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Evaluating the efficacy of the selective orexin 1 receptor antagonist nivasorexant in an animal model of binge-eating disorder

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Abstract

Objective: Test the efficacy of the selective orexin 1 receptor (OX1R) antagonist (SO1RA) nivasorexant in an animal model of binge-eating disorder (BED) and study its dose–response relationship considering free brain concentrations and calculated OX1R occupancy. Compare nivasorexant's profile to that of other, structurally diverse SO1RAs. Gain understanding of potential changes in orexin-A (OXA) neuropeptide and deltaFosB (ΔFosB) protein expression possibly underlying the development of the binge-eating phenotype in the rat model used.

Method: Binge-like eating of highly palatable food (HPF) in rats was induced through priming by intermittent, repeated periods of dieting and access to HPF, followed by an additional challenge with acute stress. Effects of nivasorexant were compared to the SO1RAs ACT-335827 and IDOR-1104-2408. OXA expression in neurons and neuronal fibers as well as ΔFosB and OXA-ΔFosB co-expression was studied in relevant brain regions using immuno- or immunofluorescent histochemistry.

Results: All SO1RAs dose-dependently reduced binge-like eating with effect sizes comparable to the positive control topiramate, at unbound drug concentrations selectively blocking brain OX1Rs. Nivasorexant's efficacy was maintained upon chronic dosing and under conditions involving more frequent stress exposure. Priming for binge-like eating or nivasorexant treatment resulted in only minor changes in OXA or ΔFosB expression in few brain areas.

Discussion: Selective OX1R blockade reduced binge-like eating in rats. Neither ΔFosB nor OXA expression proved to be a useful classifier for their binge-eating phenotype. The current results formed the basis for a clinical phase II trial in BED, in which nivasorexant was unfortunately not efficacious compared with placebo.

Public Significance: Nivasorexant is a new investigational drug for the treatment of binge-eating disorder (BED). It underwent clinical testing in a phase II proof of

Michel Alexander Steiner and Luca Botticelli contributed equally to this study.

concept trial in humans but was not efficacious compared with placebo. The current manuscript investigated the drug's efficacy in reducing binge-like eating behavior of a highly palatable sweet and fat diet in a rat model of BED, which initially laid the foundation for the clinical trial.

KEYWORDS
animal model, binge-eating disorder, deltaFosB, hypocretin, nivasorexant, orexin, orexin 1 receptor antagonist, rats

1 | INTRODUCTION

Orexin neuropeptides are best known for their wakefulness-stabilizing effects (Adamantidis et al., 2007). While mice and dogs with an absent or dysfunctional orexin 2 receptor (OX2R) show prominent dysregulations of sleep and wake (Hungs et al., 2001; Willie et al., 2003), this is, however, not the case for mice lacking the orexin 1 receptor (OX1R) (Hondo et al., 2010). It is believed that the role of the OX1R is to modulate reward- and anxiety-related processes (Hopf, 2020; Sargin, 2019). First clinical proof of concept (POC) trials with the selective OX1R antagonists (SO1RAs) JNJ-61393215 (Salvadore et al., 2020) and nivasorexant (Kaufmann et al., 2021) on CO_2 challenge-induced panic anxiety and in major depressive patients with anxious distress (ClinicalTrials.gov, NCT04080752), nevertheless, have yielded ambiguous results. A proven translation of the anxiolytic effects discovered in animals to human still remains to be shown.

The largest part of the preclinical research on the function of the OX1R has so far been conducted with the SO1RA SB-334867, which was discovered early on by GlaxoSmithKline (Smart et al., 2001). Unfortunately, this molecule showed later off-target activity at adenosine and serotonin receptors as well as on monoamine transporters and may decompose easily to OX1R-inactive, but possibly otherwise active, metabolites (Gotter et al., 2012; McElhinny Jr. et al., 2012). Therefore, it is important to confirm findings with SB-334867 with newer SO1RAs designed for clinical testing that were examined for having a clean off-target profile. Besides JNJ-61393215, nivasorexant is the first SO1RA recently entering human testing. It showed no relevant off-target activity at therapeutic concentrations on more than 130 selected proteins (Suppl. Table 1), including the targets in question for above mentioned SB-334867.

OX1Rs are located within brain nuclei directly involved in reward processing and dopamine signaling, including the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC) and amygdala, but also in nuclei at the edge of the mesolimbic dopamine system, such as the locus coeruleus (LC), paraventricular nucleus of the thalamus (PVT) and bed nucleus of the stria terminalis (BNST) (Darwinkel et al., 2014; Lei et al., 2019; Marcus et al., 2001). From a behavioral perspective, pharmacological OX1R blockade reduces reward seeking in animals (primarily under high effort demand conditions) in relation to almost all known classes of drugs of abuse (Hopf, 2020; James et al., 2017). Seeking of sweet and palatable foods and liquids can also be under control of OX1R signaling (Borgland et al., 2009; Buczek et al., 2020), especially during fasting and expectation of food availability (Akiyama et al., 2004; Johnstone et al., 2006; Mieda et al., 2004; Sakurai et al., 1998). The involvement of orexins in the motivation to obtain food (Borgland et al., 2009) and in foraging behavior (Akiyama et al., 2004; Hassani et al., 2016) opens the possibility for a potential role in pathological eating behaviors.

Binge-eating disorder (BED) is characterized by episodes of excessive eating within short periods of time, associated with feelings of guilt and a loss of control over one's eating pattern (American Psychiatric Association, 2013; Giel et al., 2022). It can be precipitated by stressful life events, repeated periods of dieting, or genetic predisposition (Braun et al., 2019; Giel et al., 2022; Lie et al., 2021; Manfredi et al., 2021). BED is a complex psychiatric disorder and not easy to model in animals; however, several attempts have been made (Corwin et al., 2011; Razzoli et al., 2017). One model stands out because of its large face, construct, and presumed predictive validity. Here, binge-like eating is provoked in female rats primed through intermittent periods of fasting, access to highly palatable food (HPF), and an acute additional stressor (Cifani et al., 2009). The locomotor activation triggered during this stress exposure (Cifani et al., 2020; Micioni Di Bonaventura et al., 2017), might be related to orexins' arousal-promoting effects (Tsujino & Sakurai, 2013). Some experiments have provided first evidence of SO1RAs being able to reduce binge-like drinking or eating in rodents (Alcaraz-Iborra et al., 2014; Piccoli et al., 2012; Rodriguez-Ortega et al., 2019; Vickers et al., 2015). Moreover, intermittent fasting may increase or alter orexin neuron activity (Horvath & Gao, 2005; Pankevich et al., 2010), and possibly render the system more susceptible to pharmacological manipulation.

We used the aforementioned rat model of binge-like HPF consumption (Cifani et al., 2009), to (i) investigate the potential of nivasorexant (Kaufmann et al., 2020) and, for confirmatory purposes, other SO1RAs for reducing binge-like eating, and to link efficacy to calculated unbound drug concentrations at the brain OX1R, (ii) explore nivasorexant's effects under chronic treatment and in an advanced model setup, and (iii) try to obtain a deeper understanding at the protein level of the potential underlying plasticity changes within (orexin-A, OXA) and beyond (delta FosB, ΔfosB) the orexin system responsible for the binge-eating phenotype. Changes in the expression of ΔfosB, a transcription factor up-regulated in various brain regions following repeated exposure to drugs of abuse or stress (Lobo et al., 2013; Nestler, 2015), were studied as a marker of long-term neuronal adaptations and plasticity.

