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S Brooks[1,2], RGJA Zuiker[1], C Bleys[3], D Ziagkos[1], JA Moyer[4], L van Nueten[3], P Bonaventure[5], WC Drevets[5], JMA van Gerven[1,2], G Salvadore[4*] and GE Jacobs[1,6*]

Abstract

Background: Up to 40% of patients suffering from anxiety disorders do not benefit from currently available pharmacological treatments. Overactivity of the orexin-1 receptor (OX1R) has been implicated in anxiety- and panic-related states.

Aim & methods: We investigated the pharmacokinetics and characterized the pharmacodynamic (PD) profile of the OX1R antagonist JNJ-61393215 using a battery of central nervous system assessments investigating relevant functional domains such as alertness, attention, (visuo)motor coordination, balance, subjective effects and resting-state electroencephalography in a single ascending dose placebo-controlled study in doses from 1 to 90 mg inclusive, assessing PD up to 10 h after dosing, safety and pharmacokinetic in 48 healthy male subjects.

Results: Average time to maximal plasma concentration (T_{max}) ranged between 1.0 and 2.25 h; average half-life ranged from 13.6 to 24.6 h and average maximum plasma concentration ranged from 1.4 to 136.8 ng/mL in the 1 and 90 mg groups, respectively. JNJ-61393215 did not demonstrate any statistically significant or clinically meaningful effects on any PD endpoint at any dose investigated at T_{max} nor over the total period up to 10 h post-dose and was well tolerated. The reported somnolence rate was 16.7% (which was attributable to the cohorts receiving 6 mg and higher doses) compared to 12.5% in placebo.

Conclusion: This observation is in line with our knowledge about the OX1R in preclinical studies, where only inconsistent and non-dose-dependent changes in electroencephalography or other behavioural measures were observed under non-challenged conditions, potentially exemplifying the need for a challenged subject.

Keywords

Anxiety, orexin 1 antagonist, pharmacodynamics

What is already known about this subject:

- Overactivity of the orexin-1 receptor pathway has been linked to anxiety- and panic-related states.
- Antagonizing the orexin-1 receptor mediated pathway represents a promising novel therapeutic strategy for different neuropsychiatric disorders characterized by hyperarousal.

What this study adds:

- This is the first systematic investigation of the clinical pharmacology of the central nervous system (CNS) penetrating, selective orexin-1 receptor antagonist JNJ-61393215 in healthy humans.
- JNJ-61393215 did not affect attention, coordination, electroencephalography (EEG) parameters or subjective ratings in healthy subjects under unchallenged conditions despite adequate exposure.
- Since JNJ-61393215 demonstrated anxiolytic effects in a carbon dioxide (CO_2) induced model for panic in a separate clinical study, the functional and behavioural effects of orexin-1 antagonism in humans should ideally be examined under an experimentally induced hyperarousal state.

Introduction

Anxiety disorders encompass a group of phenomenologically diverse fear- and anxiety-related conditions (American Psychiatric Association, 2013). Within the anxiety disorders, panic disorder represents a distinct entity characterized by recurring panic attacks, symptoms such as dyspnoea and feeling of suffocation, autonomic hyperactivation including excessive perspiration and hot flushes or chills, nausea, trembling, paraesthesia, derealization, fear of losing control and/or dying. Pharmacological treatment of panic disorder is primarily based

[1]Centre for Human Drug Research, Leiden, The Netherlands
[2]Leiden University Medical Center, Leiden, The Netherlands
[3]Janssen Research & Development, LLC, Beerse, Belgium
[4]Janssen Research & Development, LLC, Titusville, NJ, USA
[5]Janssen Research & Development, LLC, San Diego, CA, USA
[6]Department of Psychiatry, Leiden University Medical Center, Leiden, The Netherlands

*Joint last authors.

Corresponding author:
Brooks, Centre for Human Drug Research, Zernikedreef 8, Leiden, 2333 CL, The Netherlands.
Email: sbrooks@chdr.nl

on monoamine neurotransmitter modulating compounds, such as the selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors, which often are supplemented using benzodiazepines (BZDs) (e.g. alprazolam, clonazepam) (Baldwin et al., 2014). However, up to a third of patients do not respond to treatments with different pharmacological agents and augmentation strategies, or they may experience unacceptable side effects (Farach et al., 2012; Taylor et al., 2012). Moreover, panicolytic effects may only be evident after 8–12 weeks of treatment, which puts patients at risk for non-compliance and increased suicidality (Cassano et al., 2002). Since mono-aminergic neurons project widely throughout the central nervous system (CNS), the panicolytic effects of currently available drugs may result from more efficient cortico-limbic modulation of fear and anxiety-related neurocircuitry due to structural adaptations over time (Felten and Sladek, 1983; Ferguson, 2001). Together, a therapeutic shift towards compounds that target more proximal neurocircuits involved in emotion regulation could prove beneficial in the treatment of fear and panic.

The orexins (alternatively known as hypocretins) are excitatory neuropeptides that can act as neurotransmitters, and they have been shown to influence CNS functions related to arousal and emotion regulation such as sleep-wake regulation and emotional valence. Orexinergic nuclei are primarily located in the lateral and posterior hypothalamus with efferent axons projecting to the cerebral cortex, nucleus accumbens, mesocorticolimbic ventral tegmental area and the amygdala (Sakurai, 2007). In addition, neuronal projections to brainstem nuclei such as the locus coeruleus and raphe nuclei are involved in modulating noradrenergic and serotonergic neurotransmission, leading to indirect modulation of anxiety/panic-related behaviour in mammals (Sakurai, 2007). The orexin-1 receptors (OX1R) are expressed in brain regions implicated in the regulation of fear that include the prefrontal and infralimbic cortex, hippocampus, paraventricular thalamic nucleus, ventromedial hypothalamic nucleus, dorsal raphe nucleus and locus coeruleus (Marcus et al., 2001). Likewise, OX1R signalling has been implicated in responses similar to anxiety in rodents (Johnson et al., 2010, 2012a, 2012b). Moreover, in line with the anatomical sites involved with OX1R, optogenetic stimulation of orexin neurons increases anxiety in rats (Heydendael et al., 2014) as well as the physiological reactions associated with anxiety in rats (Bonnavion et al., 2015). In humans, higher orexin concentrations were demonstrated in the cerebrospinal fluid (CSF) of panic disorder patients compared to healthy controls (Johnson et al., 2010). Early preclinical studies have shown that anxiety-like behaviour induced by the inverse BZD agonist FG-7142 was attenuated by selectively blocking OX1R (Johnson et al., 2012a). Similarly, administration of selective OX1R antagonists decrease anxiety responses as well as hyperlocomotion elicited by lactate challenge in rodents (Johnson et al., 2010, 2012b). Importantly, selective antagonism of OX1R does not affect the sleep-wake cycle in rodents, in contrast with sleep-promoting effects of non-selective OX1R/OX2R or selective OX2R antagonists (Bonaventure et al., 2015, 2017). These preclinical data support OX1R as a hypothetical novel target in the treatment of anxiety disorders in general. It should be noted, however, that OX2R is also implicated in fear and anxiety (Arendt et al., 2014; Li et al., 2021; Staton et al., 2018). Together, these findings support the treatment of anxiety disorders by targeting OX1R's, since selective OX1R antagonism is anticipated to be devoid of the undesired sleep-promoting effects that are associated with selective OX2R or dual OX1R/OX2R antagonism.

