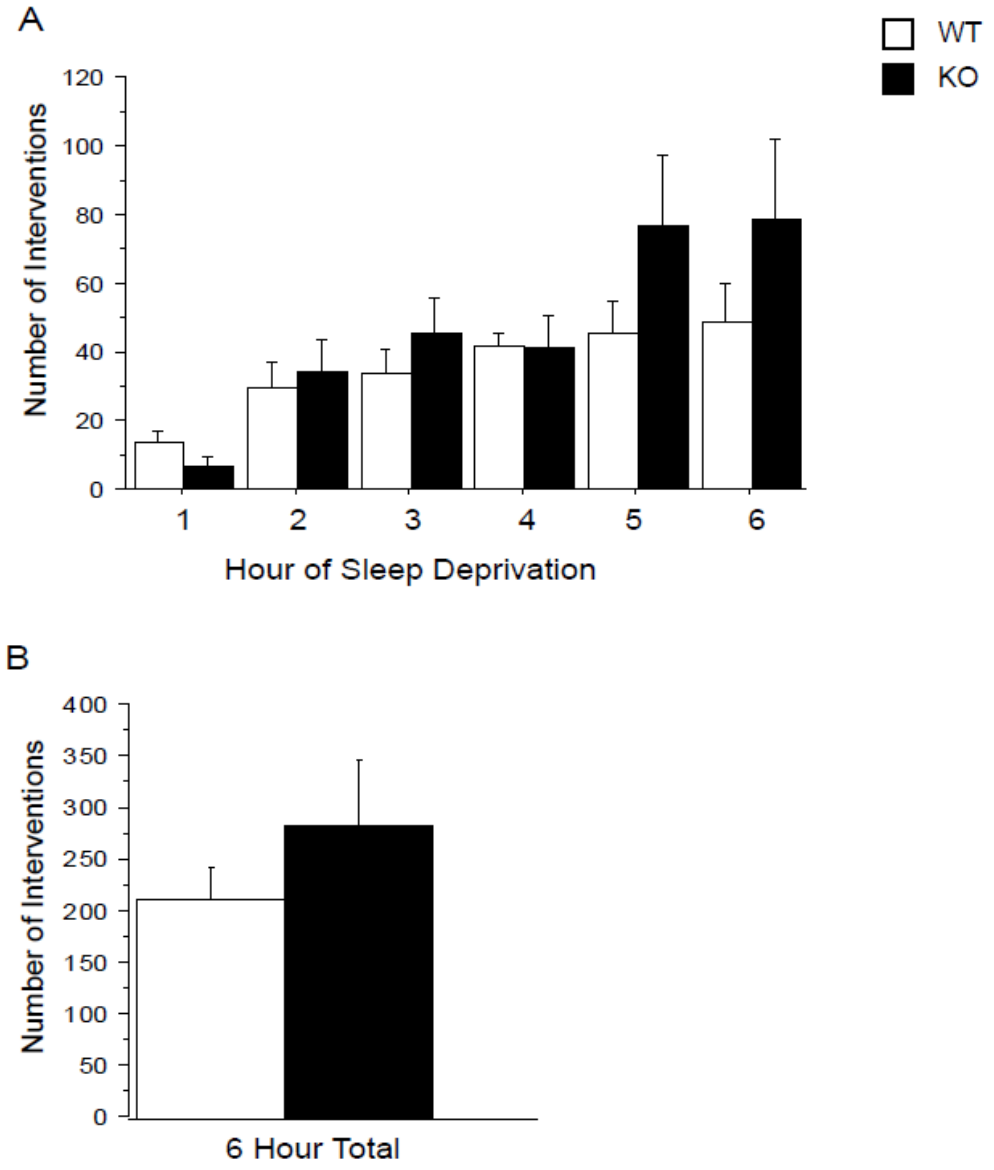


Supplementary Figure A3. Gel Electrophoresis of PCR Products: 2% agarose gel electrophoresis of DNA samples stained with ethidium bromide. Lane 1 contains a 1000 base-pair ladder. Lane 2, 3, and 4 show PCR with amplified DNA representing the 3 possible genotype respectively: STOP KO (1 band at 600 base-pairs), WT (1 band at 300 base-pairs), and heterozygous (1 band at 600 base-pairs, and 1 band at 300 base-pairs). Shaded circle in center agarose gel artifact ie. air bubble.