2 | METHOD

2.1 | Animals

Female Sprague Dawley rats (Charles River, Calco [LC], Italy; 200–225 g, 9 weeks old at the beginning of the experiments) were singly housed at the animal facility of the University of Camerino under controlled temperature and humidity and a regular 12 h light/dark cycle (lights on at 8:00 a.m.) with free access to water and food (unless stated otherwise). Only female rats were used, in alignment with the high prevalence for eating disorders among adolescent and young, adult women (Swanson et al., 2011). Experiments were authorized by the Italian Ministry of Health (authorization n° 692/2021-PR).

2.2 | In vivo pharmacology experiments

The binge-eating protocol was performed as previously established (Cifani et al., 2009). Briefly, rats were primed during three 8-day-cycles of repeated periods of dieting (66% of chow intake on days 1–4 and free feeding on days 5–8 of each cycle), during which they had intermittent access to HPF (Nutella mixed with ground rat chow and water; 52%, 33%, and 15% weight, respectively). When then additionally challenged on the test day (day 25) with 15 min of prior frustration stress, during which rats could see and smell the HPF but had no access to it, they showed a binge-eating phenotype during 2 h of free HPF access. Besides this binge-eating condition (*R*estriction and *S*tress, R + S), various control conditions, that did not lead to binge-like eating, were applied: *N*either food *R*estriction *N*or *S*tress (NR + NS), food *R*estriction but *N*o *S*tress (R + NS), or food ad libitum (*N*o *R*estriction) and *S*tress (NR + S). To increase the face validity of the model, in one experiment, we also tested *R*epeated frustration *S*tress (RS) exposure during the priming period (Suppl. Figure 1). Rats exposed to frustration stress were housed separately from the other rats to prevent spreading of the HPF's smell.

Once established, binge-like eating behavior remains stable over time when intermittent periods of food restriction are continued (Piccoli et al., 2012; Pucci et al., 2022). Thus, in two experiments, testing of binge-like HPF intake was repeated at day 35: after one day off at the end of the first test (day 25), all rats remained in the same experimental group and received an additional 8-day cycle. The following day (day 35, second test), only the NR + S and R + S groups were exposed to stress.

We conducted in total three independent experiments (Exp.) with $n = 144$ rats each. In Exp. 1, we tested the acute (1 h pretreatment time) effect of the SO1RA ACT-335827 (100 or 300 mg/kg), an initial drug development candidate of Idorsia (Steiner et al., 2013), on HPF intake at day 25 under several control conditions and the binge-eating condition (R + S). In Exp. 2, we tested the acute (−1 h) effects of three SO1RAs under one control (NR + NS) and the binge-eating (R + S) condition: nivasorexant (1.5, 5, 15, or 50 mg/kg) (Kaufmann et al., 2020) at day 25, and IDOR-1104-2408 (5, 15, or 50 mg/kg) (example 60 in Riether et al., 2017) and a low dose (30 mg/kg) of ACT-335827 both at day 35. In Exp. 3, we tested an efficacious dose of nivasorexant (15 mg/kg) acutely (−1 h) at day 25 under various control conditions, and the binge-eating condition, as well as in the model setup with repeated stress (Suppl. Figure 1). We continued to treat a subset of rats tested at day 25 for another consecutive 10 days with daily administrations of nivasorexant (15 mg/kg) and retested them at day 35, 1 h after the last treatment, to investigate whether the drug effect was maintained. At the end, following 1 week of drug washout, some rats from Exp. 2 and 3 were used for pharmacokinetic evaluations and ex vivo immunohistochemistry.

The estrous phase of the rats was determined according to cytology of vaginal smears (Suppl. Methods) immediately after testing for HPF intake on days 25 or 35 by an experimenter blinded to treatment.

2.3 | Drugs and formulations

Nivasorexant (ACT-539313), ACT-335827C, and IDOR-1104-2408 (example 60 in Riether et al., 2017), were synthesized at Idorsia Pharmaceuticals Ltd. Topiramate (Topamax® tablets) was from Janssen-Cilag SpA, Cologno Monzese (MI), Italy. Drugs were formulated as aqueous solution or suspension in different vehicles and formulated for oral gavage at 5 mL/kg body weight (see Suppl. Methods for details). Dose ranges of SO1RAs were chosen such as to achieve free drug brain concentrations with the lowest dose starting approximately at or few fold above the equilibrium dissociation constants (appK_b and K_b) at the OX1R at the time of testing and higher doses increasing by half log units.

The anticonvulsant drug topiramate was used as a positive control at 60 mg/kg, which was previously validated (Cifani et al., 2009; Piccoli et al., 2012). Topiramate is frequently prescribed off-label for treating BED (McElroy, 2017) and has demonstrated clinical efficacy in five randomized, placebo-controlled trials in BED or bulimia nervosa (Arbaizar et al., 2008; McElroy, 2017).

2.4 | Pharmacokinetics

Rats were killed by decapitation at different time points after oral drug treatment. Drug concentrations in collected plasma (ng/mL) and brain homogenate (ng/g of tissue) were determined by liquid chromatography coupled with tandem mass spectrometry. See Suppl. Methods for more details.

2.5 | Immunohistochemistry

A sub-selection of rats from Exp. 3 (chronically vehicle- and nivasorexant-treated control (NR + NS) and binge-eating (R + S) rats; $n = 7$ per treatment and condition; $n = 28$ total), which were not in the estrous phase on test day 35 (to reduce variability in the ex vivo markers as response to their HPF intake) were euthanized through isoflurane inhalation on day 36, 24 h after the last vehicle or drug

administration. Following transcardial perfusion of rats and post-fixation of brains with 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, brains were shipped to Neuroscience Associates (NSA; Knoxville, TN, USA) for sectioning and staining. The ΔFosB and OXA IHC were performed through the entire length of the left hemisphere (minus olfactory bulbs). ΔFosB signal was quantified in the medial PFC (mPFC), NAc core (NAcC) and shell (NAcSh), the ventral, lateral, intermediate, medial, and total caudate putamen (CPu), paraventricular hypothalamic nucleus (PVN) and VTA (Suppl. Figure 11 and 13). OXA signal (Figure 4a–c) was quantified in the mPFC (incl. the prelimbic, PR, and infralimbic, IL cortices), the BNST, the VTA, the LC (Suppl. Figure 12), the central amygdala (CeA; Figure 4d), the dorsal raphe nucleus (DRN; Figure 4e) and the PVT (Figure 4f). ΔFosB/OXA double immunofluorescent histochemistry was performed through the entire lateral hypothalamus (LH). The number of ΔFosB-positive cells, OXA-positive cells and ΔFosB/OXA co-expressing neurons was quantified in three hypothalamic areas (LH, perifornical area, PeF, and dorsomedial hypothalamus, DMH) where OXA-positive neurons are predominantly located (Figure 5a–e). Signals were averaged across all brain sections stained for each region. See Suppl. Methods for experimental details.

2.6 | In vitro pharmacology

The potency (appK_b and K_b) and selectivity of the three SO1RAs were determined in two types of OXA mediated OX1R and OX2R antagonist curve shift assays, one based on calcium mobilization, the other on myo-inositol-1-phosphate (IPOne) accumulation, in CHO and HEK293 cells, recombinantly expressing the OXRs (see Suppl. Methods for details).