JNJ-61393215 is a selective OX1R antagonist (human in vitro pK_i value for OX1R 8.17 vs 6.12 for OX2R), which is currently under development for the treatment of fear and anxiety-related disorders, mood disorders and addiction. Its pharmacological characteristics have been characterized in healthy volunteers following single and multiple dose administration, as well as in a human experimental panic model. In these studies, JNJ-61393215 demonstrated a favourable safety profile and anxiolytic properties in an experimental carbon dioxide (CO_2) inhalation paradigm for panic as described recently (Salvadore et al., 2020). The pharmacodynamic (PD) effects of the single ascending dose study have not been published yet. The current paper describes PD characterization of single oral doses of JNJ-61393215 in these healthy male subjects using the NeuroCart (Centre for Human Drug Research (CHDR), Leiden, The Netherlands). By quantifying body sway (postural stability), saccadic peak velocity (visuomotor coordination), adaptive tracking (sustained attention, alertness) effects on EEG and Visual Analogue Scales (VASs) (subjective drug effects), the NeuroCart has been instrumental in demonstrating central PD effects of both marketed and novel experimental compounds in numerous past studies (Groeneveld et al., 2016). With regard to the orexin system, previous studies with dual orexin receptor antagonists (DORAs)-like almorexant, ACT-462206 (Hoch et al., 2014), and daridorexant (Groeneveld et al., 2016; Muehlan et al., 2019a) have demonstrated dose-dependent PD effects, including alertness, visuomotor and motor coordination and subjective drug effects. Also, other drugs with an anxiolytic profile in humans have demonstrated effects on NeuroCart. These include various histaminergic non-selective and experimental subtype selective (ant)agonists (Baakman et al., 2019; Baas et al., 2009; Heuberger et al., 2016), and numerous other (experimental) anxiolytic and/or sleep-promoting compounds, including both the non-selective BZD gamma-aminobutyric acid $(GABA)_A$ receptor positive allosteric modulators and subtype selective $GABA_A$ agonists, such as AZD6280 (Chen et al., 2015), AZD7325 (Chen et al., 2014, 2019) and PF-06372865 (Van Amerongen et al., 2019). In summary, the NeuroCart has been shown to be sensitive to the effects of compounds acting on neurotransmitters that are believed to be modulated by the orexin system as well as other compounds with an anxiolytic profile. Here, we describe the single dose PD effects of a single dose of JNJ-61393215 across a broad dose range on adaptive tracking, body sway, eye movements, EEG and various subjective measures.

Methods

Study design and subjects

Eight cohorts of eight healthy male subjects (aged 18–54 years inclusive) were included in a randomized, placebo-controlled, double blind, single ascending dosing (SAD) study with the selective OXR1 inhibitor JNJ-61393215. Subjects were excluded if they had a history of renal or kidney disease, substance abuse, positive human immunodeficiency virus, hepatitis B or C status or significant allergies. Subjects with anxiety or panic disorder or a family history of anxiety or panic disorder were excluded. Each

subject provided written informed consent before any screening procedures were performed. The study was conducted at the CHDR in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands) and was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. This study was registered on ClinicalTrial.gov under number NCT02812251.

Randomization and study procedures

Eligible subjects were randomly assigned to JNJ-61393215 or placebo using a computer-generated randomization schedule based on a 3:1 ratio whereby in each cohort of eight subjects six subjects were allocated to receive JNJ-61393215 and two subjects were allocated to receive placebo.

All subjects underwent a medical screening 21–2 days before first dosing, consisting of medical history, physical examination, blood chemistry and haematology, urinalysis and electrocardiogram (ECG). Upon admission for the study period (Day-1), an alcohol breath and urine drug were performed, and eligibility was reconfirmed.

At regular intervals, PD measurements were performed as described later. Safety examination and pharmacokinetic (PK) sampling were performed as described earlier (Salvadore et al., 2020).

Study drug and dosing rationale

Detailed information on the study drug and dosing regimen can be found elsewhere (Salvadore et al., 2020). In short, JNJ-61393215 was provided as a 25 mg/vial and a 250 mg/vial white to light pink solid for reconstitution for oral use. The doses administered were 1, 2, 6, 15, 30, 45, 60 and 90 mg JNJ-61393215. Dose escalation decisions were made upon review of the safety, tolerability and PK results at the end of each cohort.