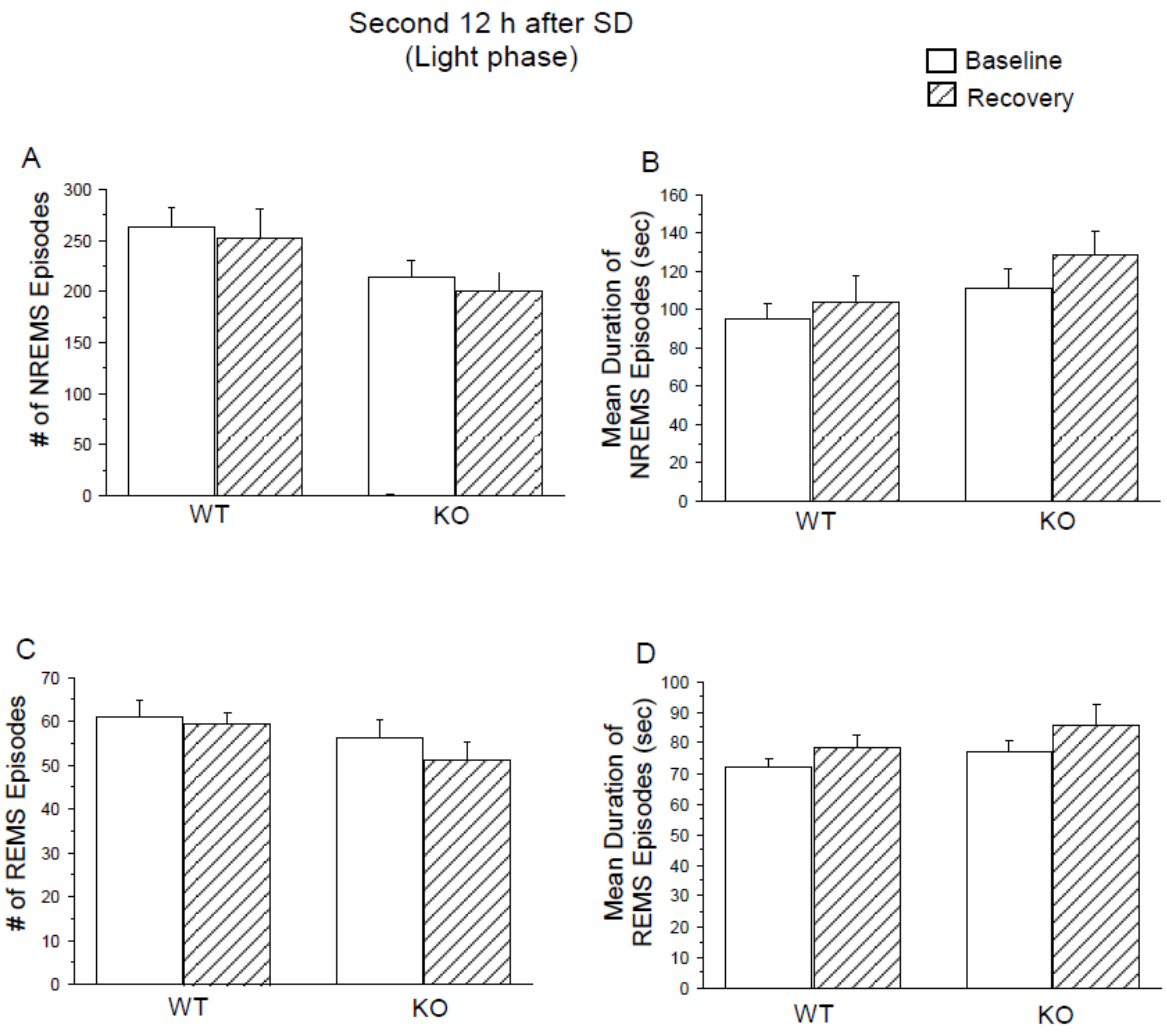


Supplementary Figure A4. Scoring of Sleep-Wake States: Typical EEG/EMG Recording in a WT mouse.

From top to bottom: wake, NREMS, and REMS. For each vigilance state, the top trace shows the EEG. The second trace shows the EMG. The bottom panel shows the EEG power spectrum, with colour-coded frequency bands. Delta power (Hz) dominates NREMS, and theta power (Hz) dominates REMS. EEG/EMG units are in microvolts. Vigilance states were determined for each 10-s epoch of the 54 h EEG/EMG recording, as described in the Methods section.



Supplementary Figure A5. Number of interventions during sleep deprivation in the STOP KO and WT mice**:** Mean (+SEM) number of interventions at every h of sleep deprivation (A), and a total number over the entire 6 h period (B). The number of interventions increased with each h of sleep deprivation, without any significant differences between genotypes. N=8 WT N=7 KO.



Supplementary Figure A6. Numbers (left) and mean durations (right) of episodes of NREMS (A and B) and REMS (C and D) in the second 12 h period (light phase) of recovery (hatched bars) following sleep deprivation, and during the time-matched baseline period (white bars) in the STOP KO and WT mice.

There were no significant differences between the STOP KO mice and WT mice in the second 12 h following SD, and no significant differences between recovery and time-matched baseline period within genotypes for either the number or mean duration of NREMS or REMS episodes N=8 WT N=7 KO