Effects of pharmacological manipulation of the serotonergic/cholinergic systems on sleep structure in two 5-HT_{1A} genotypes: Implications for a model of depression

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Abstract

The serotonergic and cholinergic systems are jointly involved in regulating sleep but this balance is theorized to be disturbed in depressed individuals (Janowsky 1972, Jouvet 1972). One potential cause of disturbed neurotransmission is genetic predisposition. The G(-1019) allele of the 5-HT_{1A} receptor predicts an increased risk for depression compared to the wild-type C(-1019) allele.

The goal of this study was to use pharmacological probes in normal controls to model the serotonergic/cholinergic imbalance of depression and its associated abnormalities in sleep structure while controlling for 5-HT_{1A} receptor genotype.

Seventeen healthy female participants homozygous for either C (n=11) or G (n=6) alleles, age 18-27 years were tested on four non-consecutive nights. Participants were given galantamine (an anti-acetylcholinesterase), buspirone (a serotonergic agonist), both drugs together, or placebos before sleeping.

Buspirone suppressed tonic REM: there was a significant increase in REM latency ($p<0.001$). Galantamine increased tonic REM sleep, leading to more time spent in stage REM ($p<0.001$) and shorter REM latency ($p<0.01$). Galantamine and buspirone given together tended to negate the effects of each other on REM sleep measures but disrupted sleep more than either drug alone, showing lower SE and N3% and increased awakenings, Wake% and N1% ($p<0.019$). There was no main effect of genotype nor was there a significant multivariate interaction between genotype and drug condition.

These findings are partially consistent with the literature about sleep in depression, notably short REM latency, higher percentage of total sleep time spent in

REM, and increased sleep fragmentation. The C/G mutation in the 5-HT_{1A} receptor does not appear to cause noticeable differences in the sleep patterns of healthy young females.

Keywords: Sleep, Depression, Sleep Structure, REM, Serotonin, Acetylcholine, Buspirone, Galantamine, Neurotransmission, 5-HT_{1A} receptor, genetic risk factors.

Résumé

Les systèmes sérotonergiques et cholinergiques sont ensemble responsables pour le contrôle du sommeil, mais cet équilibre est supposément perturbé chez les individus souffrant de dépression. Une cause possible de ce dérangement est une prédisposition génétique. L'allèle G(-1019) du récepteur 5-HT_{1A} prédit un risque accru de dépression en comparaison à l'allèle type naturel C(-1019).

L'objectif de cette étude était d'utilisesr des agents pharmacologiques chez des sujets normaux pour modeler le déséquilibre sérotonergique/cholinergique de la dépression et ses anormalies dans la structure du sommeil tout en contrôlant pour le génotype du récepteur 5-HT_{1A}.

Dix-sept participantes en santé homozygotes pour l'allèle C (n=11) ou G (n=6), âgées de 18 à 27 ans, ont été enregistrées en laboratoire durant quatre nuits non-consécutives. Les participantes ont reçu successivement de la galantamine (un anti-acétylcholinestérase), du buspirone (un agoniste sérotonergique), les deux médicaments ensemble, ou un placebo avant le sommeil.

Le buspirone a supprimé le sommeil paradoxal tonique : il y a eu une augmentation significative de la latence au sommeil paradoxal ($p<0.001$). La galantamine a augmenté le sommeil paradoxal tonique, menant à une augmentation du temps consacré au stage du sommeil paradoxal ($p<0.001$) et un délai plus court avant l'apparition du sommeil paradoxal ($p<0.01$). Lorsque combinés, la galantamine et le buspirone ont eu tendance à annuler leurs effets sur le sommeil paradoxal mais à déranger le sommeil plus que les deux médicaments pris individuellement. Cela s'est manifesté par une efficacité réduite du sommeil, une proportion réduite de stage N3, une augmentation

d'éveils, de la proportion d'éveil et de stage N1 ($p<0.019$). Il n'y a pas eu d'effet du génotype et il n'y a pas eu d'interaction multivariée significative entre le génotype et la condition médicamentée.

Ces observations sont en partie congruentes avec la littérature sur le sommeil et la dépression, notamment un délai plus court avant le sommeil paradoxal, un plus haut pourcentage du temps total endormi consacré au sommeil paradoxal, et une fragmentation accrue du sommeil. La mutation C/G dans le récepteur 5-HT_{1A} ne semble pas engendrer de différences apparentes dans le rythme du sommeil de jeunes femmes en santé.

Mots clés : Sommeil, Dépression, Structure du sommeil, Sommeil paradoxal, Sérotonine, Acétylcholine, Buspirone, Galantamine, Neurotransmission, récepteur 5-HT_{1A}, Facteurs de risque génétiques.

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Article 1

Article 2

Legend

5-HT: Serotonin

ACh: Acetylcholine

CNS: Central nervous system

DRN: Dorsal raphe nucleus

LDT/PPT: Laterodorsal and the pedunculopontine tegmental nuclei

MDD: Major Depression Disorder

N1%: Percentage of time asleep spent in stage N1

N2%: Percentage of time asleep spent in stage N2

N3%: Percentage of time asleep spent in stage N3

PGO: Pontine-geniculate-occipital

RD: Rem density

REM: Rapid eye movement

REM%: Percentage of time asleep spent in REM

RL: REM latency

SE: Sleep efficiency

SNP: Single nucleotide polymorphism

SOL: Sleep onset latency

SSRI: Selective Serotonin Reuptake Inhibitor

SWS: Slow wave sleep

TST: Total sleep time

Wake%: Percentage of time in bed after sleep onset spent awake

WASO: wake after sleep onset

Preface

Authorial contributions:

Kathleen Biard: refined study design, recruited and screened participants, performed the NPSGs, scored the sleep studies and collected data, performed and interpreted the statistical analysis, drafted and revised the manuscripts.

Alan Douglass: provided initial study design, arranged funding from a grant from the IMHR, performed medical screening for participants, supervised lab work and research, interpreted the statistical analysis, revised the manuscripts.

Rébecca Robillard: interpreted the statistical analysis, revised the manuscripts.

Joseph De Koninck: supervised the research project, interpreted the statistical analysis, revised the manuscripts.

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Introduction

Overview

The past 40 years have marked the discovery of biological abnormalities in patients with mood disorders, including Major Depression Disorder (MDD). Some of these have been in three related areas: the physiology of Rapid Eye Movement (REM) sleep, the functioning of the neuroendocrine system, and genetic discoveries in neurochemical receptor and transporter systems.

Serotonin (5-HT) has been heavily implicated in the pathophysiology of depression. Its effects on REM sleep are also well known: 5-HT acts primarily at the 5-HT_{1A} receptor to suppress REM sleep and reduce REM density (RD) (Datta 2007). The cholinergic system, also implicated in depression, has also been discovered to be involved in the regulation of REM sleep (Sitaram 1982, Riemann 1994a). The serotonergic and cholinergic systems are believed to normally be in balance, jointly controlling REM sleep in healthy individuals: this balance is disturbed in depressed individuals, leading to observable disturbances in REM sleep (Palagini 2013). The exact nature of this disturbance has never been thoroughly explored: no study has yet employed both serotonergic and cholinergic drugs in a repeated measures design using multiple drugs in the same subjects.

While it has long been known that genetics have some effect on risk of mood disorders, recent genetic research has begun to pinpoint specific genes implicated in major depression. One such genetic risk factor is the allele of the 5-HT_{1A} receptor. An individual's possession of the G(-1019) allele of the 5-HT_{1A} receptor predicts an increased risk for depression compared to the native C(-1019) allele (Robinson 1990, Kawashini 1998, Lesch 2004). This effect appears to be even stronger for persons homozygous for the "G" allele,

while homozygosity for the "C" allele appears to confer some protection against depression. The importance of these findings may explain some of the contradictory findings in previous studies that looked at the effects of serotonergic drugs on REM sleep without taking the genotypes of their subjects into account.

The goal of this project was to examine the effects on sleep structure of a serotonergic agonist (buspirone), and a cholinergic agonist (galantamine) while controlling for the genotype of the 5-HT_{1A} receptor. This was a relatively small feasibility study on normal subjects. The intent was to determine whether these two drug probes can alter sleep in a systematic way that supports the concept of a serotonergic-cholinergic balance that controls REM sleep. Controlling for this highly relevant genotype could potentially make this effect much clearer than in previous studies. The ultimate goal was to improve sleep physiology markers for susceptibility to MDD and to obtain some physiological insight into how the depressive state occurs.

Previous Research

The first links between depression and abnormalities in sleep structure were noted in the 1970s. Depression was seen to affect tonic measures of sleep such as reduced sleep continuity, reduced non-REM (NREM) sleep (Benson 1989; Kupfer 1984) and reduced REM latency (RL)(Keshavan 1990; Kupfer 1976) as well as phasic measures of sleep such as REM density (RD)(Clark 1998; Foster 1976; Wichniak 2002). RD was usually measured as either "raw" RD (number of rapid eye movements per minute of REM sleep) or as "Kupfer RD", a semi-quantitative method of estimating RD at a glance. RD was rarely analysed in-depth because of the impracticality of identifying every rapid eye movement in a study.

Further research found that some of these findings were not unique to depression but were shared by several other mental illnesses (Benca 1996; Benca 1992; Peterson 2006;

Zarcone 1987). Some schizophrenic patients have a shortened RL and mildly increased RD, as do substance abuse patients (Chouinard 2004). Bipolar patients often have markedly higher RD and altered tonic measures of sleep (Gillin 1994). It has also been noted that there is a subset of depressed patients who do not have a shortened RL (Buysse 1990). Most patients with depression, however, have consistently elevated RD and pathologically shortened RL.

The sleep control system. Sleep disturbances are seen in patients with depression (and in many other psychiatric disorders) due to the neurotransmitter systems which regulate mood, cognition, and mental functioning also regulating sleep and wake: it is almost impossible to have a neurophysiological imbalance severe enough to affect mental health without also affecting sleep (Lee 2010).

Acetylcholine (ACh) is a stimulating system that promotes wakefulness and REM sleep when activated. Cholinergic neurons project from the LDT/PPT towards the hippocampus, thalamus, and neocortex (Brown 2012). These neurons have been found to fire fastest during wakefulness and REM sleep – both states of cortical activation and conscious awareness (Perry 1999). Activation of cholinergic neurons increases fast EEG rhythms such as theta and gamma waves and decreases slow oscillations like the delta waves seen in stage N3 sleep (Steriade 2004).

Serotonin, in contrast, suppresses ACh activity in the LDT/PPT and reduces gamma and theta activity without boosting slow oscillations: the 5-HT system promotes a "quiet waking state with reduced cortical activation" (Brown 2012). 5-HT neurons are more active during wake periods but also fire during NREM sleep, ceasing during REM sleep: 5-HT agonists tend to decrease sleep but especially repress REM, likely caused by 5-HT1a receptor-mediated inhibition of the LDT/PPT REM-promoting cholinergic neurons (Boutrel

2002). Thus, even though both ACh and 5-HT are wake-promoting neurotransmitter systems they have opposite effects on REM sleep in particular: ACh promotes REM sleep while 5-HT reduces it by suppressing ACh activity (Saper 2010).

5-HT_{1A} receptor binding and depression. Many links have been drawn between altered serotonin transmission and depression (Rosa-Neto 2004, Delgado 1994). The serotonin hypothesis of depression suggests that depression is caused by a relative or absolute lack of serotonin neurotransmission. Reduced 5-HT synthesis in the areas of the brain responsible for regulating mood is one possible cause of decreased 5-HT transmission. Another mechanism that could be contributing to the decrease is abnormal 5-HT transmitter proteins. Several recent studies have found evidence that the 5-HT_{1A} receptor is heavily implicated in faulty neurotransmission. MDD patients have been shown to have a lower response to 5-HT_{1A} agents than normal controls. Positron Emission Tomography (PET) and post-mortem studies have found that patients with MDD have altered 5-HT_{1A} binding. Altered 5-HT_{1A} binding has also been reported in post-mortem brain studies of suicide victims with depression (Stockmeier 1998). The suicide victims in this study were found to have significantly higher rates of 5-HT_{1A} binding density in the raphe region than gender- and age-matched controls, specifically in the dorsal and ventrolateral subnuclei. Increased binding on 5-HT_{1A} autoreceptors causes decreased serotonin transmission, thus linking a deficiency of 5-HT transmission with suicide in MDD patients.