Pharmacokinetic assessments

Analysis of the PK assessments are described in detail elsewhere (Salvadore et al., 2020). In short, blood samples for PK analysis were sampled at regular time points pre-dose and until 12 h post-dose up. Plasma samples were analysed to determine concentrations of JNJ-61393215 using a scientific validated, specific and sensitive liquid chromatography-mass spectrometry (LC-MS/MS) method (Janssen Research and Development, Beerse, Belgium). Sample processing was performed by means of protein precipitation using a sample volume of 20 µL plasma to which 20 µL of methanol was added, followed by 20 µL dimethyl sulfoxide containing 200 ng/mL of the internal standard (JNJ-55681964) and subsequent addition of 200 µL acetonitrile. After vortex-mixing at room temperature, the sample extract was centrifuged for 10 min at approximately 5000–6000×*g* and 20°C. The sample extract was then transferred to a HPLC autosampler (Shimadzu SIL-HTC) for injection to the LC-MS/MS analyzer. Separation between metabolites and interfering endogenous compounds is achieved by injecting 2 µL sample extract onto a XBridge BEH Shield RP18 column (50×2.1 mm, 3.5 µm, Waters) at 45°C and using 10 mM ammonium carbonate solution as mobile phase A, and methanol as mobile phase B. A gradient elution at a flow rate of 0.50 mL/min was applied using the following programme: 70% A and 30% B were the starting conditions, and increased to 90% B until 3.00 min, followed by an additional increase to 98% B at 3.01 min, which was held constant up to 4.00 min. At 4.01 min, the eluents were set back to their initial conditions to re-equilibrate the column until 5.00 min. An API-4000 triple quadrupole mass spectrometer (Sciex) equipped with a turbo ion spray source is used for analyte detection in positive ion mode using electrospray ionization. The mass spectrometer was operated under the following conditions: Gas 1 (nebulizer gas) flow (N_2), 50 arbitrary units; Gas 2 (heater gas) flow (N_2), 40 arbitrary units; ion source voltage 5.0 kV; capillary temperature 600°C; and a dwell time of 150 ms. Curtain and collision gas (N_2) was 30 L/min and 6.0 L/min, respectively. The mass spectrometer was optimized for the quantification of JNJ-61393215 (multiple reaction monitoring (MRM) transition m/z 461.1 > 201) and the internal standard (MRM transition m/z 440.2 >183). A calibration curve ranging from 0.200 to 10,000 ng/mL JNJ-61393215 was prepared in blank human ethylenediaminetetraacetic acid (EDTA) plasma. Quality control (QC) samples at 0.400, 4.00, 40.0, 400 and 4000 ng/mL were prepared from separate analyte stock solutions that was spiked to blank human EDTA plasma and were stored under similar conditions as the study samples. Calibration standards, QC and study samples were processed at the same time.

A log-log linear regression model was used, and log-transferred peak area ratios of the analyte to its internal standard were plotted against the log-transferred analyte concentrations. The concentrations in samples were calculated by interpolation from the standard curve. The cumulative accuracy (%bias) from lower level of quantification to upper level of quantification (0.200–10,000 ng/mL) was −5.0% to 7.0% and the cumulative precision (%CV) was 2.9%–7.7%. The performance of QC samples during sample analysis for inter-run %CV was 6.2%–7.7%, and for intra-run %CV it was 0.0%–8.1%. For analysis of the unbound fraction, plasma samples were fortified with ^{14}C-JNJ-61393215 (230 ng/mL, 1.04 kBq/mL) before equilibrium dialysis for in vitro determination. After dialysis, the concentration of ^{14}C-JNJ-61393215 in buffer and plasma was determined using liquid scintillation counting.

Pharmacodynamic assessments

Functional CNS effects were measured using the NeuroCart (CHDR, Leiden, The Netherlands). The NeuroCart is an integrated battery of tests for a wide range of CNS domains which was developed to assess various CNS-active drugs and extensively described in the past (Chen et al., 2012; Groeneveld et al., 2016). Neurophysiologic functioning was measured with saccadic peak velocity and smooth pursuit, postural stability with body sway and attention and eye-hand coordination with adaptive tracking. Effects on brain activity were measured using EEG. The Bond and Lader VAS was used to assess alertness, mood and calmness, the VAS Bowdle was used to assess psychedelic effects, the Swiss Narcolepsy Scale to detect narcoleptic symptoms and the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue to assess tiredness and fatigue. PD assessments were performed pre-dose and at 0.5-, 1-, 2-, 3-, 6- and 10-h post-dose.

Eye movements. Saccadic peak velocity is one of the most sensitive parameters for sedation, and it is also highly sensitive to DORAs (Hoch et al., 2014; Hoever et al., 2010; Muehlan et al., 2018, 2019b). The use of a computer for measurement of saccadic eye movements was originally described by Baloh et al. (1975). Recording of eye movements was performed in a quiet room with dimmed lightning. Recording and analysis of saccadic eye movements was conducted with a microcomputer-based system for sampling and analysis of eye movements using software by Cambridge Electronic Design (CED Ltd., Cambridge, UK), an amplifier by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, MA, USA). Sampling and analysis scripts were developed at CHDR (Leiden, The Netherlands). Disposable silver-silver chloride electrodes (Ambu Blue Sensor N) were be applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kOhm before measurements. Head movements were restrained using a fixed head support. The target consisted of a moving dot that was displayed on a computer screen. This screen was fixed 58 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15° to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 s. Saccadic peak velocity of all correct saccades was used as a parameter of interest for the statistical analyses. The same system was used for saccadic eye movements. During smooth pursuit eye movements, the target moved at a frequency ranging from 0.3 to 1.1 Hz, by incremental steps of 0.1 Hz. The amplitude of target displacement corresponded to 22.5° eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as a parameter of interest for statistical analyses.

Postural stability. The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Postural stability is also affected by DORAs (Hoch et al., 2014; Hoever et al., 2010; Muehlan et al., 2018, 2019b). Body sway was measured with a pot string meter (celesco) based on the Wright ataxiameter as described earlier (Wright, 1971). With a string attached to the waist, all body movements over a specified period of time are integrated and expressed as mm sway. Before starting a measurement, subjects were asked to stand still and be comfortable, with their feet approximately 10 cm apart and their hands in a relaxed position alongside the body and eyes closed. Subjects were not allowed to talk during the measurement. The total period of body sway measurement was 2 min.

Adaptive tracking. The adaptive tracking is also highly sensitive to DORAs (Hoch et al., 2014; Hoever et al., 2010; Muehlan et al., 2018, 2019b). The test was performed, using customized equipment and software (based on TrackerUSB hard-/software (Hobbs, 2004, Hertfordshire, UK)) (Borland and Nicholson, 1984). The average performance and the standard deviation of scores over a 3.5-min period was used for analysis. This 3.5-min period was including a run-in time of 0.5 min in which no data was stored. A circle moved randomly about a screen. The subject needed to keep a dot inside the moving circle by operating a joystick. If this effort was successful, the speed of the moving circle increased. Conversely, the velocity was reduced if the test subject could not maintain the dot inside the circle.

Each test was preceded by three training sessions and included two baseline measurements. After 4 to 6 practice sessions, learning effects were limited.

Electroencephalography. Pharmaco-EEG during rest was used to monitor the effects of JNJ-61393215 on brain electrical activity, to investigate evidence of central penetration and brain PD effects. DORAs have also been shown to produce advantageous changes in sleeping patterns without clear changes in EEG as expressed by analysis of the treatment effect on EEG and power spectral density (Hoever et al., 2013; Ma et al., 2014). Resting-state EEG recordings with alternating periods of open and closed eyes were made using gold electrodes, fixed with EC2 paste (Astromed) at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). Each period of open or closed eyes lasted 64 s. Subjects were instructed not to stare, not to move their head and eyes and to suppress eye blinks during the open-eyes period. The electrode resistances were kept below 5kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 s and a low pass filter at 100 Hz. Data collection and analysis were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artefacts were identified by visual inspection and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the very low (0.5–2 Hz), delta- (2–4 Hz), theta- (4–7.5 Hz), alpha- (7.5–13.5 Hz), beta- (13.5–35 Hz) and gamma-(35–48.9 Hz) frequency ranges. The total test lasted approximately 2 min.

