mGlu2 Receptor Agonism, but Not Positive Allosteric Modulation, Elicits Rapid Tolerance towards Their Primary Efficacy on Sleep Measures in Rats

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Abstract

G-protein-coupled receptor (GPCR) agonists are known to induce both cellular adaptations resulting in tolerance to therapeutic effects and withdrawal symptoms upon treatment discontinuation. Glutamate neurotransmission is an integral part of sleep-wake mechanisms, which processes have translational relevance for central activity and target engagement. Here, we investigated the efficacy and tolerance potential of the metabotropic glutamate receptors (mGluR2/3) agonist LY354740 versus mGluR2 positive allosteric modulator (PAM) JNJ-42153605 on sleep-wake organisation in rats. In vitro, the selectivity and potency of JNJ-42153605 were characterized. In vivo, effects on sleep measures were investigated in rats after once daily oral repeated treatment for 7 days, withdrawal and consecutive re-administration of LY354740 (1–10 mg/kg) and JNJ-42153605 (3–30 mg/kg). JNJ-42153605 showed high affinity, potency and selectivity at mGluR2. Binding site analyses and knowledge-based docking confirmed the specificity of JNJ-42153605 at the mGluR2 allosteric binding site. Acute LY354740 and JNJ-42153605 dose-dependently decreased rapid eye movement (REM) sleep time and prolonged its onset latency. Sub chronic effects of LY354740 on REM sleep measures disappeared from day 3 onwards, whereas those of JNJ-42153605 were maintained after repeated exposure. LY354740 attenuated REM sleep homeostatic recovery, while this was preserved after JNJ-42153605 administration. JNJ-42153605 enhanced sleep continuity and efficiency, suggesting its potential as an add-on medication for impaired sleep quality during early stages of treatment. Abrupt cessation of JNJ-42153605 did not induce withdrawal phenomena and sleep disturbances, while the initial drug effect was fully reinstated after re-administration. Collectively, long-term treatment with JNJ-42153605 did not induce tolerance phenomena to its primary functional effects on sleep measures, nor adverse effects at withdrawal, while it promoted homeostatic recovery sleep. From the translational perspective, the present rodent findings suggest that mGluR2 positive allosteric modulation has therapeutic potential based
on its superior long term efficacy over agonists in psychiatric disorders, particularly of those commonly occurring with REM sleep overdrive.

Introduction

Abnormalities in glutamate balance have been recently implicated in the mechanisms underlying neuropsychiatric illnesses. The glutamate signalling through the metabotropic glutamate receptor 2 (mGluR2) is actively pursued in academia and pharmaceutical groups as a promising therapeutic approach to normalize excessive glutamate flow [1–9]. Given the potential for induction of tolerance with GPCR agonists, an important question remains unanswered regarding efficacy and safety following longer term use of the mGluR2 agonist, as well as the duration of its effectiveness. Preclinical studies have reported behavioral data with differential effects regarding the potential for tolerance development following chronic dosing with mGluR2 agonists, depending on behavioural pharmacology assessed: LY379268 had motor depressant effect, to which animals developed rapid tolerance following repeated administration [10–11]. In drug abuse model, repeated administration of LY379268 reduced toluene-induced hyperlocomotion [12], whereas repeated dosing with LY379268 had no effect on PCP-induced hyperlocomotor activity [10]. In addition, subchronic treatment with LY354740 failed to reverse ketamine-evoked prepulse inhibition deficits and hyperlocomotion [6]. Moreover, acute LY379268 was effective in pain models; while tolerance developed against its analgesic effect upon repeated dosing [13].

In mammals and submammalian species, sleep is regulated by homeostatic and circadian factors. The brain structures actively involved in waking are located in the basal forebrain, hypothalamus and brainstem pons, whereas the active sleep mechanisms mainly reside in the preoptic/anterior hypothalamic area. Glutamate is, in addition to acetylcholine, monoamines and hypocretin/orexin, an important factor with dual function in waking and sleeping. Glutamate release follows daily fluctuation rhythms, with peak levels during waking and Rapid Eye Movement (REM) sleep. Suprachiasmatic nucleus (SCN) projections to the ventromedial and ventrolateral regions of the preoptic area use both glutamate and GABA as stimulatory and inhibitory inputs, respectively for the control of the sleep-wake rhythms [14]. The glutamate input from SCN to paraventricular neurons [15,16] is believed to stimulate melatonin synthesis [17]. In addition, microdialysis studies demonstrated an enhancement of glutamate release in the orbitofrontal cortex during REM sleep in rats [18]. Moreover, real-time biosensor measurement of neurotransmitters release across the sleep-wake cycle revealed increases in glutamate level in the pre-frontal cortex during wakefulness, decreases during sleep episodes and spike increases during REM sleep [19]. Furthermore, several research groups investigated the effects of mGluR modulation on sleep [20–24]. mGluR2’s are highly expressed in the limbic amygda-loid nuclei [25–27], known to play a pivotal role in the regulation of REM sleep [23,28]. The role of mGluR2 in the regulation of the sleep-wake cycle has been supported by using specific pharmacological agents on this receptor. Great consistency was found across all studies with respect to the suppressing effect on REM sleep following the activation of mGluR2 [20,21,23], whereas blockade of mGluR2 elicited waking [21,29]. The specific suppression effect on REM sleep has been confirmed in WT but not mGluR2 (-/-) mice [20]. Collectively, these studies provide evidence for a strong relationship between glutamate neurotransmission and sleep mechanisms. In addition, empirical data showed a clear-cut relationship between sleep disturbances and mental disorders involving glutamatergic abnormalities, which lends credence for
the study of glutamate abnormalities as a potential common factor. Therefore, sensitive experimental paradigms such as the sleep-wake model can be advantageous applied in translational research to screen potential mGluR2-based therapeutic compounds.

Recently we have developed a specific PAM JNJ-42153605 with good metabolic stability, displaying high in vitro potency and good selectivity for the mGluR2 with an acceptable pharmacokinetic profile in both rodent and non-rodent species [30]. The present studies were aimed at investigating whether chronically activated mGluR2 results in rapid tolerance towards its primary functional effects on sleep and arousal behavior. To this end, after characterisation of the test compound’s specificity, selectivity, molecular binding and docking characteristics, the efficacy and tolerance potential of effects on sleep-wake behaviour in rats after once daily dosing was compared between the mGluR2/3 agonist LY354740 and the PAM JNJ-42153605.

Material and Methods

1. In vitro pharmacology

For functional [35S]GTPγS binding, Chinese Hamster Ovary (CHO) cells expressing the human or rat mGluR2 were grown until 80% confluence, washed in ice-cold phosphate-buffered saline and homogenized. Protein concentrations were measured by the Bio-Rad protein assay using bovine serum albumin as standard. Final assay mixtures contained 7 (human mGluR2) or 10 (rat mGluR2) μg of membrane protein were pre-incubated with JNJ-42153605 alone (determination of agonist effects) or together with an EC20 concentration (4 μM) of glutamate (determination of PAM effects) for 30 min at 30°C. [35S]GTPγS was added at a concentration of 0.1 nM and Filter-bound radioactivity according to an earlier protocol [31].

For mGluR2 competition binding, membranes from human mGluR2-CHO cells were homogenized and suspended in assay mixtures with 2 nM [3H]LY341495 and appropriate concentrations of JNJ-42153605. Non-specific binding was determined in the presence of 1 mM glutamate and was about 10% of total binding.

For mGluR2 selectivity, Ca2+ assays (Fluorescent Drug Screening system, FDSS, Hamamatsu) were used to assess the activity at the human mGluR1, 3, 5, 7 or 8 receptor and [35S]GTPγS binding experiments were performed to evaluate the activity of JNJ-42153605 on the rat mGluR6 and human mGluR4 using the protocol described elsewhere [31].

JNJ-42153605 was furthermore tested for its inhibition of radioligands binding to a battery of neurotransmitter and peptide receptors, ion channels and transporters (CEREP, Celle L’Evescault, France).

2. Building an mGluR2 receptor homology model

A model of the active state 7TM domain of the human mGluR2 (Uniprot code Q14416) bound to G protein was built using several structural templates. The crystal structure of human mGluR5 (PDB 4OO9, [32]) was used to model all 7TM helices except TM6. The β2AR (PDB ID 3SN6, [33]) active structure was used to model both the distinct open TM6 conformation as well as the G protein. Extracellular loop 2 (ECL2) is not refined in the mGluR5 X-ray structure and was therefore modelled based on the mGluR1 crystal structure (PDB 4OR2, [34]). The sequence alignment for the model building is provided in S1 Fig. The mGluR2 7TM monomer can be activated upon PAM binding [35]. Overall sequence identity between mGluR2 and mGluR5 7TM’s was 51%. The initial model was constructed in MOE v2014.9 (Chemical computing group Inc., Montreal, QC, Canada) and then Maestro (Schrodinger LLC, New York, NY, USA) was used for structure preparation. Amino acid numbering is based on recent recommendations [36].
2.1 Docking of JNJ-42153605. The ligand was prepared for docking using Maestro. Conformational sampling was performed with ConfGen and multiple conformers were docked into the mGlu2 active state model using Glide XP. The docking grid was centered on the ligand position in the mGluR1 structure. Sampling was increased in the Glide docking by turning on expanded sampling and passing 100 initial poses to post-docking minimisation. All other docking parameters were set to the defaults.