Research in primates has found that serotonergic neurons originating in the dorsal and median raphe nuclei project extensively throughout the central nervous system (CNS), selectively innervating different cortical areas (Wilson 1991). The 5-HT_{1A} receptor is found throughout the CNS, with the highest receptor density in the hippocampal formation, the neocortex and the raphe (Aznar 2003, Hall 1997). In the dorsal and median raphe the 5-HT_{1A}

receptors are situated on the pre-synaptic cell bodies and dendrites and act as autoreceptors: when they are activated they reduce the firing of the 5-HT neurons which results in a suppression of serotonin synthesis and release in the projection areas. Post-synaptic 5-HT_{1A} receptors are found mainly in the limbic regions like the hippocampus and septum, the frontal cortex and the pontine tegmentum (Hall 1997, Staner 2006). It is worth noting that the pontine tegmentum contains the "REM on/off switch" and is responsible for controlling REM sleep (Jun 2006). The 5-HT_{1A} receptors in this area decrease the firing rate of the post-synaptic neurons, inhibiting REM sleep. The 5-HT_{1A} receptor can therefore act as a pre-synaptic autoregulator as well as a post-synaptic regulator in the serotonin system.

The C(-1019)G polymorphism. The gene that codes for the 5-HT_{1A} receptor is found on chromosome 5q11.2-113 (Fargin et al., 1988). It is an intronless gene that codes a 422-amino acid protein. There are several low frequency single nucleotide polymorphisms (SNPs) that have been found on this gene but only the frequent C(-1019)G SNP has been associated with psychiatric disorders such as depression and anxiety, and suicide (Nakhai 1995, Erdmann 1995, Kawanishi 1998, Lemonde 2003).

The G polymorphism prevents binding of the transcriptional repressor NUDR throughout development and into adulthood resulting in enhanced 5-HT_{1A} receptor expression in raphe neurons. Increased expression of the 5-HT_{1A} somatodendritic autoreceptors reduces serotonergic tone, a condition associated with MDD (Albert 2004). The deleterious effect of the polymorphism appears to be strongest in subjects who are homozygous for the G allele: Lemonde (2003) compared the frequency of the C and G alleles in 129 patients with MDD and 134 healthy controls and found that there was a twofold increase in frequency of the homozygous GG genotype in depressed patients compared to controls. The same study examined the frequencies of C and G allelles among

102 suicide completers compared to 116 controls and found that the GG genotype was four times as common among suicide completers. The G allele has also been linked to "neurotic" personality traits on the NEO test in normal subjects, primarily due to Neuroticism's Anxiety and Depression subscales. Neuroticism has been identified as one of the major risk factors for anxiety and depression (Angst 2003, Wilhelm 1999, Ormel 2001, Fanous 2002, Maier 1995).

Several studies have found that the presence of this polymorphism can affect treatment outcomes, especially in drugs that target the $5HT_{1A}$ receptors. In a study of 188 MDD patients, Lemonde (2004) found that patients with the GG genotype were twice as likely as patients with the CC genotype to be nonresponders to drug treatment with antidepressants. In their study, flibanserin (a $5HT_{1A}$ agonist) also resulted in less clinical improvement in GG patients than CC or CG patients. Findings such as these have not been entirely consistent, however. Serretti and colleagues (2004) assessed the severity of depressive symptoms in 151 patients with major depression and 111 bipolar patients before and following 6 weeks of treatment with the SSRI fluvoxamine and demonstrated that in bipolar disorder, but not in unipolar depression, patients homozygous for the C variant of the polymorphism showed a better response compared to carriers of the G allele. A retrospective study of 209 patients with MDD (Noro 2010) found no association between a clinical response to antidepressants and the C1019G polymorphism, though the authors note that their statistical power, which varied from 0.07 to 0.18, was too limited to rule one out.

These findings suggest that in normal subjects the G allele acts as a genetic risk factor for depression. The C(-1019)G SNP seems to play a key role in the development of anxious and depressive traits as well as anxiety disorders, depression, psychosis and substance abuse (Lesch 2004) and may affect treatment outcomes in psychiatric patients.

Effects of 5-HT$_{1A}$ agonists and antagonists on REM sleep. REM sleep is inhibited by the serotonergic neurons originating in the dorsal raphe nucleus (DRN) which project into the cholinergic cells of the pontine nuclei (Jun 2006). REM sleep can be increased via microdialysis perfusion of 5-HT$_{1A}$ agonists into the DRN: the 5-HT$_{1A}$ receptors in the DRN are autoreceptors and activating them causes a decrease in 5-HT activity. This reduces the 5-HT neurotransmission in the DRN's projection areas, the laterodorsal and the pedunculopontine tegmental nuclei (the LDT and PPT), which control REM sleep. While direct application of 5-HT$_{1A}$ agonists to the DRN increase REM, however, direct perfusion of 5-HT$_{1A}$ agonists in the LDT / PPT will reduce REM sleep, which is consistent with findings that 5-HT$_{1A}$ receptors in these areas are post-synaptic and inhibitory (McCarley 1995).

An early study with an oral 5-HT$_{1A}$ agonist (ipsapirone) showed strongly suppressed total REM sleep time, an increased RL and a reduction in RD in both normal controls and in depressed patients (Seifritz 1997). The ipsapirone was presumed to be acting most strongly at the post-synaptic 5-HT$_{1A}$ receptors in the LDT / PPT. Because this effect was seen in both normal and depressed patients, the researchers concluded that the post-synaptic receptors in the LDT / PPT were not the cause of the elevated RD and lower RL in the depressed patients (the genetic alleles of the subjects in this study were, however, unknown).

Fesinoxan is a more selective 5-HT$_{1A}$ receptor agonist. When injected into the DRN it causes an increase in REM sleep and RD and a lower RL by activating the inhibitory autoreceptors. This causes the DRN to downregulate the REM-inhibiting 5-HT signal that it is sending to the LDT/PPT (Portas 2008). When pindolol, a 5-HT$_{1A}$ antagonist, is injected into the DRN the opposite occurs: there is a dose-related suppression of REM sleep, higher RL and decreased RD (Seifritz 1997). Pindolol is an antagonist of both 5-HT$_{1A}$ and 5-HT$_{1B}$

receptors (as well as a mixed beta-1 and beta-2 adrenoceptor agonist). Pindolol is believed to inhibit REM via antagonistic action at the 5-HT_{1A} autoreceptors in the DRN, thus upregulating the 5-HT signal being sent to the LDT/PPT.

When the 5-HT_{1A} agonists buspirone or eptapirone are given orally, REM sleep is suppressed (Wilson 2005). This finding supports the hypothesis that the post-synaptic 5-HT_{1A} receptors in the LDT/ PPT are being activated and suppressing REM sleep. Both drugs also increased sleep fragmentation. Although eptapirone had the strongest REM-suppressing effects, buspirone appears to be the oral drug with the fewest side effects in humans. Buspirone is primarily an anxiolytic though it can also be prescribed to depressed patients, usually in conjunction with another antidepressant to increase the other drug's effect (Loane 2012). It has a strong affinity for the post-synaptic $5HT_{1A}$ receptors in the LDT/PPT where it acts as a partial agonist: it is currently suspected that buspirone binds to the $5HT_{1A}$ receptors and dislocates inhibitory G-proteins. There is some evidence that buspirone has a low affinity for the dopamine D_2 autoreceptor as an antagonist (McMillen 1983) as well as a weak affinity for $5HT_2$ receptors (Pecknold 1994), but the main neuropharmalogical effects are believed to be mediated via the $5HT_{1A}$ receptors. Because it reliably suppresses REM and is well tolerated in human subjects, we chose buspirone as the serotonergic probe for the present study. Buspirone's suggested therapeutic dose is 20–30 mg in 2 to 3 divided doses, mainly to avoid daytime sedation. Starting dose is suggested at 5 mg three times per day (CPHA 2008). Since sedation is not an issue in a sleep study and since we needed to guarantee measureable effects throughout the night, a dose of 15 mg was selected.

Effect of cholinergic agonists and antagonists on REM sleep. Serotonin is not the only neurotransmitter that directly affects REM sleep control systems. Acetylcholinergic agonists and antagonists also play a role in REM sleep regulation. An experiment using three

muscarinic antagonists in rats found that scopolamine, trihexyphenidyl and biperiden all decreased the amount of REM sleep and RD (Zoltoski 1993). Scopolamine and trihexyphenidyl are mixed M1 and M2 type muscarinic antagonists. These also affected the amount of slow wave sleep (SWS) in rats. Biperiden, a selective M1 muscarinic antagonist, only affected REM sleep and RD. A follow-up study in humans found that biperiden, given orally, had similar effects: REM sleep was decreased and RL was increased (Salin-Pascual 1993). RD was not strongly affected by the biperiden in humans, however.

A third study by the same group found that M2 agonists increase RD significantly when injected directly into the LDT/PPT (Velazquez-Moctezuma 1989). The conclusion drawn from the three experiments was a model for the of the REM control system: Phasic REM events (RD) as well as the associated EEG spikes in the pontine-geniculate-occipital areas (PGO waves) are promoted by cholinergic projections from the parabrachial regions of the pons (LDT/PPT). REM sleep is tonically suppressed (tonic REM is defined by EEG changes and lack of muscle tone, its measures include REM time and RL, whereas phasic REM refers to bursts of eye movements and is commonly measured by RD) by the noradrenergic and serotonergic neurons in the DRN and locus coeruleus by way of their inhibition of the LDT/PPT. The LDT/PPT also has internal cholinergic trigger or "burst" activity, mediated by a cholinergic M2 receptor. Therefore, the timing of sleep is controlled separately from the bursts of phasic activity in REM: tonic sleep is hastened by M1 agonists and delayed by M1 antagonists, while phasic RD is increased by M2 agonists and reduced by M2 antagonists. REM induction using other cholinergic agonists supports this model. The agonists arecoline, RS-86, and pilocarpine were all able to produce the expected phenomena in patients with mild depression and in normal controls (Lauriello 1993).

Another study found an inverse correlation between phasic activity and the cerebrospinal fluid levels of a serotonin metabolite, 5-HIAA, in psychiatric patients (Benson 1983). This observation combined with the cholinergic REM induction data suggests that there may be an imbalance between cholinergic and serotonergic REM controls systems in psychiatric patients (Seifritz 1998).

For our cholinergic probe we chose a slightly more modern drug than those listed above. Galantamine, commonly used for the treatment of mild to moderate dementia in Alzheimer's disease, is a short-acting anti-acetylcholinesterase that enhances central cholinergic activity (Jiang 2013). Acetlycholine is an agonist at muscarinic receptors (M1 and M2), as well as an allosteric modulator of nicotinic receptors (Jiang 2013, Riemann 1994). Animal studies have found that tonic REM is primarily mediated by muscarinic receptors, while phasic pontine-geniculate occipital (PGO) waves associated with REMs are mediated by nicotinic receptors (Gillin 1993). Previously, Riemann et al. (1994) found that galantamine, taken before sleeping, shortens RL, increases RD and suppresses SWS in healthy participants; and recommended its use for future pharmacological challenge studies. Galantamine has in the past been sold commercially in both immediate-release and slow-release forms. The geriatric starting dose is either 8 or 16 mg for slow-release galantamine (CPHA 2008). Since our participants were all healthy young adults, the slow-release formula was selected in order that its effects on REM sleep would still be occurring in the latter part of the night and the 16mg dose was chosen to maximize the potential response (Riemann 1994).

Rationale

Disturbed 5-HT neurotransmission is considered to be a key physiological component of MDD. The G(-1019) allele in the 5-HT_{1A} gene's promoter region has been shown to play

a significant role in 5-HT_{1A} function, potentially acting as a marker for higher risk of depression and psychopathology. Recent longitudinal studies have also found that abnormal REM sleep measures such as elevated RD and lower RL can predict the onset and course of depression in adolescents and adults (Modell 2005). Most research in this area uses cross sectional samples, however, and not all patients with mood disorders display these REM sleep abnormalities. One reason for the variance in results could be that most sleep studies measure the crude RD and RL without controlling for the genotype of the participants. Few studies have probed the system with both cholinergic and serotonergic agents.