Subjective drug effects. The Bond and Lader VAS is a self-report instrument used to measure subjective drug effects in the domains of mood, self-control and sedation (Bond and Lader, 1974). These VAS scores have been used to quantify subjective effects for various DORAs, showing significant sedative effects (Hoch et al., 2014; Hoever et al., 2010; Muehlan et al., 2018, 2019b). The Bowdle VAS is a self-report instrument used to evaluate psychedelic effects (Bowdle et al., 1998), which are not typically found with DORAs; however, for OX1R antagonists, this PD effect has not yet been assessed (Hoever et al., 2013).

The Swiss Narcolepsy Scale is a short questionnaire that screens for the occurrence of several behavioural symptoms that may be associated with sedative drugs (Sturzenegger et al., 2018).

FACIT-Fatigue is a questionnaire that assesses self-reported tiredness, weakness and difficulty conducting usual activities due to fatigue. The total FACIT-Fatigue score ranges from 0 to 52, with a higher score indicating less fatigue (Webster et al., 2003).

Safety evaluations

During the study, safety evaluations were performed through assessment of clinical laboratory tests (haematology, biochemistry

Table 1. Subject characteristics.

Characteristic	Placebo ($n=16$)	JNJ-61393215 1–90 mg ($n=48$)
Mean age, (years) (range)	26.1 (19–41)	25.6 (18–52)
Mean BMI (kg/m²) (range)	22.2 (18.6–27.8)	22.7 (18.9–28.5)
Race *n* (%)		
White	13 (81)	42 (88)
Black	2 (13)	1 (2)
Mixed	1 (6)	5 (10)

BMI: body mass index; *n*: number.

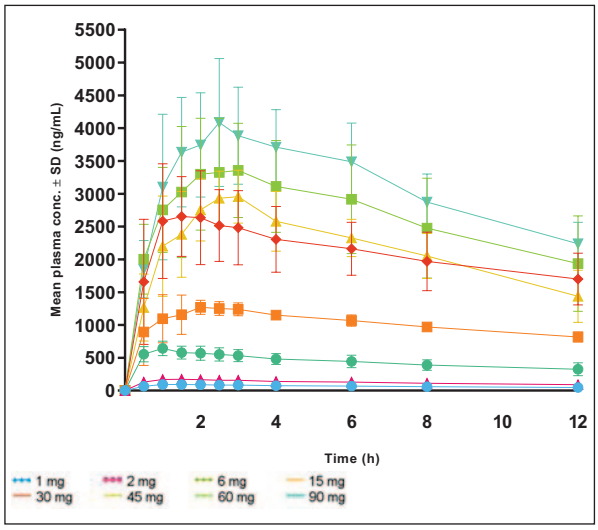

Figure 1. Mean plasma concentration ± SD (ng/mL) of JNJ-61393215 up to 12 h post-dose.

and urinalysis), vital signs (supine and standing), 12-lead ECGs. Physical and neurological examination were assessed and all treatment emergent adverse events (TEAEs; monitored from signing the informed consent until the follow-up visit) were recorded.

Statistics

Repeatedly measured PD data were analysed with a mixed model analysis of covariance (ANCOVA) with fixed factors treatment, time and treatment by time, random factor subject and the baseline value as covariate. Differences between active drug and placebo were estimated both at 2 h and at 3 h post-dose, as this coincided with the anticipated T_{max}, as well as over the entire assessment period. A table of the analysis results per variable was generated with estimates of the difference of the different contrasts and a back transformed estimate of the difference in percentage for log transformed parameters, 95% confidence intervals (CI) (in percentage for log transformed parameters) and least square means (LSMs) (geometric means for log transformed parameters, LS means), and the *p* value of the contrasts. LSMs graphs were generated, with the LS means of the analysis, and with the LSMs of the analysis of the data as change from baseline. Testing for an overall treatment effect was performed on a two-sided alpha level of 0.05 (uncorrected, for each test). Subsequent exploration of differences between placebo and any JNJ-61393215 dose group was carried out at the 0.05 two-sided significance level. For EEG, differences between placebo and JNJ-61393215 in each frequency band were assessed. All calculations were performed using SAS for Windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Subject characteristics and disposition

Sixty-four subjects met eligibility criteria and were randomized to eight cohorts of eight individuals, of which 18 received placebo and 48 a single dose of JNJ-61393215 (Table 1). All randomized subjects completed the full trial period. Baseline characteristics of the JNJ-61393215 and placebo group were similar (Table 1).

Pharmacokinetics

JNJ-61393215 was rapidly absorbed after oral administration. A single dose of 1 mg up to 30 mg reached C_{max} with a median time (t_{max}) of 1.00–1.50 h (Figure 1). Doses of 45 mg up to 90 mg took slightly longer to reach C_{max} at a median of 2.25 h (Figure 1). Average total C_{max} was 97.4 ± 10.1 ng/mL after a single dose of 1 mg JNJ-61393215, and increased dose-proportionally up to 2850 ± 701 ng/mL after 30 mg. Higher dose levels (i.e. 45 mg, 60 mg and 90 mg) showed a less than dose-proportional increase in C_{max} (Figure 1). The mean total C_{max} in the 90 mg cohort was 4497 ± 664 ng/mL. The concentration of the unbound fraction was markedly lower ranging from 1.40 ± 0.36 ng/mL to 136 ± 30 ng/mL in the 1 mg and 90 mg cohorts, respectively. Based on extrapolations from preclinical models, the predicted receptor occupancy at peak concentration was above 95% in the 90 mg cohort (Salvadore et al., 2020).

Pharmacodynamics

LSM and estimated difference from placebo over a period of 10 h for saccadic peak velocity, body sway, adaptive tracking, smooth pursuit, VAS Alertness and VAS Feeling high are depicted in Table 2, panels A to F. In Figure 2 panels (a)–(f) the LSM change from baseline are depicted. Overall, JNJ-61393215 administration did not result in a clinically meaningful difference on any of the PD endpoints compared with placebo. Although limited statistically significant effects were found, these were dose/

Table 2. Estimate of the difference compared with placebo (95%CI) for pharmacodynamic outcome measures, period up to 10 h post-dose.