2.7 | Statistics

All rats retrospectively determined to have been in the estrous phase during testing at day 25 or 35 were excluded from the statistical analyses because spontaneous eating in rats decreases during estrus (Asarian & Geary, 2013) and their binge-eating phenotype becomes weaker under hormonal influence (Klump et al., 2008; Micioni Di Bonaventura et al., 2017), similarly to variation of binge-eating rates across the menstrual cycle in women (Klump et al., 2014). Individual HPF intake per time period was divided by the rat's body weight and multiplied by HPF's caloric value of 3.6296 kcal/g. Body weights between different rat cohorts or treatment groups were compared using one-way analysis of variance (ANOVA) or Student's *t*-test. HPF intake between the vehicle and the different treatment groups were analyzed separately for each test day (i.e., day 25 or 35) and condition (e.g., NR + NS, R + S, etc.) by two-way ANOVA for the factors treatment and time (test-time, 0–120 min; repeated measure) followed by Sidak's (single dose experiments) or Dunnett's (multiple dose experiments) post-hoc test. Effects of treatment and priming on OXA and ΔFosB expression were analyzed separately for each region and marker by ANOVA (whole region) or mixed model (across different bregma levels). Significance level was set at $p < .05$.

3 | RESULTS

Approximately 25% of rats were excluded from the analyses because they were in estrous phase at the time of testing, and their binge-eating phenotype is then not as prominent (see Suppl. Tables 2–4 for the final group sizes). However, even when rats in estrous were included in the analyses, the overall findings regarding efficacy of treatments under different test conditions (see below) remained the same (Suppl. Figures 2–4). The temporary body weight loss experienced by some rats during the repeated fasting episodes throughout the priming period was fully recovered by the times of testing (Suppl. Figure 5–7). The mean body weights of rats assigned to the various treatment groups within each specific experimental condition at the test days were comparable (Exp. 1: $p > .72$; Exp. 2: $p > .72$; Exp. 3: $p > .24$; Suppl. Tables 2–4).

3.1 | Experiment 1

As shown previously (Cifani et al., 2009), priming rats with intermittent periods of food restriction, and exposing them to additional frustration stress before testing, resulted in increased (by approximately 60%) binge-like intake of HPF in vehicle-treated rats in comparison to rats submitted to any of the three control conditions (compare the vehicle groups in panel D to those in panels A–C in Figure 1). The majority of food intake happened during the first 15 min of the 2 h test period. ACT-335827, a previous drug development candidate of Idorsia (Steiner et al., 2013) but abandoned because of unfavorable DMPK properties for human use, was the first SO1RA that we tested. ACT-335827 and topiramate had no meaningful effect on HPF intake in any of the control conditions (Figure 1a-c). However, under binge-eating conditions, both drugs reduced HPF intake ($p < .001$; Figure 1d; see Suppl. Table 5 for detailed statistics) to an extent equivalent to intake levels observed in control conditions.

3.2 | Experiment 2

To corroborate results obtained with ACT-335827 in Exp. 1, we tested two additional, structurally different SO1RAs. This time, we used only one control condition (NR + NS), given that ACT-335827 had not affected HPF intake in any of the previously tested control conditions. Both nivasorexant and IDOR-1104-2408 reduced binge-like intake of HPF in a dose-dependent manner to an extent similar to that achieved with topiramate ($p < .001$, Suppl. Table 5; Figure 2b, d). Neither SO1RA affected HPF intake under control conditions (Figure 2a, c). The lowest efficacious dose of nivasorexant was 5 mg/kg and that of IDOR-1104-2408 was 15 mg/kg. ACT-335827 at 30 mg/kg, a lower dose than used in Exp. 1, was still efficacious.

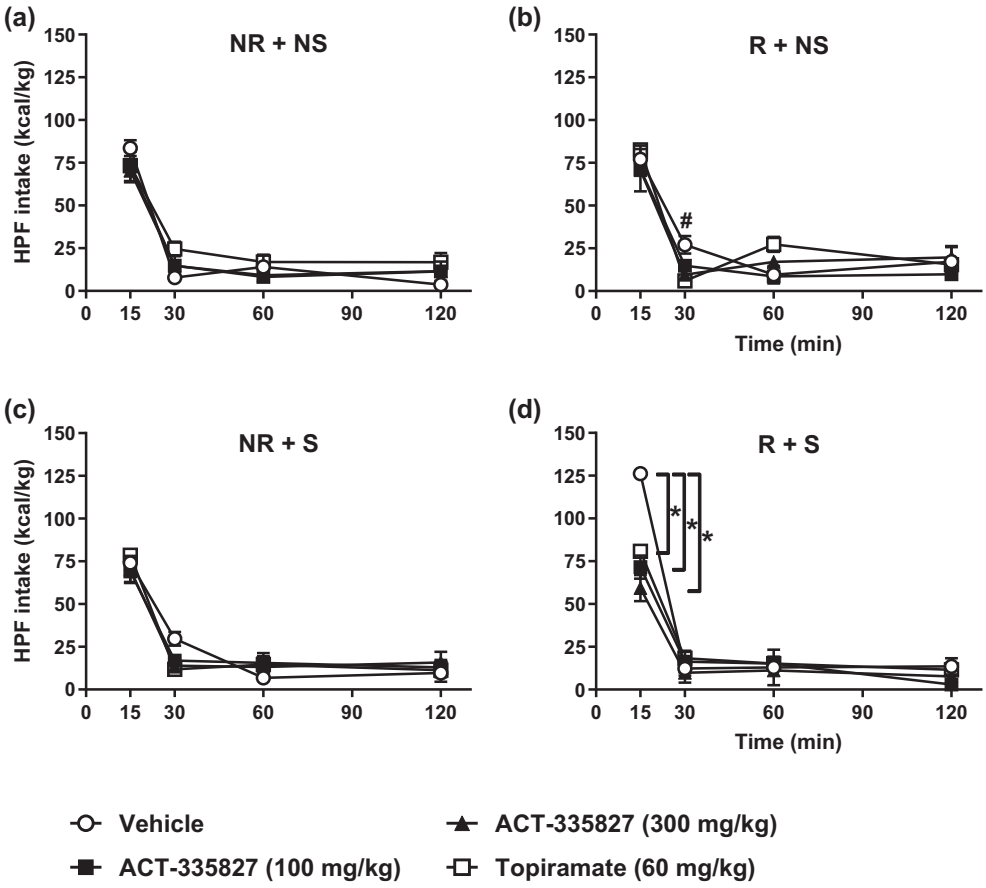

FIGURE 1 Effects of the SO1RA ACT-335827 on binge-like eating behavior in rats. Starting 1 h after oral treatment, highly palatable food (HPF) intake was measured as kcal/kg body weight during a 2 h test period under different control (non-restricted + non-stressed [NR + NS; a], restricted + non-stressed [R + NS; b], non-restricted + stressed [NR + S; c]), or binge eating (restricted + stressed [R + S; d]) conditions. Data are mean ± SEM. $N = 6–7$/group. $^{*}p < .0001$ versus vehicle at the 15 min time-point, $^{\#}p < .05$ for topiramate versus vehicle at the 30 min time-point (Dunnett's post hoc test following ANOVA). SEM, standard error of the mean; SO1RA, selective orexin 1 receptor antagonist.