This study is comprised of two main analyses. In the first analysis, presented in the paper titled "The effects of galantamine and buspirone on sleep structure: Implications for understanding sleep abnormalities in major depression," we examine the effects of a 5-HT_{1A} agonist and a cholinergic agonist on sleep. Using a four-night experimental model we look at the effects of each drug separately as well as the combined effects of the two drugs compared to a baseline night. We hypothesized that the addition of a 5-HT_{1A} agonist would up-regulate post-synaptic 5-HT levels and lead to tonic suppression of REM sleep throughout the night. We also hypothesized that the cholingeric system would be the primary driver of phasic REM activity and also promote REM tonically. Consequently, administering our cholinergic agonist should have lead to an increase in phasic REM events during the night as well as lowering RL and increasing the amount of time spent in REM.

In the second analysis, described in the paper titled "5-HT_{1A} receptor genotypes and REM sleep sensitivity to serotonergic/cholinergic imbalance in humans: A pharmacological model of depression," we divide the subjects into groups based on their C(-1019) or G(-1019) alleles to observe whether this mutation has any detectable effect on baseline sleep measures as well as whether this genotype affects the functioning of the cholinergic and

serotonergic agonists. Our hypothesis was that the serotonergic drug would be less effective in the participants with the mutant version of the receptor to which the drug is intended to bind, leading to a stronger suppression of tonic REM in wild-type participants. We also hypothesized that participants with defective serotonergic systems (the GG mutants) were going to be overwhelmed by the cholinergic effect, leading to a stronger boost in phasic activity from the cholinergic agonist.

The effects of galantamine and buspirone on sleep structure: Implications for understanding sleep abnormalities in major depression

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Abstract

Rationale

The serotonergic and cholinergic systems are jointly involved in regulating sleep but this balance is theorized to be disturbed in depressed individuals.

Objective

The goal of this study was to use biological probes in healthy participants, to model the serotonergic/cholinergic imbalance of depression and its associated abnormalities in sleep structure.

Methods

We tested 20 healthy female participants 18–30 years of age on four non-consecutive nights. Participants were given galantamine (a cholinergic agent), buspirone (a serotonergic agonist), both drugs together, or placebo before sleeping.

Results

Buspirone suppressed tonic REM: there was a significant increase in REM latency ($p < 0.001$). Galantamine increased tonic REM sleep, leading to more time spent in REM ($p < 0.001$) and shorter REM latency ($p < 0.01$). Galantamine and buspirone given together were not significantly different from the placebo night by REM sleep measures, but disrupted sleep more than either drug alone.

Conclusions

These findings are partially consistent with the cholinergic literature about sleep in depression, notably short REM latency, higher percentage of total sleep time spent in REM, and increased sleep fragmentation. The prolonged REM latency and reduced percentage of REM with buspirone resembled the effect of selective serotonin reuptake inhibitor antidepressants on REM sleep.

Keywords: Sleep, Depression, Sleep Structure, REM, Serotonin, Acetylcholine, Buspirone, Galantamine

Introduction

One of the most commonly reported symptoms of depression is disturbed sleep, with up to 90% of patients suffering from depression reporting symptoms of insomnia or hypersomnia (Riemann et al. 2001). Modern polysomnographic sleep research has repeatedly found altered sleep structure associated with depression such as decreases in slow wave sleep (SWS) and increases in the duration and intensity of rapid eye movement (REM) sleep (Palagini 2013). Recent longitudinal studies have found that abnormal REM sleep measures such as elevated REM density (RD, the number of rapid eye movements (rems) per minute of REM sleep) and shorter REM latency (RL, the amount of time it takes to enter REM sleep after sleep onset) can predict the onset and course of depression in adolescents and adults (Modell et al. 2005; Augustinavicius et al. 2014). It is possible that the altered sleep structure is not necessarily caused by depressive episodes but may reflect an underlying neuropathology; the neural mechanisms governing sleep regulation are inextricably linked with those regulating cognitive and affective systems (Riemann et al. 2001).

The cholinergic-aminergic imbalance hypothesis (Janowsky et al., 1972; Jouvet, 1972) is one framework for understanding the links between sleep and depression. The serotonergic and cholinergic systems are believed to be normally in balance, jointly controlling REM sleep. This balance is disturbed in depressed individuals, leading to abnormalities in REM sleep (Foster et al. 1976; Buysse et al. 1990; Benca 1996).

Serotonin (5-HT) has been heavily implicated in the pathophysiology of depression and its effects on REM sleep are also well known. REM sleep is inhibited by the

serotonergic neurons originating in the dorsal raphe nucleus (DRN) which projects into the cholinergic cells of the pontine nuclei (Jun et al., 2006) where the REMs are generated. Here, 5-HT acts primarily at the 5-HT_{1A} receptor to suppress REM sleep and reduce REM% (Palagini, 2013).

The cholinergic system has also been shown to be involved in the regulation of REM sleep. Cholinomimetics have been found to shorten RL and increase RD, an effect that has been found to be stronger in healthy relatives of depressed patients than in controls, implying that sensitivity to cholinergic stimulus could be a trait marker for depression (Rieman et al, 1994; Sitaram et al. 1982).

Most research in this area uses cross-sectional samples, however, and not all patients with mood disorders display these REM sleep abnormalities. Few studies have simultaneously probed the system with both cholinergic and serotonergic agents, especially in a repeated measures design with the same subjects.

The goal of this study was to use biological probes in normal individuals to gain a clearer picture of the link between a serotonergic/cholinergic imbalance and its associated abnormalities in sleep structure. We used the 5-HT_{1A} agonist buspirone to suppress REM sleep and a drug that increases acetylcholine, galantamine, to increase REM sleep in a crossed design, with a placebo control night.

Buspirone is a 5-HT_{1A} agonist most commonly used to either to treat anxiety symptoms or as an antidepressant, sometimes given in conjunction with other antidepressants to augment their effects (Altamura et al., 2013; Robinson et al. 1990). When it is administered orally it suppresses REM sleep, meaning that it must act more strongly at the post-synaptic receptors in the laterodorsal tegmentum and the pedunculopontine (LDT/PPT) region than at the autoreceptors in the DRN, which would otherwise reduce 5-HT

transmission (Wilson et al. 2005). This finding supports the hypothesis that the post-synaptic 5-HT_{1A} inhibitory receptors in the LDT/ PPT are being activated, thereby suppressing REM sleep. Buspirone also increased sleep fragmentation.

For our cholinergic probe we used Galantamine, commonly prescribed for the treatment of mild-to-moderate dementia in Alzheimer's disease. Galantamine is a short-acting anti-acetylcholinesterase that enhances central cholinergic activity. Acetlycholine is an agonist at muscarinic receptors (M1 and M2), as well as an allosteric modulator of nicotinic receptors (Jiang et al., 2013; Riemann et al., 1994). Animal studies have found that tonic REM (electroencephalogram (EEG) changes and atonia) is primarily mediated by muscarinic receptors, while phasic pontine-geniculateoccipital (PGO) waves associated with REMs are mediated by nicotinic receptors (Gillin et al., 1993). Previously, Riemann et al. (1994) found that galantamine, taken before sleeping, shortens RL, increases RD and suppresses SWS in healthy participants; and recommended its use for future pharmacological challenge studies. Galantamine is now available in a long-acting form ideal for sleep research and was used as our cholinergic probe in this study.

Our hypothesis was that our cholinergic probe would alter sleep in a way that mimics sleep patterns commonly found in major depressive disorder (MDD) while our serotonergic probe would cause the opposite effects, similar to the effects of SSRIs on sleep. We also hypothesized that giving both probes together might cause them to mitigate each other's effects.

Predictions

1. The 5-HT_{1A} agonist buspirone would suppress REM sleep leading to a reduced REM% in the night and longer RL.

2. The anti-cholinesterase galantamine would increase RD and shorten RL.

3. There would be an interactive effect in that galantamine and buspirone given together may mitigate each other's effects on tonic REM sleep (RL and REM%) but the REM periods would still have an elevated RD from the galantamine.

4. Both drugs would increase sleep fragmentation, leading to lower sleep efficiency (SE) and a larger amount of wake after sleep onset (WASO).

Research Protocol and Experimental Design

We recruited 22 female participants age 18–27, through the University of Ottawa's online recruitment system, the Integrated System for Participation in Research (ISPR). Participants were chosen from the same gender and age group, in order to reduce variance in the dependent variables. Participants were asked to fill out three questionnaires: Sleep Disorders Questionnaire (Douglass et al., 1994), Beck Depression Inventory 2 (BDI-2) (Beck et al.,1996) and Epworth Sleepiness Scale (Johns, 1991). Participants were also asked about their general health and their immediate family's psychiatric history, to determine eligibility. Exclusion criteria included a family history of major mental illness, a score above 12 on the BDI-2 (Kjaergaard et al., 2014), use of psychotropic drugs, significant health problems, and pregnancy or lactation.

Eligible participants were screened for psychiatric illness by the investigator, using the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, (DSM-IV) Structured Clinical Interview for DSM-IV, Non-Patient version (SCID-NP) (APA, 2000). They were assessed by a physician prior to their participation in the procedure, via physical examination, measurement of blood pressure and pulse rate in the sitting position, and

clinical laboratory tests (lactate dehydrogenase (LDH), Aspartate Aminotransferase (SGOT), bilirubin, serum creatinine, thyroid free triiodothyronine (FT3), thyroid-stimulating hormone (TSH), urinalysis and a complete blood count (CBC) including platelets). Participants with normal results on these measures underwent a screening nocturnal polysomnogram (NPSG), which ruled out sleep disorders such as sleep apnea. Because this study also included 8 hours of electrocardiogram (EKG) on two channels, it also served to exclude any participants with cardiac arrhythmias or other EKG abnormalities. The screening NPSG also served as an acclimatization to the sleep laboratory, to eliminate the 'first-night effect' from the subsequent drug trial nights (Israel et al., 2012).

Participants were recorded for four non-consecutive study nights in the sleep laboratory. Brief (non-validated) laboratory questionnaires were given, to assess sleepiness and perceived sleep quality at bedtime and in the morning.

On each of the study nights, participants received 2 double-blind test medications in identical coloured capsules; galantamine 16 mg (slow release), buspirone 15 mg, or placebo in a randomized double-blind design. The order of the treatments was randomized with a latin square design, to avoid order effects of the treatments.

Galantamine has in the past been sold commercially in both immediate-release and slow-release forms. As of 2008, the manufacturer removed the immediate-release forms from the market due to the possibility of gastrointestinal (GI) side-effects in elderly patients; accordingly, the geriatric starting dose is now either 8 or 16 mg slow-release galantamine (CPHA 2008). Because our participants were all healthy young adults, the 16 mg slow-release dose was selected in order that its effects on REM sleep would still be occurring in the latter part of the night and the 16-mg dose was chosen to maximize the potential response (Riemann et al.,1994). Buspirone's suggested therapeutic dose is 20–30 mg in 2 to 3 divided

doses, mainly to avoid daytime sedation. Starting dose is suggested at 5 mg three times per day to avoid sedation (CPHA 2008). Since sedation is not an issue in a sleep study and since we needed to guarantee measureable effects throughout the night a dose of 15 mg at bedtime was selected.

The half-lives of the drugs (galantamine at 8 hours and buspirone at 2 to 10 hours) require a 48 hour wash-out period after each night in the sleep lab in order to allow 5 half-lives to elapse before the next test drug (CPHA 2008). Therefore the subjects slept for *at least* one night at home between each of the lab nights 2, 3, 4, and 5 (the median number of nights at home between study nights was seven; mean 11.2 (SD 12.4)). To further reduce variance between subjects, they were asked not to use any drugs, alcohol, or caffeine after noon for the duration of the study. Strenuous physical activity was not permitted within 4 hours of bedtime on sleep study nights to avoid possible induction of initial insomnia. The medications were taken orally in the laboratory 1 to 2 hours before bedtime and at least 2 hours after the last meal, for optimal timing and absorption. Participants chose their bedtime between 9 pm and midnight, according to when they usually fell asleep at home.