3. In vivo pharmacology

3.1 Animals, surgery and polysomnography recordings. All animal studies have been carried out in accordance with guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), and of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by Janssen Pharmaceutical Ethical Committee. Every effort was made to minimize animal use and disturbances in animal well-being and experimental animals were euthanized at the end of the study by common rodents CO2 procedure. Sixty-four male Sprague Dawley rats (Crl:SD, Charles River, France) were housed in controlled environmental conditions throughout the study: 22°C ± 2°C ambient temperature, relative humidity 60%, standard 12:12 light cycle regime (lights on from 12:00 a.m. to 12:00 p.m., illumination intensity: ~ 100 lux (i.e. at the height of the recording box, while a recessed lighting consoles managed diffuse and uniform light levels below 60 lux within cages, a software-controlled dimmer handled a gradual transition between light and dark cycles). Standard rodent pellets and tap water were provided ad libitum.

Surgery was performed using the protocol described earlier [20]. In short, a mixture of 30% O2, 70% N2O and 5% isoflurane was administered to animals as an initial induction for 2 minutes. Then, the animals were mounted in a stereotaxic apparatus and were given a continuous constant mixture of O2, N2O and 2% isoflurane. An analgesic Piritramide (dipidolor) was administered before the incision over the total length of the head. The oval area of the scalp was removed, and the uncovered skull was cleared of the periosteum in order to place 4 fixing stainless steel screws for the recording of the frontal and parietal electroencephalogram (EEG). For the recording of the electro-oculogram (EOG) and electromyogram (EMG), stainless steel wires were placed in peri-orbital and into the nuchal muscle, respectively. Electrodes were fitted into an 8 holes connector and were fixed with dental cement to the cranium.

After a recovery period of at least 10 days, animals were gently handled twice a day for one week: rats were quietly held and gently stroked by hand and were increasingly allowed to explore and become familiar with the experimenter’s hands. Afterwards, the rats were systematically habituated to their surroundings and recording procedure. These habituation procedures enable a reduction in anxiety and stress that may occur during the experimental procedure and therefore reduce subsequent consequences for data collection. A welfare monitoring of each animal was managed by using an animal inventory system taking to consideration regular general observations of behavior and physical health. During the adaptation period and recordings, animals were carefully connected via a rotating swivel to a bipolar recorder amplifier (Embla, MedCare Flaga, Iceland) for recording of polygraphic signals with an input range of +/- 500 mV.

All experiments were performed in a large scale EEG laboratory setting under controlled conditions, in which animals were kept in their home cage placed in recording boxes six hours before the start of the first baseline recording and throughout the chronic experiments in order to avoid any stress that may result from cage changes and displacement of home-cages from the holding room to recording room and vice-versa. The first recording session started at 14:00 pm (i.e. end of the second hour of the light period) and lasted 20-h after the administration of
saline in vehicle and drug-treated groups. The selection of this post-acrophase of sleep for the timing of pharmacological administration, is taken as phase reference when sleep pressure is neither maximal nor minimal, thus allowing for assessment and observation of subtle drug effects on sleep parameters. The consecutive recordings were performed for the same duration and circadian conditions following repeated treatments. In the first group of animals (n = 32), polygraphic recordings were performed during 7 consecutive days following once daily administration of LY354740 (1, 3 and 10 mg/kg) and of vehicle for the control group (n = 8 for each condition). In the second group of animals (n = 32), recordings were performed during 7 consecutive days following once daily administration of JNJ42153605 (3, 10 and 30 mg/kg) and of vehicle for the control group (n = 8 for each condition), during the 3 days after withdrawal, and during 2 subsequent days after re-administration of the compound or vehicle.

Although the level of occupancy required for REM inhibition may be different for each pharmacological mechanism, the dose-ranges for both compounds were selected to ensure equipotent dose effects for JNJ-42153605 and LY354740 on REM sleep inhibition. Given the relative short half-life ($T_{1/2}$) values of both drugs given at a single dose (JNJ42153605 at 10 mg/kg: 2.7 ± 0.2-h; and LY354740 at 1 mg/kg: 0.91 ± 0.3-h), a once 24-h dosing schedule allows for comparing development of tolerance while taking pharmacokinetic parameters into consideration.

3.2 Vigilance states analysis. A sleep-wake analysis system was applied for 20 continuous hours following acute and sub-chronic administration of LY354740 or JNJ-42153605. As previously described [20], the discriminative analysis uses classification rules to assign the sleep-wake stages based on 6 EEG frequency domain values ($\delta$: 0.5–4 Hz, $\theta$: 4.2–8 Hz, $\alpha$: 8.2–12 Hz, $\sigma$: 12.2–14 Hz, $\beta$: 14.2–30 Hz, $\gamma$: 30.2–50 Hz), integrated EMG, EOG and body activity level.

Six vigilance states were classified as being indicative of respectively active wakefulness, passive wakefulness, light sleep, deep sleep, intermediate stage or REM sleep. Briefly, different vigilance states were characterized as follows: Active wake: low-voltage fast EEG activity, high EMG activity, numerous eye movements and high body activity; Passive wake: low-voltage fast EEG activity, high to moderate EMG activity, numerous eye movements and absence of body activity; Light sleep: high-voltage slow cortical waves interrupted by low-voltage fast waves and reduced EMG activity; Deep sleep: continuous high-amplitude slow-wave activity in EEG in absence of EMG, EOG and body activity; Intermediate sleep: transient spindle activity with theta rhythm, absence of EOG and body movements; REM sleep: low-voltage fast cortical waves with a regular theta rhythm, presence of rapid eye movements and absence of muscular and body movements. The scores were synchronized in time with the EEG signal and different sleep-wake parameters were calculated, such as the amount of time spent in each vigilance state, the number and duration of episodes in each state, latencies for deep sleep and REM sleep and the number of shifts from one state to another one. For each sleep state, the latency was defined as the time between the beginning of the recording and the appearance of the first sleep period lasting at least 20 consecutive seconds. To determine whether LY354740- and JNJ-42153605 induced inhibition of REM sleep during the light phase would influence the pattern of late REM sleep recovery, the total time spent in REM sleep over the entire dark phase of the circadian time were assessed.

4. Drugs

(+)-2-aminobicyclo [3.1.0]hexane-2,6-dicarboxylate (LY354740) and 1,2,3-triazolo[4,3-a]pyridine (JNJ-42153605) were synthesized at Janssen Research and Development laboratories. For oral administration in vivo studies, LY354740 was prepared in 10% Cyclodextrin + NaOH, while JNJ-42153605 was dissolved in 20% Cyclodextrin + HCl. All drugs were given at a volume of 10 ml/kg of body weight in rat. An equivalent volume of vehicle was administered in control animals.
5. Statistical analysis

The time course of different sleep variables following drug treatment were expressed as the mean ± S.E.M averaged within each treatment group and presented as mean values over periods of 60 min. The Wilcoxon Mann–Whitney Signed Rank test with Bonferroni correction tests was used to compare the values of sleep parameters. Probabilities of less than 0.05 were considered statistically significant. Some derived sleep variables (e.g. REM sleep time) were calculated over 4-h post-treatment: the rational for this 4-h period is based on pharmacokinetic parameters such as half-lives of both compounds indicating that direct effects were expected to occur over this time interval. In addition, a mixed-model ANOVA was used to further analyse the dose–response changes in time spent in each vigilance states. Post hoc tests were done comparing the treatment group (drug) to the reference treatment level (vehicle) at each time point. Raw p-values were computed and adjusted for multiplicity by false discovery rate procedure.

Results

1. In vitro pharmacology

Fig 1A represents the chemical structure of the mGluR2 PAM JNJ-42153605. The pharmacological specificity of JNJ-42153605 was confirmed in binding and functional studies. At the human mGlu2R, JNJ-42153605 potentiates [35S]GTPγS binding induced by 4 μM of glutamate (∼ EC20) up to 285 ± 34%, with an EC50 of 17 ± 6 nM (n = 10; Fig 1B). JNJ-42153605 also activates the receptor on its own, although with a lower potency and to a lower extent compared to its treatment combined with low concentrations of glutamate (EC50 = 270 ± 65 nM, Emax = 67 ± 8%; n = 9).

Consistent with the effects of an allosteric potentiator, JNJ-42153605 shifts the concentration–response curve of glutamate to the left (Fig 1C left panel), increasing the potency of glutamate up to ∼ 25-fold. At the human mGluR2, the EC50 of glutamate decreases from ∼ 10 to 0.5 μM with the addition of 3 μM JNJ-42153605. Similar results were obtained in [35S]GTPγS assays using CHO cells expressing the rat mGluR2 receptor variant (Fig 1C, right panel).

To confirm whether JNJ-42153605 binds at a site distinct from the glutamate recognition site, we evaluated the potency of the compound to displace binding of [3H]LY341495, an orthosteric mGluR antagonist [37]. [3H]LY341495 binding to the human mGluR2 was inhibited by LY404039, an mGluR2/3 orthosteric agonist, but not by JNJ-42153605 (Fig 1D), clearly indicating that JNJ-42153605 does not bind to the orthosteric mGluR2 binding site.

JNJ-42153605 is a selective mGluR2 PAM, devoid of agonist or antagonist activity at the human mGluR1, 4, 5, 6, 7 or 8 up to 30 mM (data on file). However, JNJ-42153605 showed some hmGluR3 PAM activity (EC50 = 770 nM), but with an about 50-fold lower potency compared to the hmGluR2. Moreover, JNJ-42153605 was found inactive in a battery of GPCRs and ion channels tested in a broad CEREP profiling (data on file). These results demonstrate that JNJ-42153605 is a highly selective and potent PAM at the mGluR2.

2. Docking of JNJ-42153605 into the mGluR2 7TM Model

JNJ-42153605 was docked into the active state mGluR2 homology model (Fig 2) and the preferred binding pose is shown. The triazopyridine scaffold binds in the hydrophobic cluster formed between amino acids L6393.32a.36c, F6433.36a.40c, L7325.43a.44c, W7736.48a.50c and F7766.51a.53c. The cyclopropylmethyl substituent interacts deeper in the receptor with F7766.51a.53c. Aromatic residues L6393.32a.36c and F6433.36a.40c interact on one face of the triazopyridine scaffold and L7325.43a.44c and W7736.48a.50c on the other. Amino acid N7355.47a.47c acts as an H-bond donor to the nitrogen acceptor in the triazo ring of the scaffold. Tryptophan
W7736.48a.50c is conserved in the mGluRs and its sidechain adopts an outwards orientation pointing to the membrane in the mGluR5 crystal structure. Further modelling work reveals this sidechain to be flexible and it is shown in Fig 2 in an inwards orientation. The cyclopropyl group makes a steric interaction with W7736.48a.50c at approximately 3 Å distance. The CF₃ group sits in a small cavity between TM3 and TM5 formed above N7355.47a.47c, it interacts with S7315.42a.43c. The 4-phenylpiperidine substituent points towards the extracellular side of receptor and the distal phenyl interacts with H723ECL2. The predicted binding mode for JNJ-42153605 overlaps with the allosteric site in mGluR1 whereas the mGluR5 modulator goes deeper into the receptor, see S2 Fig.

3. In vivo pharmacology

**LY354740: acute and subchronic effects on sleep-wake measures.** Treatment day 1: Acute subcutaneous administration of LY354740 (1, 3 and 10 mg/kg) produced significant
changes in the distribution of sleep-wake states over the entire recording period, notably a significant decrease in total amount of REM sleep (“treatment x time” interaction: F(12, 1232) = 5.9, p < 0.0001) (Fig 3).

Closer analysis of time distribution among arousal and sleep stages indicated that LY354740 (1, 3, and 10 mg/kg) dose-dependently reduced REM sleep (“treatment x time” interaction: F(9, 212) = 5.9, p < 0.0001) for up to 4-h following dosing on day 1 (Fig 4A).

The suppression of REM sleep was derived from a reduction in both numbers and mean durations of this vigilance state. Consequently, decreases in total sleep time and sleep efficiency resulted from direct effect of LY354740 on REM sleep mechanisms (Fig 4B, inset). Concomitant to changes in REM sleep state, LY354740 consistently lengthened REM sleep onset latency F(3, 28) = 12.8, p < 0.0001) (Fig 4A, inset).

Treatment days 2 till 7: LY354740 consistently reduced the duration of REM sleep up to 4-h post-administration on subsequent day 2 (“treatment” F(3, 28) = 13.3, p < 0.0001), “time” F(3, 212) = 39.4, p < 0.0001), however the ANOVA interaction “treatment x time” did not reach significance level; F(9, 212) = 1.23, p = 0.27) (Fig 4A). Similarly, the intermediate state was reduced (“treatment” F(3, 28) = 6.1, p = 0.0026), “time” F(3, 212) = 22.2, p < 0.0001), however, the ANOVA interaction “treatment x time” did not reach significance level; F(9, 212) = 1.24, p = 0.27) (Fig 4A).

Total sleep time and sleep efficiency were decreased only at the highest dose of the compound. However, as from day 3 onwards LY354740 did not lead to significant changes in total amount of different vigilance states and sleep parameters including REM sleep (Fig 4A). Hence, no withdrawal and re-administration of the mGluR2 was considered afterwards.

**JNJ-42153605: acute and subchronic effects on sleep-wake measures.** Treatment day 1: Oral administration of JNJ-42153605 (3, 10 and 30 mg/kg) significantly reduced REM sleep
JNJ-42153605 consistently decreased REM sleep and intermediate stage sleep ("treatment x time" interaction: F(12, 1232) = 10.6, p < 0.0001) and intermediate stage sleep ("treatment x time" interaction: F(12, 1232) = 14.3, p < 0.0001) (Fig 5).

JNJ-42153605 consistently increased REM sleep and intermediate stage sleep during the first 4-h post-administration ("treatment x time" interaction: F(9, 212) = 3.6, p = 0.0003) and ("treatment x time" interaction: F(9, 212) = 5.9, p < 0.0001), respectively (Fig 6A). Concomitant to changes in REM sleep, JNJ-42153605 consistently lengthened REM sleep onset latency (F(3, 28) = 11.8, p < 0.0001) (Fig 6A, inset). Interestingly, JNJ-4215605 enhanced total sleeping time during the first 2 days following the administration (F(3, 28) = 5.4, p = 0.005) and (F(3, 28) = 2.9, p = 0.04) and increased sleep efficiency (Fig 6B and B inset) likely associated with the effects of the compound on deep sleep. JNJ-42153605 did not modify sleep parameters and total number of transitions from any sleep stage towards wakefulness.
Treatment days 2 till 7: Subsequent oral administration of JNJ-42153605 was accompanied with consistent reduction of REM sleep (“treatment x time” interaction: F(9, 212) = 3.07, p = 0.001) and enhancement of deep sleep (“treatment x time” interaction: F(9, 212) = 11.2, p = 0.0001). Repeated oral administration up to 7 days of JNJ-42153605 consistently reduced REM sleep and enhanced deep sleep during the first 4-h post-administration (Fig 6A). JNJ-42153605 maintained efficacy on REM sleep onset latencies over repeated treatment (Fig 6A, inset). Interestingly, JNJ-42153605 enhanced sleep maintenance and sleep efficiency, particularly with the higher dose of the compound (Fig 6B, inset), which may suggest a potential for improving sleep quality at early stage of the treatment.

Withdrawal days 8 till 10: During discontinuation of JNJ-42153605, the amount of different vigilance states was normalized and sleep parameters returned to baseline at cessation of treatment indicating that discontinuation of the compound did not result in withdrawal phenomena and sleep disturbances (Fig 6A and 6B).

Treatment days 11 till 12: In the subsequent re-administration 2 days, JNJ-42153605 elicited consistent enduring modifications of sleep-wake architecture similar to those observed in the initial subchronic treatment (Fig 6A and 6B). JNJ-42153605 reduced REM sleep during the first 4-h post-treatment (“treatment x time” interaction: F(9, 212) = 3.22, p = 0.001) and (“treatment x time” interaction: F(9, 212) = 3.26, p = 0.001), respectively, as well as lengthened REM sleep onset latency (F(3, 28) = 5.66, p = 0.003) and (F(3, 28) = 19.8, p < 0.0001).

Fig 4. Sleep parameters following repeated dosing with LY354740 (1, 3, 10 mg/kg) or vehicle on experimental days (D1 first treatment day till D7 seventh treatment day). Total sleep time expressed in minutes (Panel A) and sleep efficiency expressed in percentage (Panel A inset). REM sleep amount during the first 4-h of recording sessions after repeated administration (Panel B) and REM sleep latency (Panel B inset). Total amount of REM sleep expressed in minutes that was recovered during the subsequent 10-h dark period (Panel C). The data are presented as means ± S.E.M. Significant differences between the vehicle and LY354740 group are indicated by the drug-dose symbol.