The drug effects on reducing HPF intake were greatest at the beginning of testing, within 1 h and 1 h and 15 min after drug administration. The corresponding, calculated free brain concentrations of ACT-335827 and nivasorexant at their lowest efficacious doses at 1 h after administration were around the OX1R K_b in the FLIPR and IPOne assays (corresponding to approximately 50%–80% of estimated OX1R occupancy); the concentrations of IDOR-1104-2408 were around 50- to 100-fold the K_b, or >98% OX1R occupancy (Table 1). Similar calculations showed that the OX2R was not engaged at those doses by any SO1RA (OX2R occupancy <3%). The total plasma concentration of topiramate at 60 mg/kg in rats (Suppl. Table 9) matched that achieved in human patients at doses of around 400 mg/day that are effectively reducing binge-eating (Christensen et al., 2001; Garnett, 2000).

3.3 | Experiment 3

We extended the investigations on nivasorexant using additional experimental settings (Suppl. Figure 1). Nivasorexant did not influence regular HPF intake under any control condition (Figure 3a–c, e, g), including those not previously tested in Exp. 2 (Figure 3b, c, e). Its efficacy in reducing binge-like eating under the regular protocol (Figure 2b) was confirmed ($p < .001$, Suppl. Table 5; Figure 3d) and extended to a protocol variation involving repeated stress ($p < .001$; Figure 3f), which resulted in a nominally 20% higher HPF intake in the first 15 min. Nivasorexant's efficacy was preserved upon chronic treatment ($p < .001$; Figure 3h). For this, the dose of 15 mg/kg of nivasorexant was chosen to provide the longest possible duration of action while remaining selective for OX1R blockade. Nivasorexant brain concentrations after repeated and acute dosing were comparable (Suppl. Table 10).

3.4 | Experiment 4

We investigated whether binge-eating rats would show differences in OXA or ΔFosB protein expression (Figures 4 and 5) in brain regions involved in reward-processing and binge-eating behavior (Kessler et al., 2016; Quansah Amissah et al., 2020; Romano et al., 2020) that are either innervated by OXA fibers (Nambu et al., 1999) and/or react prominently to external stimuli with ΔFosB expression (Perrotti et al., 2008). Moreover, we assessed ΔFosB co-expression in OXA producing neurons in the hypothalamus. The truncated, long-lived immediate early gene (IEG) transcription factor ΔFosB is frequently used as a protein marker of continuous, long-lasting neuronal activity (Nestler, 2015), and OXA expression varies depending upon environmental influences such as continuous exposure to drugs of abuse (Thannickal et al., 2018).

Neither priming for binge-eating nor chronic nivasorexant treatment affected the amount of OXA-fiber staining in the mPFC, BNST,

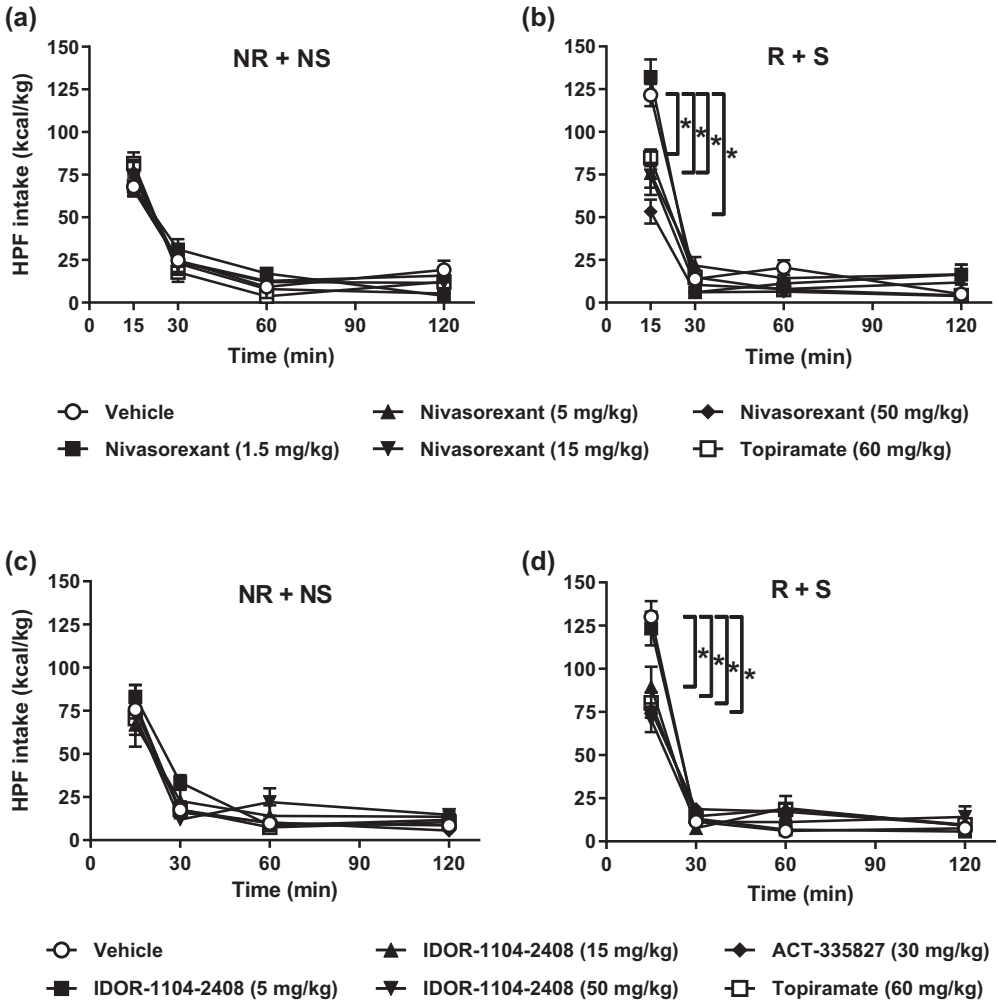

FIGURE 2 Effects of the SO1RAs nivasorexant, IDOR-1104-2408, and ACT-335827 on binge-like eating behavior in rats. Starting 1 h after oral treatment, highly palatable food (HPF) intake was measured as kcal/kg body weight during a 2 h test period under control (non-restricted + non-stressed [NR + NS]) or binge eating (restricted + stressed [R + S]) conditions. The same rats were tested repeatedly, first for acute effects of nivasorexant (a, b) at day 25, and second, after 10 days of drug-washout, for acute effects of IDOR-1104-2408 and low dose of ACT-335827 (c, d) at day 35. Data are mean ± SEM. $N = 7–11$/group. *$p < .0001$ versus vehicle at the 15 min time-point (Dunnett's post hoc test following ANOVA). SEM, standard error of the mean; SO1RA, selective orexin 1 receptor antagonist.

TABLE 1 Mean apparent K_b and mean K_b values of different SO1RAs as determined in immortalized cell lines expressing human OX1R and OX2R and fold-ratios/receptor occupancies in relation to calculated free drug brain concentrations at 1 h after oral administration at lowest efficacious doses in a rat binge-eating model.