Sleep Methodology

Sleep was recorded by a 10-electrode EEG array (C3-A2, C4-A1, O1-A2, O2-A1, EOG, EKG lead II and EMG) and scored visually from the C3/A2 electrode trace using standard AASM (Iber et al 2007). Standard nocturnal polysomnography instrumentation was used on night 1 to screen out participants suffering from a clinical sleep disorder such as sleep apnea or periodic limb movement disorder. This equipment included a microphone to detect snoring, a motion sensor, finger pulse oximetry, respiration belts, pressure and

temperature airflow sensors on the upper lip, and electrodes on the anterior tibial areas for leg movements. None of our subjects were found to have a clinical sleep disorder.

On night 2 through night 5 only sensors for sleep scoring, EMG, EEG, and EOG electrodes were used. Sleep waveforms were recorded on the Somnologica® computerized sleep recording system (Embla Systems, Ottawa, ON, Canada) using digital amplification and filtering. Sleep staging on 30-second epochs was done visually by a single experienced researcher using the Stellate Harmonie® system, version 6.1 (Stellate Systems, Montreal, QC, Canada). Individual REMs were identified using Harmonie's proprietary software, which searches for inverse mV/sec slope rates in the two EOG channels, employs a noise reduction algorithm and requires that a total duration criteria be met for the REM to be counted. The algorithms for this eye movement detection are published (Agarwal and Gotman, 2001).

Statistical Analysis

All variables were assessed for normal distribution via normality plots and measures of skewedness and kurtosis using the SPSS statistical program. Variance stabilizing transformations (logit and natural log) were done if required. These data were appropriate for a repeated measures analysis of variance (ANOVA) with repeated measures on subjects. Since these measures are known to vary with age we first ran a MANCOVA with age as a covariate. Age was found to have no significant effect on the measures, likely due to the small age range of the participants. Participants' menstrual cycles were sufficiently randomized by the study design that they did not need to be considered in the analysis.

We then performed a multivariate repeated measures MANOVA on the following variables: RL, RD, the percentage of time asleep spent in REM (REM%), the percentage of

sleep time spent in stage N1 (N1%), which is a usually transitory stage often described as a light doze; the percentage of sleep time spent in N2 (N2%), which is fully but not deeply asleep; the percentage of sleep time spent in N3 (N3%), which is a deeper stage of sleep previously referred to as slow wave sleep; WASO; sleep onset latency (SOL); total sleep time (TST); percentage of time spent awake (Wake%); and the number of wakenings during the night. There were four levels of the within-group variable, "drug condition": baseline (placebo), buspirone, galantamine, and the combination of the two drugs. The MANOVA was significant, leading us to perform univariate ANOVAs on each measure. Pairwise comparisons of the drug nights versus placebo nights were made when the univariate ANOVA was significant. The significance level required for post-hoc comparisons was adjusted by the Bonferroni method.

Results

The overall repeated-measures MANOVA found a significant effect of drug condition on sleep measures ($F_{33,147} = 4.574$, $p<0.001$). The result of the univariate ANOVA performed on WASO is illustrated in Figure 1 and the univariate results for the rest of the individual sleep measures are shown in Table 1.

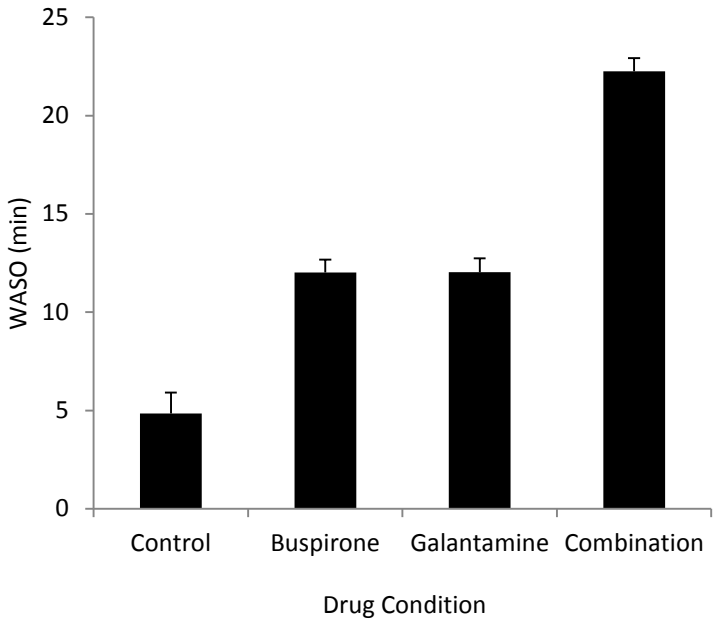

Figure 1. Wake after sleep onset (WASO) in minutes (error bars show standard deviation) for each drug condition. WASO was significantly higher in the combination night compared to the other three nights, and higher in both the buspirone and galantamine nights compared to control night.

	Control		Buspirone		Galantamine		Galantamine and Buspirone		ANOVA	
	mean	SE	**mean**	SE	**mean**	SE	**mean**	SE	*F* (3,57)	*p*
Sleep Efficiency (%)	**95.95**	16.34	**94.38**	15.61	**93.80**	15.30	**91.45***	16.57	4.21	0.009
Awakenings (number)	**2.76**	0.49	**4.17***	0.50	**4.62***	0.54	**4.52***	0.46	3.59	0.019
Total Sleep Time (min)	491.1	12.0	487.0	10.9	485.8	10.9	461.4	15.4	1.47	NS
Wake%	**0.68**	0.43	**1.13***	0.43	**1.08***	0.46	**1.67***	0.57	9.58	<0.001
N1 %	**0.67**	0.49	**1.25***	0.37	**1.42***	0.52	**1.61***	0.42	11.07	<0.001
N2 %	44.42	1.70	45.86	2.23	42.51	1.90	44.12	1.91	1.01	NS
N3 %	**31.80**	2.01	**31.18**	2.19	**23.84***	1.64	**26.02***	1.97	12.88	<0.001
REM%	**21.28**	1.06	**19.54**	1.50	**28.38***	1.23	**23.81**	1.39	11.05	<0.001
RL (min)	**92.20**	7.86	**143.58***	12.45	**68.38***	3.41	**102.48**	7.25	17.40	<0.001
RD (rems/min)	7.06	0.72	6.17	0.79	7.33	0.63	7.29	0.74	1.79	NS
SOL (min)	10.62	0.58	10.87	0.69	12.89	0.67	13.36	0.68	0.50	NS

Table 1 Results from the univariate ANOVA post-hoc analyses. The *asterisk* indicates a significant difference compared to the control night in pair-wise comparisons. ANOVA: analysis of variance; N1%, N2%, N3%: percentage of time asleep spent in Stage N1, N2 or N3; NS: not significant; RD: REM density; REM: rapid eye movement; REM%: percentage of time asleep spent in REM; RL: REM latency; SE: sleep efficiency, which is TST divided by time in bed; SOL: sleep onset latency; TST: total sleep time; Wake%: percentage of time in bed spent awake.

Buspirone

The 5-HT_{1A} agonist buspirone suppressed REM sleep: there was a significant increase in RL ($p < 0.001$) on the buspirone night compared to the control night. REM% and RD trended lower on the buspirone night but these differences were not significant. Buspirone also disrupted sleep, approximately doubling WASO, Wake%, N1% and the number of awakenings during the night.

Galantamine

The anti-cholinesterase galantamine did not significantly increase RD. It did increase tonic REM sleep, leading to a higher REM% ($p < 0.001$) and lower RL ($p < 0.01$) on galantamine nights compared to control. Galantamine also disrupted sleep, significantly increasing WASO, Wake%, N1% and the number of awakenings during the night as well as lowering N3%.

Buspirone and galantamine combination

Galantamine and buspirone given together negated the effects of each other on REM sleep measures: the drug combination nights were not significantly different in REM% or RL compared to the placebo night.

Galantamine and buspirone together increased sleep fragmentation and lowered sleep quality more than either did when taken alone: the combination drug night had a higher number of awakenings ($p < 0.05$), WASO ($p < 0.001$), N1% ($p < 0.001$), N3% ($p < 0.001$) as well as a significantly lower sleep efficiency ($p < 0.01$) compared to the control night. As shown in Figure 1 the amount of WASO was significantly higher in the combination night than in either of the single-drug nights.

Sleep disruption and dreaming

Two participants had to be removed from the analysis due to galantamine disrupting their sleep too much: one awoke after three hours of sleep with symptoms of nausea and vomiting on both galantamine nights and withdrew from the study; the second also awoke after approximately three hours of sleep on both of the galantamine nights and could not get back to sleep before morning. Of the remaining 20 participants 7 reported nausea in the morning after taking galantamine with buspirone and 3 reported nausea in the morning after galantamine alone. Responses on the morning questionnaire indicated that the subjective sleep perception accurately corresponded to their sleep recording, in all drug conditions.

Galantamine was also found to dramatically increase the number of dreams reported as well as increasing their unpleasantness. On the control night 20 subjects reported a total of 10 dreams, one of which was “a stressful disaster”. On the buspirone night there were 16 dreams, one of which was “scary”. On the galantamine night subjects reported 32 dreams, with 5 subjects reporting dreams that were “weird”, “scary”, or “nightmares.”

Galantamine also appeared to induce dreams of false awakenings with 5 participants reporting “inception-style” layered dreams or dreaming that they awoke in the lab and found something wrong (e.g., they were “sick and mute” or “afraid of the technician”). The combination night had a similar effect of increasing dreams and false awakenings with 42 dreams reported in total, 5 participants reporting dreams of false awakenings, and 6 reporting dreams that were unpleasant or nightmares.

Discussion

This study demonstrates that the effects of a serotonergic agonist and a cholinergic agent on sleep support the predictions of the cholinergic-aminergic imbalance hypothesis of depression.

Our serotonergic agonist, buspirone, suppressed REM sleep by increasing REM latency. Total REM% was not significantly lower but this may have been because buspirone was not available in a slow-release preparation and was wearing off in the second half of the night when the majority of REM sleep occurs. Previous studies using this drug found that it decreased the amount of time spent in REM sleep (Wilson et al., 2005). In animals, direct perfusion of 5-HT_{1A} agonists in the LDT / PPT reduced REM sleep, which is consistent with findings that 5-HT_{1A} receptors in these areas are post-synaptic and inhibitory (McCarley, Greene, Rainnie, & Portas, 1995). Our findings support the hypothesis that, in humans, it is

primarily the post-synaptic 5-HT_{1A} receptors in the LDT/ PPT that are being activated by buspirone and subsequently suppressing REM sleep.

Our cholinergic agent, galantamine, decreased N3%, increased REM% and decreased RL but had no significant effect on RD. To explain its different effects between tonic and phasic REM activity, it is helpful to examine animal models of REM sleep. A study by Velazquez-Moctezuma et al. (1989) found that M2 agonists increase RD significantly when injected directly into the LDT/PPT. Their model for the REM control system hypothesized that phasic REM events (RD) as well as the associated EEG spikes in the pontine-geniculate-occipital areas (PGO waves) are promoted by cholinergic projections from the parabrachial regions of the pons (LDT/ PPT). They postulated that REM sleep is tonically suppressed (lower total REM%, increased RL) by the noradrenergic and serotonergic neurons in the DRN and locus coeruleus by way of their inhibition of the LDT/PPT. The LDT/PPT also has internal cholinergic trigger or "burst" activity, mediated by a cholinergic M2 receptor. Therefore, the timing of REM sleep is controlled separately from the bursts of phasic activity in REM: tonic REM sleep is hastened by M1 agonists and delayed by M1 antagonists, while phasic RD is increased by M2 agonists and reduced by M2 antagonists.

This model explains our findings and implies that, while galantamine increases ACh, it acts mainly at the M1 receptors. Galantamine also has an alerting effect, due to its allosteric action on the nicotinic acetylcholine receptor alpha four subunit (CHRNA4), which could be the mechanism of its disruption of sleep (Jiang et al., 2013). Nicotinic receptors have also been linked to the generation of PGO waves in animals; and galantamine's nicotinic properties have been proposed as a mechanism for increased RD (Riemann et al., 1994). While we did not see effects on RD in contrast to Riemann's proposal that there is a

nicotinic effect via galantamine, we did find that there was an increase in alertness shown by a higher Wake%, more numerous awakenings, and less Stage N3 'deep' slow-wave sleep.

Our prediction regarding the interaction between the two drugs was validated. Galantamine and buspirone given together tended to cancel out each other's effects on REM sleep latency and REM% while their deleterious effects on sleep quality appeared to be cumulative: both drugs increased sleep fragmentation, leading to lower sleep efficiency and a higher percentage of time in bed spent awake. The higher cholinergic tone led to more stage REM sleep, similar to the finding that depressed patients have a higher percentage of total sleep time spent in REM. The way buspirone counteracted this effect was similar to how antidepressants act in depressed patients: by lowering the proportion of sleep spent in REM.

Sleep was fragmented by both drugs, something to be conscious of when prescribing these medications clinically. Galantamine, particularly, was found to disturb sleep although it is a drug that patients often take at bedtime to avoid the side effect of nausea. It is unclear from this study whether sleep disruption is a long-term side effect or if it would cease to be a problem after a patient had adapted to the drug. Nightmares and insomnia can be disturbing to patients as well as their caregivers. Being aware of these effects on sleep might encourage patients to compensate with other means of improving sleep quality or quantity.

The finding that a cholinergic agent caused nightmares and interrupted sleep is consistent with previous findings: galantamine and other cholinergic agonists can also increase the rate of dreaming, especially lucid dreams, as well as increasing the risk of sleep paralysis (Corbo et al., 2003; Rogers et al., 1998). A recent study with Alzheimer patients found that galantamine did not have a significant effect on insomnia and there was only a "mild" increase in reported nightmares (Stahl 2004). Our findings contradict the latter, possibly because our subjects were younger than the usual geriatric patient population and

may have had stronger adverse reactions. Another possible reason for the discrepancy in reported nightmares is that Stahl's numbers for dreams were taken from physicians' accounts whereas our participants were asked about sleep quality and dreams immediately upon waking; the Alzheimer's patients may have forgotten their unpleasant dreams or considered them not important enough to report, artificially lowering the numbers. In the future, similar research efforts might benefit from administering a validated dream questionnaire to their participants, to more thoroughly assess the effects of drugs on dreaming.

We found no change in crude REM density. While it is possible that a more subtle method of analysis that examines the patterns of discrete events, such as a Markov analysis (Douglass 1992, Boukadoum 1988), would find an effect of the buspirone or galantamine on the rapid eye movements in REM sleep, it is more probable that RD is controlled by some neurotransmitter system other than ACh under normal physiological conditions It has been suggested that dopamine (DA) affects the relationship between ACh and RD (Tandon et al., 1999). A repeated measures study that manipulated ACh and DA levels simultaneously could offer valuable insight on the effects of that interaction on sleep.

This research had several limitations. Our participants did not include those of male gender, older participants, nor patient populations; so our results cannot be generalized to these populations without further study. We were also unable to record the serum levels of galantamine and buspirone before each study night, to ensure there was complete washout of the drugs. Future studies may wish to include this as part of their procedure, for extra rigor.

Conclusion

This study provided a direct test of the aminergic-cholinergic imbalance model of depression in human participants, for the first time. Our findings demonstrate that the effects of buspirone and galantamine on sleep largely support the predictions of the cholinergic-aminergic imbalance hypothesis: Buspirone suppressed REM sleep, while galantamine increased it. These findings were consistent with the literature about the effects of depression on sleep structure, except for the absence of RD findings. With both drugs, sleep was fragmented, which is something to be conscious of when prescribing these medications.

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A pilot study of 5-HT$_{1A}$ receptor genotypes and REM sleep sensitivity to serotonergic/cholinergic imbalance in humans: A pharmacological model of depression

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Abstract

Rationale

The serotonergic and cholinergic systems are jointly involved in regulating sleep but this system is theorized to be disturbed in depressed individuals. We previously reported that cholinergic and serotonergic agents induce sleep changes partially consistent with monoamines models of sleep disturbances in depression. One potential cause of disturbed neurotransmission is genetic predisposition. The G(-1019) allele of the 5-HT_{1A} receptor promoter region predicts an increased risk for depression compared to the wild-type C(-1019) allele.

Objective

The goal of this study was to use pharmacological probes in normal controls to model the serotonergic/cholinergic imbalance of depression and its associated abnormalities in sleep structure while controlling for 5-HT_{1A} receptor genotype.

Methods

Seventeen healthy female participants homozygous for either C (n=11) or G (n=6) alleles, age 18-27 years were tested on four non-consecutive nights. Participants were given galantamine (an anti-acetylcholinesterase), buspirone (a serotonergic agonist), both drugs together, or placebos before sleeping.

Results

As reported in previously, buspirone significantly increased REM latency (RL; $p<0.001$), as well as awakenings, Wake%, and N1% ($p<0.019$). Galantamine increased awakenings, Wake%, N1%, and REM%, and decreased RL and N3% ($p<0.019$). Galantamine plus buspirone given together disrupted sleep more than either drug alone, lowering SE and N3%

and increasing awakenings, Wake% and N1% ($p<0.019$). There was no main effect of genotype, nor was there a significant multivariate interaction between genotype and drug condition.

Conclusions

These findings are partially consistent with the literature about sleep in depression, notably short REM latency, higher percentage of total sleep time spent in REM, lower N3%, and increased sleep fragmentation. The C/G mutation in the 5-HT_{1A} receptor promoter region does not appear to cause noticeable differences in the sleep patterns of a relatively small sample of healthy young females. Future studies with larger sample sizes are required.

Keywords: Sleep, Depression, Sleep Structure, REM, Serotonin, Acetylcholine, Buspirone, Galantamine, Neurotransmission, Serotinon 1A receptor, genetic risk factors.

Introduction

While it has long been known that genetics have some effect on risk of mood disorders, recent genetic research has begun to pinpoint specific genes implicated in major depression. One such genetic risk factor is the allelic variation of the 5-HT_{1A} receptor (Stockmeier et al., 1998). The G(-1019) allele of the 5-HT_{1A} receptor predicts an increased risk for depression compared to the wild-type C(-1019) allele. This effect appears to be even stronger for persons homozygous for the "G" allele, while homozygosity for the "C" allele appears to confer some protection against depression. Pharmacological probes could be used to gain a clearer picture of the link between a serotonergic/cholinergic imbalance and abnormalities in sleep structure (Janowsky 1972, Jouvet 1972) in individuals with different 5-HT_{1A} receptor genotypes.

The gene that codes for the 5-HT_{1A} receptor is found on chromosome 5q11.2-113 (Fargin et al., 1988). There are several low frequency single nucleotide polymorphisms (SNPs) that have been found on this gene but the more common C(-1019)G SNP has been associated with anxious and depressive traits as well as anxiety disorders, depression, psychosis, substance abuse, and suicidality (Nakhail 1995; Erdmann 1995; Kawanishi 1998; Lesch 2004). The C(-1019)G SNP is in an upstream, regulatory region of the gene and modulates 5-HT_{1A} transcription: while the C allele correctly binds to repressing regulatory factors, the G allele insufficiently represses transcription of the 5-HT_{1A} receptor, some of which are found in post-synaptic sites while others are autoreceptors on serotonin neurons. An excess of autoreceptors would be expected to lead to overall reduced serotonergic neurotransmission (Lemonde 2003; Koller 2006).

Serotonin (5-HT) has been heavily implicated in the pathophysiology of depression and its effects on REM sleep are also well known. REM sleep is inhibited by the

serotonergic neurons originating from the dorsal raphe nucleus (DRN) which project to the cholinergic cells of the pontine nuclei (Jun 2006) where the rapid eye movements (rems) are generated. Here, 5-HT acts primarily at the 5-HT_{1A} receptor to suppress REM sleep and reduce REM% (Palagini 2013).

Depression has also been linked to cholinergic hypersensitivity (Dilsaver 1986). Importantly, the cholinergic system is involved in the regulation of REM sleep (Riemann 1994). Cholinomimetics have been found to shorten REM latency (RL: the amount of time it takes to enter REM sleep after sleep onset) and increase rem density (RD: the number of rapid eye movements (rems) per minute of REM sleep), effects that have been found to be stronger in depressed patients than in controls (Riemann (2), 1994). Consequently, the sensitivity of REM sleep to cholinergic stimulus could be a trait marker for depression.

Using pharmacological probes in healthy adults, we recently reported that a serotonergic agonist increases REM latency, while a promoter of cholinergic activity shortens REM latency and increases REM sleep duration (Biard 2015). These changes in REM sleep are well-aligned with the potential role of monoamine deregulation in the emergence of sleep disturbances in depression. Recent longitudinal studies have found that abnormal REM sleep measures such as elevated RD and shorter RL can predict the onset and course of depression in adolescents and adults (Modell et al. 2005; Augustinavicius et al. 2014). It is possible that these REM alterations are not necessarily caused by depressive episodes but may reflect an underlying neuropathology; the neural mechanisms governing sleep regulation are closely linked with those regulating cognitive and affective systems (Riemann et al. 2001). However, not all patients with mood disorders display these REM sleep abnormalities. It may be postulated that specific genetic variations such as the C(-

1019)G SNP in the case of the 5-HT_{1A} receptor could modulate the association between REM alterations and the risk for depression.

The main objective of the present study was to determine whether 5-HT_{1A} genotype may affect REM sleep sensitivity to cholinergic and serotonergic manipulations. We aimed to gain some insight into some of the possible genotypic and sleep-related pathophysiological mechanisms which could be linked to the risk for depression. We used a 5-HT_{1A} agonist (buspirone) to suppress REM sleep and a drug that increases acetylcholine (ACh) (galantamine) to increase REM sleep.

Buspirone is a 5-HT_{1A} agonist most commonly used either as an anxiolytic or an antidepressant, and sometimes given in conjunction with other antidepressants to augment their effects (Altamura 2013, Robinson 1990). When it is administered orally, it suppresses REM sleep, suggesting that it acts more strongly at the post-synaptic 5-HT1A inhibitory receptors in the laterodorsal tegmental and pedunculopontine nuclei (LDT/PPT) than at the autoreceptors in the DRN (which would be expected to reduce 5HT transmission and increase REM)(Wilson 2005). Buspirone also increases sleep fragmentation. Because it reliably suppresses REM and is well tolerated in human subjects, buspirone was chosen as the serotonergic drug for this study.

For our cholinergic drug, we used galantamine, commonly prescribed for the management of mild to moderate dementia in Alzheimer's disease. Galantamine is a short-acting anti-acetylcholinesterase that enhances central cholinergic activity. Acetylcholine is an agonist at muscarinic receptors (M1 and M2) whereas galantamine is also an allosteric modulator of nicotinic receptors (Riemann 1994, Jiang 2013). Animal studies have found that tonic REM (EEG changes and atonia) is primarily mediated by muscarinic receptors while phasic ponto-geniculo-occipital waves (associated with rapid eye movements) are

mediated by nicotinic receptors (Gillin 1993). Previously, Riemann (1994) found that galantamine taken before sleeping shortened RL, increased RD, and suppressed SWS in healthy participants, and recommended its use for future pharmacological challenge studies. Galantamine is now available in a long-acting form that is ideal for sleep research and was used as the cholinergic probe in this study.

To model the genetic predisposition for depression, we focused on healthy subjects with two genotypes: the homozygous "protective" CC alleles and the homozygous "at risk" GG alleles. Our hypothesis was that the GG participants would be less affected by the serotonergic drug due to impaired 5-HT neurotransmission and more strongly affected by the cholinergic drug. More specifically, we predicted that:

1. Buspirone would have a stronger effect on REM sleep in the CC group than in the GG group due to better serotonergic in the CC group.

2. There would be an interaction effect of the two genotype groups with galantamine, in that this drug would increase RD more prominently in the GG group than in the CC group, illustrating reduced serotonergic tone at the LDT / PPT nuclei (the generator of bursts of rems) in the GG group.

3. There would be an interaction effect of the two genotype groups with combined galantamine and buspirone. In the combined drug condition, the GG group would have a higher RD, shorter RL and an increased REM percentage compared to the CC group, illustrating their normal sensitivity to cholinergic drive but reduced serotonergic tone despite the administration of 5-HT_{1A} agonist. This is the direct test of the serotonergic/ cholinergic balance theory of depression: subjects with weaker serotonergic systems were expected to be more affected by the cholinergic challenge.

Methods

Participants

Seventeen female participants aged 18-27 years were recruited through the University of Ottawa's online recruitment system, the Integrated System for Participation in Research (ISPR), and through notices in local classified advertisements. Participants were asked to fill out the Sleep Disorders Questionnaire (Douglass et al. 1994) as a pre-screening measure for sleep disorders, as well as the Beck Depression Inventory 2 (BDI-2; Beck et al. 1996). Participants were also asked about their general health and their immediate family's psychiatric history to determine eligibility. A family history of major mental illness was an exclusion criterion. Subjects with scores above 12 on the BDI-2 were excluded (Kjaergaard et al. 2014), as were subjects currently using psychotropic drugs, subjects with significant health problems, and pregnant or lactating subjects. The 5-HT_{1A} G(-1019)C polymorphism genotype was determined using Oragene-DNA (OG-250) saliva sample collection (Nunes, 2012) and only homozygous participants were included: 11 "CC" and 6 "GG" participants. The two groups did not significantly differ by t-test in age or BDI scores: the CC participants had a mean age of 20.5 years (SD 2.4) and a BDI-2 of 5.4 (SD 3.5), while the GG group had a mean age of 19.5 years (SD 2.3) and a BDI-2 of 2.5 (SD 3.3).

Eligible subjects were screened for psychiatric illness using the Structured Clinical Interview for DSM-IV, Non-Patient version (SCID-NP) (APA, 2000). They were assessed by a physician prior to their participation via physical examination, measurement of blood pressure and pulse rate in the sitting position, and clinical laboratory tests (LDH, SGOT, bilirubin, serum creatinine, thyroid FT3, TSH, urinalysis, and CBC including platelets). Subjects with normal results on these measures underwent a screening nocturnal

polysomnogram (NPSG) which ruled out sleep disorders such as sleep apnea or periodic limb movement disorder. Sleep electroencephalography (EEG) was recorded by a 10-electrode array (C3/C4, O1/O2, A1/A2, EOG, EKG (lead II)), as well as a microphone to detect snoring, a motion sensor, finger pulse oximetry, respiration belts, pressure and temperature airflow sensors on the upper lip, and electrodes on the anterior tibial areas for leg movements. This NPSG included 8 hours of EKG on 2 channels so it also served to exclude any subjects with cardiac arrhythmias or other EKG abnormalities.

The screening NPSG also served as an acclimatization to the sleep laboratory to eliminate the "first-night effect" from the subsequent drug trial nights. Subjects were asked not to use any drugs, alcohol, or coffee for the duration of the study and to avoid strenuous physical activity after 16:00h on sleep study nights.

Procedure

Subjects were recorded for four non-consecutive study nights in the sleep laboratory (nights ranged from 48 hours apart to several weeks, depending on the participant's availability). On each of the study nights, participants received two identical capsules at bedtime in a counter-balanced randomized double-blind design: i.) one 16 mg capsule of galantamine (slow release) and one placebo (sugar pill); ii.) one 15 mg capsule of buspirone and one placebo capsule; iii.) one capsule of galantamine plus one of buspirone; or iv.) two capsules of the placebo. The order of the drug conditions was randomized with a latin square design to avoid order effects of the drug conditions. The capsules were taken orally 1 to 2 hours before bedtime and at least 2 hours after the last meal, for optimal timing and absorption.

Galantamine has in the past been sold commercially in both immediate-release and slow-release forms. The geriatric starting dose is either 8 or 16 mg for slow-release

galantamine (CPHA 2008). Since our participants were all healthy young adults, the slow-release formula was selected in order that its effects on REM sleep would still be occurring in the latter part of the night and the 16mg dose was chosen to maximize the potential response (Riemann 1994).

Buspirone's suggested therapeutic dose is 20–30 mg in 2 to 3 divided doses, mainly to avoid daytime sedation. Starting dose is suggested at 5 mg t.i.d. to avoid sedation (CPHA 2008). Since sedation is not an issue in a sleep study and since we needed to guarantee measureable effects throughout the night, a dose of 15 mg was selected.

The half-lives of the drugs (galantamine = 8 hr, buspirone = 2-10 hr with a mean of 2.8 hr) required a 48 hour wash-out period after each night in the sleep lab in order to allow 5 half-lives to elapse before the next test drug (CPHA 2008). Therefore the participants slept for *at least* one night at home between each of the lab nights 2, 3, 4, and 5 (median number of nights at home between study nights was 7, mean 11.2, S.D. 12.4). Plasma concentrations of the drugs were not measured but the half-lives of the drugs are sufficiently long that the effects would persist until the participants woke up. Both drugs have been used in previous sleep studies and have demonstrated acute, one-time effects on sleep measures.

Participants were self-reported good sleepers with regular sleep schedules. To avoid circadian misalignment, they chose their bedtimes between 9 pm and midnight according to when they usually fell asleep at home and awoke between 6 and 8 am according to their preferences and routines.

Sleep Methodology

On nights 2 through 5, EEG, EMG, EKG, and EOG electrodes were used, with an acquisition rate of 200 Hz. Sleep was recorded on the Somnologica® computerized sleep recording system (Embla Systems, Ottawa, ON) using digital amplification and AASM

recommended filters (EEG and EOG: low 0.3Hz, high 35Hz; ECG: low 0.3Hz, high 70Hz; EMG: low 10Hz, high 100Hz). Sleep stages were scored visually on 30 s epochs from the C3/A2 and O2/A1 electrode traces using standard AASM criteria (Iber 2007) with the Stellate Harmonie® system, version 6.1 (Stellate Systems, Montreal, QC). Harmonie uses proprietary algorithms to identify individual rems (Agarwal 2001).

The analyzed sleep variables were: sleep onset latency (SOL), RL, RD, total sleep time (TST), percentage of time spent awake (Wake%), percentage of time asleep spent in each sleep stage (N1%, N2%, N3%, REM%), number of awakenings during the night (Awakenings), time spent awake after sleep onset (WASO), and sleep efficiency (SE).

Ethics approval was granted by the Research Ethics Board of the Royal Ottawa Mental Health Center; experimental administration of the drugs was approved by Health Canada. The study ran from June 2010 to January 2014. Polysomnographic data for the entire sample of participants (irrespective of genotype) was previously published in a report on the effects of galantamine and buspirone on sleep (Biard 2015).

Statistical Analysis

All variables were assessed for normal distribution via normality plots and measures of kurtosis and skew using the SPSS statistical program (IBM, Armonk, New York, U.S.A.). Variance stabilizing transformations were done if the plot showed significant deviation from a normal distribution (logit and natural log transforms were used) and if the z-scores of the skew or kurtosis were higher than 1.96. The non-normal variables were: SOL, Wake%, N1%, awakenings, and SE. These variables were normalized with a natural log transform except for SE, which required a logit transform.

These data were submitted to a multivariate mixed design repeated-measures analysis of variance (MANOVA), with independent genotype groups and repeated measures over

drug conditions. A second analysis was done using night instead of drug condition to check for order effects.

Results

The main effects of drug conditions across the entire sample of participants (i.e. irrespective of genotype) are published elsewhere (Biard 2015). Averages and univariate results taking in account genotypes are presented in table 1. In brief, consistent with previous analyses, the mixed design repeated-measures MANOVA (with repeated measures across drug nights and between measures of genotype groups) revealed a significant effect of drug condition on sleep ($F = 3.575$, $p<0.001$). Compared to placebo, buspirone increased awakenings, Wake%, N1%, and RL (all $p<0.019$), while galantamine increased awakenings Wake%, N1% and REM%, and decreased N3% and RL (all $p<0.019$). Compared to placebo, the combination galantamine and buspirone lowered SE and N3%, and increased awakenings, wake% and N1% (all $p<0.019$). No order effects were found.

The mixed design MANOVA showed no significant main effect of genotype on sleep measures ($F=0.456$, $p=0.869$). There was no significant interaction between drug condition and genotype at the multivariate level ($F=1.053$, $p= 0.409$). At the univariate level, repeated measure ANOVAs indicated a significant interaction between drug and genotype on N2% ($p<0.01$) and Wake% ($p<0.05$) as shown in Figure 1 and Figure 2. N2% was significantly higher in the CC wildtype group compared to the GG group on both the buspirone night and the buspirone plus galantamine night; Wake% was significantly higher in the GG group than in the CC group on the galantamine night, but higher in the CC group on the galantamine plus buspirone night. The univariate results for the rest of the individual sleep measures are shown in Table 1.

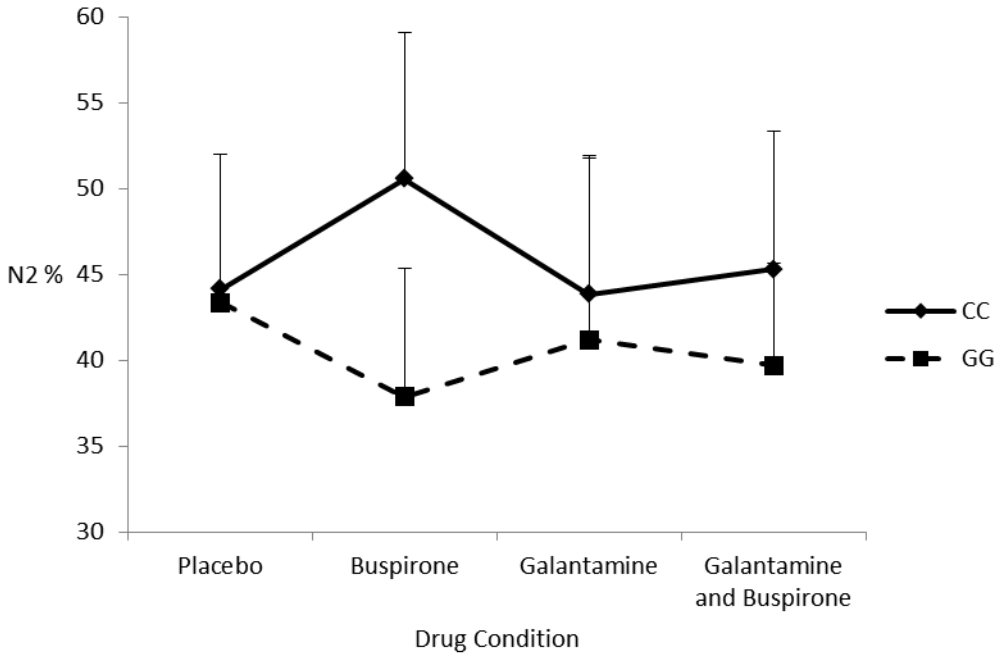

Figure 1. Percentage of sleep spent in stage N2 in wild-type (CC) and mutant (GG) participants (interaction p=0.010, η^2=0.215) with standard deviation error bars. Both buspirone and the combination night show a significantly higher N2% in wild-type subjects.

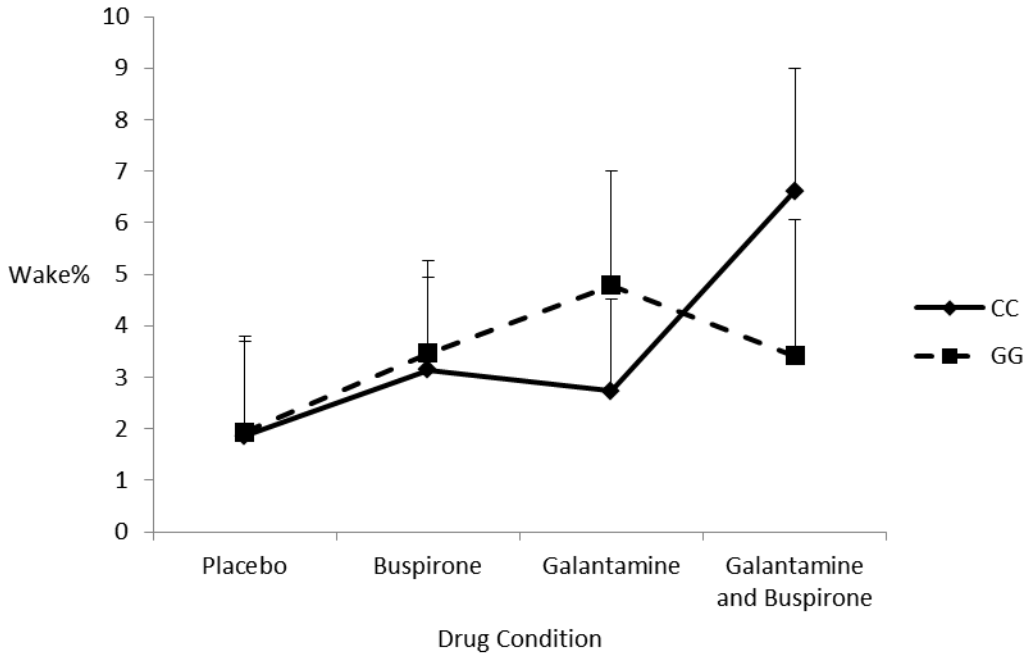

Figure 2. Percentage of time in bed spent awake (Wake%) in wild-type (CC) and mutant (GG) participants (interaction p=0.045, η^2=0.117) with standard deviation error bars. The GG group spent significantly more time awake on the galantamine night than the CC group, and the CC group spend significantly more time awake on the combination night.

	Genotype	Placebo		Buspirone		Galantamine		Galantamine and Buspirone		ANOVA		
		mean	SD	mean	SD	mean	SD	mean	SD	F (3,114)	p	η^2
Sleep Efficiency (%)	CC	96	0.72	95	0.69	94	0.59	90	0.73	1.03	0.385	0.051
	GG	97	0.71	95	0.71	92	0.71	94	0.72			
Awakenings (number)	CC	2.52	2.02	4.57	1.96	4.33	2.10	5.08	1.72	1.69	0.183	0.077
	GG	2.94	2.23	4.35	2.24	7.87	2.28	3.98	2.73			
Total Sleep Time (min)	CC	476.63	58.69	482.04	43.35	473.04	52.33	461.63	73.91	0.17	0.914	0.011
	GG	501.00	46.96	494.08	66.08	502.83	30.91	467.33	63.85			
N1 %	CC	1.75	2.49	3.38	1.55	4.77	2.05	5.49	1.61	0.25	0.875	0.009
	GG	1.80	1.61	3.75	1.95	5.12	1.78	4.52	2.45			
N3 %	CC	30.69	9.43	27.01	8.02	21.52	7.63	23.79	7.18	1.85	0.151	0.058
	GG	35.82	8.59	38.64	10.25	26.51	6.85	30.96	8.28			
REM%	CC	22.72	5.03	18.77	6.04	28.89	5.76	24.76	7.25	0.51	0.674	0.020
	GG	18.84	3.87	18.92	5.37	26.40	5.54	22.63	4.51			
RL (min)	CC	82.00	24.09	149.81	61.09	67.68	17.49	101.59	36.96	0.43	0.731	0.014
	GG	107.5	48.48	149.66	53.11	72.00	13.59	110.66	27.94			
RD (rems/min)	CC	6.41	3.54	6.20	3.44	6.70	2.58	7.19	3.71	1.05	0.379	0.061
	GG	7.57	2.35	6.47	4.36	8.85	3.22	6.91	2.66			
SOL (min)	CC	10.41	2.23	11.27	3.15	16.17	1.83	15.90	2.74	0.53	0.660	0.033
	GG	10.34	2.99	11.60	2.98	14.30	3.16	9.18	3.72			
WASO (min)	CC	4.59	4.04	12.23	2.43	10.95	2.39	29.54	2.64	1.47	0.233	0.059
	GG	4.01	4.30	11.54	3.53	20.64	3.74	14.01	3.19			

Table 1 Results from the univariate genotype-by-drug interaction post-hoc analyses. SE: sleep efficiency (=TST/time in bed); TST: total sleep time; N1%, N3%, REM%: percentage of time asleep spent in stage N1, N3 or REM; RL: REM latency; RD: rem density; SOL: sleep onset latency.

Discussion

The current study identified no marked differences in sleep structure between 5-HT_{1A} genotypes. This could be due to the small sample size and the expected subtlety of an effect of genotype. While it is possible that a larger sample might highlight small differences, it is also likely that the C/G mutation is not enough to cause noticeable differences in the sleep patterns of healthy young adults. For instance, sleep may be sensitive to combinations of genotypic factors with other risk factors or systemic stressors.

Similarly, we did not find any multivariate interaction between the drug effects and genotype. Two of the univariate post-hoc ANOVAs indicated significant interactions: N2% and Wake%. Buspirone appeared to increase the proportion of sleep spent in stage N2 more in the CC wild-type group than in the GG group. While this could be interpreted as being due to buspirone more easily affecting the intact serotonergic system in the CC group and therefore having a stronger effect on those participants it bears repeating that the overall multivariate interaction was not significant and this effect was not seen in other measures previously shown to be affected by buspirone (such as RL). The Wake% interaction is harder to interpret, with galantamine appearing to disrupt sleep in the GG group more than in the CC group, but the buspirone plus galantamine drug night having a stronger effect on the wildtype CC participants.

Buspirone appears to affect participants with the GG polymorphism in a similar way to those with CC polymorphism, or at least well enough that the difference in the effect on REM and SWS sleep structure is negligible. This is an encouraging finding: if buspirone had noticeably less effect on individuals with the GG genotype, this would have suggested that patients should be tested for their 5-HT_{1A} receptor genotype before using buspirone as part of their treatment plan to adjust dosage.

The effects of the drugs on sleep structure are discussed more in depth in our previous article (Biard 2015), but it is worth noting here that both drugs affected sleep as predicted by current models of the serotonergic/cholinergic imbalance in depression. Our serotonergic agonist, buspirone, suppressed REM sleep by increasing REM latency. Our cholinergic agent, galantamine, caused an increase in alertness shown by a higher Wake%, more numerous awakenings, and less Stage N3 'deep' slow-wave sleep.

Galantamine and buspirone given together tended to cancel out each other's effects on REM sleep latency and REM% while their deleterious effects on sleep quality appeared to be additive: both drugs increased sleep fragmentation, leading to lower sleep efficiency and a higher percentage of time in bed spent awake. The higher cholinergic tone led to more stage REM sleep, similar to the finding that depressed patients have a higher percentage of total sleep time spent in REM. The way buspirone counteracted this effect was similar to how antidepressants act in depressed patients: by lowering the proportion of sleep spent in REM. With regards to the C(1019)G polymorphism, increased cholinergic drive on the galantamine plus buspirone night was expected to overwhelm impaired serotonergic systems in those participants with the GG polymorphism but this effect was not shown. This suggests that 5-HT_{1A} receptors may possibly not be as integral to sleep regulation as other serotonergic and cholinergic mechanisms. Performing a similar push-pull experiment with cholinergic and serotonergic drugs in populations with different relevant mutations might prove to be more effective in finding an interaction between genotype and cholinergic/serotonergic imbalance effects on sleep.

In addition to the small sample size, one of this study's limitations is the restricted demographic characteristics of the participants. They were all healthy young women, so these findings may not be generalizable to older, male, or patient populations. Future studies

in this area should replicate this design in a clinical population, such as in patients with MDD, in order to see the effect of the drugs on participants whose sleep is endogenously affected by a serotonergic-cholinergic imbalance.

Conclusion

While the C(1019)G 5-HT_{1A} receptor genotype is a risk factor for depression, it had limited effects on sleep structure in the healthy females in our experimental design. REM sleep parameters of mutant and wildtype participants did not differ when taking the 5-HT_{1A}–targeting buspirone or when taking the cholinergic agent galantamine, and only univariate trends were found for other sleep variables. However, because this small pilot study had limited statistical power to assess the above sleep effects, it is possible that larger studies might find that the G/G mutation does have a small but statistically significant effect. Future research into genetic risk factors for mood disorders should investigate the effects on sleep structure of this and other mutations in the serotonin system in order to better our understanding of biomarkers for depression.

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General Discussion

This study set out to test the cholinergic-aminergic model of depression by examining the effects of a serotonergic agent and a cholinergic agent on sleep structure in normal controls. This research also sought to determine the role played by a relevant and common genetic mutation in the serotonin transport system and to determine how useful it might be as a potential biomarker for mood disorders or whether it should be examined when considering treatment options.

One weakness in the current literature was a lack of controlled, repeated-measures administrations of the different drugs on the same subjects while also using full polysomnography techniques to thoroughly examine the changes seen in sleep. This research sought to answer two main research questions: first, whether the cholinergic-aminergic model of depression could predict the effects of two relevant drugs on normal controls, and second, whether or not an apparently relevant genetic mutation in the 5-HT_{1A} receptor would affect the functioning of the drugs or the sleep of the participants.

The main empirical findings are detailed in the two research papers already presented. The first paper, "The effects of galantamine and buspirone on sleep structure: Implications for understanding sleep abnormalities in major depression," examined the first of the two research questions. Using the cholinergic-aminergic model to explain the altered sleep patterns commonly found in depression we made predictions about how the sleep of our participants would be altered in the three drug conditions.

The first drug condition was the administration of buspirone. We expected that the serotonergic agonist would mimic the action of the serotonergic neurons originating in the dorsal raphe nucleus (DRN), which inhibit REM sleep by activating the 5-HT_{1A} post-synaptic receptors on the cholinergic pontine nuclei. We found that REM sleep was tonically

inhibited, with a much longer REM latency. Some subjects appeared to skip their first REM period entirely which is consistent with observations of patients on anti-depressants. We also expected to find lower REM% and lower RD but while there were trends in the data for both of these findings neither was significant. Overall the drug's effects on sleep were consistent with the model.

The second drug condition was galantamine. Galantamine, an anti-cholinesterase, is capable of enhancing central cholinergic activity. According to the cholinergic-aminergic model, increasing cholinergic activity should cause the participants' sleep to resemble that of depressed patients, including lowering the amount of restorative SWS they experience as well as specific changes to the structure of REM sleep.

To briefly reiterate the rationale behind our expectations regarding the effects on REM sleep: Noradrenergic and serotonergic pathways from the LC and the DRN act on post-synaptic receptors on the cell bodies of the cholinergic neurons of the LDT-PPT to inhibit REM sleep (Datta 2007). The LDT/PPT also has an internal cholinergic trigger for phasic "burst" activity, mediated by a cholinergic receptor. Previous research (Reimann, 1994) led us to expect that galantamine would increase RD. Instead we found that it increased tonic REM measures but did not affect phasic activity. This finding still supports the overall model which predicted that a global anti-cholinesterase would augment the REM drive, though it has implications for the specific mechanism of galantamine: considered in combination with other research that shows galantamine has, in the past, also been able to increase RD it is possible that this drug behaves slightly differently in different populations and also potentially at different dosages. Riemann's study used two different dosages for galantamine: 10mg and 15mg. Interestingly, the researchers only found an increase in RD for the 10mg night and not for the 15mg night. It is possible that galantamine has a non-linear relationship

with RD and that our dose of 16mg was too high to provoke an increase in REMs during the night.

There is also the possibility that ACh alone is not sufficient to increase RD. Animal studies have found that rapid eye movements are directly linked to a cortical phenomenon known as PGO waves: synchronized electrical field potentials in the pons, lateral geniculate nucleus, and occipital cortex (Brown 2012). PGO waves occur immediately before REM onset as well as in bursts during REM sleep and are also associated with the rapid eye movements associated with dream imagery. Cholinergic input to the thalamus from neurons in the LDT/PPT appears to be a requirement of PGO wave generation (Brown 2012), but that non-cholinergic neurons in the brainstem (specifically the subcoeruleus/parabrachial area) are responsible for generating the initial pontine component of the PGO waves (Datta 1997).

Our finding, combined with other studies' results from observing the effects of cholinergic agents on sleep, seems to indicate that while cholinergic mechanisms are certainly involved in REM sleep regulation they appear not to be the primary driver of phasic activity. Without a higher pontine drive for PGO waves, it is possible that increasing the cholinergic tone is not sufficient to increase the total amount of REMs during REM sleep, which may explain why galantamine failed to increase RD in our healthy participants. Galantamine's expected effect of lowering the percentage of sleep spent in N3 was seen, however.

The final drug condition was a mixed treatment of both buspirone and galantamine. Working under the assumption that the two recommended starting treatment doses would push the cholinergic-aminergic balance in opposite directions but with similar amounts of force we predicted that the effects of the two drugs on REM sleep would cancel each other out, with the exception of higher RD from the galantamine. We did see that the tonic

measures of REM in this condition were not significantly different than baseline measures, though again we did not see the expected surge in RD from galantamine's cholinergic action. Once again galantamine also significantly reduced the amount of slow wave sleep compared to baseline conditions.

Overall we found that the push-pull of the two opposing regulatory systems was demonstrated, answering our first research question: the cholinergic-aminergic model successfully predicted the effects of the serotonergic and cholinergic agents on the sleep structure of healthy young women.

Our second research question of whether or not the G(-1019)C 5-HT_{1A} receptor mutation affects sleep in this context was addressed with the empirical findings described in the second paper, "5-HT_{1A} receptor genotypes and REM sleep sensitivity to serotonergic/cholinergic imbalance in humans: A pharmacological model of depression." Those results showed no significant link between the 5-HT_{1A} G(-1019)C polymorphism and the observed measures of sleep structure. Baseline sleep did not differ between the two genotypes and there was no overall interaction between genotype and drug effect.

In that article it is noted that two of the univariate measures, percentage of time spent awake and in stage N2, did show significant interactions between genotype and drug condition. Both the buspirone night and the combination night showed a significantly higher N2% in CC participants compared to GG participants. An increase in N2 sleep has been seen in the literature before: Wilson's 2005 study found that both buspirone and eptapirone increased the N2% of their participants, though their results were just shy of statistical significance ($p=0.06$), they did note the trend. It is possible that the increase in N2% is a real but subtle effect of the buspirone. The second interaction we found, that the amount of time spent awake was higher in the GG group for the galantamine night but higher for the CC

group in the combination night, is more difficult to interpret. Since it is both unexpected and unexpected and came from post-hoc analysis, it seems likely that this result is a false positive.

As neither interaction occurs in a measure that we most expected to be affected by a mutation in the serotonergic transport system, such as REM%, RL, or RD, and as their effect sizes are also small, it seems most prudent to not put too much emphasis on these results in the absence of corroborating findings.

We can therefore answer our second research question with a qualified negative: the 5-HT_{1A} G(-1019)C single nucleotide polymorphism may be linked to clinical outcomes such as depression, anxiety, and suicide, but we found no evidence that suggests it affects baseline sleep structure, nor does it mediate the effects of either buspirone or galantamine on REM or slow wave sleep in healthy young women.

Theoretical and clinical implications

The results of this study have theoretical implications for the aminergic/cholinergic model of depression as well as clinical implications for the study drugs. The first and clearest take-home message is that our results support the theory of an aminergic/cholinergic imbalance, which is at least partly responsible for the sleep physiological changes seen in depression. As seen in depression, higher cholinergic tone led to increases in REM sleep and decreases in slow wave sleep. Conversely, increasing the serotonergic tone suppressed REM sleep and countered the effects of the cholinergic agent.

What the model did not predict, however, was the lack of any effect of either drug on rem density. From previous research as well as our own study it seems likely that rem density is regulated primarily by another neurotransmitter or group of them. Cholinergic agonists have had a mixed range of effects on RD in the literature. A group of studies by

Riemann in 1994 examined the effects of five different cholinergic agonists on REM sleep. Interestingly, his study found that while all of the agonists lowered RL only one of them – galantamine – increased RD, and even then only with the 10mg dose but not with the higher 15mg dose. Another cholinergic agonist, SDZ-210-086, actually decreased RD while the other three (Physostygmine, Tacrine, and RS 86) had no effect on it at all. Riemann concluded that the effect of individual cholinergic agonists on RD was likely dependant on their specific mechanisms on M1 and M2 receptors and their nicotinic properties.

Returning to our own results, we can perhaps extend Riemann's conclusion – that the effects of cholinergic agents on RD depend on the specific drug and its mechanism – to add that it also appears to depend on the participants of the study and/or the dose. While his team found an increase in RD where we did not, it is possible that this was because his participants included men (6 males, 12 females) and were much older (mean age of 38.7, sd 8.3 years) than ours, and so they reacted differently to the drug. The discrepancy might also be explained by dosage: Riemann's group found the elevated RD only in the moderate dose of 10mg and not in the group with the 15mg dose. We used 16mg as our dose, so perhaps the relation between galantamine and RD is not a linear one.

Taken together, the literature and our own research appear to indicate a link between cholinergic tone and RD but not as RD's primary controller. Another neurotransmitter is likely involved in the regulation of RD. One suggestion is that dopamine, as a part of the dopamine/acetylcholine balance, plays a strong role in controlling RD (Sasai 2012). Further studies in human subjects are needed to clarify the links between antidepressant drugs and changes in RD.

There are also clinical implications of our findings. One of our unexpected results was that galantamine disrupted sleep and caused significant morning nausea in our

participants, an effect not seen previously in other subject groups. Galantamine is generally well tolerated in older patients (Stahl 2004) and older non-patient groups (Douglass, in preparation). While previous research has found slight increases in nightmares and sleep disruption the rates of increase for both complaints was small enough to be considered negligible from a clinical point of view (Stahl 2004). In our own younger, all-female population, however, galantamine showed much stronger negative side effects. As described in the first article, nearly half of our participants complained of nausea in the morning and two of our original twenty-two participants had their sleep so severely disrupted by galantamine that they could not be included in the analysis. Most participants complained that the galantamine sleep was not restful and that they intended to nap later that day to recover from their night. There was also a marked increase of nightmares and disturbing imagery in dreams on galantamine nights, another unexpected finding. The increase in unpleasant dreams may have been caused by the cholinergic neurons providing strong excitatory input to the dopaminergic midbrain pathways involved in the reward process: Solms (2002) argues that these pathways are linked to dreaming and, if the increase in cholinergic tone was activating them, then this may explain why galantamine was provoking vivid dreams in participants. If galantamine were ever to be considered as a treatment option for younger patients the increased severity of the side effects would have to be considered as a potential deterrent in a patient's willingness to comply with treatment, though it is unclear whether or not younger patients could acclimatize to galantamine or whether these side effects would persist: there is some evidence to indicate that rapid titration of galantamine can cause or exacerbate anxiety and nightmares (Corbo 2013) and our research model did not allow for gradual acclimatisation to the drug. While these results are fortunately not from the

demographic that galantamine is generally used in, it is still worth taking note of how age can alter not only the effects of certain drugs but also their side effects as well.

Buspirone, for its part, fared much better in the younger demographic. While it did cause statistically significant disruption of sleep in our healthy participants, the size of the effect was not large and participants generally awoke feeling refreshed after the buspirone nights. This was an encouraging finding as buspirone is more likely to be considered as a useful treatment option in younger adult patients than galantamine since it targets anxiety and depression as opposed to Alzheimer's dementia. These results, coupled with the finding that the fairly-common G(1019) genotype did not interfere with buspirone's apparent effectiveness (as measured by its effects on REM and SWS) both bode well for buspirone's overall potential usefulness in different demographics. Future research in different patient populations would be needed to confirm this, however.

Study limitations and future research

In addition to studying the effects on patient populations, future studies could also expand on different demographics with healthy participants. Considering how age and gender have both been shown to mediate the effect of different medications it would be interesting to see how these drugs, or related ones, altered sleep structure in older female participants and also in male participants.

Similarly, while the genotype we examined had no obvious effect on sleep structure or drug function, other genes implicated in sleep regulation certainly might. Performing a similar push-pull experiment with cholinergic and serotonergic drugs in a population such as narcoleptics with faulty orexin control or in participants identified to have mutations in the circadian clock genes might prove to be more effective in finding an interaction between genotypes and drug effects on sleep.

A final suggestion for future research is that we examine our own data from this study in more depth. One way in which we could do this is to perform a spectral analysis of each night and compare them across genotypes and drug conditions. Of particular interest would be the delta waves in SWS. It has been suggested that reduced delta power is a good candidate as a biomarker for the depressive trait, similar to increased RD (Wichniak 2013). Another way in which we might examine our own data further would be to analyze rapid eye movements using a novel Markovian statistical approach. This approach was derived by Boukadoum and Ktonas (1988) to address the shortcomings of traditional time-series analyses and while such an analysis was beyond the scope of these two papers it would be a potentially useful undertaking with this dataset. This method appears to be more stable, reliable, and repeatable than crude RD as it examines the time intervals between each rapid eye movement and creates a statistic describing the rem density patterns (the Markovian state transition probability or STP). Previous studies using this method have successfully demonstrated significant differences between normals, patients with schizophrenia, and patients with MDD (Douglass, Benson, Hill, & Zarcone, 1992). We could use this method to search for differences between C and G(1019) genotypes as well as to determine whether or not the drug conditions had any effect on an individual's Markovian STPs.

While there are many interesting potential suggestions for future research suggested by our findings our own study has successfully demonstrated the push-pull relationship between serotonin and acetylcholine described by the aminergic-cholinergic model of sleep regulation in depression. The model successfully predicted the effects of the drugs on slow wave sleep as well as tonic measures of REM sleep in a novel repeated-measures study using both serotonergic and cholinergic agents. Rem density remains only partially explained by this model, as has been noted previously in the literature, and our findings support the notion

that it is primarily regulated by another neurotransmitter system. Our investigation of a common mutation in the 5-HT_{1A} receptor gene showed that it did not appear to interfere with the effects of either drug on REM or SWS – a null finding, but still an interesting one as it suggests that future research into the effects of specific genes on sleep structure would most benefit from examining other genetic variations implicated in depression and sleep dysregulation.

Appendix A – Pre-sleep questionnaire

Centre de sante mentale
RoyalOttawa
Mental Health Centre

Sleep Disorders Service

Recent Patient History Questionnaire

Name: _____

Date: _____

1. List **all** your current medications. **Circle** any that you did **not** take today and will **not** take **by the time you go to bed**:

2. List any over-the-counter medications, herbal supplements, vitamins, minerals, or recreational drugs (ie. Marijuana) you have taken today:

3. What time did you **get into** bed last night? _____
   What time did you **get out of** bed this morning? _____
   How many hours of sleep do you think you got? _____

5. Rate the quality of your sleep last night:
   Poor ☐ Typical ☐ Sound ☐

6. Did you take any naps today? Yes ☐ No ☐
   If yes, how many _____ , and how long was each? _____
   And at what time did you take your nap?_____

7. Are you a smoker ☐ or a non-smoker ☐ ?
   If you smoke, how many cigarettes did you have today? _____
   And at what time did you have your last one? _____

8. Do you ever drink alcohol? Yes ☐ No ☐
   If yes, did you have any alcohol today? Yes ☐ No ☐
   If yes, how many drinks did you have today? _____
   And at what time did you have your last drink? _____

9. Did you drink any caffeinated beverages today? Yes ☐ No ☐
   If yes, please state **what kind** and **how many** (eg. 1 large Starbuck's coffee and 2 cans of root beer): _____
   And at what time did you have your last caffeinated beverage? _____

10. How tall are you _____, and what is your current weight? _____

11. Please state any changes in your medical condition since you last saw the sleep doctor:

Appendix B – Post-sleep questionnaire

Services de santé RoyalOttawa Health Care Group

Sleep Disorders Centre
Post – Sleep Questionnaire

Name: _____ **Date:** _____

1. a) How long do you think it took you to fall asleep last night?_____
   b) How long does it usually take you to fall asleep at home?_____

2. a) How long does it feel like you slept last night?_____
   b) How long do you usually sleep at home? _____

3. a) Please estimate how many times you recall waking up last night: _____
   b) If you know what caused these awakenings, please indicate: _____

4. a) Did you take anything to help you sleep last night? Yes_____ No_____
   b) If yes, what did you take? _____
5. Were your legs restless (did they bother you) last night? Yes _____ No _____

6. Please choose the best word to describe the quality of your sleep last night: (circle one)

   Poor Average Sound

7. a) Was last night's sleep typical of your sleep at home? Yes _____ No _____
   b) If no, please indicate how your sleep differed from home: _____

8. Does it feel like you got enough sleep last night? Yes _____ No _____

9. Please rate how rested you felt after waking up this morning:

   1 2 3 4 5 6 7 8 9 10
   Not at all Very well rested

10. a) Was it difficult for you to wake up this morning? Yes _____ No _____ Somewhat ____
    b) If you know what woke you up this morning, please indicate: _____
11. a) How many dreams do you recall having last night? _____
    b) If you have any comments about last night's dreams, please indicate:

12. Do you have any other comments about last night's sleep? (you may write on the back).

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