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During the 10-h dark period, total REM sleep amounts in rats treated with JNJ-42153605 showed significantly higher REM sleep recovered than the control vehicle treated animals (Fig 6C). Overall, the repeated recovery effects that were seen predominantly during the dark (active) phase did not persist in the subsequent light phase.

**Discussion**

In the present study, JNJ-42153605 showed high affinity, potency and selectivity at mGluR2. We further report perspective on the allosteric binding site of mGluR2, the molecular docking analysis and the specificity of JNJ-42153605 at the mGluR2 allosteric binding site as compared with other mGluRs.

**Proposed binding mode of JNJ-42153605 at the mGluR2**

Multiple mutagenesis studies concluded the presence of the 7TM allosteric binding site at mGluR2 [38–42]. In a recent report from our labs we published results from a large site
directed mutagenesis study to clarify the binding mode of multiple mGluR2 PAMs [38]. JNJ-42153605 was part of that study although results were not included in the manuscript for reasons of space. That work confirmed a role for mutants F643A, H723V, L732A, N735D, W773A, and F776A on the PAM activity of JNJ-42153605. Recently, the mGluR1 and mGluR5 crystal structures have surpassed mutagenesis data by revealing the specifics of ligand receptor interaction. Comparing the binding mode of JNJ-42153605 (see S2 Fig) reveals good shape and volume overlap with that of FITM in the mGluR1 structure. However, in the case of the mGluR5, Mavoglurant penetrates deeper into the receptor due to a single amino acid difference (P6553.36a.40c in mGluR5). This amino acid opens a narrow channel suitable for binding of the aryl acetylene motif which is common in mGlu5 allosteric modulators. The same position in mGlu2 corresponds to F643 which does not allow the ligand to bind so deep into the receptor. Hence, small changes in amino acids can make a difference to the binding site and contribute to specificity.

Comparison of the binding site amino acids (see S3 Fig) can further explain the specificity of JNJ-42153605. In particular, a key hydrogen bond is formed between the ligand which acts as acceptor and N7355.47a.47c which behaves as donor. Interestingly, this amino acid is an aspartate in mGlu3 and hence being deprotonated is unable to act as an H-bond donor, or may have a different adjacent water network. Considering the structure activity relationships of mGluR2 PAMs and the presence of an H-bond acceptor in this position, along with the small adjacent
substituent (in this case CF₃) is crucial for activity. Hence it seems likely that this key interaction with N735.47a.47c and the insertion of the small hydrophobic substituent towards S731.42a.43c, is likely a key interaction motif of mGlu2 PAMs. The combination of shape complementarity, changes in pocket volume, and specific amino acid interactions contribute to the specificity of JNJ-42153605.

Recent work in our labs has focused on the detailed characterization of the radioligand [³H]-JNJ-46281222 [43]. A detailed modelling study was included in that work which took advantage of SAR knowledge and experimental mutagenesis data reported therein and previously [38], to derive a binding mode for the JNJ-46281222. In this report we show that JNJ-42153605 competitively displaces [³H]-JNJ-46281222 in occupancy assays. This is understandable given the similarity in structure between the two ligands, and the similar predicted binding modes (see S4 Fig). The ligands overlap very well and form the same interactions between the cyclopropylmethyl groups, the scaffold, the H-bond to N735.47a.47c as well as the CF₃ group interaction towards S731.42a.43c in TM5. There is small structural deviation in the ligand position at the extracellular side of the binding site. This is as expected due to the larger cavity and the increased flexibility of JNJ-46281222 compared to JNJ-42153605 which arises due to the methylene spacer between the scaffold and the piperidine nitrogen.

Thus, this docking modeling was highly valuable for the sleep-wake study: by better defining the JNJ-42153605 compound in its role as an appropriate mGluR2 PAM, the in vivo outcomes and comparison with the LY354740 agonist is more reliable.

The present in vivo findings indicate that acute activation of mGluR2 by the agonist LY354740 and acute allosteric modulation by the specific PAM JNJ-42153605 consistently and dose-dependently reduced REM sleep and intermediate stage during the first 4-h post-administration and lengthened REM onset latency.

**Activation of mGluR2 affects REM sleep state**

The glutamatergic system is fundamental for physiological brain processes and any dysfunction in glutamate neurotransmission may play an important role in the pathophysiology of psychiatric disorders. Glutamatergic tone exhibits a circadian burst across sleep wake states, being maximal in the orbitofrontal cortex and rostromedulla nuclei during wakefulness and REM sleep [18,44]. Therefore, alteration of glutamatergic neurotransmission is expected to affect the organization of vigilance states. Accordingly, local administration of glutamate in the brainstem pontine nuclei elicited REM sleep [45]; while blockade of NMDA increased the homeostatic need for sleep through neuroendocrine pulses controlled by hypothalamic sleep centres [46]. mGluR2 is of particular interest because of its unique distribution and regulatory roles in synaptic neurotransmission [25,26,47]. The effects of mGluR2 agents on sleep has been examined by several research groups, and a great consistency across all these studies has been found with respect to the suppressing effect on REM sleep. Systemic or central activation of mGluR2 selectively suppress REM sleep and prolong its onset latency [20,21,23], whereas blockade of mGluR2 promoted wakefulness [21,48]. The specificity of the changes has been supported by the findings in mGluR2 (-/-), in which activation of mGluR2 specifically suppressed REM sleep in WT animals but not mGluR2 (-/-) mouse strain [20]. Thus, our present results are consistent with the profile of central activation of mGluR2, resulting in a common changes in sleep-wake organization of mice and rats i.e. dose-response inhibition of REM sleep occurrence and lengthening of REM sleep onset latency.

There have been attempts to further dissect the neural basis of the effects of mGluR2 agents on sleep. On one hand, acetylcholine, serotonin and GABA neurotransmission play a pivotal role in the regulation of sleep behaviour, and mGluR2 activation modulates the levels of these
neurotransmitters in several brain regions [10,49,50]. Consequently, activation or inhibition of the GABA A receptors decreased and increased REM sleep, respectively [28].

On the other hand, mGluR2 are highly expressed in the basolateral and central nuclei of the amygdala [25–27,51]. These limbic structures send direct projections to brain stem nuclei known to control the mechanisms of sleep and arousal [28,52,53]. Activation of mGluR2 within the basolateral amygdaloid region selectively suppressed REM sleep [23]. Therefore, activation of mGluR2 might affect the organization of vigilance states through other pathways than direct glutamatergic system. A promising candidate is the sublaterodorsal nucleus (SLD), which contains glutamatergic neurons that are crucial for the generation of atonia during REM sleep [54]. Acetylcholine is thought to participate in the activation of these descending atonia pathways as increases in glutamatergic input to SLD neurons occur during REM sleep when the levels of acetylcholine in the dorsal pons are highest [55]. LY354740 has been shown to inhibit the electrically-evoked endogenous acetylcholine release in brain slices [56] as well as the NMDA-evoked [3H]-choline release from synaptosomes and acetylcholine release in brain slices [57]. While depletion of acetylcholine inhibits REM sleep and blocking acetylcholine degradation promotes REM sleep [58], it is possible that reduced REM sleep may result from LY354740-induced inhibition of acetylcholine release.

mGluR2 agonist but not PAM elicited tolerance to its primary functional activity on REM sleep

During repeated dosing, the efficacy of LY354740 rapidly vanished as from day 3 onwards indicating rapid development of tolerance to REM sleep suppression. In contrast, the specific mGluR2 PAM elicited enduring significant inhibitory effects on REM sleep measures up to day 7. During discontinuation of JNJ-42153605, all vigilance states were normalized indicating a lack of withdrawal adverse side effects at cessation of the treatment. In the subsequent re-administration days, JNJ-42153605 consistently suppressed REM sleep variables indicating that no rapid development of tolerance to REM sleep suppression action took place.

There is evidence that agonists rapidly lose their efficacy after repeated dosing. Receptor desensitization and internalization are two early events that are thought to mark the onset of tolerance [59].

Our findings are consistent with the hypothesis that mGluR2 agonists function best as acute, time-limited interventions and highlighted a possible role of receptor desensitization and/or down regulation as a limiting factor for mGluR2/3 agonist therapies. However, caution should be taken as the expression levels, receptor activation, desensitization, internalization, recovery from desensitization of mGluR2/3 receptors were not assessed in the present study. Thus, the results obtained in this preclinical translational model shed lights on possible tolerance development to a primary functional activation with the mGluR2 agonist.

REM sleep recovery was differently affected by LY354740 and JNJ-42153605

During the subsequent 10-h dark period, the increase gain in REM sleep over vehicle was greater in animals treated with JNJ-42153605 but not with LY354740.

Sleep is regulated by a homeostatic drive that increases sleep propensity in proportion to the duration of prior waking and a circadian drive that alternately promotes sleep and waking across the day [60]. Sleep loss is known to induce a compensatory increase that is proportional to the duration of sleep suppression and to the stage of sleep that was lost e.g. REM sleep [61]. LY354740 powerfully and specifically suppresses spontaneous REM sleep and its homeostasis recovery during the light and dark phases, respectively. The mechanisms underlying the
reduced recovery of REM sleep after the administration LY354740 are not well understood. The basal forebrain is one key brain region implicated in sleep recovery after sleep loss [62,63], and it is possible that mGluR2/3 activation may act on the basal forebrain and its projections to the cortex to alter homeostatic need of REM sleep generation. Another potential mechanism that could explain the reduced ability of LY354740 to inhibit and to recover REM sleep in subsequent days could be related to diminished efficacy of mGluR2s trafficking including their internalization and desensitization in response to repeated stimulation as described earlier for the serotonin 5-HT7 receptor [64]. Future studies need to investigate the detailed molecular mechanisms and relationship between intrinsic agonist efficacy and agonist-induced internalization and/or desensitization, and to address in future clinical work whether the loss of efficacy after mGluR2 agonism may be related to clinical tolerance issues. Until such studies are performed, the current findings underscore possible unresponsiveness to long term use of mGluR2 agonists.

It is noteworthy that the JNJ-42153605-induced inhibition of REM sleep during the light phase was followed by a delayed compensatory increase during the following dark phase. This time course is consistent with molecular dynamics that can mediate genomic expression of proteins involved in sleep-wake regulatory nuclei including brainstem neurons, basal forebrain, thalamus and hypothalamus/preoptic area, that are thought to promote REM sleep. Consistent with these possibilities, mGluR2 activation modulates c-Fos expression markers of neuronal activation in sleep and wake regulatory areas such as the amygdala and thalamic nuclei [23,64–66]. Consistent with a homeostatically driven increase in REM sleep, the acute homeostatic increase in REM sleep response was preserved throughout the subchronic treatment and following withdrawal.

The ability of LY354740 to attenuate the homeostatic need for REM sleep, while JNJ-42153605 promoted recovery sleep raises the potential benefit from recovery sleep in relation to performance and cognitive function, which may be an important factor in differentiating these two compounds and related mechanism of action. These data do not preclude the possibility that other mGluRs including mGluR3 may also be implicated in the lack of REM sleep recovery. Nevertheless, the present data point clearly point to a differential timing of REM recovery to preserve the normal light-dark REM sleep distribution and suggest the mGluR2 signalling as a mechanism for REM sleep homeostasis.

mGluR2 PAM had an early deep sleep promoting effect

Interestingly, JNJ-42153605 appears to enhance deep sleep, at least during the first days of treatment. JNJ-42153605 had positive effects on sleep continuity and efficiency, which may be beneficial at early stages of the treatment in psychiatric disorders associated with impaired sleep quality.

Sleep disturbances are prevalent in psychiatric diseases and poor sleep quality is associated with decrements in cognitive performance [67]. The anatomical and functional interactions between mGluR2 and 5HT2A receptors have been reported to form heterodimeric complex that modulate G-protein-mediated intracellular signaling differentially compared to mGluR2 and 5-HT2A homomers [68–71], and both human and animal pharmacological studies demonstrated that an interaction with 5-HT2A receptor can improve comorbid sleep disturbance associated with mood disorders and schizophrenia [72–75]. Similarly, the functional interactions between mGluR2 and NMDA receptors signaling has been evidenced by the inhibitory effects of the more potent mGluR2 agonist LY379268 and the PAM CBiPES, which modulate the ketamine evoked increases in glutamate release in the medial prefrontal cortex [75], as well as histamine release the limbic brain regions [76] point towards a reduction in central excitatory levels.
Therefore, the early effects of JNJ-42153605 on deep sleep and sleep efficiency suggest a putative functional interaction response that may result in a greater early benefit on sleep regulating emotional brain reactivity and functioning.

Effectiveness of different mGluR2 PAMs on sleep-wake pharmacodynamics index

The Table 1 shows a summary of half-lives (T1/2), brain receptor occupancy and the relative contribution of mGluR2 versus 5-HT2A receptor in the sleep-wake pharmacodynamics model of published mGluR2 PAMs (JNJ-40411813, JNJ-40068782 and JNJ-42153605). All mGluR2 PAMs showed comparable mean T1/2 values (ranging from 2.3 to 2.4-h) following oral administration in rats.

**JNJ-40411813.** JNJ-40411813 is an mGluR2 PAM with a moderate antagonist activity at the 5HT2A receptor, dose-dependently decreased REM sleep and increased deep sleep in rats [77]. Over a period of 4-h, JNJ-40411813 was effective at the Lowest Active Dose (LAD) of 3 mg/kg for REM sleep and 10 mg/kg for deep sleep. This corresponds to plasma levels of 50–180 ng/ml and 25–45% mGluR2 occupancy. Exposure levels of JNJ-40411813, that were needed to maintain effects on REM sleep at the lowest efficacious plasma level in this model was 40 ng/ml, corresponding to 50% receptor occupancy.

**JNJ-41329782 versus JNJ-40411813.** JNJ-41329782 acts as a mixed mGluR2 PAM-5-HT2A antagonist with similar affinity and activity at both receptors [31]. JNJ-41329782 is brain penetrant and occupies rat brain mGluR2 with an ED50 of 5.5 mg/kg, corresponding to a plasma concentration of 850 ng/ml, while the 5-HT2A occupancy was reached at lower plasma concentrations (EC50 54 ng/ml). JNJ-41329782 is comparable in potency to JNJ-40411813, however, a more than 3-fold difference in corresponding ED50 was found for 5-HT2A receptor occupancy after oral administration (1.6 and 9.5 mg/kg, respectively). JNJ-41329782 seems somewhat more potent than JNJ-40411813, although both compounds are very comparable in their pharmacological profile. JNJ-41329782 had no activity towards other mGluR subtypes up to a concentration of 10 μM. Similar to JNJ-40411813, JNJ-41329782 reduced REM sleep during the first 4-h post-administration with a LAD of 3 mg/kg; corresponding to ~30% receptor occupancy and enhanced deep sleep with a LAD of 10 mg/kg, corresponding to plasma levels of 320 ng/ml.

**JNJ-42153605 versus JNJ-40411813.** Compared to JNJ-40411813, JNJ-42153605 is about 10-fold more potent in vitro on mGluR2. JNJ-42153605 showed a binding as well as functional mGluR2 affinity in the range of 15 nM. JNJ-42153605 increased glutamate efficacy up to 3-fold and enhances glutamate potency up to 25-fold at both the cloned human and rat mGluR2. In contrast to JNJ-40411813, which shows moderate 5-HT2A antagonist activity, JNJ-42153605

| JNJ-40411813 | JNJ-4139782 | JNJ-42153605 |
|---------------|-------------|---------------|
| T1/2 (h)      | 2.3 ± 0.5   | 2.4 ± 0.3     | 2.3 ± 0.5     |
| Occupancy ED50 (mg/kg) |     |               |               |
| mGluR2        | 16          | 5.5           | 5.5           |
| 5HT2A         | 9.5         | 1.6           | > 40          |
| Pharmacodynamic index ED50 (mg/kg) |     |               |               |
| Deep sleep    | 3           | 3             | 3             |
| REM sleep     | 10          | 10            | 10            |

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shows >100-fold selectivity versus other targets. Similar to JNJ-40411813, JNJ-42153605 suppressed REM sleep over a period of 4-h at a LAD of 3 mg/kg and enhanced deep sleep at 10 mg/kg, which corresponds to plasma levels of 40–170 ng/ml and 50–90% mGluR2 occupancy. Overall, a comparative analysis of the different, published mGluR2 PAMs JNJ-40411813, JNJ-40068782, JNJ-42153605 shows a functional effectiveness on sleep wake pharmacodynamics at similar LAD.

Functional significance

There has been pharmacological evidence indicating the potential of mGluR2 agents in animal models of anxiety and stress [5,78]. Sleep is regulated by multiple ascending and descending pathways including the limbic structure, which is critically implicated in the control of anxiety and emotion [79]. Sleep disturbance are prevalent symptoms in depression, anxiety and stress related disorders and improvement of sleep has often been considered as one of the first signs of impending recovery [80–83]. Additionally, sleep-EEG variables such as elevated REM density and REM latency are used as fundamental biomarkers and diagnostic criteria in major depressive disorders [84,85]. Moreover, a sustained suppression of REM sleep variables and lengthening of REM latency are common features found in healthy, depressed subjects and laboratory animals after chronic treatment with clinically effective antidepressants [86–92]. Therefore, the intimate relationship between glutamate signalling and sleep mechanisms highlights the value of sleep-wake measurements as a reliable sensitive index of the mGluR2 target engagement. Potentiating the mGluR2 response to glutamate by using selective PAMs may offer advantages over orthosteric mGluR2 agonists to achieve superior mGluR2 selectivity [5,93], which may translate to an improved efficacy and tolerability profile in the clinic. Additionally, potentiators may exhibit lower potential for the induction of receptor desensitization and tolerance that can occur with GPCR agonists during long-term dosing. The present results suggest that mGluR2 modulators may have superior therapeutic efficacy over agonists to counter-act excessive glutamate flow in psychiatric disorders associated with REM sleep overdrive.

Overall, repeated treatment with LY354740 elicited tolerance phenomena to its primary acute functional effect, whereas JNJ-42153605 resulted in sustained efficacy on sleep measures in rats. JNJ-42153605 promoted homeostatic recovery sleep and had an early beneficial effect on sleep efficiency, which may serve as an early add-on treatment of impaired sleep quality in psychiatric disorders commonly associated with REM sleep overdrive. From the translational perspective, the present findings should raise awareness that long term therapeutic application of an mGluR2 agonist, but not of a PAM might lead to tolerance development to its primary acute functional activity.

Supporting Information

S1 Fig. Sequence Alignment used to construct mGluR2 7TM model. (TIF)

S2 Fig. Comparison of the predicted JNJ-42153605 binding mode with that of NAMs in the mGluR1 (top, yellow) and mGluR5 (bottom, orange) X-ray structures. Top: mGluR1 (yellow) compared to mGluR2 model (purple). Position of allosteric ligand and selected amino acid side chains can be seen. mGluR1 X-ray structure is from PDB code 4OR2. Bottom: mGluR5 (orange) compared to mGluR2 model (purple). Position of allosteric ligand and selected amino acid side chains can be seen. mGluR5 X-ray structure is from PDB code 4OO9. (TIF)
S3 Fig. Binding site amino acid comparison, human mGluR2 is highlighted in the red box. Amino acids are non-sequential and identified by selecting within 6Å radius of ligand in mGluR1 and mGluR5 crystal structures, selected examples from mGluR2 are labelled. (TIF)

S4 Fig. Overlap of the proposed binding mode of JNJ-42153605 (magenta) binding mode with that of JNJ-46281222 (blue). (TIF)

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Author Contributions
Conceived and designed the experiments: AA HL WD. Performed the experiments: AA HL. Analyzed the data: AA HL. Contributed reagents/materials/analysis tools: GT JC. Wrote the paper: AA HL GT JC WD.

References
1. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci. 2006; 7:137–151. PMID: 16429123
2. Chaki S, Hikichi H. Targeting of metabotropic glutamate receptors for the treatment of schizophrenia. Curr Pharm Des. 2011; 17:94–102. PMID: 21355835
3. Conn PJ, Lindsley CW, Jones CK. Activation of the metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. Trends Pharmacol Sci. 2009; 30:25–31. doi: 10.1016/j.tips.2008.10.006 PMID: 19058862
4. Linden AM, Schoepp DD. Metabotropic glutamate receptor targets for neuropsychiatric disorders. Drug Discov Today 2006; 3:507–517.
5. Palucha A, Pilc A. Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. Pharmacol Ther. 2007; 115:116–47. PMID: 17582504
6. Schoepp DD, Marek GJ. Preclinical pharmacology of mGluR2/3 receptor agonists: novel agents for schizophrenia? CNS. Neurolog. Disord Drug Targets 2002; 1: 215–225.
7. Sodhi M, Wood KH, Meadow-Woodruff J. Role of glutamate in schizophrenia: integrating excitatory avenues of research. Expert Rev. Neurotherapy 2008; 8:1389–1406.
8. Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov. 2005; 4:131–144. PMID: 15665858
9. Witkin JM, Marek GJ, Johnson BG, Schoepp DD. Metabotropic glutamate receptors in the control of mood disorders. CNS Neurolog Disord Drug Targets 2007; 6: 87–100.
10. Cartmell J, Monn JA, Schoepp DD. Tolerance to the motor impairment, but not to the reversal of PCP-induced motor activities by oral administration of the mGlu2/3 receptor agonist, LY379268. Naunyn Schmiedebergs Arch Pharmacol. 2000; 361: 39–46. PMID: 10651145
11. Imre G, Fukkemaas DS, Ter Horsta GJ. Subchronic administration of LY354740 does not modify ketamine-evoked behavior and neuronal activity in rats. Eur J Pharmacol. 2006; 544: 77–81. PMID: 16860791
12. Riegel AC, Ali SF, French ED. Toluene-induced locomotor activity is blocked by 6-Hydroxydopamine lesions of the nucleus accumbens and the mGluR2/3 agonist LY379268. Neuropsychopharmacol. 2003; 28:1440–1447.
13. Jones CK, Eberle EL, Peters SC, Monn JA, Shannon HE. Analgesic effects of the selective group II (mGlu2/3) metabotropic glutamate receptor agonists LY379268 and LY389795 in persistent and inflammatory pain models after acute and repeated dosing. Neuropharmacol. 2005; 49:206–218.
14. Sun X, Whitefield S, Rusak B, Semba K. Electrophysiological analysis of suprachiasmatic nucleus projections to the ventrolateral preoptic area in the rat. Eur J Neurosci. 2001; 14:1257–1274. PMID: 11703455

15. Cui LN, Codere E, Renaud LP. Glutamate and GABA mediate suprachiasmatic nucleus inputs to spinal-projecting paraventricular neurons. Am J Physiol Regul Integr Comp Physiol. 2001; 281:R1283–R1289. PMID: 11557637

16. Hermes ML, Codere EM, Buijs RM, Renaud LP. GABA and glutamate mediate rapid neurotransmission from suprachiasmatic nucleus to hypothalamic paraventricular nucleus in rat. J Physiol Lond. 1996; 496:749–757. PMID: 8930841

17. Perreau-Lenz S, Kalsbeek A, Pévet PRM, Buijs RM. Glutamatergic clock output stimulates melatonin synthesis at night. Eur J Neurosci. 2004; 19:318–324. PMID: 14725626

18. Lopez-Rodriguez F, Medina-Ceja L, Wilson CL, Jhung CL, Morales-Villagram A. Changes in extracellular glutamate levels in rat orbitofrontal cortex during sleep and wakefulness. Arch Med Res. 2007; 38:52–55. PMID: 17147723

19. Naylor E, Allon DV, Gabbert S, Harmon H, Johnson DA, Wilson GS, et al. Simultaneous real-time measurement of EEG/EMG and L-glutamate in mice: A biosensor study of neuronal activity during sleep. J Electroanalytical Chem. 2011; 656:106–113.

20. Ahnaou A, Dautzenberg FM, Geys H, Imogai H, Gibelin A, Moechars D, et al. Modulation of group II metabotropic glutamate receptor (mGlu2) elicits common changes in rat and mice sleep-wake architecture. Eur J Pharmacol. 2009; 603:62–72. doi: 10.1016/j.ejphar.2008.11.018 PMID: 19046965

21. Feinberg I, Schoepp DD, Hsieh KC, Darchia N, Campbell IG. The metabotropic glutamate (mGlu)U2/3 receptor antagonist LY341495 [2S-2-amino-2-(1S,2S-carboxycyclopentyl-1-yl)-3-(xanth-9-yl)propionic acid] stimulates waking and fast electroencephalogram power and blocks the effects of the mGluU2/3 receptor agonist 3R,4R-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate in rats. J Pharmacol Exp Ther. 2005; 312:826–33. PMID: 15383637

22. Fell MJ, Wilkin JM, Falcone JF, Katner JS, Perry KW, Hart J, et al. N-(4-((2-(trifluoromethyl)-3-hydroxy-4-(isobutyryl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide (THIIC), a novel metabotropic glutamate 2 potentiator with potential anxiolytic/antidepressant properties: in vivo profiling suggests a link between behavioral and central nervous system neurochemical changes. J Pharmacol Exp Ther. 2011; 336:155–77. doi: 10.1124/jpet.110.172957 PMID: 20947638

23. Dong E, Wellman LL, Yang L, Sanford LD. Effects of microinjections of Group II metabotropic glutamate agents into the amygdala on sleep.Brain Res. 2012; 1452:85–95. doi: 10.1016/j.brainres.2012.03.003 PMID: 22453124

24. Parmentier-Batteur S, O’Brien JA, Doran S, Nguyen SJ, Flick RB, Uslaner JM, et al. Differential effects of the mGluR5 positive allosteric modulator CDPPB in the cortex and striatum following repeated administration. Neuropharmacology 2012; 62:1453–60. doi: 10.1016/j.neuropharm.2010.11.013 PMID: 21112344

25. Ohishi H, Shigemoto R, Nakashiba I, Mizuno N. Distribution of the messenger RNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. Neurosciences 1993; 53:1009–1018.

26. Gu G, Lorrain DS, Wei H, Cole RL, Zhang X, Daggett LP, et al. Distribution of metabotropic glutamate 2 and 3 receptors in the rat forebrain: Implication in emotional responses and central disinhibition. Brain Res. 2008; 1197:47–62. doi: 10.1016/j.brainres.2007.12.057 PMID: 18245587

27. Tamaru Y, Nomura S, Mizuno N, Shigemoto R. Distribution of metabotropic glutamate receptor (mGlu2) elicits common changes in rat and mice sleep-wake architecture. Eur J Pharmacol. 2009; 603:62–72. doi: 10.1016/j.ejphar.2008.11.018 PMID: 19046965

28. Sanford LD, Parris B, Tang X, GABAergic regulation of the central nucleus of the amygdala: implications for sleep control. Brain Res. 2002; 956:276–87. doi: 10.1016/S0006-8993(02)00516-6 PMID: 12445696

29. Ahnaou A, Ver Donck L, Drinkenburg WH. Blockade of the metabotropic glutamate (mGluR2) modulator elicits arousal through vigilance states transitions: evidence from sleep-wake EEG in rodents. Behav Brain Res. 2014; 270:56–67. doi: 10.1016/j.bbr.2014.05.003 PMID: 24821401

30. Cid JM, Tresadem G, Vega JA, de Lucas A, Mateus E, Iturriño L, et al. Discovery of 3-Cyclopropylmethyl-7-(4-phenylpiperidin-1-yl)-8-trifluoromethyl[1,2,4]triazolo[4,3-a]pyridine (JNJ-42153605): A Positive Allosteric Modulator of the Metabotropic Glutamate 2 Receptor. J Med Chem. 2012; 55: 8770–8789. doi: 10.1021/jm3010724 PMID: 23072213

31. Lavrysen H, Langlois X, Ahnaou A, Drinkenburg W, Te Riele P, Biesmans I, et al. Pharmacological characterization of JNJ-4068782, a new potent, selective and systemically active positive allosteric modulator of the mGlu2 receptor and its radioligand [3H]JNJ-4068782. J Pharmacol Exp Ther. 2013; 346:514–27. doi: 10.1124/jpet.113.204990 PMID: 23766542
32. Doré AS, Okrasa K, Patel JC, Serrano-Vega M, Bennett K, Cooke RM, et al. Structure of class C GPCR metabotropic glutamate receptor 5 transmembrane domain. Nature 2014; 511: 557–62. doi:10.1038/nature13396 PMID: 25042998

33. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Koblika TS, et al. Crystal structure of the β2 adrenergic receptor-Gs protein complex. Nature 2011; 477:549–55. doi:10.1038/nature10361 PMID: 21772288

34. Wu H, Wang C, Gregory KJ, Han GW, Cho HP, Xia Y, et al. Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. Science 2014; 344:58–64. doi:10.1126/science.1249489 PMID: 24603153

35. El Moustaine D, Granier S, Doumazane E, Scholler P, Rahmeh R, Bron P, et al. Distinct roles of metabotropic glutamate receptor dimerization in agonist activation and G-protein coupling. Proc Natl Acad Sci USA. 2012; 109: 16342–7. doi: 10.1073/pnas.1205838109 PMID: 22988116

36. Isberg V, de Graaf C, Bortolato A, Cherezov V, Katritch V, Marshall FH, et al. GPCR residue numbers—aligning topology maps while minding the gaps. Trends Pharmacol Sci. 2015; 36:22–31. doi:10.1016/j.tips.2014.11.001 PMID: 25541108

37. Kingston AE, Ornstein PL, Wright RA, Johnson BG, Mayne NG, Burnett JP. LY341495 is a nanomolar potent and selective antagonist of group II metabotropic glutamate receptors. Neuropharmacol. 1998; 37:1–12.

38. Farinha A, Lavreysen H, Peeters L, Russo B, Masure S, Trabanco AA, et al. Molecular determinants of positive allosteric modulation of the human metabotropic glutamate receptor 2. Br J Pharmacol. 2015; 172:2383–96. doi:10.1111/bph.13065 PMID: 25571949

39. Hemstapat K, Da Costa H, Nong Y, Brady AE, Luo Q, Niswender CM, et al. A novel family of potent negative allosteric modulators of group II metabotropic glutamate receptors. J Pharmacol Exp Ther. 2007; 322:254–64. PMID: 17416742

40. Lundström L, Bissantz C, Beck J, Wettstein JG, Woltering TJ, Wichmann J, et al. Structural determinants of allosteric antagonism at metabotropic glutamate receptor 2: mechanistic studies with new potent negative allosteric modulators. Br J Pharmacol. 2011; 164: 521–7. doi:10.1111/j.1476-5381.2011.01409.x PMID: 21470207

41. Rowe BA, Schaffhauser H, Morales S, Lubbers LS, Bonnefoi C, Karnenecke TM, et al. Transposition of three amino acids transforms the human metabotropic glutamate receptor (mGlur)-3-positive allosteric modulation site to mGlur2, and additional characterization of the mGlur2-positive allosteric modulation site. J Pharmacol Exp Ther. 2008; 326:240–51. doi:10.1124/jpet.108.138271 PMID: 18430863

42. Schaffhauser H, Rowe BA, Morales S, Chavez-Noriega LE, Yin R, Jachec C, et al. Pharmacological characterization and identification of amino acids involved in the positive modulation of metabotropic glutamate receptor subtype 2. Mol Pharmacol. 2003; 64:798–810. PMID: 14500736

43. Doornbos MLJ, Pérez-Benito L, Tresadern G, Mulder-Krieger T, Biesmans I, Trabanco AA, et al. Molecular mechanism of positive allosteric modulation of the metabotropic glutamate receptor 2 by JNJ-46281222. 2015 Manuscript submitted and under revision.

44. Kodama T, Lai YY, Siegel JM. Enhanced glutamate release during REM sleep in the rostromedial medulla as measured by in vivo microdialysis. Brain Res. 1998; 780:178–81. PMID: 9497097

45. Datta S, Spoley EE, Patterson EH. Microinjection of glutamate into the pedunculopontine tegmentum induces REM sleep and wakefulness in the rat. Am J Physiol Regul Integr Comp Physiol. 2001; 280: R752–9. PMID: 11171654

46. Campbell IG, Feinberg I. NREM delta stimulation following MK-801 is a response of sleep systems. J Neurophysiol. 1996; 76:3714–20. PMID: 8985869

47. Wright RA, Arnold MB, Wheeler WJ, Ornstein PL, Schoepp DD. [3H]LY341495 binding to group II metabotropic glutamate receptors in rat brain. J Pharmacol Exp Ther. 2001; 298: 453–460. PMID: 11454905

48. Hikichi H, Kaku A, Karasawa J, Chaki S. Stimulation of metabotropic glutamate (mGlu) 2 receptor and blockade of mGlu1 receptor improve social memory impairment elicited by MK-801 in rats. J Pharmacol Sci. 2013; 122:10–6. PMID: 23603933

49. Smolders I, Lindekens H, Clinkers R, Meurs A, O’Neill MJ, Lodge D, et al. In vivo modulation of extra-cellular hippocampal glutamate and GABA levels and limbic seizures by group I and II metabotropic glutamate receptor ligands. J Neurochem. 2004; 88:1066–77. PMID: 15096663

50. Pisani A, Bonsi P, Catania MV, Giuffrida R, Morari M, Marti M, et al. Metabotropic glutamate 2 receptors modulate synaptic inputs and calcium signals in striatal cholinergic interneurons. J Neurosci. 2002; 22:6176–61850. PMID: 12122076
51. Smiałowska M, Wierońska JM, Wedzony K. A search for colocalization of mglu la receptors with CRF or NPY in the rat brain amygdala. Folia Histochem Cytobiol. 2002; 40:153–4. PMID: 12056621
52. Jha SK, Ross RJ, Morrison AR. Sleep-related neurons in the central nucleus of the amygdala of rats and their modulation by the dorsal raphe nucleus. Physiol Behav. 2005; 15: 86:415–26.
53. Tang X, Yang L, Liu X, Sanford LD. Influence of tetrodotoxin inactivation of the central nucleus of the amygdala on sleep and arousal. Sleep 2005; 28:923–30. PMID: 16218075
54. Lu J, Sherman D, Devor M, Saper CB. A putative flip-flop switch for control of REM sleep. Nature 2006; 441:589–94. PMID: 16688184
55. Leonard TO, Lydic R. Pontine nitric oxide modulates acetylcholine release, rapid eye movement sleep generation, and respiratory rate. J Neurosci. 1997; 17:774–85. PMID: 8987799
56. Marti M, Paganini F, Stocchi S, Mela F, Beani L, Bianchi C, et al. Plasticity of glutamatergic control of striatal acetylcholine release in experimental parkinsonism: opposite changes at group-II metabotropic and NMDA receptors. J Neurochem. 2003; 84:792–802. PMID: 12562523
57. Mela F, Marti M, Fiorentini C, Missale C, Morari M. Group-II metabotropic glutamate receptors negatively modulate NMDA transmission at striatal cholinergic terminals: role of P/Q-type high voltage activated Ca++ channels and endogenous dopamine. Mol Cell Neurosci. 2006; 31:284–92. PMID: 16249096
58. Jones BE. Paradoxical sleep and its chemical/structural substrates in the brain. Neuroscience 1991; 40: 637–56. PMID: 2062436
59. Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. Physiol Rev. 2001; 81:299–343. PMID: 11192760
60. Borbély AA, Achermann P. Concepts and models of sleep regulation: an overview. J Sleep Res. 1992; 1:63–79. PMID: 10607028
61. Shea JL, Mochizuki T, Sagvaag V, Aspevik T, Bjorkum AA, Datta S. Rapid eye movement (REM) sleep homeostatic regulatory processes in the rat: changes in the sleep-wake stages and electroencephalographic power spectra. Brain Res. 2008; 1213:48–56. doi: 10.1016/j.brainres.2008.03.062 PMID: 18455709
62. Kalinchuk AV, McCarley RW, Stenberg D, Porkka-Heiskanen T, Basheer R. The role of cholinergic basal forebrain neurons in adenosine-mediated homeostatic control of sleep: lessons from 192 IgG-saporin lesions. Neuroscience 2008; 157:239–53. doi: 10.1016/j.neuroscience.2008.08.040 PMID: 18805464
63. Kaur S, Junek A, Black MA, Semba K. Effects of ibotenate and 192IgG-saporin lesions of the nucleus basalis magnocellularis/substantia innominata on spontaneous sleep and wake states and on recovery sleep after sleep deprivation in rats. J Neurosci. 2008; 28:491–504. doi: 10.1523/JNEUROSCI.1585-07.2008 PMID: 18184792
64. Guthrie CR, Murray AT, Franklin AA, Hamblin MW. Differential agonist- mediated internalization of the human 5-hydroxytryptamine 7 receptor isoforms. J Pharmacol Exp Ther. 2005; 313:1003–10. PMID: 15716386
65. Linden AM, Baez M, Bergeron M, Schoepp DD. Effects of mGlur2 or mGlur3 receptor deletions on mGlur2/3 receptor agonist (LY354740)-induced brain c-Fos expression: specific roles for mGlur2 in the amygdala and subcortical nuclei, and mGlur3 in the hippocampus. Neuropharmacology 2006; 51:213–28. PMID: 16733060
66. Turner JP, Salt TE. Group II and III metabotropic glutamate receptors and the control of the nucleus reticularis thalami input to rat thalamocortical neurones in vitro. Neuroscience 2003; 122:459–69. PMID: 14614910
67. Walker MP. Sleep, memory and emotion. Prog Brain Res. 2010; 185: 49–68. doi: 10.1016/B978-0-444-53702-7.00004-X PMID: 21075233
68. Marek GJ, Wright RA, Schoepp DD, Monn JA, Aghajanian GK. Physiological antagonism between 5-hydroxytryptamine (2A) and group II metabotropic glutamate receptors in prefrontal cortex. J Pharmacol Exp Ther. 2000; 292:76–87. PMID: 10604933
69. Marek GJ, Wright RA, Gewirtz JC, Schoepp DD. A major role for thalamocortical afferents in serotonergic hallucinogen receptor functions in the rat neocortex. Neurosci. 2001; 105: 379–92.
70. Gewirts JC, Marek GJ. Behavioral evidence for interactions between a hallucinogenic drug and group II metabotropic glutamate receptors. Neuropsychopharmacology 2000; 23:569–576. PMID: 11027922
71. González-Maeso J, Ang RL, Yuen T, Chan P, Weissstaub NV, López-Giménez JF, et al. Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 2008; 452:93–97. doi: 10.1038/nature06612 PMID: 18297054
72. Cohrs S. Sleep disturbances in patients with schizophrenia: impact and effect of antipsychotics. CNS Drugs 2008; 200:939–962.
73. Landolt HP, Wehrle R. Antagonism of serotonergic 5-HT2A/2C receptors: mutual improvement of sleep, cognition and mood? Eur J Neurosci 2009; 29:1795–1809. doi: 10.1111/j.1460-9568.2009.06718.x PMID: 19473234

74. Saletu-Zyhlarz GM, Arnold O, Saletu B, Anderer P. The key-lock principle in the diagnosis and treatment of nonorganic insomnia related to psychiatric disorders: sleep laboratory investigations. Methods Find Exp Clin Pharmacol. 2002; 24:37–49.

75. Lorrain DS, Baccei CS, Bristow LJ, Anderson JJ, Varney MA. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 2003; 117:697–706. PMID: 12617973

76. Fell MJ, Katner JS, Johnson BG, Khilevich A, Perry KW, et al. Activation of metabotropic glutamate (mGlu)2 receptors suppresses histamine release in limbic brain regions following acute ketamine challenge. Neuropharmacology 2010; 58:632–9. doi: 10.1016/j.neuropharm.2009.11.014 PMID: 19951716

77. Lavreysen H, Ahnaou A, Drinkenburg W, Langlois X, Mackie C, Pype S, et al. Pharmacological and pharmacokinetic properties of JNJ-40411813, a positive allosteric modulator of the mGlu2 receptor. Pharmacol Res Perspect. 2015; 3:e00096. doi: 10.1002/prp2.96 PMID: 25692015

78. Helton DR, Tizzano JP, Monn JA, Schoepp DD. Kallman MJ. Anxiolytic and side-effect profile of LY354740: a potent, highly selective, orally active agonist for group II metabotropic glutamate receptors. J Pharmacol Exp Ther. 1998; 284:651–660. PMID: 9454811

79. Davis M, Whalen PJ. The amygdala: vigilance and emotion. Mol Psychiatry 2001; 6:13–34. PMID: 11244481

80. Steiger A, Kimura M. Wake and sleep EEG provide biomarkers in depression. J Psychiatr Res. 2010; 44:242–252. doi: 10.1016/j.jpsychires.2009.08.013 PMID: 19762038

81. Thase ME. Depression and sleep: pathophysiology and treatment. Dialogues Clin Neurosci. 2006; 8:217–226. PMID: 16889107

82. Kahn M, Sheppes G, Sadeh A. Sleep and emotions: bidirectional links and underlying mechanisms. Int J Psychophysiol. 2013; 89:218–28. doi: 10.1016/j.ijspsycho.2013.05.010 PMID: 23711996

83. Tsuno N, Besset A, Ritchie K. Sleep and depression. J Clin Psychiatr. 2005; 66: 1254–1269.

84. American Psychiatric Association. Task Force on DSM-IV, Diagnostic and statistical manual of mental disorders: DSM-IV-TR, 2000; fourth ed., American Psychiatric Association, Washington, DC.

85. Petersson M, Benca R. Sleep in mood disorders. Psych Clin North Amer. 2006; 29:1009–1032.

86. Feuillade P, Pringuey D, Belugou JL, Robert P, Darcourt G. Trimipramine: acute and lasting effects on sleep in healthy and major depressive subjects. J Affect Disord. 1992; 24:135–145. PMID: 1573122

87. Ivarsson M, Paterson LM, Hutson PH. Antidepressants and REM sleep in Wistar-Kyoto and Sprague-Dawley rats. Eur J Pharmacol. 2005; 22:63–71.

88. Landolt HP, Raimo EB, Schnierow BJ, Kelsoe JR, Rapaport MH, Gillin JC. Sleep and sleep electroencephalogram in depressed patients treated with phenelzine. Arch Gen Psychiatry 2001; 58:268–276. PMID: 11231834

89. Mayers AG, Baldwin DS. Antidepressants and their effect on sleep. Hum Psychopharmacol. 2005; 20:533–559. PMID: 16229049

90. Neckelmann D, Bjorvatn B, Bjørkum AA, Ursin R. Citalopram: differential sleep/wake and EEG power spectrum effects after single dose and chronic administration. Behav Brain Res. 1996; 79:183–192. PMID: 8983829

91. Silvestri R, Pace-Schott EF, Gersh T, Stickgold R, Salzman C, Hobson JA. Effects of fluvoxamine and paroxetine on sleep structure in normal subjects: a home-based Nightcap evaluation during drug administration and withdrawal. J Clin Psychiatry 2001; 62:642–652. PMID: 11561938

92. Wilson S, Argyropoulos S. Antidepressants and sleep: a qualitative review of the literature. Drugs 2005; 65:927–947. PMID: 15892588

93. Fraley ME. Positive allosteric modulators of the metabotropic glutamate receptor 2 for the treatment of schizophrenia. Exp Opin Ther Pat. 2009; 19:1259–1275.