Assay	Receptor	ACT-335827	Nivasorexant	IDOR-1104-2408
FLIPR: appKb [sg], given in nM	OX1R	5.4 [1.7]	0.5 [1.6]	0.8 [1.3]
	OX2R	498 [3]	69 [1.3]	>1000
IPOne: Kb [sg], given in nM	OX1R	15 [1.7]	1.5 [2.1]	1.4 [2.1]
	OX2R	566 [1.2]	172 [1.3]	2581 [1.3]
Fold-ratio above appKb (FLIPR); receptor occupancy	OX1R	2.6; 72%	4; 80%	101; 99%
	OX2R	0.03; 3%	0.03; 3%	n.a.
Fold-ratio above Kb (IPOne); receptor occupancy	OX1R	0.9; 47%	1.3; 57%	58; 98%
	OX2R	0.02; 2%	0.01; 1%	0.03; 3%

Note: The appKb values were derived from orexin-A-SO1RA concentration response curves in the calcium release curve shift assay using the generalized Cheng-Prusoff equation. The Kb values were derived from orexin-A-SO1RA concentration response curves in the IP_1 accumulation curve shift assay using the Gaddum/Schild EC50 shift equation (Suppl. Figures 8 and 9). FLIPR = Fluorescence Imaging *Plate Reader* technology assay to measure calcium influx; IPOne = myo-inositol 1 phosphate accumulation assay. The geometric mean and geometric standard deviation [σg] are shown. Free brain concentration at lowest efficacious dose: 14 nM for ACT-335827 at 30 mg/kg, 2 nM for nivasorexant at 5 mg/kg, 81 nM for IDOR-1104-2408 at 15 mg/kg (Suppl. Tables 6–8). Free brain concentration was calculated from in vitro brain homogenate binding and total rat brain concentration measurements at 1 h after compound administration. Receptor occupancies were calculated from $appK_b$ and K_b values and free brain concentrations assuming one site saturation binding using the formula RecOcc = [free conc/$appK_b$]/[(free conc/$appK_b$) + 1] × 100%. SO1RA, selective orexin 1 receptor antagonist; n.a., not applicable.

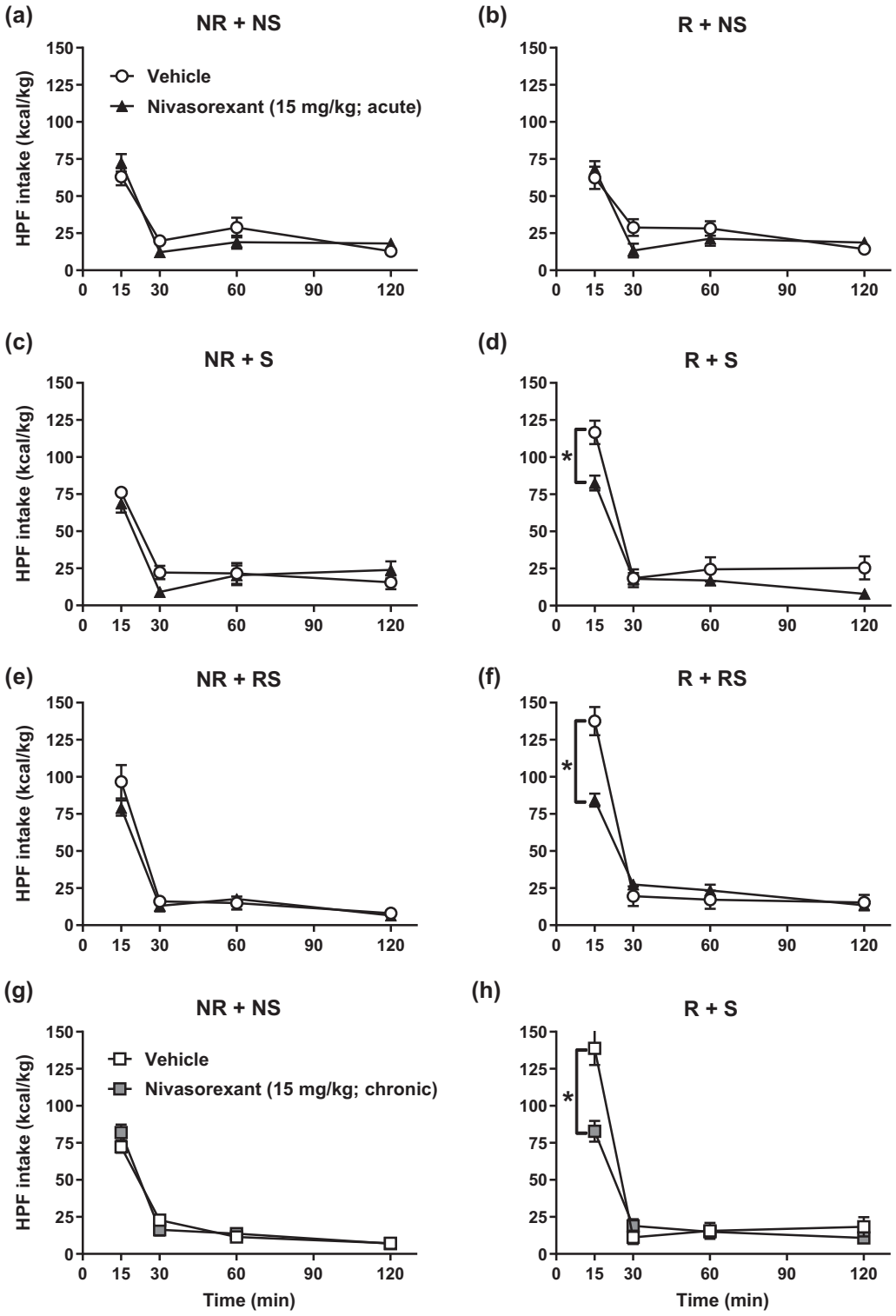

FIGURE 3 Effects of the SO1RA nivasorexant on binge-like eating behavior in rats under conditions of acute and repeated drug administration and acute and repeated stress exposure. Starting at 1 h after oral treatment, highly palatable food (HPF) intake was measured as kcal/kg body weight during a 2 h test period under regular control (non-restricted + non-stressed [NR + NS; a], restricted + non-stressed [R + NS; b], non-restricted + stressed [NR + S; c]), or binge eating (restricted + stressed [R + S; d]) conditions, or under conditions featuring repeated stress exposure: (non-restricted + repeatedly-stressed [NR + RS; e]) as control, and (restricted + repeatedly-stressed [R + RS; f]) as binge-eating condition. After the first testing of nivasorexant effects at acute, single dose on day 25 (a–f), the NR + NS and R + S groups (a, d) continued to receive daily administrations of vehicle or nivasorexant for 10 days until repeated testing on day 35 (g, h). Data are mean ± SEM. $N = 9–11$/group. *$p < .01$ versus vehicle at the 15 min time-point (Sidak's post hoc test following ANOVA). SEM, standard error of the mean; SO1RA, selective orexin 1 receptor antagonist.

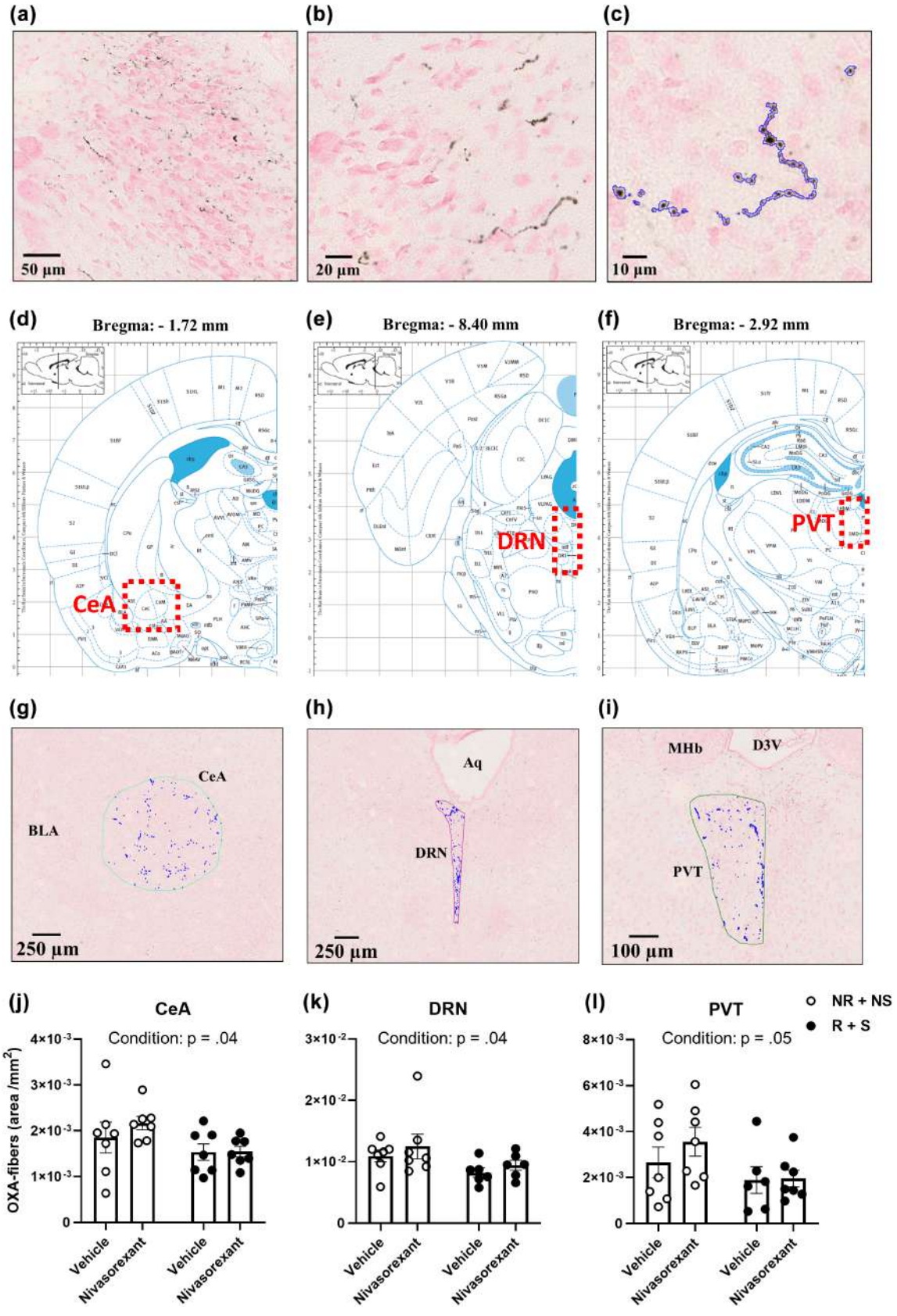

FIGURE 4 Legend on next page.

VTA and LC (Suppl. Table 11, Suppl. Figure 12) or the total number of ΔFosB-positive cells, be they of high- or low-expression profile, in the mPFC, the NAc or CPu, PVN, or VTA (Suppl. Table 12, Suppl. Figure 13). However, independent of treatment, binge-eating rats had reduced OXA fiber staining in the CeA ($F(1,24) = 4.92$, $p = .04$; Figure 4g, j) and DRN ($F(1,22) = 4.82$, $p = .04$; Figure 4h, k), and the same trend was observed in the PVT ($F(1,23) = 4.25$, $p = .051$; Figure 4i, l). The effect size was small; post-hoc comparisons showed no differences among the experimental subgroups.

Within the hypothalamus, more OXA-positive cell bodies were found in nivasorexant-treated rats, independent of priming for binge-eating, in the LH ($F(1,24) = 8.65$, $p < .01$; Figure 5c, f), the PeF ($F(1,24) = 2.90$, $p = .03$; Figure 5d, g), and nominally in the DMH ($F(1,24) = 3.57$, $p = .08$; Figure 5e, h). Post-hoc comparisons showed no further general differences among the experimental subgroups (but see Suppl. Figure 14 for differences depending on the bregma level). Similar to OXA, we also detected more ΔFosB-positive cells in nivasorexant-treated rats, independent of priming for binge-eating, in the PeF ($F(1,24) = 5,44$, $p = .03$; Figure 5d, j, Suppl. Figure 15B) and nominally in the LH ($F(1,24) = 3.07$, $p = .09$; Figure 5c, i, Suppl. Figure 15A) but not in the DMH ($p = .19$; Figure 5e, k, Suppl. Figure 15C).

Less than 15% of orexin-positive neurons in the hypothalamus co-expressed ΔFosB. Most of the co-expression was found in the DMH, less in the LH and PeF. Binge-eating rats showed a lower co-expression of OXA and ΔFosB, independent of treatment, in the DMH ($F(1,24) = 6.28$, $p = .02$, Figure 5e, n), but not in the LH (Figure 5c, l) and PeF (Figure 5d, m). Nivasorexant treatment had no effect.

4 | DISCUSSION

SO1RAs effectively reduced binge-like eating in a rat model mimicking a major endophenotype of BED, namely overeating on HPF within a short period of time beyond actual caloric demand. Herewith we corroborate previous findings in the same and other models of HPF or sweet liquid intake (Alcaraz-Iborra et al., 2014; Freeman et al., 2021; Piccoli et al., 2012; Vickers et al., 2015). Our results were consistent among three independent experiments testing three SO1RAs belonging to different chemical classes. This is essential, because almost all published literature about OX1R signaling in relation to feeding is based on the compound SB-334867 (Smart et al., 2001), which is known to have off-target activity (Gotter et al., 2012). Thus, our results convey confidence that the effects observed were truly due to OX1R blockade, particularly considering that nivasorexant and ACT-335827 have clean off-target profiles (Suppl. Table 1, and [Steiner et al., 2013]).

Based on our PK/PD relationships, binge-like eating in rats can be effectively reduced by selective OX1R blockade without involving the OX2R. Even though our calculations were based on several assumptions (e.g., unbound fraction measured in in vitro brain homogenate binding = unbound fraction in the intact brain, unbound fraction = biologically active fraction, K_b values determined in recombinant systems = K_b values at the native receptors in neurons), the observed data with the three SO1RAs suggest the behavior of the drugs in an in vivo system can relatively well be described using these surrogates. It remains somewhat obscure why a substantially higher (98% versus 50%–80%) OX1R occupancy was required for IDOR-1104-2408 than for the other two SO1RAs to be efficacious. Some of the above listed assumptions do likely not apply as closely to this very compound.

We saw no effect of OX1R blockade on HPF intake under control conditions. This is supported by the lack of effects on food intake or body weight observed during the preclinical toxicology and clinical efficacy studies with the three marketed dual OXR antagonists (DORAs) at plasma concentrations within the therapeutic range (e.g., FDA Integrated NDA review of daridorexant, 2022). Thus, in contrast to other studies suggesting an impact of orexin signaling on regular food intake (e.g., Sakurai et al., 1998), our results do not support this notion. One must consider for those prior studies potential confounding effects such as OXA-induced increases of wakefulness when given during the rats' sleep period that might have secondarily resulted in increased food intake in the home cage.

Some studies implied that orexin neuron activity, OXA expression, and signaling might display certain plasticity depending on the environmental conditions (Gao & Hermes, 2015). Chronic exposure to cocaine, for example, can increase the excitatory synaptic input

FIGURE 4 Effects of priming for binge-eating and of nivasorexant treatment on the expression of orexin A (OXA) in nerve fibers within the CeA, DRN, and PVT. Rats, primed to exert binge-eating behavior (restricted + stressed [R + S]) or not (non-restricted + non-stressed [NR + NS]), were treated for 10 days with vehicle or nivasorexant (15 mg/kg) and sacrificed one day after the last oral administration and testing for highly palatable food (HPF) intake. Brains were processed for immunohistochemistry. Representative pictures of coronal brain sections at different magnification illustrating an exemplary staining of OXA-positive fibers are shown in (a, b). Signals (an example shown as the area encompassed by the blue line in [c]) were detected using QuPath software, separated from background, and quantified as area occupied by OXA-positive fibers (in mm^2) within manually annotated brain regions of interest (in mm^2). Pictures (d–f) show the anatomical location of three areas of interest (within the red rectangles) at one exemplary, selected, bregma level for each region, and exemplary pictures (g–i) show the respective area occupied by positive OXA staining (blue color) within those regions (encircles by lines). The basolateral amygdala (BLA, g), acqueduct (Aq, h) dorsal 3rd ventricle (D3V, i), and medial habenular nucleus (MHb, i) were mentioned in the respective pictures only as reference points for the visualization of the brain regions of interest. Data relating to the quantification of the staining for the different treatment groups are shown individually (scatter) and as mean ± SEM (bars) (j–l). Parameters of the statistical analyses of variances (ANOVAs) are shown, where the effects were close to or showed statistical significance. CeA, central nucleus of the amygdala; DRN, dorsal raphe nucleus; PVT, paraventricular nucleus of the thalamus.

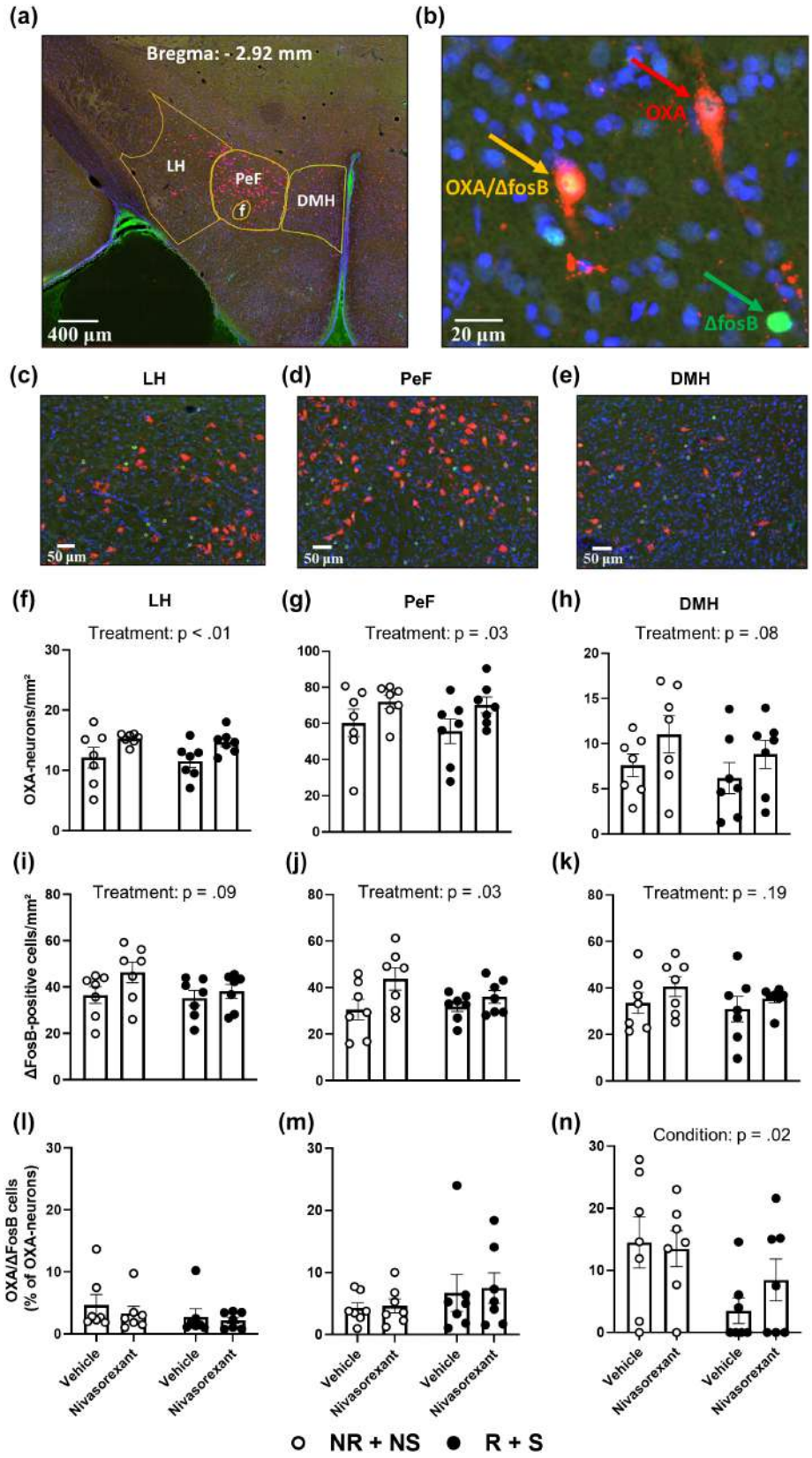

FIGURE 5 Effects of priming for binge-eating and of nivasorexant treatment on the amount of orexin A (OXA)-expressing neurons, ΔFosB-positive cells and on the percentage of cells in the LH, PeF, and DMH showing OXA-ΔFosB colocalization. Rats, primed to exert binge-eating behavior (restricted + stressed [R + S]) or not (non-restricted + non-stressed [NR + NS]), were treated for 10 days with vehicle or nivasorexant (15 mg/kg) and sacrificed one day after the last oral administration and testing for highly palatable food (HPF) intake. Brains were processed for immunofluorescence and quantification of OXA and ΔFosB. A representative picture (a) of a coronal brain section at Bregma: −2.92 mm is shown illustrating the subdivision of the hypothalamus in the lateral hypothalamus (LH), perifornical area (PeF) and dorsomedial hypothalamus (DMH) according to the rat brain atlas. The fornix (f) was included as a reference point for the visualization of the hypothalamic subregions. (b) Shows an exemplary high magnification picture of the hypothalamus illustrating the differential fluorescent staining of cells expressing OXA (red arrow), ΔFosB (green arrow), or both (yellow arrow). The number of OXA- and ΔFosB-positive cells per area, and the percentage of cells displaying colocalization of OXA and ΔFosB (calculated with respect to the total number of OXA-positive neurons) was determined for the LH, PeF, and DMH. Representative images at high magnification are shown in (c–e). Data relating to the quantification of the staining for the different treatment groups are shown individually (scatter) and as mean ± SEM (bars) (f–h for OXA, i–k for ΔFosB, and l–n for colocalized cells). Parameters of the statistical analyses of variances (ANOVAs) are shown, where the effects were close to or showed statistical significance.

to orexin neurons in mice (Rao et al., 2013), and chronic exposure to opiates can increase OXA peptide expression in the hypothalamus, in animals and humans (Thannickal et al., 2018). Temporary food restriction in rodents increases glutamatergic synapses on orexin neurons (Horvath & Gao, 2005) and hypothalamic OXA mRNA expression (Sakurai et al., 1998). Because we observed efficacy of SO1RAs

selectively in rats primed for BED, we investigated whether any changes in orexin peptide expression occurred. We hypothesized that areas involved in binge eating might become more strongly innervated by orexin fibers, making them susceptible to OX1R blockade. Surprisingly, we did not detect many changes by IHC. Against our expectations, orexin fibers projecting to the CeA, DRN, and PVT showed slightly lower OXA expression in binge-eating animals. Possibly, this is a compensatory mechanism for a subpopulation of orexin neurons projecting to those areas which might have become hyperactive through priming for binge-eating. This would require exploration through future electrophysiology studies. To our knowledge, only one study has shown decreased OXA peptide levels in brain regions outside of the hypothalamus (Mondal et al., 1999), and this was the consequence of prolonged fasting.

Despite its high expression in relevant brain regions including the striatum, NAc, PFC and amygdala (Espitia-Bautista & Escobar, 2021), the transcription factor ΔFosB has received little attention in research on eating disorders. We know that orexin neurons can become activated for a short while (as indicated through cFOS expression) by dieting and HPF exposure (Diano et al., 2003; Valdivia et al., 2015), as do various, specific brain regions in the binge eating rat model we used (Romano et al., 2020). Therefore, we assumed that long-lasting changes in neuronal activity might become visible using ΔFosB. However, we did not observe any changes in ΔFosB expression in the brains of binge-eating rats. This contrasts with changes in ΔFosB in the mPFC, NAc, VTA and PVN in another rat model of binge eating (Quansah Amissah et al., 2020), based on different conditions: sucrose-solution drinking in the dark, repeated dieting, and sessions of foot-shock stress. We are not aware of any other study that investigated ΔFosB in animal models of BED. Thus, it is difficult to further conclude.

We are the first to investigate long-term changes of OXA expression in orexin neurons after combination of prolonged intermittent dieting and HPF presentations. One day after the last HPF intake, we found no change in the number of OXA-positive neurons in the different regions of the hypothalamus. Previous studies reported either decreases (Alcaraz-Iborra et al., 2014; Olney et al., 2015) or increases (Olszewski et al., 2009) of OXA mRNA or protein levels after repeated sucrose or HPF exposure, or an increased proportion of orexin neurons positive for cFOS (Valdivia et al., 2015). However, measurements were always performed up to 4 h after the last drink/food presentation. As we did not include a non-HPF intake control group, we can only conclude that the additional exposure to dieting periods and stress did not lead to increased, long-lasting hypothalamic OXA peptide expression and recruitment of the proposed "reserve pool" of orexin neurons (James & Aston-Jones, 2022) as was previously reported in response to chronic opiate or cocaine exposure (James et al., 2019; Thannickal et al., 2018). Chronic exposure to alcohol, however, for example, does also not change OXA expression in the hypothalamus of rats (McGregor et al., 2023).

The higher number of OXA-positive neurons in nivasorexant- as compared to vehicle-treated rats suggests a compensatory mechanism on the level of protein expression. The organism potentially tried to counter-act the chronic OX1R signaling inhibition through production of more ligand. Contrasting our findings, the chronic administration of a high dose of the DORA suvorexant to mice led to a long-lasting down-regulation of OXA within the whole brain (Kaushik et al., 2021), which seems counterintuitive. The reasons for this discrepancy are unclear but might reside in differences of compensatory mechanisms in response to DORAs versus SO1RAs or pool of OXA investigated (i.e., intracellular vs. intra- + extracellular, hypothalamic vs. whole brain).

Besides OXA, we also observed a slightly higher hypothalamic ΔFosB expression. The difference in absolute cell numbers and distribution along the rostro-caudal extension of the hypothalamus suggests that additional hypothalamic cells besides orexin neurons were affected by the treatment. ΔFosB/OXA co-localization was very low, and impacted by priming for binge-eating, which reduced the number of co-localized cells selectively in the DMH. Diminished activation of medial hypothalamic orexin neurons, which is associated to an impairment of response inhibition (Freeman & Aston-Jones, 2020), could possibly facilitate binge-like eating.

There are several limitations of our study: (i) we did not confirm the efficacy of SO1RAs in models using other environmental triggers or types of HPF to induce binge-like eating; (ii) we looked at the main endophenotype of BED, intake of large amounts of food within a short period of time but did not investigate other endophenotypes, such as compulsive intake despite negative consequences; (iii) we used the easier to obtain and previously validated topiramate as positive control, used off-label for treating BED (Himmerich et al., 2023), rather than lisdexamfetamine, a schedule II controlled drug approved by the FDA for BED; (iv) protein expression was used as surrogate for investigating neuronal plasticity changes, and detection by immunohistochemistry provides only a semi-quantitative assessment within a limited linear range. Functional electrophysiology readouts might have been more appropriate.

Unfortunately, putting the efficacy of nivasorexant to the test in a clinical setting has revealed a lack of translation (McElroy et al., 2023). It could be that a complex, psychiatric disease like BED cannot be completely mimicked in animals and that higher order brain functions involved in the motivation and urge to binge in humans, including feelings of anxiety, guilt, worthlessness, and so forth. are not captured in a rat model. Alternatively, the placebo response in BED patients was too large for an effect of nivasorexant to emerge, or the drug was possibly not taken regularly during the trial; compliance in BED patients is a serious issue (Towell et al., 2001). More speculative is the possibility that unlike OX2R, OX1R functionality might not be as conserved between human and rodents as we think. For example, OXA levels in microdialysate from human amygdala were not much influenced by anticipation of or during eating, while they were highly elevated by positive emotions and social interaction (Blouin et al., 2013).

In conclusion, we showed that SO1RAs can effectively reduce binge-like eating of HPF in rats, and that priming for binge eating and chronic SO1RA treatment can change the expression pattern of OXA in certain brain areas. It will be interesting to further explore and compare the preclinical profile of nivasorexant or other clinical SO1RA

candidates with that of lisdexamfetamine in additional models of binge-eating, such as punished conflict (Heal et al., 2016) or behavioral economic paradigms (Freeman et al., 2021) to try and better understand the specific mechanisms of both types of drugs in relation to different endophenotypes of binge-eating pathology.

AUTHOR CONTRIBUTIONS

Michel Alexander Steiner: Conceptualization; data curation; formal analysis; methodology; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Luca Botticelli:** Formal analysis; investigation; methodology; visualization; writing – review and editing. **Giorgio Bergamini:** Conceptualization; data curation; supervision; validation; visualization; writing – review and editing. **Emanuela Micioni Di Bonaventura:** Investigation; methodology. **John Gatfield:** Conceptualization; resources; writing – review and editing. **Jodi T. Williams:** Conceptualization; resources; writing – review and editing. **Alexander Treiber:** Conceptualization; writing – review and editing. **Catherine Vaillant:** Investigation; methodology; supervision; visualization; writing – review and editing. **Carlo Cifani:** Conceptualization; investigation; methodology; supervision; validation; writing – review and editing. **Maria Vittoria Micioni Di Bonaventura:** Formal analysis; investigation; methodology; supervision; validation; visualization; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors MAS, GB, JG, JTW, AT, and CV are employees and shareholders of Idorsia Pharmaceuticals Ltd, Switzerland, which has developed ACT-335827 and nivasorexant for clinical testing. LB, CC, and MVMDB received funding from Idorsia Pharmaceuticals Ltd. for carrying out part of the experiments; they have no other competing interests to declare.

DATA AVAILABILITY STATEMENT

ACT-335827 and nivasorexant, as well as extended raw data (in addition to those provided in the Supporting Information) are made available upon reasonable request.

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