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Saccadic peak velocity	**LSM**	**Est. difference from placebo (deg/s) (95%CI)**	***p*-value**
Placebo	498.8	NA	NA
1 mg	494.7	−4.10 (−20.50; 12.30)	0.6185
2 mg	489.8	−9.00 (−25.40; 7.40)	0.2763
6 mg	499.3	0.51 (−15.92; 16.95)	0.9502
15 mg	491.3	−7.47 (−23.87; 8.93)	0.3652
30 mg	496.2	−2.58 (−18.98; 13.81)	0.7533
45 mg	491.0	−7.74 (−24.15; 8.67)	0.3484
60 mg	510.9	12.12 (−4.27; 28.51)	0.1439
90 mg	492.3	−6.52 (−23.00; 9.95)	0.4310
B. Body sway.			
Body sway	**LSM**	**Est. difference from placebo (mm) (95%CI)**	***p*-value**
Placebo	254.8	NA	NA
1 mg	274.0	7.5 (−18.4; 41.8)	0.6006
2 mg	267.0	4.8 (−20.5; 38.2)	0.7362
6 mg	281.3	10.4 (−16.3; 45.6)	0.4752
15 mg	285.6	12.1 (−15.1; 48.0)	0.4140
30 mg	288.3	13.2 (−14.2; 49.3)	0.3753
45 mg	313.6	23.1 (−6.8; 62.5)	0.1398
60 mg	285.6	12.1 (−15.1; 48.0)	0.4126
90 mg	324.8	27.5 (−4.1; 69.4)	0.0926
C. Adaptive tracking.			
Adaptive tracking	**LSM**	**Est. difference from placebo (%) (95%CI)**	***p*-value**
Placebo	32.23	NA	NA
1 mg	31.74	−0.487 (−2.693; 1.718)	0.6595
2 mg	32.97	0.740 (−1.437; 2.917)	0.4984
6 mg	31.81	−0.414 (−2.599; 1.770)	0.7053
15 mg	29.28	−2.943 (−5.130; −0.756)	**0.0093**
30 mg	31.48	−0.745 (−3.052; 1.561)	0.5197
45 mg	30.39	−1.838 (−4.032; 0.357)	0.0989
60 mg	31.19	−1.040 (−3.221; 1.140)	0.3430
90 mg	31.49	−0.736 (−2.926; 1.455)	0.5036
D. Smooth pursuit.			
Smooth pursuit	**LSM**	**Est. difference from placebo (%) (95%CI)**	***p*-value**
Placebo	43.3	NA	NA
1 mg	44.4	1.15 (−1.50; 3.80)	0.3886
2 mg	42.8	−0.48 (−3.08; 2.11)	0.7118
6 mg	40.8	−2.51 (−5.11; 0.10)	0.0590
15 mg	45.0	1.78 (−0.89; 4.45)	0.1866
30 mg	41.6	−1.71 (−4.39; 0.96)	0.2052
45 mg	43.4	0.10 (−2.56; 2.76)	0.9401
60 mg	43.1	−0.17 (−2.77; 2.43)	0.8972
90 mg	43.6	0.30 (−2.31; 2.91)	0.8181
E. VAS Alertness.			
VAS Alertness	**LSM**	**Est. difference from placebo (mm) (95%CI)**	***p*-value**
Placebo	50.3	NA	NA
1 mg	50.0	−0.34 (−1.70; 1.03)	0.6218

(Continued)

Table 1. (Continued)

2 mg	50.2	−0.05 (−1.42; 1.32)	0.9386
6 mg	50.1	−0.19 (−1.56; 1.17)	0.7782
15 mg	49.4	−0.84 (−2.22; 0.53)	0.2231
30 mg	48.8	−1.44 (−2.84; −0.05)	**0.0432**
45 mg	49.7	−0.64 (−2.00; 0.72)	0.3505
60 mg	48.6	−1.65 (−3.07; −0.22)	**0.0243**
90 mg	50.0	−0.34 (−1.71; 1.03)	0.6228

F. VAS Feeling high.

VAS Feeling high	LSM	Est. difference from placebo (mm) (95%CI)	*p*-value
Placebo	0.332	NA	NA
1 mg	0.335	0.0030 (−0.0618; 0.0677)	0.9269
2 mg	0.311	−0.0214 (−0.0874; 0.0447)	0.5196
6 mg	0.311	−0.0214 (−0.0867; 0.0439)	0.5143
15 mg	0.331	−0.0014 (−0.0674; 0.0647)	0.9674
30 mg	0.392	0.0597 (−0.0063; 0.1258)	0.0754
45 mg	0.360	0.0278 (−0.0411; 0.0968)	0.4215
60 mg	0.358	0.0255 (−0.0398; 0.0908)	0.4375
90 mg	0.322	−0.0103 (−0.0764–0.0558)	0.7557

Data are presented as LSMs difference from placebo (95%CI). Bold *p* values represent significant results ($p<0.05$).
VAS: Visual Analogue Scale; LSM: least square mean.

exposure independent and inconsistent over the total period up to 10 h post-dose nor at the time points corresponding approximately to the T_{max} of the compound (2 h (Supplemental Table S1) and at 3 h (Supplemental Table S2) post-dose). The subjective outcomes VAS Alertness (Table 2E) and VAS Feeling high (Table 2F) also did not show a clinically meaningful effect compared with placebo. Furthermore, JNJ-61393215 did not demonstrate any consistent, dose-dependent differences compared to placebo in any of the EEG leads as expressed by the power in each frequency band (Table 3 showing estimates of the difference and 95% confidence, Supplemental Table S3 showing LSM changes and the *p* values). The Swiss Narcolepsy Scale did not reveal any effects on narcoleptic and cataplexic symptoms (Table 4 and Figure 3). Furthermore, dosing with JNJ-61393215 did not cause a significant increase in fatigue symptoms in the FACIT-Fatigue scale (Table 4 and Figure 3).

Safety

A more extensive description of the safety results is reported elsewhere (Salvadore et al., 2020). In short, no serious or severe TEAEs were reported. The most frequently occurring TEAEs were somnolence (16.7%) and headache (6.3%) and nasopharyngitis (8.3%). Somnolence also was reported in 2 out of 16 subjects (12.5%) dosed with placebo (Table 5).

