Psychoneuroendocrinology 106 (2019) 183–194

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Ghrelin signalling within the rat nucleus accumbens and skilled reach foraging

Jesper Vestlund[a], Filip Bergquist[a], Daniel Eckernäs[a], Valentina Licheri[b], Louise Adermark[b], Elisabet Jerlhag[a,*]

[a] Department of Pharmacology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
[b] Addiction Biology Unit, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

ARTICLE INFO

Keywords:
Gut-brain axis
GABA
Skilled reach foraging
Nucleus accumbens shell
Electrophysiology

ABSTRACT

Motivation alters behaviour in a complex manner and nucleus accumbens (NAc) shell has been implied as a key structure regulating such behaviour. Recent studies show that acute ghrelin signalling enhances motivation when assessed in a simple motor task. The aim of the present study was to define the role of ghrelin signalling on motivation in a more complex motor behaviour. Rats were tested in the Montoya staircase, an animal model of skilled reach foraging assessed by the number of sucrose pellets consumed. Electrophysiological recordings were conducted to explore the neurophysiological correlates of ghrelin signalling. The initial electrophysiological results displayed that *ex vivo* administration of ghrelin increased NAc shell output in brain slices from drug- and training-naïve rats. In rats with an acquired skilled reach performance, acute as well as repeated treatment with a ghrelin receptor (GHSR-1 A) antagonist (JMV2959) decreased the number of sucrose pellets consumed. Moreover, infusion of JMV2959 into NAc shell reduced this consumption. Sub-chronic, during ten days, JMV2959 treatment during training on the Montoya staircase reduced the number of pellets consumed, whereas ghrelin improved this behaviour. In addition, field potential and whole cell recordings were conducted in NAc shell of rats that had been treated with ghrelin or GHSR-1 A antagonist during training on the Montoya staircase. Sub-chronic administration of ghrelin during motor-skill learning selectively increased the frequency of inhibitory transmission in the NAc shell, resulting in a net suppression of accumbal output. Collectively these data suggest that ghrelin signalling in NAc shell enhances skilled reached foraging tentatively by increasing the motivation.

1. Introduction

The neurocircuits underlying motivated behaviours are complex and involve multiple neurotransmitters. When assessing the motivation as well as learning of a complex motor behaviour, the Montoya staircase test can be used as a valid model of skilled reach foraging for sucrose pellets (Montoya et al., 1991). Learning and motivational processes can be studied during the acquisition of Montoya skilled reach performance, whereas the performance of trained rats reflects motivation. Studies investigating the neuronal circuits involved in skilled reach foraging show that the initial learning involves projections from the medial prefrontal cortex (mPFC) to the dorsomedial striatum (DMS) (Licheri et al., 2018), whereas the dorsolateral striatum (DLS) is an important structure during consolidation of a motor skill (Balleine and Dickinson, 1998; Barnes et al., 2005; Corbit and Balleine, 2003; Jog et al., 1999; Yin et al., 2006). Furthermore, extensive research has established that motivated behaviours also involve nucleus accumbens (NAc) shell, a key area of the mesolimbic dopamine circuit (Robinson and Berridge, 1993).

The orexigenic peptide ghrelin regulates feeding behaviours via both hedonic and homeostatic circuits (for review see (Muller et al., 2015). Ghrelin has also been attributed an ability to activate the dopamine reward circuit through stimulation of ghrelin receptors (growth hormone secretagogue receptor, GHSR-1 A) expressed in key reward areas of the mesolimbic dopamine system such as the ventral tegmental area (VTA) (Abizaid et al., 2006; Jerlhag, 2008; Jerlhag et al., 2006, 2007; Quarta et al., 2009; Wellman et al., 2012) and NAc (Landgren et al., 2011a). Acute systemic administration of ghrelin increases,

* Corresponding author at: Department of Pharmacology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Medicinaregatan 13A, SE-405 30 Gothenburg, Sweden.
E-mail address: Elisabet.Jerlhag@pharm.gu.se (E. Jerlhag).

https://doi.org/10.1016/j.psyneuen.2019.04.008
Received 5 February 2019; Received in revised form 12 March 2019; Accepted 6 April 2019

whereas a GHSR-1 A antagonist decreases, the motivation to consume sucrose or alcohol in the operant lever-pressing paradigm, an effect linked to ventral tegmental GHSR-1 A (Landgren et al., 2012, 2011b; Skibicka et al., 2011, 2012). We therefore hypothesise that ghrelin signalling alters motivation via NAc shell, where the expression of the GHSR-1 A has been detected (Landgren et al., 2011a; Skibicka et al., 2011). In the present line of research, a combination of behavioural and electrophysiological studies is used to identify the role of ghrelin signalling in areas of importance for complex motor behaviours as exemplified by skilled reach foraging.

Ex vivo electrophysiology was performed to define the acute effects by ghrelin or a GHSR-1 A antagonist, JMV2959, on neurotransmission in NAc shell, mPFC, DLS and DMS, in drug and training naïve rats. In addition, to further link the behavioural outcome of sub-chronic, ten days of repeated, effects by ghrelin or GHSR-1 A antagonist to accumbal neurotransmission *ex vivo* electrophysiology was performed in NAc shell in rats with an acquired skilled reach performance. The Montoya staircase test was used to evaluate the effects of altered ghrelin signalling on the motivation of skilled reach foraging in rats with an acquired skilled reach performance as well as in rats without prior Montoya experience. In addition, the influence of local infusion of a GHSR-1 A antagonist into NAc shell on behaviour in the Montoya staircase test was studied in rats with an acquired skilled reach performance.

2. Material and methods

2.1. Experimental procedure

A combination of electrophysiological recordings and behavioural assessments were used to address the role of ghrelin signalling in skilled reach foraging (Supplementary table 1). Field potential and whole cell recordings were conducted to evaluate possible neurocircuits underlying behaviours regulated by ghrelin signalling. The effects of repeated ghrelin or JMV2959 treatment on the motivation, as measured by the number of pellets consumed as well as the improvement of success rate, of skilled reach performance in the Montoya staircase test. To exclude that treatment influences gross motor performance the Rotarod test was used. To achieve groups with similar motor capacities the results of the Rotarod was used for stratification. In addition, to confirm the orexigenic properties of ghrelin food intake measurements were undertaken.

2.2. Animals

Outbred male Wistar Rcc Han rats (160–190 g; Envigo, Horst, Netherlands) were group-housed (total of n = 92, in experiments with systemic administration) or housed individually (n = 12, in the experiment with local injections). Wistar Rcc Han was used since this strain shows a robust ability to pick sucrose pellets in the Montoya staircase test. They were kept on a 12/12 h light/dark cycle at 20 °C with 50% humidity with free access to water. Food availability in the home-cage after behavioural assessments was restricted during night (8% of body weight in chow per day, which correspond to 80% of the daily free-feeding) to increase the motivate for skilled reach performance. All experiments were approved by the Gothenburg Animal Research Ethics Committee (151–2015) and conducted during the light time cycle. All behavioural experiments used an independent set of rats.

2.3. Drugs

Acylated rat ghrelin (Bionuclear, Bromma, Sweden) was diluted in vehicle (0.9% NaCl) for intraperitoneal (IP) use and was administered 10 min prior to initiation of the experiments as this time frame has been found to be required for initiation of the behavioural effects (Jerlhag, 2008; Landgren et al., 2011b; Skibicka et al., 2012). The selected dose (0.33 mg/kg = 100 nmol/kg) activates the mesolimbic dopamine system and increases sucrose intake (Jerlhag, 2008; Landgren et al., 2011b; Skibicka et al., 2012). JMV2959, a potent and selective GHSR-1 A antagonist (Moulin et al., 2007), was synthesised at the Institut des Biomolécules Max Mousseron (IBMM; UMR5247, CNRS, Montpellier 1 and 2 Universities, France). For systemic as well as intra-NAc injection, JMV2959 was administered 20 min prior to test since this time is necessary for its pharmacological effect to be initiated (Jerlhag et al., 2012; Landgren et al., 2012, 2011b; Prieto-Garcia et al., 2015). The selected systemic dose (3 mg/kg = 5.9 μmol/kg, IP) was dissolved in vehicle (0.9% NaCl), and was used since it attenuates alcohol-mediated behaviours and has no effect per se on gross behaviour nor accumbal dopamine release in rats (Jerlhag et al., 2012; Landgren et al., 2012, 2011b). For local and bilateral infusion into NAc shell JMV2959 was dissolved in vehicle (Ringer solution: NaCl 140 mM, Ca Cl2 1.2 mM, KCl 3.0 mM and MgCl2 1.0 mM). The selected dose of JMV2959 (10 μg = 20 nmol in 0.5 μl per side) reduces food intake in mice (Prieto-Garcia et al., 2015) and alcohol intake in rats (Abtahi et al., 2018) without affecting gross behaviour of the mice (Prieto-Garcia et al., 2015). In the electrophysiology experiments the response to three different doses of ghrelin (10, 100 or 5000 nM) was evaluated, while 1 μM of JMV2959 was used to block the effect.

2.4. Guide canula implantation

To enable accumbal injections, guides (stainless steel, length 10 mm, with an o.d./i.d. of 0.6/0.45 mm) were implanted two days prior to the drug challenge. The rat was anaesthetized with isofluran (Isofluran Baxter, Kronans Apotek, Gothenburg, Sweden) using a pump (Univentor 400 Anaesthesia Unit, Univentor Ldt., Zejtun, Malta), placed in a stereotaxic apparatus (David Kopf Instruments; Tujunga, CA, USA) and kept on a heating pad to prevent hypothermia. Two drops of Xylocain (10 mg/ml) adrenalin (5 μg/ml) (Pfizer Inc, Kronans Apotek) applied locally on the skull surface, were used for local anaesthesia. The skull bone was exposed after an incision and three holes were drilled, two for the guide and one for the anchoring screw. The NAc shell coordinates relative to bregma was: AP + 1.85 mm, ML ± 1.0 mm, DL 7.8 mm (Paxinos and Watson, 1998) The tip of each guide was inserted 1 mm into the brain and they were anchored to the screw and the skull with dental cement (DENTALON® plus; Agntho's AB, Lidingö, Sweden). Carprofen (5 mg/kg, SC, Rimadyl®; Astra Zeneca, Kronans Apotek) was administered once directly following surgery to relieve pain. One hour prior to the injection, a dummy cannula was carefully inserted and retracted from the guide to remove clotted blood and hamper spreading depression. At the time of the experiment, a cannula was inserted another 6.8 mm ventrally beyond the tip of the guide aiming for drug administration into the NAc shell. The drug was administered over one minute and the cannula was left in place for another minute before it was retracted. The injections sites were verified post mortem (Supplementary Fig. 1) and only rats with correct placements were included in the statistical analysis.

2.5. Montoya staircase test

Three sucrose pellets (45 mg; BioServ, Frenchtown, NJ, USA) were placed on each staircase of Montoya apparatus (9 × 6 × 30 cm, Campden Instruments Ltd; Loughbrough, United Kingdom). At each session the rat was placed inside the Montoya for 15 min and the number of sucrose pellets consumed as well as the success rate, reflecting motivation of skilled reach performance was measured. There was two treatment/test free days between session 5 and 6.

2.5.1. Treatment effects in rats with an acquired skilled reach performance

Rats were subjected to vehicle treatment and trained in the Montoya staircase for the initial five days (session 1–5) allowing rats to acquire a skilled reach performance (Soderlund et al., 2015). This baseline behaviour was used to divide rats into future treatment groups with equal

skilled reach performance. In these experiments the rats received pharmacological treatment or relevant vehicle in combination with the Montoya staircase after session 5.

In experiment one, rats received either JMV2959 or vehicle for four days (session 6–9) and were treated acutely with ghrelin (treatment group: JMV2959-ghrelin) or vehicle (treatment group: vehicle-vehicle) at session 10. Separate rats received bilateral infusion of JMV2959 or vehicle into NAc shell at session 6 and 7 (experiment two). To further study how ghrelin influences motivation in rats with high or no skilled reach performance, rats were treated with either ghrelin or vehicle for five days (session 6–10) (experiment three).

2.5.2. Treatment effects in rats treated during acquisition of skilled reach performance

Two separate experiments (experiment four and five) were conducted in rats without prior exposure to the Montoya staircase test. In these experiments the rats received treatment in combination with training in the Montoya staircase.

In the fourth experiment, rats were treated daily with JMV2959 or vehicle for ten days (session 1–10). A subset of rats, exposed to either JMV2959 or vehicle for seven days, were included in the electrophysiological recordings allowing identifications of neurocircuits involved in the behavioural effects observed.

In experiment five, rats were treated daily for ten days with ghrelin or vehicle (session 1–10). To evaluate if the effect of ghrelin treatment is transient, the rats were then exposed to the Montoya staircase test for three additional days with only vehicle treatment (session 11–13). To test if acute JV2959 reduces the motivation in rats with an acquired skilled reach performance, the GHSR-1 A antagonist was administered at session 14 (treatment groups ghrelin-vehicle-JMV2959 and vehicle-vehicle-JMV2959). A subset of rats, exposed to either ghrelin or vehicle for seven days, were included in the electrophysiological recordings allowing identifications of neurocircuits involved in the behavioural effects observed.

2.6. Rotarod test

Rats were trained to stay on a rotating and accelerating (from 4 to 40 rpm) rotarod device (LE-8500, Panlab S.L.U.; Barcelona, Spain) placed in a ventilated and sound attenuating cupboard for 10 min per session. The mean time of four daily trials on the rod indicates the rat's gross motor performance. Rats with similar gross motor performance were thereafter divided into future treatment groups (vehicle or pharmacological manipulations). This design was used to reduce the possible effect of altered gross motor performance on the consumption of sucrose pellets in the Montoya staircase test. In addition, rats were trained on the rod following drug exposure at session 10 (experiment one), at session 7 (experiment two), at session 10 (experiment three) and at session 0, 5 and 10 (experiment four and five).

2.7. Food intake measurements

As ghrelin is an orexigenic peptide the effect of ghrelin manipulations was also evaluated with food intake measurements. Following the Montoya staircase test, where the rats consume sucrose pellets, a subset of rats that were injected with JMV2959 or vehicle (experiment four) were transferred to an individual empty cage, which they had habituated to earlier. The cage contained pre-weighed rat chow ad libitum. Food intake was measured in other rats that were injected systemically with ghrelin or vehicle (experiment five) during the Montoya staircase test. The food was re-weighed after one hour. The rats were thereafter transferred back to their home cage (group-housed condition).

2.8. Electrophysiological recordings

2.8.1. Brain slice preparation

Brain slice preparation was performed as previously described (Adermark et al., 2016). In brief, animals were anesthetized and the brain quickly removed and submerged in ice-cold modified artificial cerebrospinal fluid (aCSF) containing (in mM); 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 d-glucose, continuously bubbled with 95% O_2/ 5% CO_2. Coronal brain slices (300 μm) were transferred to conventional aCSF containing (in mM); 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH2PO_4 and 10 d-glucose, continuously bubbled with a gas mixture of 95% O2/5% CO_2. Slices were incubated in aCSF for 30 min at 30 °C and then at room temperature for the remainder of the day.

2.8.2. Field potential recordings

Field potential recordings were performed to investigate the acute and sub-chronic effects elicited by ghrelin and JMV2959 on neurotransmission. For field potential recordings, one hemisphere of a slice was transferred to a recording chamber and perfused with pre-warmed aCSF kept at 30 °C as previously described elsewhere (Morud et al., 2016). Population spikes (PS) were activated by paired pulse stimulation (50 ms interpulse interval) at a frequency of 0.05 Hz. Stimulus intensity (0.01 − 0.04 mA) was adjusted so that the PS amplitude was approximately half the size of the maximal response. Signals were amplified by a custom-made amplifier, filtered at 3 kHz, and digitized at 8 kHz.

To assess the acute effects displayed by ghrelin, PSs were monitored in NAc shell, DLS, DMS and mPFC of treatment-naïve rats (Fig. 1A, I). Following a stable baseline, ghrelin (10–5000 nM) or JMV2959 (1 μM) was administered via the perfusion system. In a subset of recordings, slices were pre-treated with JMV2959 for at least 20 min before ghrelin administration. The paired pulse ratio (PPR) was calculated to estimate changes in release probability (PS2/PS1) elicited by ghrelin. To evaluate the sub-chronic effect by ghrelin and JMV2959 field potential recordings were performed in animals trained on the Montoya staircase for seven days during treatment. This allows the assessment of neuroadaptations caused by treatment in combination with training. Changes in excitability were measured in the NAc shell and DMS by stepwise increasing the stimulation strength (18–72 μA), and by calculating PPR.

2.8.3. Whole cell recordings

To further assess the origin of neuroadaptations elicited by seven days of training combined with drug-treatment, whole cell recordings in voltage-clamp mode were performed in the NAc shell and DMS as previously described in detail (Licheri et al., 2018). Only animals with an acquired skilled reach performance in the Montoya staircase test were used. Spontaneous inhibitory currents (sIPSCs) were isolated by blocking NMDA and AMPA receptors using 50 μM AP5 (dissolved in H_2O; 28 mM) and 10 μM CNQX (dissolved in H_2O; 20 mM). For recording spontaneous excitatory postsynaptic currents (sEPSCs), brain slices were perfused with the $GABA_A$ receptor antagonist bicuculline (20 μM; dissolved in H_2O, 20 mM). NAc shell and DMS were identified using a 10x/0.30 objective attached to a Nikon Eclipse FN-1 microscope, while a 40x/0.80 water-immersion objective was used to localize medium spiny neurons (MSNs). Recording pipettes with a resistance ranging from 2.5 to 4.5 MΩ were prepared from borosilicate glass (Sutter Instruments; Novato; CA, USA) and filled with an internal solution containing (in mM): 150 CsCl, 10 Hepes, 2 $MgCl_2$, 0.3 Na_2GTP, 3 MgATP, and 0.2 BAPTA, 5 lidocaine N-ethyl bromide (QX314), pH adjusted to 7.2 with CsOH, with the osmolarity adjusted with sucrose to 292–294 mOsm. MSNs were voltage clamped at −70 mV, and a stable baseline response was observed over 5–10 min before spontaneous activity was recorded for 5 min. Data was amplified using an Axopatch 700B amplifier (Axon Instruments; Foster City, CA, USA), filtered at

2 kHz, digitized at 5 kHz, and acquired using Clampex 10.2 (Molecular Devices, Foster City, CA, USA). All recordings were performed under constant flow of pre-warmed aCSF (33–34 °C, 2 ml/min). Only recordings with a stable series resistance that varied less than 20% and did not exceed 25 MΩ were included in the analysis.

2.9. Statistical analysis

The data from the Montoya staircase test, rotarod as well as one hour food intake was analysed using repeated measure two-way ANOVA. In addition, an unpaired *t*-test was used to analyse the data from rats receiving acute drug treatments in the Montoya staircase test. Data from electrophysiological recordings were analysed using Clampfit (Molecular Devices, Foster City, CA), Minianalysis 6.0 software (Synaptosoft; Decatur, GA, USA) and Graph Pad Prism. Treatment-effects were analysed using two-way ANOVA, or *t*-test when applicable. All data is presented as mean values ± SEM, and the level of significance was set to $p < 0.05$.

3. Results

3.1. Effects of acute administration of ghrelin on ex vivo accumbal output in training and treatment naïve rats

The electrophysiological recordings confirmed that ghrelin acts in the NAc shell, as acute perfusion of physiologically relevant ghrelin concentrations *ex vivo* onto brain slices from treatment and training-naïve rats significantly modulated evoked PS amplitude in the NAc shell in a concentration dependent manner (one-way ANOVA: $F(1.1, 38.5) = 378$, $P < 0.001$; Fig. 1B), while JMV2959 (10 μM) had no effect (two-way ANOVA: $F(1,11) = 0.72$, $P > 0.05$; Fig. 1D). The dose of 100 nM, which was the lowest concentration to produce a change in neurotransmission and that correlates relatively well with the dose used for previous (Jerlhag, 2008; Landgren et al., 2011b; Skibicka et al., 2012), as well as the present *in vivo* experiments, was therefore used in the subsequent electrophysiological recordings. Pre-treatment with JMV2959 prevented the increase in PS amplitude induced by 100 nM ghrelin (two-way ANOVA: $F(1,21) = 5.24$, $P < 0.05$; Fig. 1E), showing that the effect is mediated via GHSR-1 A. The increase in PS amplitude was accompanied with a decrease in PPR, indicating that ghrelin facilitates transmitter release (*t*-test: $t = 2.75$, $df = 13$, $P < 0.05$; Fig. 1G). The change in PPR was not present in JMV2959-pretreated slices (*t*-test: $t = 0.23$, $df = 14$, $P > 0.05$; Fig. 1G). Ghrelin (100 nM) did not modulate PS amplitude as compared to JMV2959 + ghrelin in the DLS (two-way ANOVA: $F(1,11) = 0.03$, $P > 0.05$; Fig. 1H). Ghrelin (100 nM) did also not produce any significant increase in PS amplitude (two-way ANOVA: $F(1,12) = 2.08$, $P = 0.18$; Fig. 1I) or PPR in the DMS (paired *t*-test: $t = 0.25$, $df = 9$, $P = 0.81$; Fig. 1J). Ghrelin (100 nM) did not modulate PS amplitude as compared to JMV2959 + ghrelin in the mPFC (two-way ANOVA: $F(1,08) = 0.02$, $P > 0.05$; Fig. 1L).

3.2. Effects of systemic or NAc JMV2959 on the number of pellets consumed and success rate in rats with an acquired skilled reach performance

Rats established an acquired skilled reach performance as demonstrated by an increase in the number of sucrose pellets consumed (treatment $F(1,35) = 0.28$, $P = 0.599$, time $F(4,35) = 5.17$, $P = 0.002$, interaction $F(4,35) = 0.03$, $P = 0.999$; n = 8 per group; Fig. 2A) as well as enhanced success rate (treatment $F(1,35) = 0.13$, $P = 0.722$, time $F(4,35) = 2.05$, $P = 0.109$, interaction $F(4,35) = 0.19$, $P = 0.941$; Fig. 2B) during session 1–5. Sub-chronic JMV2959 reduced the number of sucrose pellets consumed during session 6–9 (treatment $F(1,28) = 4.30$, $P = 0.047$, time $F(3,28) = 0.37$, $P = 0.774$, interaction $F(3,28) = 0.26$, $P = 0.855$; Fig. 2A) and acute ghrelin reversed the ability of JMV2959 to reduce the pellets consumption in a complex motor task to the level of vehicle ($P = 0.511$; Fig. 2A). Neither JMV2959 (session 6–9; treatment $F(1,28) = 0.51$, $P = 0.481$, time $F(3,28) = 0.49$, $P = 0.691$ interaction $F(3,28) = 0.43$, $P = 0.734$; Fig. 2B) nor ghrelin ($P = 0.95$) influenced success rate in rats with acquired skilled reach performance. There was no difference in time at the rotarod between treatment groups at baseline (treatment $F(1,28) = 0.13$, $P = 0.717$, time $F(3,28) = 2.23$, $P = 0.107$, interaction $F(3,28) = 0.83$, $P = 0.488$; Fig. 2C). Neither treatment affected gross motor performance since there were no differences in time at the rotarod between rats in treatment groups ($P = 0.847$; Fig. 2C).

In a separate experiment, rats established an acquired skilled reach performance as shown by the increase in the number of pellets consumed (treatment $F(1,25) = 0.20$, $P = 0.661$, time $F(4,25) = 9.14$, $P < 0.001$, interaction $F(4,25) = 0.23$, $P = 0.917$; n = 6 per group; Fig. 2D) and increased success rate (treatment $F(1,25) = 0.002$, $P = 0.969$, time $F(4,25) = 6.69$, $P = 0.001$, interaction $F(4,25) = 0.42$, $P = 0.795$; Fig. 2E) during session 1–5. Local and bilateral infusion of JMV2959 into NAc shell reduced the number of sucrose pellets consumed during session 6–7 compared to vehicle (treatment $F(1,10) = 5.37$, $P = 0.043$, time $F(1,10) = 0.09$, $P = 0.766$, interaction $F(1,10) = 0.29$, $P = 0.605$; Fig. 2D). There was a tendency to a reduction in success rate following JMV2959 treatment (session 6–7; treatment $F(1,10) = 3.95$, $P = 0.075$; Fig. 2E), indicating that JMV2959 into NAc shell may alter the execution of skilled reach performance. However, there was no effect of time ($F(1,10) = 0.34$, $P = 0.572$) or interaction ($F(1,10) = 0.03$, $P = 0.869$).There were no differences in time at the rotarod between treatment groups at baseline (treatment $F(1,25) = 0.0002$, $P = 0.993$, time $F(4,25) = 2.57$, $P = 0.063$, interaction $F(4,25) = 0.07$, $P = 0.991$; Fig. 2F) nor after accumbal JMV2959 or vehicle treatment ($P = 0.742$; Fig. 2F), indicating that JMV2959 into NAc shell does not alter gross motor performance.

3.3. Effects of ghrelin on the number of pellets consumed and success rate in rats that did as well as did not established an acquired a skilled reach performance

Some rats established a skilled reach performance during session 1–5 (treatment $F(1,30) = 0.10$, $P = 0.760$, time $F(4,30) = 12.56$, $P < 0.001$, interaction $F(4,30) = 0.06$, $P = 0.992$; n = 7 per group; Fig. 3A), whereas some did not (n = 5 per group; Fig. 3B). In rats with an acquired skilled reach performance sub-chronic ghrelin did not influence on the number of sucrose pellets consumed during session 6–10 (treatment $F(1,30) = 0.32$, $P = 0.577$, time $F(4,30) = 0.51$, $P = 0.727$, interaction $F(4,30) = 0.08$, $P = 0.987$; Fig. 3A). On the other hand, sub-chronic ghrelin had a tendency to increase the number of sucrose pellets consumed during session 6–10 in rats with no acquired skilled reach performance (treatment $F(1,20) = 4.31$, $P = 0.051$, time $F(4,20) = 0.33$, $P = 0.853$, interaction $F(4,20) = 0.04$, $P = 0.997$; Fig. 3B). The success rate was increased over time (session 1–5) in rats with an acquired skilled reach performance (treatment $F(1,30) = 0.08$, $P = 0.778$, time $F(4,30) = 4.61$, $P = 0.005$, interaction $F(4,30) = 0.32$, $P = 0.860$; Fig. 3C), but not in the rats with no established skilled reach performance (Fig. 3D). Sub-chronic ghrelin treatment did not alter the success rate in rats with an acquired skilled reached performance (session 6–10; treatment $F(1,30) = 3.29$, $P = 0.080$, time $F(4,30) = 0.09$, $P = 0.987$, interaction $F(4,30) = 0.39$, $P = 0.813$; Fig. 3C). However, ghrelin increased the success rate in rats with no acquired skilled reach performance (treatment $F(1,20) = 4.91$, $P = 0.038$, time $F(4,20) = 0.35$, $P = 0.842$, interaction $F(4,20) = 0.05$, $P = 0.996$; Fig. 3D).

In rats with high acquired skilled reach performance there were no differences in time at the rotarod between treatment groups at baseline (treatment $F(1,30) = 0.29$, $P = 0.597$, time $F(4,30) = 0.22$, $P = 0.923$, interaction $F(4,30) = 0.30$, $P = 0.879$; Fig. 3E) nor after ghrelin or vehicle treatment ($P = 0.678$; Fig. 3E). In rats without acquired skilled

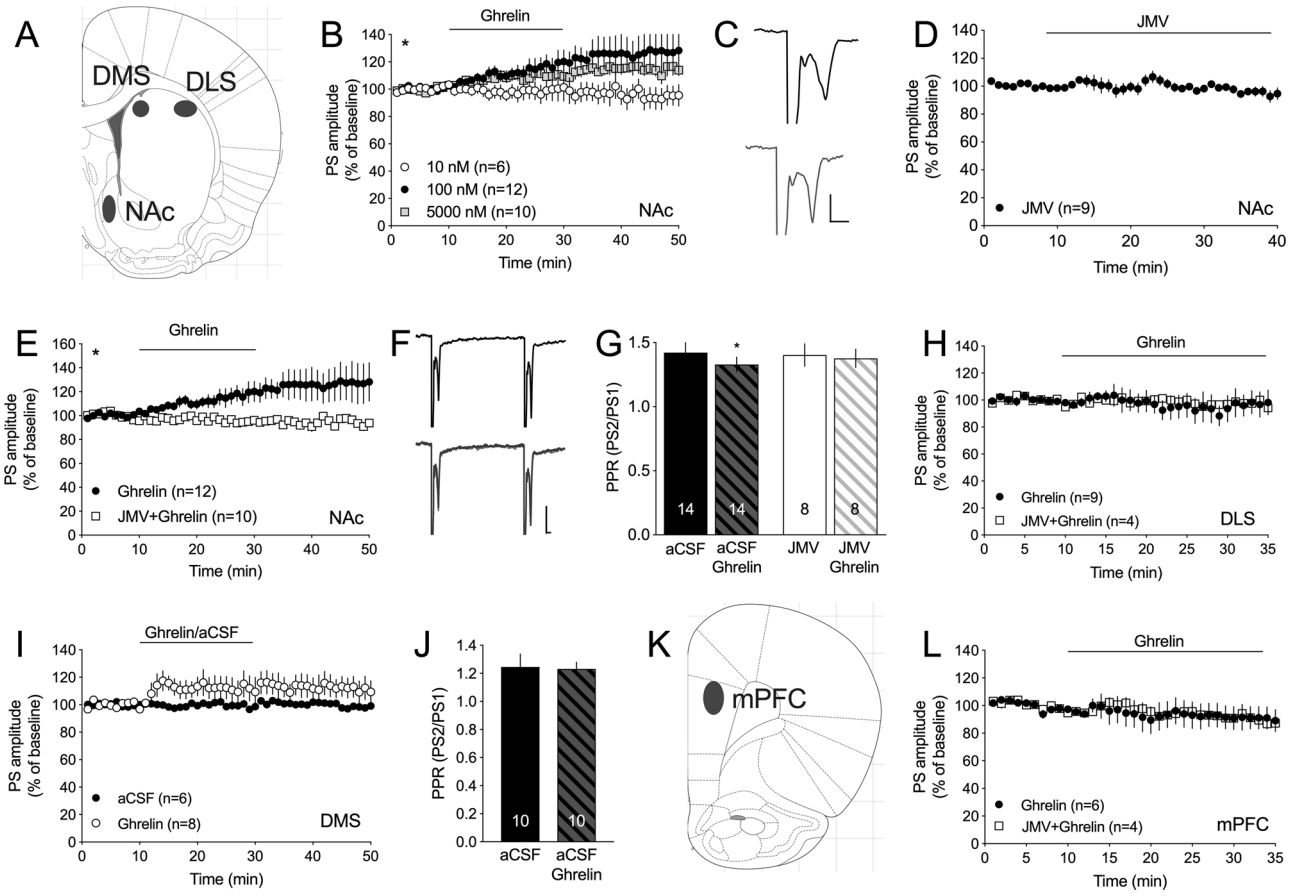

Fig. 1. Effects of acute administration of ghrelin on ex vivo accumbal output in training and treatment naïve rats.
A) Schematic slice showing the areas where field potential recordings were performed. B) Perfusion of ghrelin increases evoked population spike (PS) amplitude in the NAc shell. C) Example traces showing evoked PS at baseline (black) and following 15 min administration of ghrelin (100 nM; gray) in the NAc shell. Calibration is 0.2 mV, 2 ms. D–E) JMV2959 (JMV) did not affect PS amplitude *per se,* but prevented the effect displayed by 100 nM ghrelin. F) Example traces show paired pulse stimulation-evoked PS at JMV-treated baseline (black) and following 15 min administration of JMV + ghrelin (gray). Calibration is 0.2 mV, 2 ms. G) The increase in PS amplitude induced by 100 nM ghrelin was accompanied with a decrease in PPR in aCSF-treated slices, but not in JMV pre-treated slices. H) PS amplitude in the DLS was not modulated by 100 nM ghrelin perfusion as compared to JMV + ghrelin. I) Perfusion of 100 nM ghrelin did not modulate PS amplitude in the DMS as compared to aCSF. J) Ghrelin did not alter pre-pulse ratio in aCSF pre-treated slices. K) Schematic slice showing the area where field potential recordings were performed in the mPFC. L) PS amplitude in the mPFC was not modulated by 100 nM ghrelin perfusion as compared to JMV + ghrelin. Data are presented as mean values ± SEM. n = number of brain slices taken from rats from at least 3 different litters. * $P < 0.05$, significant from control.

reach performance there were no differences in time at the rotarod between treatment groups at baseline (treatment $F(1,20) = 2.85$, $P = 0.107$, time $F(4,20) = 0.23$, $P = 0.916$, interaction $F(4,20) = 0.28$, $P = 0.890$; Fig. 3F) nor after ghrelin or vehicle treatment ($P = 0.541$; Fig. 3F), collectively indicating that ghrelin does not alter gross motor performance.

3.4. Effects of daily JMV2959 treatment on the number of pellets consumed and success rate

Compared to vehicle, daily JMV2959 decreased the number of pellets consumed (treatment $F(1,90) = 48.16$, $P < 0.001$, time $F(9,90) = 2.61$, $P = 0.010$, interaction $F(9,90) = 0.99$, $P = 0.455$; n = 10 per group; Fig. 4A) as well as reduced the success rate (treatment $F(1,90) = 18.60$, $P < 0.001$, time $F(9,90) = 1.15$, $P = 0.339$, interaction $F(9,90) = 0.53$, $P = 0.850$; Fig. 4B).

The rats were divided into treatment groups based on their baseline rotarod performance. Comparison between future treatment groups revealed similar gross motor behaviour at baseline (treatment $F(1,27) = 0.28$, $P = 0.598$, time $F(2,27) = 3.46$, $P = 0.046$, interaction $F(2,27) = 0.004$, $P = 0.996$; Fig. 4C). JMV2959 did not influence the time at the rotarod (treatment $F(1,27) = 0.41$, $P = 0.529$, time $F(2,27) = 0.65$, $P = 0.528$, interaction $F(2,27) = 0.10$, $P = 0.903$; Fig. 4C). Repeated JMV2959 treatment reduced chow intake (treatment $F(1,60) = 10.24$, $P = 0.002$, time $F(9,60) = 5.58$, $P < 0.001$, interaction $F(9,60) = 0.31$, $P = 0.968$; n = 7 per group; Fig. 4D), in rats that consumed sucrose in the preceding Montoya staircase test.

3.5. Effects of daily ghrelin treatment on the number of pellets consumed and success rate

Daily ghrelin treatment increased the number of pellets consumed compared to vehicle treatment (treatment $F(1,80) = 7.78$, $P = 0.007$, time $F(9,80) = 3.08$, $P = 0.003$, interaction $F(9,80) = 1.15$, $P = 0.339$, n = 9 per group; Fig. 5A). At the drug-free session 11–13, there was no significant difference in consumption between rats previously treated with ghrelin or vehicle (treatment $F(1,24) = 2.23$, $P = 0.149$, time $F(2,24) = 0.01$, $P = 0.995$, interaction $F(2,24) = 0.05$, $P = 0.951$; Fig. 5A), indicating that the ghrelin-induced potentiation of motivation of skilled reach foraging is transient and disappears when the drug is no longer present. In comparison to the prior non-treatment session 13, acute JMV2959 injection decreased the number of pellets consumed in both groups of rats ($P = 0.001$ for both ghrelin-vehicle-JMV and vehicle-vehicle-JMV, Fig. 5A). JMV2959 reduced the number of pellets

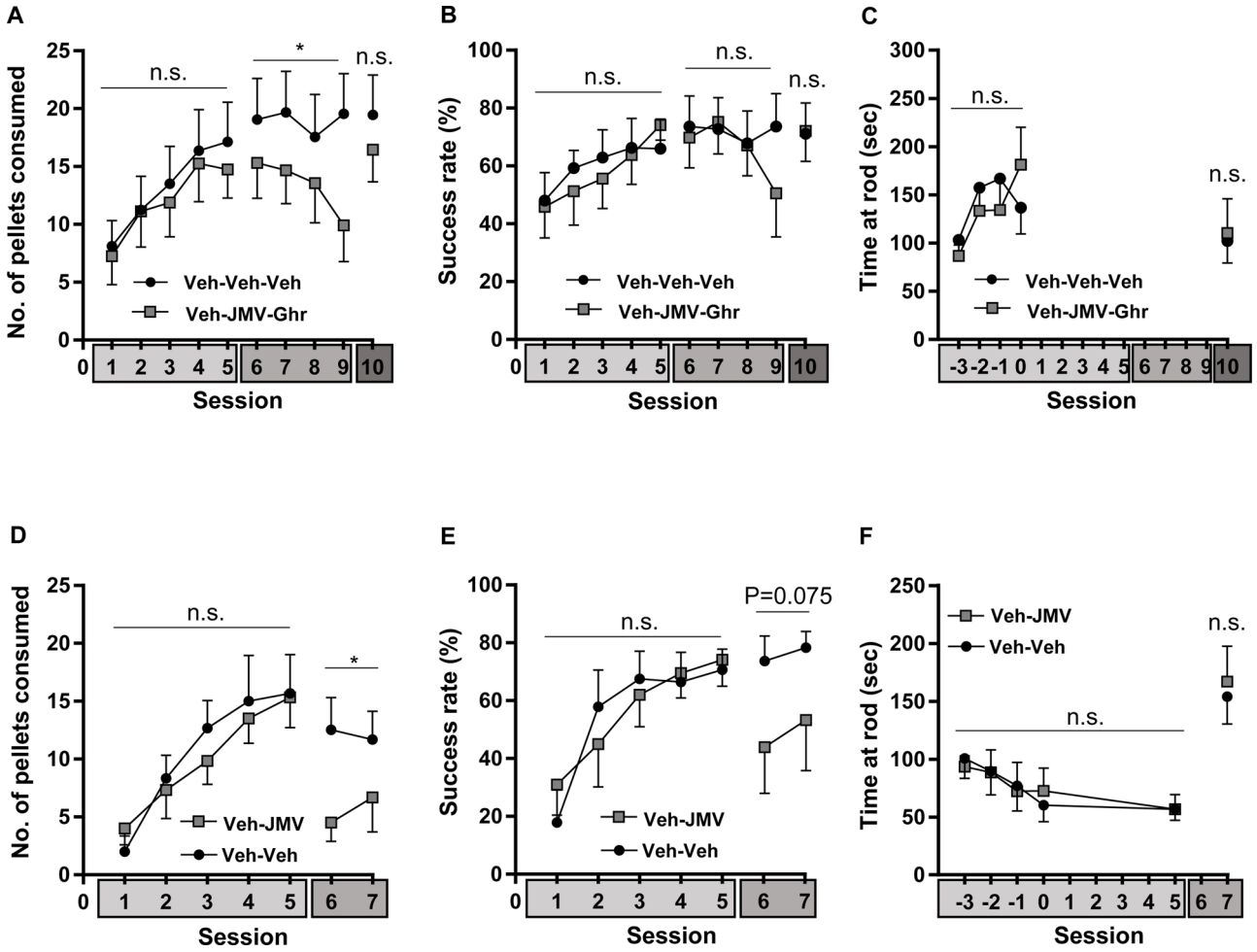

Fig. 2. Effects of JMV2959, repeated systemically or into NAC shell, on the motivation of skilled reach foraging in rats with an acquired skilled reach performance. A) Rats in both future treatment groups established similar acquired skilled reach performance during the first vehicle (Veh)-treatment period (session 1–5). Repeated JMV2959 (JMV) treatment (n = 8) during session 6–9 decreased the number of pellets consumed in rats with an acquired skilled reach performance compared to vehicle (n = 8). At session 10, there was no difference in number of pellets consumed between acute vehicle or ghrelin (Ghr) treatment, indicating that ghrelin reversed the ability of JMV2959 to reduce the number of sucrose pellets consumed. B) There were no differences in success rate during the five first vehicle days, between repeated JMV2959 or vehicle treatment nor between acute vehicle and ghrelin treated rats C) There were no differences in time at the rotarod (sec) between groups at baseline nor after drug treatment, indicating that treatment does not influence gross motor performance. D) Rats later infused locally with JMV2959 or vehicle into NAc shell established similar acquired skilled reach performance during the first vehicle (Veh)- treatment period (session 1–5). Infusion of JMV2959 (JMV, n = 6) into NAc shell decreased the number of pellets consumed in rats with an acquired skilled reach performance compared to vehicle (n = 6)(session 6–7). E) Rats later infused locally with JMV2959 or vehicle into NAc shell established similar success rate during the five first vehicle days. JMV2959 treatment into NAc shell had a tendency to reduce the success rate compared to vehicle F) There were no differences in time at the rotarod (sec) between groups at baseline nor after accumbal JMV2959 or vehicle treatment, indicating that accumbal JMV2959 does not influence on gross motor performance. Data are presented as mean ± SEM; *P < 0.05 and n.s. = not significant.

consumed similarly in both groups since there was no difference in response between the two group of rats at session 14 (P > 0.05). This confirmed the hypothesis that JMV2959 reduced the motivation to consume sucrose pellets in rats with an acquired skilled reach performance. On the other hand, ghrelin administration at session 1–10 did not alter the success rate (treatment F(1,80) = 1.86, P = 0.176, time F(9,80) = 1.75, P = 0.091, interaction F(9,80) = 0.77, P = 0.642, Fig. 5B). The rats were divided into treatment groups based on their baseline rotarod performance, comparison between groups at baseline (treatment F(1,24) = 0.60, P = 0.447, time F(2,24) = 4.10, P = 0.029, interaction F(2,24) = 1.42, P = 0.260; Fig. 5C). Sub-chronic ghrelin did not alter gross motor performance as treatment did not alter the time at the rotarod (treatment F(1,24) = 2.05, P = 0.166, time F(2,24) = 0.32, P = 0.727, interaction F(2,24) = 0.16, P = 0.853; Fig. 5C). In rats previously consuming sucrose in the Montoya staircase test, ghrelin increased chow consumption (treatment F(1,70) = 19.99, P < 0.001, time F(9,70) = 1.83, P = 0.078, interaction F(9,70) = 0.41, P = 0.926, n = 8 per group; Fig. 5D), supporting the established orexigenic role of ghrelin.

3.6. Effects of sub-chronic administration of ghrelin during skilled reach foraging on neuroadaptations in the NAc shell

To assess long-lasting effects of ghrelin on neurotransmission underlying the ability of ghrelin to enhance the motivation of skilled reach foraging, electrophysiological field potential recordings and whole cell recordings in voltage clamp mode were performed in animals trained on the Montoya Staircase test for seven days during administration of ghrelin, JMV2959 or vehicle. Field potential recordings performed in the NAc shell showed that animals receiving ghrelin during training had a significantly lower PS amplitude as compared to vehicle-treated controls (two-way ANOVA F(1,73) = 4.80, P < 0.05; Fig. 6B), while PPR was not significantly modulated (unpaired *t*-test: vehicle vs. ghrelin: t = 1.04, df = 81, P > 0.05; Fig. 6C). In addition, sIPSC

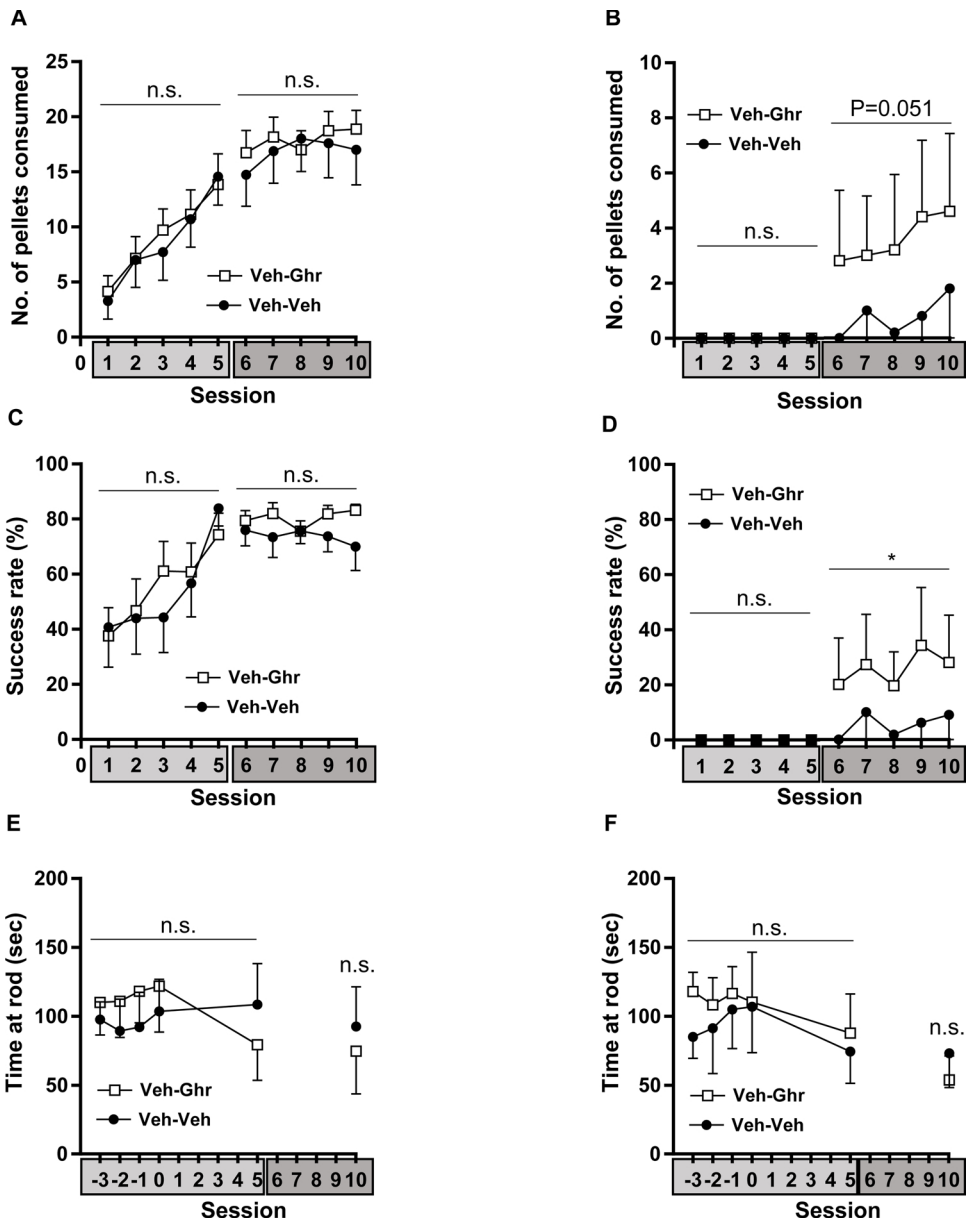

Fig. 3. Effects of ghrelin on the motivation of skilled reach foraging in rats with no or high acquired skilled reach performance.
Some rats A), but not all B) established an acquired skilled reach performance during the first vehicle (Veh)- treatment period (session 1–5). A) Repeated ghrelin (Ghr) treatment (n = 7) did not alter the number of pellets consumed in rats with an acquired skilled reach performance compared to vehicle (n = 7)(session 6–10). B) Repeated ghrelin treatment (n = 5) tended to increase the number of pellets consumed in the rats that did not established a skilled reach performance during session 1–5, compared to vehicle (n = 5)(session 6–10). C) Among rats that established an acquired skilled reach performance there were no differences in success rate during the five first vehicle treatment days. Repeated ghrelin did not alter success rate compared with vehicle in these rats (session 6–10). D) Among rats that did not establish an acquired skilled reach performance during the five first vehicle treatment days, repeated ghrelin increased success rate compared to vehicle (session 6–10). E) In rats with high acquired skilled reach performance (session 1–5) there were no difference in time at the rotarod (sec) between groups at baseline nor after ghrelin or vehicle treatment, indicating that ghrelin does not influence on gross motor performance. F) In rats without acquired skilled reached performance (session 1–5) there were no difference in time at the rotarod (sec) between groups at baseline nor after ghrelin or vehicle treatment, indicating that ghrelin does not influence on gross motor performance. Ghrelin did not influence the time at the rotarod (sec) compared to vehicle, indicating that treatment does not influence gross motor performance. Data are presented as mean ± SEM; *P < 0.05 and n.s. = not significant.

frequency was significantly increased in the NAc shell of rats treated with ghrelin (sIPSC frequency: t = 2.30, df = 13, P < 0.05; sIPSC amplitude: t = 1.38, df = 13, P > 0.05; Fig. 6D–E), while neither frequency nor amplitude of recorded sEPSCs were affected (sEPSC frequency: t = 0.00, df = 10, P > 0.05; sEPSC amplitude: t = 0.27, df = 10, P > 0.05; Fig. 6F-G). In addition, repeated administration of JMV2959 did not affect input/output function (F(1,63) = 0.355, P > 0.05; Fig. 6B) nor sIPSC frequency (t = 0.24, df = 9, P > 0.05; Fig. 6D). Sub-chronic-treatment of ghrelin did not produce neuroadaptations in the DMS as compared to vehicle-treated controls trained in parallel (Input/Output: F(1,75) = 0.31, P = 0.579; PPR: t = 1.57, df = 20, P = 0.13; sIPSC frequency: t = 0.10, df = 10, P = 0.92; sIPSC amplitude: t = 0.25, df = 10, P = 0.81; Fig. 6H-K).

4. Discussion

The data presented here suggest that the orexigenic peptide ghrelin may regulate motivation of skilled reach foraging via activation of accumbal circuits. Firstly, our electrophysiological recordings revealed that ghrelin strengthens evoked field potentials in NAc shell, putatively by facilitating accumbal transmitter release, in brain slices from drug- and training-naïve rats (Fig. 1B, G). This increase in excitatory neurotransmission by ghrelin was only apparent in the NAc shell, a key area involved in motivation (Robinson and Berridge, 1993), but not in other brain regions investigated including DMS, DLS and mPFC (Fig. 1H, I, L) which are involved in learning and consolidation of motor skill performance (Balleine and Dickinson, 1998; Barnes et al., 2005; Corbit and Balleine, 2003; Jog et al., 1999; Yin et al., 2006). These electrophysiological findings may thus provide identification of a tentative neurochemical substrate mediating previous data demonstrating that acute systemic administration of ghrelin increases, whereas JMV2959 reduces, the number of lever presses for sucrose as well as alcohol in an operant self-administration model (Gomez et al., 2015; Landgren et al., 2012, 2011b; Skibicka et al., 2012). A physiological role of ghrelin signalling in NAc shell is further substantiated by the data showing that GHSR-1 A are expressed in NAc (Landgren et al., 2011a; Skibicka et al., 2011) and that pharmacological or genetic manipulations of NAc shell-GHSR-1 A modulates the intake of chow, palatable food, high fat diet as well as alcohol (Abtahi et al., 2018; King et al., 2016; Naleid et al., 2005; Prieto-Garcia et al., 2015). Even though the expression of GHSR-1 A have been detected in NAc, the present study cannot determine whether the acute effects of ghrelin are mediated by presynaptic or

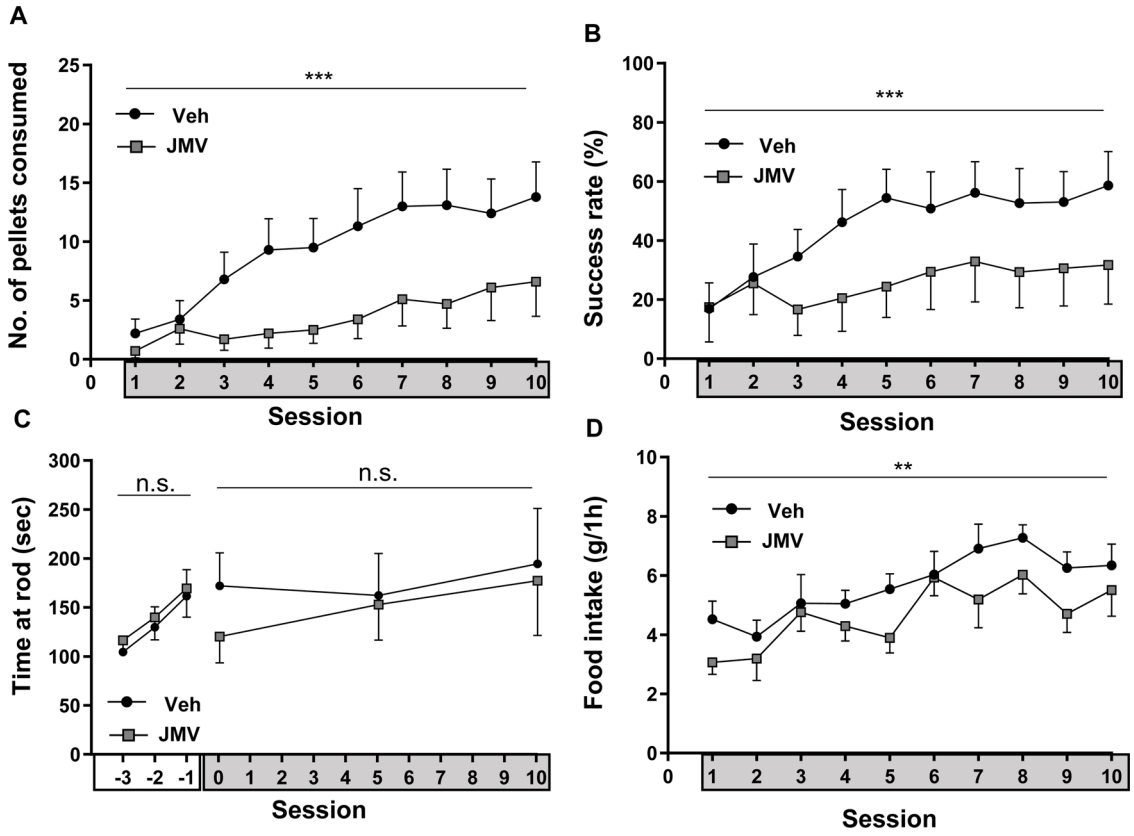

Fig. 4. Effects of daily JMV2959 treatment on the motivation to skilled reach foraging.
A) Sub-chronic JMV2959 (JMV, n = 10) treatment decreased the number of pellets consumed compared to vehicle (Veh, n = 10) in the Montoya staircase. B) Repeated JMV2959 treatment decreased the success rate compared to vehicle. C) There were no differences in time at the rotarod (sec) between groups at baseline nor after JMV2959 or vehicle treatment, indicating that JMV2959 does not influence on gross motor performance. D) Daily injection of JMV2959 decreased 1 h food intake compared to vehicle in rats that consumed sucrose during the preceding Montoya staircase test. Data are presented as mean ± SEM; ***P < 0.001, **P < 0.01 and n.s. = not significant.

postsynaptic mechanisms. Therefore, future studies should evaluate the location of GHSR-1 A within NAc shell.

Secondly, the results obtained herein expand on these previous studies that are investigated in the operant self-administration models, which is considered a simpler motor skill task (Gomez et al., 2015; Landgren et al., 2012, 2011b; Skibicka et al., 2012). Indeed, we demonstrated in rats with an acquired skilled reach performance that acute (Fig. 5A) as well as repeated (Fig. 2A) JMV2959 treatment decreased the motivation of skilled reach foraging, where rats with more established skilled reached performance tentatively display a more robust inhibitory effect of JMV2959. The data also indicate that acute ghrelin restored the JMV2959-mediated reduction in motivation (Fig. 2A). When it comes to ghrelin administration to rats with an acquired skilled reach performance, we found somewhat surprisingly that ghrelin did not alter the motivation of skilled reach foraging (Fig. 3A). However, in rats with no prior learning of the task during session 1–5, ghrelin administration during session 6–10 increased the motivation of skilled reach foraging (Fig. 3B). Collectively these findings indicate that ghrelin only increase the motivation in rats with low or no acquired skilled reach performance. It should however be considered that a pharmacological or behavioural ceiling effect may influence the outcome in this experiment. We further established that ghrelin signalling in NAc shell is involved in this behaviour in rats with acquired skilled reach performance since infusion of JMV2959 into NAc shell reduced the consumption of sucrose pellets (Fig. 2D).

Thirdly, we found that sub-chronic treatment of a GHSR-1 A antagonist reduced (Fig. 4A) the motivation of skilled reach foraging, while repeated ghrelin injection increased (Fig. 5A) this behaviour in rats treated throughout the entire behavioural testing (session 1–10). The electrophysiological results conducted in these rats suggest that sub-chronic administration of ghrelin reduced accumbal output by selectively increasing sIPSC frequency (Fig. 6B, D). Considering previous findings showing that GABA in the NAc shell participates in the central regulation of feeding behaviour, and that intra accumbal administration of the $GABA_A$ receptor agonist muscimol enhances food intake (Khaimova et al., 2004; Newman et al., 2013; Stratford and Kelley, 1997), increased inhibitory transmission could indirectly enhance performance by increasing the reward-value of the pellets. Considering the putative role of dopamine in mediating structural and synaptic plasticity in inhibitory MSNs (Adermark, 2011; Fasano et al., 2013; Villalba and Smith, 2013), these findings might be connected to region specific effects by ghrelin on dopaminergic signalling. However future studies should investigate the underlying mechanisms to this sustained effect on sIPSC frequency in NAc shell MSNs induced by repeated ghrelin treatment during motor skill behaviour. Systemic ghrelin has repeatedly been shown to exert a strong modulation over neuronal activity in the VTA resulting in accumbal dopamine release (Abizaid et al., 2006; Jerlhag, 2008; Sommer and Hauber, 2016; van der Plasse et al., 2015), while the effect by ghrelin on nigro-striatal circuitry is less well studied. Even though it remains to be determined, it is therefore possible that ghrelin elevates dopamine to a greater extent in the NAc compared to striatal circuitry, thereby facilitating long-lasting specific neuroadaptations in this brain subregion. In support for this contention are our data revealing that the inhibitory neurotransmission was not altered in the DMS. We therefore postulate that the ability of sub-chronic ghrelin treatment during skilled reach foraging to augment motivation and performance is linked to accumbal neuroadaptations elicited by repeated administration of ghrelin during the Montoya

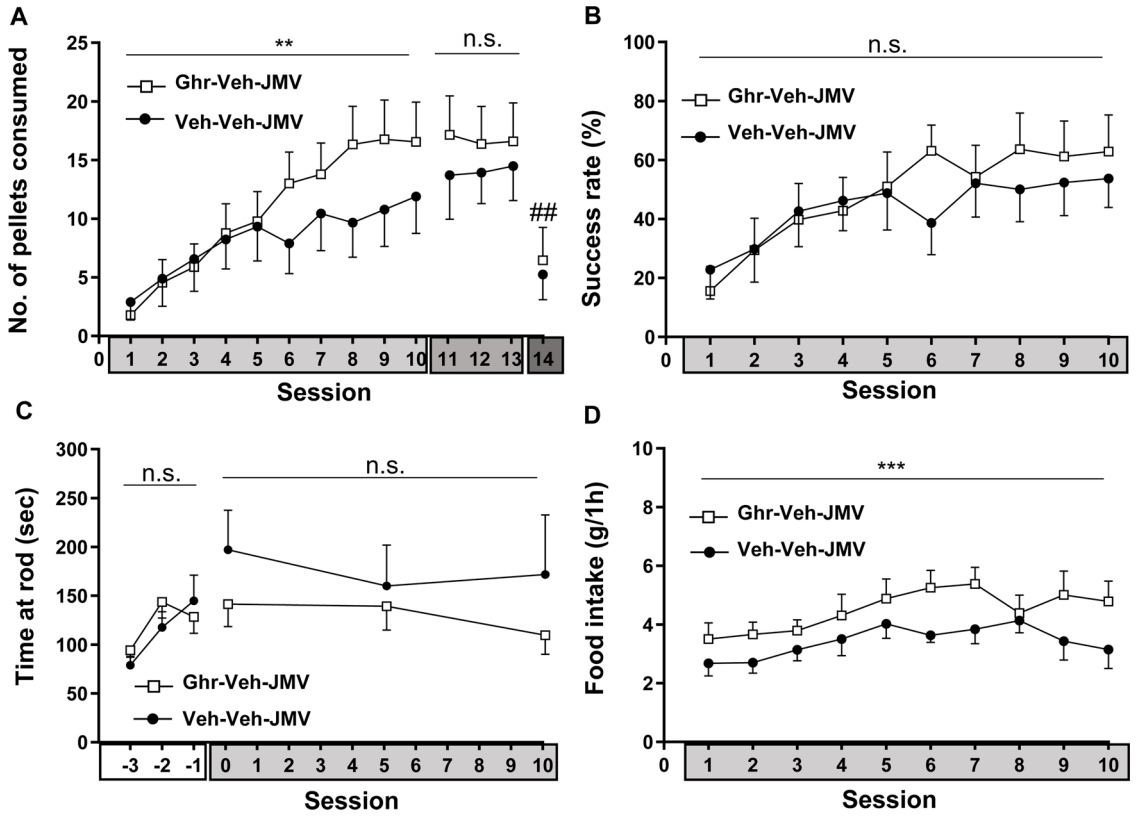

Fig. 5. Effects of daily ghrelin treatment on the motivation of skilled reach foraging.
A) Repeated ghrelin (Ghr, n = 9) treatment increases the number of pellets consumed compared to vehicle (Veh, n = 9) in the Montoya staircase during session 1–10. There are no differences between groups during the subsequent three sessions with vehicle treatment (session 11–13), indicating that the ghrelin-induced potentiation of motivation of skilled reach foraging is short-lasting, and disappears when the drug is no longer present. On the last day (session 14), acute JMV2959 injection decreased the number of pellets consumed compared with values at session 13 in both groups of rats. B) Daily injection of ghrelin did not influence the success rate compared to vehicle. C) There were no differences in time at the rotarod (sec) between groups at baseline nor after ghrelin or vehicle treatment, indicating that ghrelin does not influence gross motor performance. D) Ghrelin administered repeatedly increased 1-hour food intake compared to vehicle, in rats that consumed sucrose during the proceeding Montoya staircase test. Behavioural data are presented as mean ± SEM; ***P < 0.001, **P < 0.01 and n.s. = not significant. ## P < 0.01 for acute JMV2959 treatment in both Ghr-Veh-JMV or Veh-Veh-JMV versus the proceeding vehicle-treatment day (session 13).

staircase test. However, it should also be considered that ghrelin signalling within other areas, not investigated in the present electrophysiological studies, may also be involved in regulating the motivation to engage in a complex motor skill function (Balleine and Dickinson, 1998; Gremel and Costa, 2013).

As further shown in the conducted electrophysiological experiments JMV2959 blocks ghrelin's effects on neurotransmission, but does not affect neurotransmission in investigated brain areas *per se*. The difference in response between ghrelin and the GHSR-1 A antagonist on outcome measurement is in line with findings that ghrelin increases whereas JMV2959 by it self does not alter dopamine release in NAc (Abizaid et al., 2006; Jerlhag et al., 2006, 2007). We therefore speculate that pharmacological GHSR-1 A suppression does not interact with the mesolimbic dopamine system in absence of reinforcers but prevents the system from being activated by external stimuli such as consumption of sucrose pellets. In support for this contention are previous data showing that JMV2959, which have no effect on gross behaviour nor accumbal dopamine transmission *per se*, reduces sexual behaviour as well as prevents drug reinforcement in rodents (Egecioglu et al., 2016; Jerlhag et al., 2010, 2009; Prieto-Garcia et al., 2015).

The well-documented orexigenic role of ghrelin signalling (for review see (Muller et al., 2015)) is supported by our present data showing that sub-chronic ghrelin administration increases, whereas JMV2959 reduces, food intake in rats. It is known that energy status affects the outcome of ghrelin (Alen et al., 2013) and it should therefore be taken into consideration that all rats used in the present study were food deprived. It is therefore possible that elevated ghrelin levels observed in food restriction and hunger (Bhatti et al., 2006) enhance the motivation of skill reached behaviour. This are a potential limitation in interpretation of the data and upcoming studies using food restriction should correlate endogenous plasma levels of ghrelin to motivation of skilled reach behaviour.

The behavioural outcome in the complex motor task used herein is likely to be influenced by various factors other than motivation. One of these is alteration in calorie intake since we in addition to sucrose consumption demonstrate an effect on regular chow intake. However ghrelin signalling also modulates reinforcers without caloric content such as cocaine, amphetamine, nicotine and saccharin (for review see (Morris et al., 2018). It is unlikely that JMV2959 and ghrelin alter the consumption of sucrose pellets by affecting gross motor performance since neither treatment affected the time spend on the rod in the Rotarod test. Moreover, these Rotarod findings suggest that performance on the rod is independent of ghrelin signalling. The possibility that behaviours such as novelty seeking, impulsivity and anxiety, which ghrelin influences (Anderberg et al., 2016; Hansson et al., 2011, 2012) may affect the obtained results should also be considered. It should also be consider that the process of prediction error learning could be involved in the behavioural outcome of the present studies (Schultz and Dickinson, 2000). Factors such as sex (Clegg et al., 2007; Johnson et al., 2016) may affect the behavioural outcome of ghrelin, and therefore future studies should investigate the role of ghrelin signalling for the motivation of skilled reach performance in female rats. Contrarily to rats with an acquired skilled reach performance, learning processes may in all probability influence the obtained data in rats without prior

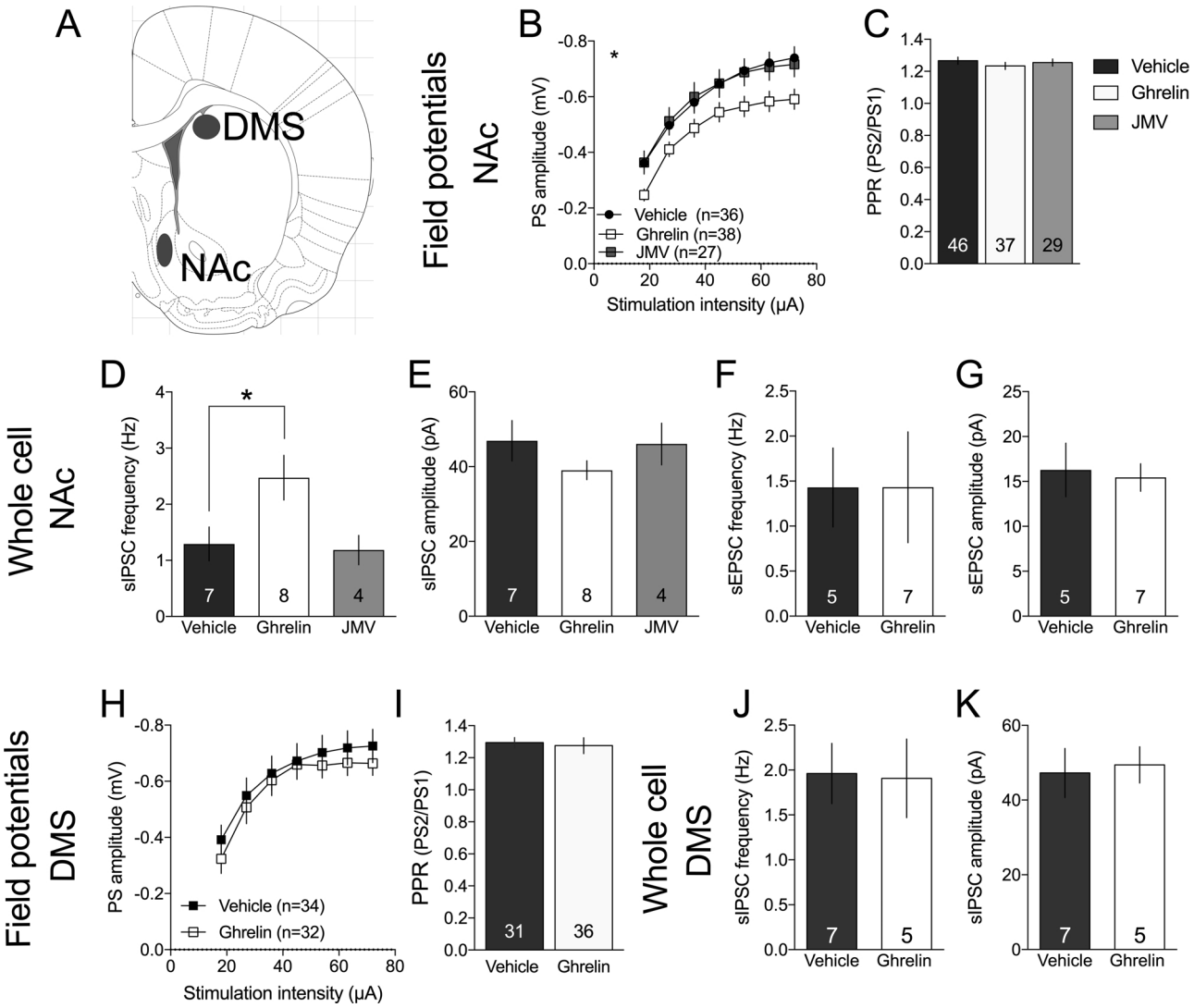

Fig. 6. Effects of sub-chronic administration of ghrelin during skilled reach foraging on neuroadaptations in the NAc shell.
Schematic drawing showing the area from which electrophysiological measures were conducted. B) Input/output function was suppressed in brain slices from animals previously treated sub-chronically during the Montoya staircase test with ghrelin, but not JMV2959 (JMV), as compared with vehicle treated controls. C) PPR was not modulated by ghrelin or JMV treatment. D) sIPSC frequency was enhanced in slices from ghrelin-treated rats. E–G) Neither sIPSC amplitude, sEPSCs frequency nor sEPSCs amplitude were affected by ghrelin treatment. H–I) Input output function and PPR were not altered in the DMS of ghrelin-treated rats as compared to vehicle-treated control. J–K) Whole cell voltage clamp recordings conducted in the DMS further showed no effect on sIPSC frequency or amplitude in ghrelin-treated rats. Electrophysiology data are presented as mean values ± SEM. n = number of brain slices taken from rats from at least 3 different animals/treatment. * $P < 0.05$, significant from control.

Montoya experience. Indeed, we found that daily JMV2959 treatment reduced the number of pellets consumed (Fig. 4A) as well as success rate (Fig. 4B) during the initial sessions. On the other hand, ghrelin treatment to rats without prior Montoya experience did not alter the success rate (Fig. 5B) and only enhances sucrose pellets consumption during session 6–10 (Fig. 5A), implying that the behavioural output of ghrelin in this model are more influenced by motivation than learning. This is substantiated by the electrophysiology data showing that ghrelin alters neurotransmission in NAc shell, but not DMS.

In summary, the present data contribute to further understand the pleiotropic effects of ghrelin as we demonstrated that ghrelin signalling regulate the motivation to complex behaviours such as skilled reach foraging via limbic neurocircuits, which supports the emerging literature suggesting that ghrelin signalling increases the incentive salience of motivated behaviours. Taken together with previous studies establishing that pharmacological suppression of the GHSR-1 A attenuate alcohol-mediated behaviours in rodents (for review see (Jerlhag, 2018)), the clinical use of such compounds, which are well tolerated and tested in patients with alcohol use disorder (Denney et al., 2017; Lee et al., 2018), would consist of an interesting future treatment perspective for various addictive disorders.

Funding and disclosure

The study is supported by grants from the Swedish Research Council (2015-03219; 2018-02814), Swedish Society for Medical Research, Stiftelsen psykiatriska forskningsfonden, The Swedish brain foundation, LUA/ALF (grant no. 240071) from the Sahlgrenska University Hospital, the foundations NovoNordisk and the Swedish Society of Medicine. EJ has received financial support from the Novo Nordisk Foundation. This does not alter the authors' adherence to any of the journal's policies on sharing data and materials. The remaining authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.04.008.

References

Abizaid, A., Liu, Z.W., Andrews, Z.B., Shanabrough, M., Borok, E., Elsworth, J.D., Roth, R.H., Sleeman, M.W., Picciotto, M.R., Tschop, M.H., Gao, X.B., Horvath, T.L., 2006. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J. Clin. Invest. 116, 3229–3239.

Abtahi, S., Howell, E., Currie, P.J., 2018. Accumbal ghrelin and glucagon-like peptide 1 signaling in alcohol reward in female rats. Neuroreport 29, 1046–1053.

Adermark, L., 2011. Modulation of endocannabinoid-mediated long-lasting disinhibition of striatal output by cholinergic interneurons. Neuropharmacology 61, 1314–1320.

Adermark, L., Morud, J., Lotfi, A., Danielsson, K., Ulenius, L., Soderpalm, B., Ericson, M., 2016. Temporal rewiring of striatal circuits initiated by nicotine. Neuropsychopharmacology 41, 3051–3059.

Alen, F., Crespo, I., Ramirez-Lopez, M.T., Jagerovic, N., Goya, P., de Fonseca, F.R., de Heras, R.G., Orio, L., 2013. Ghrelin-induced orexigenic effect in rats depends on the metabolic status and is counteracted by peripheral CB1 receptor antagonism. PLoS One 8, e60918.

Anderberg, R.H., Hansson, C., Fenander, M., Richard, J.E., Dickson, S.L., Nissbrandt, H., Bergquist, F., Skibicka, K.P., 2016. The stomach-derived hormone ghrelin increases impulsive behavior. Neuropsychopharmacology 41, 1199–1209.

Balleine, B.W., Dickinson, A., 1998. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37, 407–419.

Barnes, T.D., Kubota, Y., Hu, D., Jin, D.Z., Graybiel, A.M., 2005. Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. Nature 437, 1158–1161.

Bhatti, S.F., Hofland, L.J., van Koetsveld, P.M., Van Ham, L.M., Duchateau, L., Mol, J.A., van der Lely, A.J., Kooistra, H.S., 2006. Effects of food intake and food withholding on plasma ghrelin concentrations in healthy dogs. Am. J. Vet. Res. 67, 1557–1563.

Clegg, D.J., Brown, L.M., Zigman, J.M., Kemp, C.J., Strader, A.D., Benoit, S.C., Woods, S.C., Mangiaracina, M., Geary, N., 2007. Estradiol-dependent decrease in the orexigenic potency of ghrelin in female rats. Diabetes 56, 1051–1058.

Corbit, L.H., Balleine, B.W., 2003. The role of prelimbic cortex in instrumental conditioning. Behav. Brain Res. 146, 145–157.

Denney, W.S., Sonnenberg, G.E., Carvajal-Gonzalez, S., Tuthill, T., Jackson, V.M., 2017. Pharmacokinetics and pharmacodynamics of PF-05190457: the first oral ghrelin receptor inverse agonist to be profiled in healthy subjects. Br. J. Clin. Pharmacol. 83, 326–338.

Egecioglu, E., Prieto-Garcia, L., Studer, E., Westberg, L., Jerlhag, E., 2016. The role of ghrelin signalling for sexual behaviour in male mice. Addict. Biol. 21, 348–359.

Fasano, C., Bourque, M.J., Lapointe, G., Leo, D., Thibault, D., Haber, M., Kortleven, C., Desgroseillers, L., Murai, K.K., Trudeau, L.E., 2013. Dopamine facilitates dendritic spine formation by cultured striatal medium spiny neurons through both D1 and D2 dopamine receptors. Neuropharmacology 67, 432–443.

Gomez, J.L., Cunningham, C.L., Finn, D.A., Young, E.A., Helpenstell, L.K., Schuette, L.M., Fidler, T.L., Kosten, T.A., Ryabinin, A.E., 2015. Differential effects of ghrelin antagonists on alcohol drinking and reinforcement in mouse and rat models of alcohol dependence. Neuropharmacology 97, 182–193.

Gremel, C.M., Costa, R.M., 2013. Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. Nat. Commun. 4, 2264.

Hansson, C., Haage, D., Taube, M., Egecioglu, E., Salome, N., Dickson, S.L., 2011. Central administration of ghrelin alters emotional responses in rats: behavioural, electrophysiological and molecular evidence. Neuroscience 180, 201–211.

Hansson, C., Shirazi, R.H., Naslund, J., Vogel, H., Neuber, C., Holm, G., Anckarsater, H., Dickson, S.L., Eriksson, E., Skibicka, K.P., 2012. Ghrelin influences novelty seeking behavior in rodents and men. PLoS One 7, e50409.

Jerlhag, E., 2008. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. Addict. Biol. 13, 358–363.

Jerlhag, E., 2018. Gut-brain axis and addictive disorders: a review with focus on alcohol and drugs of abuse. Pharmacol. Ther.

Jerlhag, E., Egecioglu, E., Dickson, S.L., Andersson, M., Svensson, L., Engel, J.A., 2006. Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. Addict. Biol. 11, 45–54.

Jerlhag, E., Egecioglu, E., Dickson, S.L., Douhan, A., Svensson, L., Engel, J.A., 2007. Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. Addict. Biol. 12, 6–16.

Jerlhag, E., Egecioglu, E., Landgren, S., Salome, N., Heilig, M., Moechars, D., Datta, R., Perrissoud, D., Dickson, S.L., Engel, J.A., 2009. Requirement of central ghrelin signaling for alcohol reward. Proc. Natl. Acad. Sci. U. S. A. 106, 11318–11323.

Jerlhag, E., Egecioglu, E., Dickson, S.L., Engel, J.A., 2010. Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. Psychopharmacology 211, 415–422.

Jerlhag, E., Janson, A.C., Waters, S., Engel, J.A., 2012. Concomitant release of ventral tegmental acetylcholine and accumbal dopamine by ghrelin in rats. PLoS One 7, e49557.

Jog, M.S., Kubota, Y., Connolly, C.I., Hillegaart, V., Graybiel, A.M., 1999. Building neural representations of habits. Science (New York, N.Y.) 286, 1745–1749.

Johnson, M.L., Saffrey, M.J., Taylor, V.J., 2016. Plasma ghrelin concentrations were altered with oestrous cycle stage and increasing age in reproductively competent wistar females. PLoS One 11, e0166229.

Khaimova, E., Kandov, Y., Israel, Y., Cataldo, G., Hadjimarkou, M.M., Bodnar, R.J., 2004. Opioid receptor subtype antagonists differentially alter GABA agonist-induced feeding elicited from either the nucleus accumbens shell or ventral tegmental area regions in rats. Brain Res. 1026, 284–294.

King, S.J., Rodrigues, T., Watts, A., Murray, E., Wilson, A., Abizaid, A., 2016. Investigation of a role for ghrelin signaling in binge-like feeding in mice under limited access to high-fat diet. Neuroscience 319, 233–245.

Landgren, S., Engel, J.A., Hyytia, P., Zetterberg, H., Blennow, K., Jerlhag, E., 2011a. Expression of the gene encoding the ghrelin receptor in rats selected for differential alcohol preference. Behav. Brain Res. 221, 182–188.

Landgren, S., Simms, J.A., Thelle, D.S., Strandhagen, E., Bartlett, S.E., Engel, J.A., Jerlhag, E., 2011b. The ghrelin signalling system is involved in the consumption of sweets. PLoS One 6, e18170.

Landgren, S., Simms, J.A., Hyytia, P., Engel, J.A., Bartlett, S.E., Jerlhag, E., 2012. Ghrelin receptor (GHS-R1A) antagonism suppresses both operant alcohol self-administration and high alcohol consumption in rats. Addict. Biol. 17, 86–94.

Lee, M.R., Tapocik, J.D., Ghareeb, M., Schwandt, M.L., Dias, A.A., Le, A.N., Cobbina, E., Farinelli, L.A., Bouhlal, S., Farokhnia, M., Heilig, M., Akhlaghi, F., Leggio, L., 2018. The novel ghrelin receptor inverse agonist PF-5190457 administered with alcohol: preclinical safety experiments and a phase 1b human laboratory study. Mol. Psychiatry.

Licheri, V., Lagstrom, O., Lotfi, A., Patton, M.H., Wigstrom, H., Mathur, B., Adermark, L., 2018. Complex control of striatal neurotransmission by nicotinic acetylcholine receptors via excitatory inputs onto medium spiny neurons. J. Neurosci. 38, 6597–6607.

Montoya, C.P., Campbell-Hope, L.J., Pemberton, K.D., Dunnett, S.B., 1991. The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats. J. Neurosci. Methods 36, 219–228.

Morris, L.S., Voon, V., Leggio, L., 2018. Stress, motivation, and the gut-brain Axis: a focus on the ghrelin system and alcohol use disorder. Alcohol. Clin. Exp. Res.

Morud, J., Adermark, L., Perez-Alcazar, M., Ericson, M., Soderpalm, B., 2016. Nicotine produces chronic behavioral sensitization with changes in accumbal neurotransmission and increased sensitivity to re-exposure. Addict. Biol. 21, 397–406.

Moulin, A., Demange, L., Berge, G., Gagne, D., Ryan, J., Mousseaux, D., Heitz, A., Perrissoud, D., Locatelli, V., Torsello, A., Galleyrand, J.C., Fehrentz, J.A., Martinez, J., 2007. Toward potent ghrelin receptor ligands based on trisubstituted 1,2,4-triazole structure. 2. Synthesis and pharmacological in vitro and in vivo evaluations. J. Med. Chem. 50, 5790–5806.

Muller, T.D., