INTRODUCTION

Sleep supports human health and brain function. However, modern lifestyles and the advent of artificial lighting have resulted in sleep problems for many individuals. Several groups have reported that sleep disturbances are caused by altered biological rhythms, mood disorders, and neurodegenerative diseases (Parry et al., 2006; Costandi, 2013). Many research groups have proposed methods of overcoming sleep problems, including regulating clock transcription factors, modulating neurotransmitters, and administering sleep aids, especially melatonin (MT) (El Helou et al., 2013; Proença et al., 2014; Wilhelmsen-Langeland et al., 2013).

Sleep is modulated by gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous system. Activation of GABA<sub>А</sub> receptors modulates sleep. Pentobarbital, a major hypnotic drug, binds to GABA<sub>А</sub> receptors and increases chloride ion influx (Lees et al., 1998; Ma et al., 2008; Shah et al., 2014). In addition, pentobarbital modulates GABA<sub>А</sub> receptor conductance by increasing the duration of inhibitory postsynaptic currents (IPSCs) in the brain and initiates sleep at low and moderate doses (Wan et al., 2003). Further more, GABA currents are modulated by MT, which stimulates glutamic acid decarboxylase, an enzyme involved in GABA synthesis (Wang et al., 2002), and directly binds to GABA<sub>А</sub> receptors (Li et al., 2001). Recent studies suggest that the relationship between MT and sleep is mediated, at least in part, through modulation of synaptic transmission by GABA.

MT (5-methoxy N-acetyltryptamine) is a neurohormone with a tryptamine structure that binds to G-protein-coupled receptors.
tors MT₁ and MT₂. A recent review of MT receptors established that the MT₁ receptor is distributed in the cerebral cortex, subcortical structures, substantia nigra, and striatum, and peripheral organs, whereas the MT₂ receptor is expressed throughout the brain (Pandi-Perumal et al., 2008). The same review described the manner in which functional MT receptors modulate the protein kinase A (PKA) pathway by activating G proteins, including Gα_i, Gα_q, Gα_s, Gα_12, and Gα_13, while MT₂ receptor activation modulates PKA and protein kinase C (PKC) signaling pathways. Recent studies show that MT regulates the activity levels of extracellular signal-regulated kinase 1 and 2 (ERK1/2) and p38 mitogen-activated protein kinase (p38 MAPK), although the precise intracellular mechanisms underlying these effects remain unclear (Bondi et al., 2008; Vilar et al., 2014).

Recently, a great deal of work has been focused on dopamine; however, little attention has been focused on the effects of D2-like dopamine receptor modulation in sleeping animals. Increasing evidence suggests an important role for D2-like dopamine receptors in various aspects of sleep (Dimpfel, 2008; Volkow et al., 2012). Recent studies have demonstrated that interaction between D2-like dopamine receptors and MT is involved in the antidepressant-like effect of MT (Zawilska and Iuvone, 1990; Binfaré et al., 2010). However, the effects of D2-like dopamine receptor activation on MT-related sleep and cell signaling in the cerebral cortex of mice during pentobarbital-induced sleep have not been studied.

In the present study, we hypothesized that MT augments pentobarbital-induced sleep in mice. Moreover, we hypothesized that D2-like receptor agonist quinpirole modulates the sleep-inducing effect of MT in mice. In addition, western blotting analyses were used to study the mechanism by which quinpirole modulates intracellular signal transduction pathways related to ERK1/2, p38 MAPK, PKA, and PKC in the cerebral cortex. Our findings provide the first evidence that D2-like receptor activation affects MT-augmented sleep in mice.

MATERIALS AND METHODS

Animals

Male CD-1 mice (3-weeks-old, 15-16 g) were purchased from Koatech (Pyeongtaek, Korea). For 1 week prior to the experiments, 10 animals were housed in each cage and allowed to access to water and food ad libitum. During the acclimation period, the animals were kept in a room maintained at constant temperature (23 ± 1°C) and humidity (55 ± 5%) under a 12-h light/dark cycle (lights on from 07:00-19:00 h). After the acclimation period, the mice were divided randomly into groups. All experiments were conducted in accordance with the NIH Guide for Laboratory Animals. The study protocol was approved by the Institutional Animal Care and Use Committee of Sungkyunkwan University.

Drugs and chemicals

MT and (-)-quinpirole were purchased from Sigma Chemical Co. (Sigma, St. Louis, MO, USA). MT was dissolved in 10% dimethyl sulfoxide (DMSO) and 5% Tween-80 in normal saline. Quinpirole was dissolved in 0.9% physiological saline. All drugs were administered intraperitoneally (i.p.). Mouse anti-β-actin antibodies for western blotting were purchased from Sigma Chemical Co. Rabbit anti-phospho-p38 MAPK (Thr180/Tyr182) and anti-p38 MAPK antibodies for western blotting were purchased from Epitomics (Burlingame, CA, USA). Rabbit anti-phospho-ERK1/2 (Thr202/Thr204), anti-ERK1/2, anti-phospho-PKA (Thr197), anti-PKA, anti-phospho-PKC (gamma Thr514), and anti-PKCB antibodies for western blotting were purchased from Cell Signaling Technology (Boston, MA, USA). All other chemicals were of analytical grade.

Pentobarbital-induced sleep test

The pentobarbital-induced sleep test was carried out according to the method described by Ma et al. (2009) with some modification. Briefly, mice were treated with quinpirole or saline for 30 min prior to administration of MT or vehicle, followed by administration of pentobarbital sodium (40 mg/kg) 30 min after the administration of MT. Following administration of pentobarbital, mice were placed in individual cages and the time to sleep onset and sleep duration were measured. The observers were blinded to the treatments. Mice that stayed immobile for more than 3 min were considered asleep. The time to sleep onset was measured from the time of pentobarbital administration to the time of sleep onset. The sleep duration was defined as the difference in time between loss and recovery of the righting reflex. Animals that failed to fall asleep within 15 min after pentobarbital treatment were excluded from the study (Ma et al., 2009).

Locomotor activity test

To test the effects of MT and quinpirole on locomotor activity, each mouse was placed in an activity cage (locomotor box: 30×30×30 cm) and habituated for 40 min. Mice were administered quinpirole (1 mg/kg, i.p.) 20 min before MT (30 mg/kg, i.p.), and the subsequent horizontal activity was recorded using a video tracking system (Neurovision, Pusan, Republic of Korea). Locomotor activity was measured for 70 min, and the distance travelled over the final 30 min in the chamber was expressed using bar graphs and tracking patterns.

Western blot analysis

Each mouse was decapitated, after which the cerebral cortex was quickly dissected on an ice-cold metal surface. The dissected brain tissue samples were homogenized in ice-cold lysis T-per tissue protein extraction buffer (Thermo Scientific, Rockford, IL, USA) containing protease and phosphatase inhibitor cocktails (Roche Diagnostics, Basel, Switzerland) and incubated on ice for 30 min. After centrifugation at 13,000×rpm for 15 min at 4°C, the supernatant was separated and stored at −70°C. Protein concentrations were determined using a protein assay kit (Thermo Scientific, Rockford, IL, USA). Protein samples (30 μg protein) were separated on 10% SDS-polyacrylamide gels, transferred to polyvinylidene difluoride transfer membranes (Pall Corporation, Pensacola, FL, USA), and blocked with 5% skim milk containing 0.5 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween-20 for 1 h at room temperature. The membranes were subsequently incubated with primary antibodies overnight at 4°C (1:1,000 dilution for anti-phospho-p38 MAPK [Thr180/Tyr182], anti-p38 MAPK, anti-phospho-PKA [Thr197], and anti-PKA; 1:2,000 dilution for anti-ERK1/2 [Thr202/Thr204], anti-ERK1/2, anti-phospho-PKC [gamma Thr514], and anti-PKCB; 1:20,000 dilution for β-actin). After three washes with Tris-buffered saline containing 0.1%
Tween-20 (TBST), the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) in TBST with 5% skim milk (1:5,000) for 1 h at room temperature. The blots were washed three times in TBST, immersed in an enhanced chemiluminescence (ECL) mixture for 5 min (Perkin Elmer, Boston, MA, USA) (reagents A and B at a 1:1 ratio), and exposed to photographic film. Each western blot was quantified by densitometry using ImageJ 1.44 software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis
All data were analyzed with Prism 6.0 software (Graphpad Software, Inc., San Diego, CA, USA). The results are expressed as the mean ± S.E.M of each group. For the assays of sleeping behavior and locomotor activity, data were analyzed using one-way analysis of variance (ANOVA) followed by Fisher’s LSD post-hoc test. Western blot data were analyzed using two-way ANOVA followed by Fisher’s LSD post-hoc test to detect intergroup differences. Results of p<0.05 were considered statistically significant.

RESULTS
MT prolonged the duration of pentobarbital-induced sleep in mice
A previous study reported that the ED₅₀ values for MT-potentiated sleeping time was 6.1 mg/kg (i.p.) in pentobarbital-in-duced sleep in mice (Sugden, 1983). Therefore, we estimated the dose of MT that prolonged the duration of pentobarbital-induced sleep in our system. Administration of MT dose-dependently decreased sleep latency and dose-dependently increased the duration of pentobarbital-induced sleep (Fig. 1). A single injection of MT (10 and 30 mg/kg) markedly increased the duration of pentobarbital-induced sleep, consistent with a previous study (Sugden, 1983). Furthermore, the group treated with 30 mg/kg MT showed a decreased time to sleep onset in comparison with that of the pentobarbital-treated group (Fig. 1A; F(3,20)=12.59, p<0.001; 10 mg/kg MT: t=3.17, p<0.01; 30 mg/kg MT: t=6.03, p<0.001; 10 mg/kg MT: F(3,20)=5.00, 30 mg/kg MT: F(3,20)=3.42, p<0.01). The most significant effects on sleep duration and onset time were produced by 30 mg/kg MT; therefore, this dose was utilized for future experiments and investigation of
In order to determine whether D2-like receptor activation increased the duration of MT-augmented pentobarbital-induced sleep in mice, Quinpirole was administered to mice before MT injection. The results showed that Quinpirole increased the duration of MT-augmented pentobarbital-induced sleep in mice. (Fig. 3)

**Fig. 3.** Effects of MT and quinpirole on locomotor activity in mice. (A) Locomotor activity was assessed by measuring the distance travelled by each mouse for 30 min after MT injection (A), as well as the distance travelled every 5 min for 70 min (B). Each column represents the mean ± S.E.M (n=8-9) (one-way ANOVA followed by Fisher’s LSD post-hoc test). SAL, saline; VEH, vehicle; QNP, quinpirole.

**Fig. 4.** Effects of quinpirole on ERK1/2, p38 MAPK, PKC, and PKA activation in the cerebral cortex. Quinpirole increased phosphorylation of ERK1/2 (A), p38 MAPK (B), and PKC (D), but not that of PKA (C), in the cerebral cortex. Each column represents the mean ± S.E.M (n=3-5). ##p<0.001 vs. the group treated with MT and pentobarbital and ##p<0.01 and ###p<0.001 vs. the group treated with quinpirole and pentobarbital (two-way ANOVA followed by Fisher’s LSD post-hoc test).
altered MT-augmented sleeping behavior, mice were pre-
treated with selective D2-like receptor agonist quinpirole 30 
mind before MT treatment. Quinpirole had no significant effect 
on sleep onset time (Fig. 2B; F(6,39) = 7.66, p < 0.001); howev-
er, quinpirole at doses of 0.3, 1, and 3 mg/kg increased the 
duration of MT-augmented sleep in a dose-dependent man-
er (Fig. 2A; F(6,39) = 37.38, p < 0.001; 0.3 mg/kg quinpirole, 
t = 5.73, p < 0.001; 1 mg/kg quinpirole, t = 9.61, p < 0.001; 3 mg/
kg quinpirole, t = 4.95, p < 0.001, compared to the group treated 
with MT + pentobarbital). Interestingly, in the absence of MT, 
quinpirole also significantly affected sleep duration (t = 6.55, 
p < 0.001, compared to the control group). Furthermore, treat-
ment with the combination of quinpirole, MT, and pentobarbital 
also prolonged the duration of sleep in comparison with quin-
pirole treatment alone (t = 6.39, p < 0.001). The sleeping time 
distribution of each mouse is shown in Fig. 2C.

MT and quinpirole did not affect locomotor activity

Sugden (1983) suggested that high doses of MT affect lo-
comotor activity in rodents. In our study, we investigated the 
effect of 30 mg/kg MT on locomotor activity in mice. Locomo-
tor activity was assessed by measuring the distance travelled 
every 5 min for 70 min, as well as the distance travelled for 30 
min after MT injection. As shown in Fig. 3A, administration of 
30 mg/kg MT did not significantly affect locomotor activity. In 
addition, the locomotor activity level of mice treated with quin-
pirole or MT was similar to that of the control mice (Fig. 3A; 
F(2,23) = 0.2151, p > 0.05).

Quinpirole increased expression of phospho-ERK1/2, 
phospho-p38 MAPK, and phospho-PKC in the cerebral 
cortex

To examine the intracellular mechanisms underlying quin-
piole-induced prolongation of sleep, we isolated the brain 
3 hours after pentobarbital injection. At this time point, mice 
treated with quinpirole showed augmented activity levels of 
ERK1/2 (Fig. 4A; F(3,12) = 24.71, t = 7.648, p < 0.001), p38 MAPK 
(Fig. 4B; F(3,6) = 117.4, t = 14.52, p < 0.001), and PKC (Fig. 4D; 
F(3,9) = 213.1, t = 18.57, p < 0.001) in the cerebral cortex in com-
parison with those of mice treated with MT and pentobarbi-
tal. Further, mice treated with quinpirole, MT, and pentobarbital 
showed up-regulated expression of p-ERK1/2 (Fig. 4A; 
t = 3.584, p < 0.01), p-p38 MAPK (Fig. 4B; t = 11.32, p < 0.001), 
and p-PKC (Fig. 4D; t = 21.97, p < 0.001) in comparison with 
that of mice treated with quinpirole and pentobarbital. Mice 
treated with quinpirole, MT, and pentobarbital showed PKA ac-
tivity similar to that of mice treated with either MT or quinpirole 
and pentobarbital (Fig. 4C; F(3,9) = 1.306; p > 0.05).

DISCUSSION

This study indicates that quinpirole modulates MT-aug-
mented sleeping behavior in mice and elucidates several in-
tracellular mechanisms underlying this action in the brain. We 
showed that MT, which alone has no hypnotic or sedative ef-
facts, enhances pentobarbital-induced sleeping behavior. The 
duration of MT-augmented sleep was further increased by a 
single administration of quinpirole. Interestingly, quinpirole af-
fected pentobarbital-induced sleep even in the absence of MT. 
In addition, quinpirole, alone or in combination with MT, did 
not affect locomotor activity, suggesting that quinpirole potentiates 
the effects of MT on sleep duration without affecting the mo-
tor system. Western blot analyses revealed increased phos-
phorylation and activation of ERK1/2, p38 MAPK, and PKC in the 
cerebral cortex following treatment with quinpirole, MT, 
and pentobarbital. However, PKA activation was not involved 
in the effect of quinpirole on the duration of MT-augmented 
sleep. Although the precise molecular mechanisms by which 
quinpirole controls ERK1/2, p38 MAPK, and PKC remain un-
known, these findings suggest a unique role for MAPKs and 
PKC in the action of quinpirole.

Consistent with a previous report, systemic administration 
of MT prolonged the duration of pentobarbital-induced sleep 
and decreased the time to sleep onset (Wang et al., 2002). 
These findings suggest that MT prolongs the duration of pen-
tobarbital-induced sleep via the GABAergic system. Furth-
more, the additional effects of MT, such as locomotor depres-
sion, anti-convulsive effects, and analgesic effects, may be 
due to interaction with benzodiazepine receptors in the brain 
(Sugden, 1983). Indeed, MT binds to the GABA<sub>α</sub> receptor and 
inhibits <sup>[3H]</sup>diazepam binding in the brain (Holmes and Sug-
den, 1982). Thus, we investigated the effect of 30 mg/kg MT 
aone on locomotor activity and determined whether this dose 
produced hypnotic effects. In our mouse model, MT had no 
significant effect on locomotor activity and did not produce 
hypnotic effects, suggesting that 30 mg/kg MT augmented 
pentobarbital-induced sleep without modulating the motor 
system or producing sedative effects.

In our experiment, quinpirole affected the pentobarbital-
sleep onset time, but not the MT-sleep onset time. Because 
quinpirole as a D2-like receptor agonist produces a sedative 
effect related to GABA receptor modulation (Canales and 
Iversen, 2000; Joung et al., 2015), quinpirole may indirectly 
affect pentobarbital-induced sleep. However, in this study, 
quenpirole did not modulate the sleep onset time in MT-admin-
istered mice. There may be two reasons why quinpirole did not 
change the sleep onset time in MT-administered mice. First, 
as you can see in Fig. 2, 1 mg/kg quinpirole, and not 3 mg/
kg quinpirole, which was the highest dose used in our study, 
showed the highest effect on MT-pentobarbital sleep duration. 
However, 0.1 to 3 mg/kg quinpirole did not affect MT-induced 
sleep onset time. This result indicates that a shortened pen-
tobarbital-induced onset time by MT has the maximum effects. 
Second, it is well known that sleep onset time may be related 
to the GABA receptor channel opening time (Kim et al., 2012). 
In our experiment, quinpirole did not change the MT-induced 
sleep onset time. This result indicates that quinpirole may not 
change the channel opening time shortened by melatonin be-
cause the channel opening time is already shortened by mela-
tonin. Therefore, quinpirole may affect MT-induced pentobar-
bital sleep behavior by prolonging channel opening without a 
change in onset time.

Interestingly, we found that mice treated with the combi-
nation of quinpirole, MT, and pentobarbital were consistently 
asleep more than 3 hours after the last injection, while the 
mice in the other groups were fully awake at that time. These 
results suggest that the combination of quinpirole, MT, and 
pentobarbital prolongs sleep duration by affecting a specific 
signaling pathway. To identify the intracellular mechanisms 
underlying the effect of the combination of quinpirole, MT, and 
pentobarbital on sleep duration, we measured the abundance 
of proteins involved in cell signaling in the brain 3 hours after 
the last injection.
We investigated phosphorylation of ERK1/2, p38 MAPK, PKA, and PKC in mice treated with the combination of quinpirole, MT, and pentobarbital. Although few studies have reported that activation of MAPKs and protein kinases directly induces sleeping behavior, several studies suggest that activation of ERK signaling enhances sleeping behavior in Drosophila (Foltenyi et al., 2007), whereas PKA activation inhibits sleeping behavior induced by GABA receptor agonist baclofen (Datta, 2007). However, the relationship between p38 MAPK activation, PKC activation, and sleeping behavior has not been studied comprehensively. Our study is the first demonstration of the effects of protein kinase activation on pentobarbital-induced sleeping behavior in mice.

The ERK pathway is one of several signal transduction pathways that propagate and amplify cellular signaling (Lim et al., 2012). Several studies have reported modulation of sleeping behavior by ERK activation. Cortical ERK is involved in sleep consolidation in cats (Dumoulin et al., 2015). When the sleep-wake cycle was altered in cats, the number of hippocampal dendritic spines was altered via an ERK-dependent mechanism (Ikeda et al., 2015). In this study, quinpirole significantly increased cortical ERK activation. Quinpirole binds to D2-like receptors coupled to Gq proteins, thus activating PLCβ and inducing phosphorylation of ERK1/2 via the PKC/Ras/Raf/MEK pathway in neurons (Yan et al., 1999). Furthermore, activated ERK1/2 signaling increases GABA release in the brain (Cui et al., 2008), suggesting that phosphorylation of ERK1/2 in response to quinpirole may increase GABA currents by increasing GABA levels in the synaptic cleft, thereby potentiating sleeping behavior.

Increased p38 MAPK expression is associated with sleep apnea and disease-induced alterations in sleep patterns (Wood et al., 2006; Dyugovskaya et al., 2012). SB203580, a selective p38 MAPK inhibitor, inhibits hydrogen peroxide-induced GABAergic miniature IPSCs (Takahashi et al., 2007), whereas quinpirole increases p38 MAPK phosphorylation (Lee et al., 2006). Consistent with these reports, we found that expression of phospho-p38 MAPK was increased in the quinpirole-treated group, suggesting that quinpirole potentiates the effects of MT on sleep duration through GABAergic activity linked to the p38 MAPK pathway.

PKC activation has been observed in the hippocampus and prefrontal cortex of sleep-deprived rats (Abrail et al., 2015). Stimulation of D2-like receptors via activation of G-protein-coupled receptors modulates GABA_A receptors via PKC-dependent signaling pathways (Di Marzo et al., 1993; Brandon et al., 2002). In cortical neurons, a PKC-dependent pathway that does not involve changes in GABA_A receptor expression modulates GABA_A receptor phosphorylation and function (Brandon et al., 2000). Our findings suggest that PKC activation may influence the effect of quinpirole on sleep duration by potentiating the function of GABA_A receptors in the cerebral cortex.

Rapid eye movement (REM) sleep duration and non-rapid eye movement (NREM) fragmentation are increased in PKA knockout mice, suggesting that PKA may regulate sleep quantity (Hellman et al., 2010). In our study, ERK, p38 MAPK, and PKC were markedly activated in the cerebral cortex of mice treated with the combination of quinpirole, MT, and pentobarbital in comparison with mice treated with the combination of MT and pentobarbital or the combination of quinpirole and pentobarbital, suggesting that ERK, p38 MAPK, and PKC may be crucial mediators of the effect of quinpirole on sleeping behavior. However, PKA was not activated in the cerebral cortex of mice treated with quinpirole or MT; this discrepancy between previous reports and the present study may be due to differences in animals and protocols.

The effect of the combination of quinpirole, MT, and pentobarbital on sleep duration was similar to those of the combinations of MT and pentobarbital and quinpirole and pentobarbital, suggesting that quinpirole and MT may have additive effects on pentobarbital-induced sleeping behavior. The effect of a drug combination is classified as synergistic, additive, or antagonistic, respectively, when the effect is greater than, equal to, or less than the sum of the individual effects of each drug (Jia et al., 2009). The additive effects of drug combinations on particular targets are classified into several subtypes. In this study, the combination of quinpirole, MT, and pentobarbital activated ERK1/2, p38 MAPK, and PKC in the cerebral cortex, suggesting that quinpirole and MT may act via similar pathways, such as those associated with ERK1/2, p38 MAPK, and PKC; however, further studies are required to illuminate the mechanisms underlying the effects of quinpirole and MT on the brain.

The specified signaling pathways investigated by us may be indirectly involved in quinpirole-augmented modulation of sleeping behavior, because signaling pathways seem synergistically affected by the combination of quinpirole, MT, and pentobarbital. Although the mechanism between sleep maintenance and intracellular signaling pathway needs to be further explored, it is possible that phosphorylation of ERK, p38 MAPK, and PKC may be more active when sleep duration is maintained than when sleep duration decreases. Signaling modulation not only affects sleep duration but also sleeping behavior. Sleep increases the activation of ERK signaling; moreover, a molecular or behavioral feedback mechanism causes persistent intracellular signaling in Drosophila (Foltenyi et al., 2007). Thus, signaling modulation examined by us may be expressed longer and higher than sleep behavior change.

A majority of sedative hypnotics produce major side effects, including psychomotor impairment; therefore, many researchers have developed drugs aimed at maintaining sleep induction and reducing side effects, such as motor dysfunction (Miyamoto, 2009). In the present study, we investigated the modulatory effects of quinpirole and MT on locomotor activity. Pretreatment with MT, with or without quinpirole, had no effect on locomotor activity in mice. In addition, pretreatment with quinpirole had no significant effect on the total distance moved in comparison with that of saline. A study reported that a single systemic injection of quinpirole (5 or 10 mg/kg) increased locomotor activity in mice (Jung and Shim, 2011), whereas lower doses (0.1 mg/kg) of quinpirole decreased locomotor activity (Schindler and Carmona, 2002). In contrast, our analysis of the effects of quinpirole on locomotor activity suggests that quinpirole has no effect on the motor system at a dose of 1 mg/kg.

Taken together, our results suggest that the combination of quinpirole and MT increased the duration of pentobarbital-induced sleep by activating signaling pathways associated with MAPKs and PKC in the cerebral cortex. These findings enhance our understanding of the manner in which D2-like receptor activation enhances sleeping behavior in the presence of MT.
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REFERENCES

Abrial, E., Bétourné, A., Etélévant, A., Lucas, G., Scarna, H., Lambās-Señas, L. and Haddjeri, N. (2015) Protein kinase C inhibition rescues manic-like behaviors and hippocampal cell proliferation deficits in the sleep deprivation model of mania. Int. J. Neuropsycho- pharmacol. 18, pyu031.

Binfaré, R. W., Mantovani, M., Budni, J., Santos, A. R. and Rodrigues, A. L. (2010) Involvement of dopamine receptors in the antidepressant-like effect of melatonin in the tail suspension test. Eur. J. Pharmacol. 639, 78-83.

Bondi, C. D., McKeon, R. M., Bennett, J. M., Ignatius, P. F., Brydon, L., Jockers, R., Melan, M. A. and Witt-Endery, P. A. (2008) MT1 melatonin receptor internalization underlies melatonin-induced morphologic changes in Chinese hamster ovary cells and these processes are dependent on G-proteins, MEK 1/2 and microtubule modulation. J. Pineal Res. 44, 288-298.

Brandon, N. J., Delmas, P., Kittler, J. T., McDonald, B. J., Sieghart, W., Brown, D. A., Smart, T. G. and Moss, S. J. (2000) GABA receptors phosphorylation and functional modulation in cortical neurons by a protein kinase C-dependent pathway. J. Biol. Chem. 275, 38856-38862.

Brandon, N. J., Jovanovic, J. N., Smart, T. G. and Moss, S. J. (2002) Receptor for activated C kinase-1 facilitates protein kinase C-dependent phosphorylation and functional modulation of GABA receptors with the activation of G-protein-coupled receptors. J. Neurosci. 22, 6353-6361.

Canales, J. J. and Iverson, S. D. (2000) Dynamic dopamine receptor interactions in the core and shell of nucleus accumbens differentially coordinate the expression of unconditioned motor behaviors. Synapse 36, 297-306.

Costandi, M. (2013) Neurodegeneration: amyloid awakenings. Nature 497, 519-529.

Cui, Y., Costa, R. M., Murphy, G. G., Elgersma, Y., Zhu, Y., Gutmann, D. H., Parada, L. F., Mody, I. and Silva, A. J. (2008) Neurofibromin regulation of ERK signaling modulates GABA release and learning. Cell 135, 549-560.

Datta, S. (2007) Activation of pedunculopontine tegmental PKA prevents GABA receptor activation-mediated rapid eye movement sleep suppression in the freely moving rat. J. Neurophysiol. 97, 3841-3850.

Di Marzo, V., Vial, D., Sokoloff, P., Schwartz, J. C. and Piomelli, D. (1993) Selection of alternative G-mediated signaling pathways at the dopamine D2 receptor by protein kinase C. J. Neurosci. 13, 4846-4853.

Dimpfel, W. (2008) Pharmacological modulation of dopaminergic brain activity and its reflection in spectral frequencies of the rat electroencephalogram. Neuropsychopharmacology 58, 178-186.

Dumoulin, M. C., Aton, S. J., Watson, A. J., Renouard, L., Coleman, T. and Frank, M. G. (2015) Extracellular signal-regulated kinase (ERK) activity during sleep consolidates cortical plasticity in vivo. Cereb. Cortex 25, 507-515.

Dyugovskaya, L., Poljakov, A., Cohen-Kaplan, V., Lavie, P. and Lavie, L. (2012) Bax/Mcl-1 balance affects neutrophil survival in intermittent hypoxia and obstructive sleep apnea: effects of p38MAPK and ERK1/2 signaling. J. Transl. Med. 10, 211.

El Helou, J., Bélanger-Nelson, E., Freyburger, M., Dorsaz, S., Curie, T., La Spada, F., Gaudreault, P. O., Beaumont, É., Pouliot, P., Lesage, F., Frank, M. G., Franken, P. and Mongrain, V. (2013) Neuro-lgin-1 links neuronal activity to sleep-wake regulation. Proc. Natl. Acad. Sci. U.S.A. 110, 9974-9979.

Foltenyi, K., Greenspan, R. J. and Newport, J. W. (2007) Activation of EGFR and ERK by thomoid signaling regulates the consolidation and maintenance of sleep in Drosophila. Nat. Neurosci. 10, 1160-1167.

Hallman, K., Hernandez, P., Park, A. and Abel, T. (2010) Genetic evidence for a role for protein kinase A in the maintenance of sleep and thalamic oscillations. Sleep 33, 19-29.

Holmes, S. W. and Sugden, D. (1982) Effects of melatonin on sleep and neurochemistry in the rat. Br. J. Pharmacol. 76, 95-101.

Ikeda, M., Hojo, Y., Komatsuzaki, Y., Okamoto, M., Kato, A., Takeda, T. and Kawato, S. (2015) Hippocampal spine changes across the sleep-wake cycle: corticosterone and kinases. J. Endocrinol. 226, M13-M27.

Jia, J., Zhu, F., Ma, X., Cao, Z., Li, Y. and Chen, Y. Z. (2009) Mechanisms of drug combinations: interaction and network perspectives. Nat. Rev. Drug Discov. 8, 111-128.

Joung, H. Y., Kang, Y. M., Lee, B. J., Chung, S. Y., Kim, K. S. and Shim, I. (2015) Sedative-Hypnotic and Receptor Binding Studies of Fermented Marine Organisms. Biomol. Ther. (Seoul) 23, 479-485.

Jung, E. Y. and Shim, I. (2011) Differential DAergic Control of D1 and D2 Receptor Agonist Over Locomotor Activity and GABA Level in the Striatum. Exp. Neurobiol. 20, 153-157.

Kim, J. W., Kim, C. S., Hu, Z., Han, J. Y., Kim, S. K., Yoo, S. K., Yeo, Y. K., Chong, M. S., Lee, K., Hong, J. T. and Oh, K. W. (2012) Enhancement of pentobarbital-induced sleep by apigenin through chloride ion channel activation. Arch. Pharm. Res. 35, 367-373.

Lee, M. Y., Heo, J. S. and Han, J. H. (2006) Dopamine regulates cycle rhythmicity of sleep-related proteins via cAMP. Carβ1/PKα, MAPKs, and NF-kB in mouse embryonic stem cells. J. Cell. Physiol. 208, 399-406.

Lees, G., Edwards, M. D., Hassoni, A. A., Ganelin, C. R. and Galanis, K. (D) (1998) Modulation of GABA(A) receptors and inhibitory synaptic currents by the endogenous CNS sleep regulator ciso,9,10-octadecenoic acid (c0A). Br. J. Pharmacol. 124, 873-882.

Li, G. L., Li, P. and Yang, X. L. (2001) Melatonin modulates γ-aminobutyric acid, receptor-mediated currents on isolated carp retinal neurons. Neurosci. Lett. 301, 49-53.

Lim, H., Jang, S., Lee, Y., Moon, S., Kim, J. and Oh, S. (2012) Enhancement of Anxiety and Modulation of TH and pERK Expressions in Amygdala by Repeated Injections of Corticosterone. Bio- mol. Ther. (Seoul) 20, 418-424.

Ma, H., Kim, C. S., Ma, X., Nam, S. Y., Kim, D. S., Woo, S. S., Hong, J. T. and Oh, K. W. (2009) Magnolol enhances pentobarbital-induced sleeping behaviors: possible involvement of GABAergic systems. Phytother. Res. 23, 1340-1344.

Ma, Y., Han, H., Nam, S. Y., Kim, Y. B., Hong, J. T., Yun, Y. P. and Oh, K. W. (2008) Cylcopidele alkaloid fraction from Zizyphi Spinosi Semen enhances pentobarbital-induced sleeping behaviors. J. Ethnopharmacol. 117, 318-324.

Miyamoto, M. (2009) Pharmacology of ramelteon, a selective MT1/MT2 receptor agonist: a novel therapeutic drug for sleep disorders. CNS Neurosci. Ther. 15, 32-51.

Pandi-Perumal, S. R., Trakht, I., Srinivasan, V., Spence, D. W., Mestrioni, G. J., Zisapel, N. and Cardinali, D. P. (2008) Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Prog. Neurobiol. 85, 335-353.

Parry, B. L., Fernández Martínez, L., Maurer, E. L., López, A. M., Sersenon, D. and Melissi, C. J. (2006) Sleep, rhythms and women’s mood: Part II. Menopause. Sleep Med. Rev. 10, 197-208.

Proença, M. B., Dombrowski, P. A., Da Cunha, C., Fischer, L., Ferraz, A. C. and Lima, M. M. (2014) Dopaminergic D2 receptor is a key player in the substantia nigra pars compacta neuronal activation mediated by REM sleep deprivation. Neuropeuropharmacology 76 PT A, 119-126.

Schindler, C. W. and Carmona, G. N. (2002) Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. Pharmacol. Biochem. Behav. 72, 857-863.

Shah, V. K., Choi, J. J., Han, J. Y., Lee, M. K., Hong, J. T. and Oh, K. W. (2014) Pachymic Acid Enhances Pentobarbital-Induced Sleeping Behaviors via GABAergic Systems in Mice. Biomol. Ther. (Seoul)
22, 314-320.
Sugden, D. (1983) Psychopharmacological effects of melatonin in mouse and rat. J. Pharmacol. Exp. Ther. 227, 587-591.

Takahashi, A., Mikami, M. and Yang, J. (2007) p38 mitogen-activated protein kinase independent SB203580 block of H2O2-induced increase in GABAergic mIPSC amplitude. Neuroreport 18, 963-967.

Vilar, A., de Lemos, L., Patraca, I., Martinez, N., Folch, J., Junyent, F., Verdaguer, E., Pallàs, M., Aulaüell, C. and Camins, A. (2014) Melatonin suppresses nitric oxide production in glial cultures by pro-inflammatory cytokines through p38 MAPK inhibition. Free Radic. Res. 48, 119-128.

Volkow, N. D., Tomasi, D., Wang, G. J., Telang, F., Fowler, J. S., Logan, J., Benveniste, H., Kim, R., Thanos, P. K. and Ferré, S. (2012) Evidence that sleep deprivation downregulates dopamine D2R in ventral striatum in the human brain. J. Neurosci. 32, 6711-6717.

Wan, X., Mathers, D. A. and Puil, E. (2003) Pentobarbital modulates intrinsic and GABA-receptor conductances in thalamocortical inhibition. Neuroscience 121, 947-958.

Wang, F., Li, J. C., Wu, C. F., Yang, J. Y., Xu, F. and Peng, F. (2002) Hypnotic activity of melatonin: involvement of semicarbazide hydrochloride, blocker of synthetic enzyme for GABA. Acta Pharmacol. Sin. 23, 860-864.

Wilhelmsen-Langeland, A., Saxvig, I. W., Pallesen, S., Nordhus, I. H., Vedaa, Ø., Lundervold, A. J. and Bjorvatn, B. (2013) A randomized controlled trial with bright light and melatonin for the treatment of delayed sleep phase disorder: effects on subjective and objective sleepiness and cognitive function. J. Biol. Rhythms 28, 306-321.

Wood, L. J., Nail, L. M., Perrin, N. A., Elsea, C. R., Fischer, A. and Druker, B. J. (2006) The cancer chemotherapy drug etoposide (VP-16) induces proinflammatory cytokine production and sickness behavior-like symptoms in a mouse model of cancer chemotherapy-related symptoms. Biol. Res. Nurs. 8, 157-169.

Yan, Z., Feng, J., Fienberg, A. A. and Greengard, P. (1999) D2 dopamine receptors induce mitogen-activated protein kinase and cAMP response element-binding protein phosphorylation in neurons. Proc. Natl. Acad. Sci. U.S.A. 96, 11607-11612.

Zawilska, J. and Iuvone, P. M. (1990) Alpha-2 adrenergic activity of bromocriptine and quinpirole in chicken pineal gland. Effects on melatonin synthesis and [3H]rauwolscine binding. J. Pharmacol. Exp. Ther. 255, 1047-1052.