Discussion

We present the pharmacological characterization of single doses of the selective OX1R antagonist JNJ-61393215 administered to healthy male volunteers in a first-in-human study. Assessments included both plasma and CSF PKs (the latter presented elsewhere) (Salvadore et al., 2020), PD characterization by means of a test battery covering several relevant functional CNS domains, and safety assessments. Doses ranged from 1 to 90 mg in a single ascending dose fashion, and they reached a predicted OX1R occupancy of >95% for the highest dose. To summarize, JNJ-61393215 did not affect vigilance or arousal, sustained attention, motor coordination, EEG parameters or subjective experience in healthy subjects despite clear dose-dependent plasma exposure associated with clear evidence for CNS penetration (Salvadore et al., 2020). In apparent contrast, somnolence was reported at doses higher than 6 mg; however, rates were comparable to somnolence reported under placebo.

The paucity of demonstrable CNS effects is unlikely due to an insufficient occupancy of OX1R's, since a CSF versus unbound plasma concentration ratio of 0.6 is expected to lead to maximal unbound CNS concentrations ranging between 0.8 and 82.2 ng/mL for 1 and 90 mg, respectively. In relation to PK findings in rodents, the half maximal effective concentration (EC_{50}) was set at 34 ng/mL, which corresponded to concentrations of the unbound fraction of 5.3 ng/mL in plasma and 5.0 ng/mL in CSF based on a brain-to-plasma ratio of 0.95; these levels are well below the CNS exposure expected to have been reached in humans in the current study. Rather, the relative lack of CNS effects could be explained by other factors. From a methodological perspective, the CNS test battery we utilized might not be suitable and/or sufficiently sensitive to detect the functional effects of OX1R antagonism in unchallenged healthy subjects. This pharmacological mechanism is a novel target in drug development, and it has only been described once before, with no relevant PD effects (Kaufmann et al., 2020). Also, the current findings seem to be in contrast to recent reports demonstrating sensitivity of the NeuroCart to single doses of OX2R antagonists and a DORA (Cruz et al., 2014; Groeneveld et al., 2016; Hoch et al., 2014; Hoever et al., 2012a), and the robust and consistent effects repeatedly demonstrated by the non-selective GABA-A agonists alprazolam and lorazepam (Chen et al., 2014, 2015, 2017; De Haas et al., 2007, 2008, 2009; Huizinga et al., 2019;

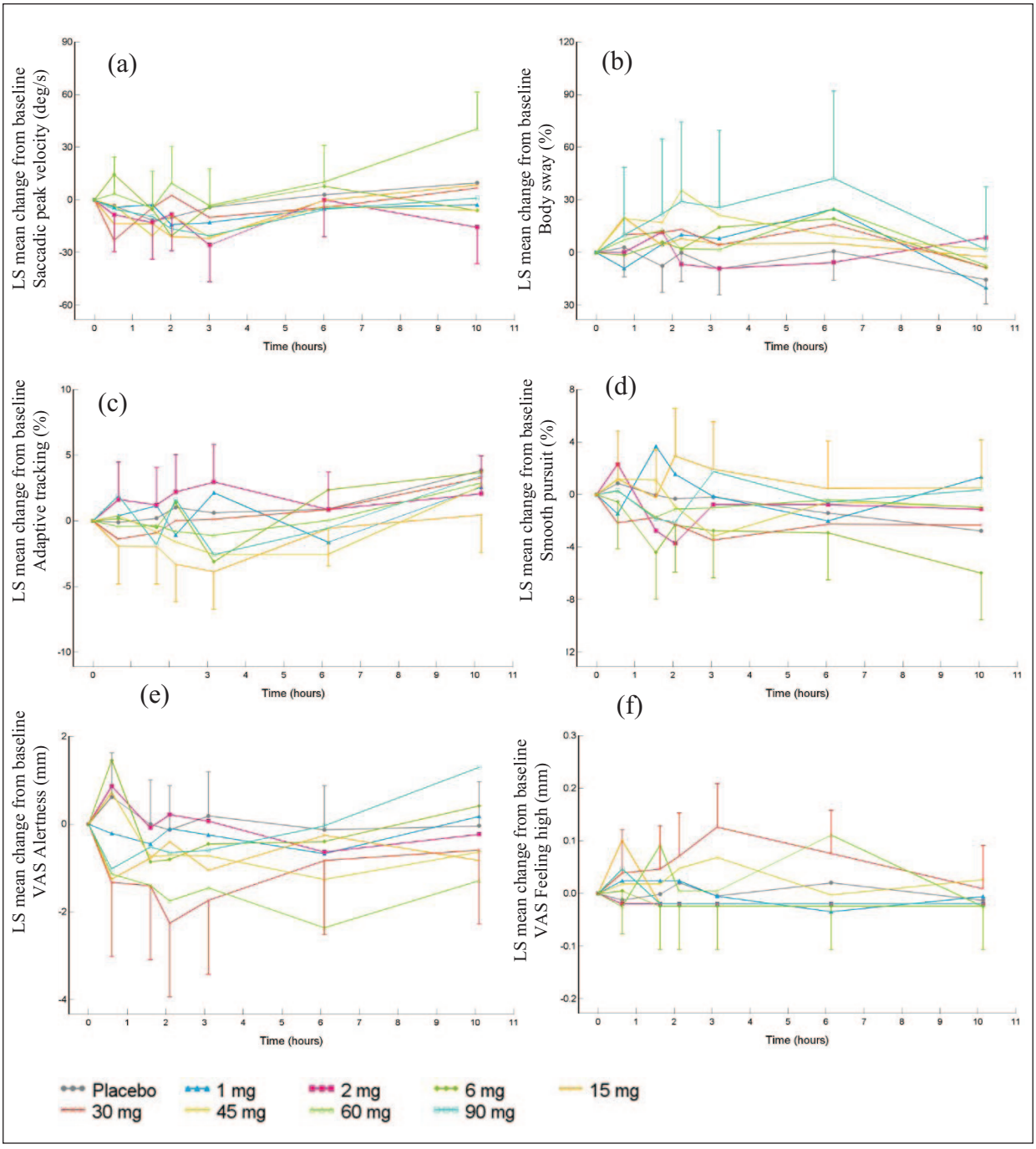

Figure 2. Least square (LS) mean change from baseline of saccadic peak velocity, (a) body sway, (b) adaptive tracking, (c) smooth pursuit, (d) Visual Analogue Scale (VAS) Alertness, (e) and VAS Feeling high, (f) up to 10 h post-dose. Same colours across figures represent same doses. Data are presented as LS mean ± 95%CI.

Zuiker et al., 2016). These compounds have shown significant, dose-dependent effects on body sway, adaptive tracking, saccadic peak velocity, smooth pursuit and VAS Bond & Lader in unchallenged healthy subjects. Together these findings are quite consistent with results from preclinical studies that demonstrate anxiolytic-like properties of OX1R inhibitors – including JNJ61393215 – in fear and anxiety challenges, but lack of behavioural or neurophysiological effects, including EEG, in unchallenged animal studies.

The human orexin system plays a crucial role in the coordination of adaptive physiological, behavioural and endocrine responses to salient stimuli or conditions such as exposure to aversive or potentially threatening or life-preserving stimuli (Johnson et al., 2010). Moreover, orexinergic overactivity has been associated with an increased sensitivity of panic and fear pathways to salient stimuli (Bonaventure et al., 2017) and compared to healthy controls (Johnson et al., 2010), patients with panic/anxiety have higher OX-A levels which are decreased after

Table 3. Estimate of the difference compared with placebo for EEG parameters (95%CI), period up to 10 h post-dose.

Dose	Alpha Fz-Cz	Alpha Pz-Oz	Beta Fz-Cz	Beta Pz-Oz	Delta Fz-Cz	Delta Pz-Oz	Gamma Fz-Cz	Gamma Pz-Oz	Theta Fz-Cz	Theta Fz-Cz
1 mg	−4.8 (−16.9;9.1)	−7.8 (−19.4;5.4)	0.7 (−9.1;11.4)	−4.4 (−13.5;5.5)	6.9 (−4.9;20.1)	−0.9 (−9.8;8.9)	3.4 (−5.5;13.2)	1.6 (−19.8;28.7)	1.3 (v9.3;13.1)	2.4 (−7.8;13.6)
2 mg	0.2 (−12.5;14.7)	−6.6 (−18.4;6.9)	−0.8 (−10.3;9.6)	−1.6 (−10.9;8.6)	−3.5 (−14.0;8.3)	−6.6 (−14.9;2.7)	−1.8 (−10.0;7.2)	1.1 (−20.2;27.9)	−6.2 (−16.0;4.7)	−3.1 (−12.7;7.5)
6 mg	5.9 (−7.6;21.3)	4.8 (−8.5;20.1)	−1.0 (−10.5;9.6)	6.7 (−3.3;17.9)	3.0 (−8.2;15.7)	4.8 (−4.8;15.3)	−1.3 (−9.6;7.7)	12.9 (−10.9;43.0)	−0.7 (−11.1;11.0)	5.2 (−5.3;16.9)
15 mg	−2.0 (−14.4;12.3)	−3.7 (−15.8;10.2)	0.7 (−8.9;11.3)	−6.0 (−14.9;3.8)	−1.9 (−12.6;10.0)	2.3 (−6.9;12.4)	−3.1 (−11.2;5.7)	−2.2 (−22.8;24.0)	−6.3 (−16.1;4.6)	7.6 (−3.1;19.4)
30 mg	−6.8 (−18.7;6.8)	−9.6 (−21.0;3.4)	−0.9 (−10.4;9.6)	−4.2 (−13.2;5.8)	1.3 (−9.9;13.8)	−4.2 (−12.9;5.4)	−1.4 (−9.6;7.5)	−0.9 (−21.7;25.5)	−1.7 (−11.9;9.8)	0.7 (−9.3;11.8)
45 mg	1.0 (−11.9;15.8)	2.7 (−10.3;17.5)	7.9 (−2.6;19.5)	4.8 (−5.1;15.8)	−7.1 (−17.3;4.4)	−7.0 (−15.4;2.2)	1.9 (−6.6;11.3)	7.5 (−15.2;36.2)	1.0 (−9.5;12.7)	4.4 (−6.0;16.0)
60 mg	−10.2 (−21.6;2.8)	**−12.7 (−23.7; 0.0)**	−3.6 (−12.8;6.6)	−6.0 (−14.9;3.9)	2.5 (−8.6;15.0)	−4.5 (−13.0;5.0)	−6.0 (−13.8;2.6)	6.3 (−16.3;35.0)	−2.5 (−12.7;8.8)	−2.9 (−12.5;7.8)
90 mg	−5.7 (−17.8;8.1)	−5.8 (−17.7;7.8)	−2.7 (−12.2;7.7)	−2.9 (−12.1;7.3)	−8.0 (−18.3;3.6)	−5.1 (−13.7;4.3)	**−8.4 (−16.0;0.0)**	11.5 (−11.9;41.1)	−6.8 (−16.5;4.1)	2.2 (−7.9;13.4)

Data are presented as LSMs difference from placebo (95%CI).
Unit is percentage (%).
Bold numbers represent significant difference from placebo.

Table 4. Estimate of the difference compared with placebo of Swiss Narcolepsy Scale and FACIT-Fatigue (95%CI), period up to 24 h post-dose.

Dose	Swiss Narcolepsy Scale			FACIT-Fatigue		
	LSM	Est. difference from placebo (mm) (95%CI)	*p*-value	LSM	Est. difference from placebo (mm) (95%CI)	*p*-value
Placebo	17.4	NA	NA	47.8	NA	NA
1 mg	15.6	−1.79 (−6.78;3.20)	0.4751	48.5	0.71 (−1.11;2.53)	0.4351
2 mg	21.8	4.42 (−0.57;9.40)	0.0814	47.8	−0.08 (−1.90;1.74)	0.9341
6 mg	17.3	−0.11 (−5.09;4.88)	0.9660	48.5	0.67 (−1.17;2.50)	0.4691
15 mg	20.9	3.53 (−1.47;8.53)	0.1625	46.1	−1.78 (−3.60;0.05)	0.0561
30 mg	21.0	3.67 (−1.32;8.65)	0.1462	47.4	−0.41 (−2.23;1.42)	0.6587
45 mg	21.7	4.36 (−0.87;9.58)	0.1005	48.4	0.62 (−1.21;2.44)	0.5009
60 mg	19.9	2.58 (−2.41;7.57)	0.3049	46.2	−1.63 (−3.60;0.33)	0.1011
90 mg	17.2	−0.16 (−5.14;4.83)	0.9498	47.7	−0.10 (−1.93;1.72)	0.9111

FACIT: Functional Assessment of Chronic Illness Therapy; LSM: least square mean.
Data are presented as LSMs difference from placebo (95%CI).

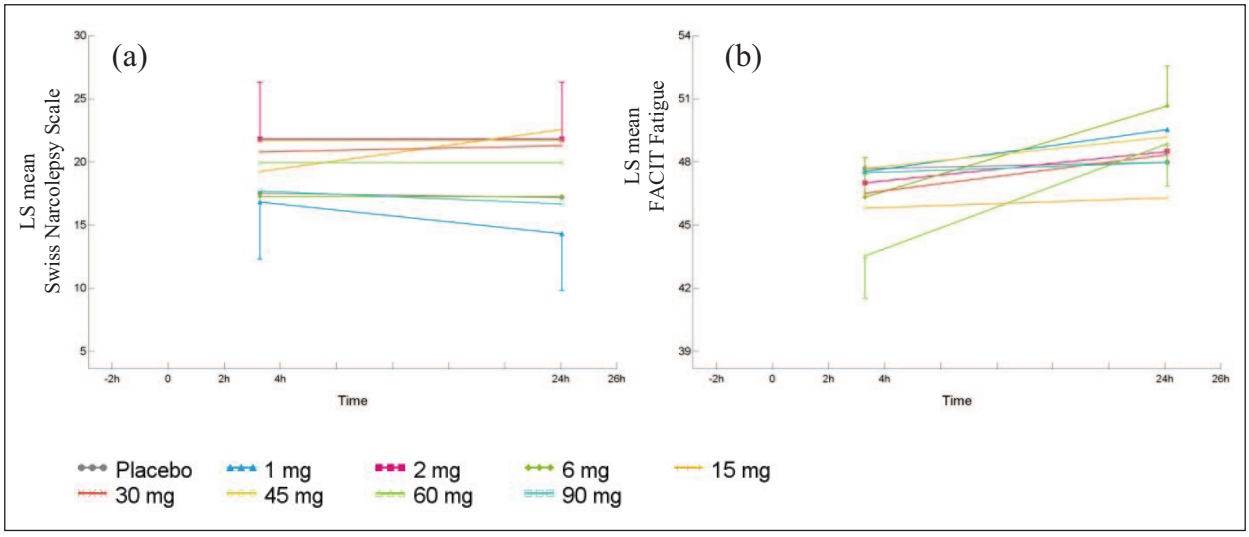

Figure 3. Least square (LS) mean of Swiss Narcolepsy Scale, (a) and Functional Assessment of Chronic Illness Therapy Fatigue, (b) up to 24 h post-dose. Same colours across figures represent same doses. Data are presented as LS mean ± 95%CI.

treatment with the SSRI sertraline (Salomon et al., 2003). In contrast, OX2R function has been related more to the regulation of circadian rhythm and energy metabolism, which are related to sleep or wakefulness. Input stimuli of OX1R consist of opportunities for reward, challenges and threats, which lead to adaptation in behaviour (Suzuki et al., 2005). Similar to what was found in preclinical experiments with JNJ-61393215, it could therefore be expected that the magnitude of OX1R signalling is relatively low in healthy individuals studied under the relaxed testing circumstances of the research laboratory in which few salient stimuli are presented. In such conditions, the functional consequences of OX1R-antagonism may not be detectable due to 'floor effects' using the test battery applied herein. In contrast, the resting behavioural condition is suitable to elicit the soporific effects of OX2 inhibition, as demonstrated by the consistent effects of DORAs and selective OX2-antagonist in healthy volunteer studies (Hoever et al., 2010, 2012b; Muehlan, 2019a; Muehlan, 2019b; van der Ark et al., 2018).

Since selective OX1R antagonism under physiological conditions does not demonstrate functional CNS effects in the current study, alternative approaches to detect PD effects relevant for potential therapeutic effects require consideration. In this light, we incorporated a validated experimental panic paradigm in the multiple ascending dose study performed following the study described herein (Salvadore et al., 2020). The results of that study which assessed the effects of JNJ-61393215, alprazolam and placebo on CO_2 inhalation challenge induced panic symptoms are described elsewhere (Salvadore et al., 2020). Those data supported the significant reduction of symptoms of panic by administering multiple doses of the selective OX1R antagonist JNJ-61393215.

To conclude, single doses of the selective OX1R antagonist JNJ-61393215 did not demonstrate PD effects following administration under non-challenged CNS-testing conditions in healthy human subjects, despite attaining relevant CNS exposure. Considering that OX1R-activation is implicated in fear and anxiety, and that reduction of fear-related phenomena arising in an experimental human CO_2 inhalation model for panic has been established (Salvadore et al., 2020), further research into the effects of antagonizing OX1R with JNJ-61393215 is warranted

Table 5. Frequency (%) of three most common treatment emergent adverse events.

Adverse event	Placebo ($n=16$)	Cohort 1 1 mg ($n=6$)	Cohort 2 2 mg ($n=6$)	Cohort 3 6 mg ($n=6$)	Cohort 4 15 mg ($n=6$)	Cohort 5 30 mg ($n=6$)	Cohort 6 45 mg ($n=6$)	Cohort 7 60 mg ($n = 6$)	Cohort 8 90 mg ($n=6$)	Total in JNJ groups
Somnolence	2 (12.5)	–	–	2 (33.3)	1 (16.7)	–	2 (33.3)	2 (33.3)	1 (16.7)	8 (16.7)
Headache	6 (37.5)	–	–	–	–	2 (33.3)	1 (16.7)	–	–	3 (6.3)
Nasopharyngitis	–	–	1 (16.7)	–	–	2 (33.3)	1 (16.7)		1 (16.7)	5 (10.4)

in patients with mood and anxiety disorders who manifest panic-related and/or fear-related symptoms.

Authors' note

The authors confirm that the PI for this paper is GE Jacobs and that he had direct clinical responsibility for the patients.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: Authors Bleys, Moyer, Van Nueten, Bonaventure and Drevets are employees of Janssen Research & Development, LLC, of Johnson & Johnson and own equity in Johnson & Johnson.

Author Salvadore was an employee and shareholder of Johnson & Johnson, LLC at the time when the study was conducted and is a current employee and shareholder of Acadia Pharmaceuticals, Inc.

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Ethics approval statement

The study was conducted at the Centre for Human Drug Research (CHDR) in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands) and was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki.

Patient consent statement

Each subject provided written informed consent before any screening procedures were performed.

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Not applicable.

Clinical trial registration

This study was registered on ClinicalTrial.gov under number NCT02812251.

ORCID iDs

S Brooks https://orcid.org/0000-0003-3539-2908

RGJA Zuiker https://orcid.org/0000-0001-5604-0157

Data availability statement

The data that support the findings of this study are available from the corresponding author, GJ, upon reasonable request.

Supplemental material

Supplemental material for this article is available online.

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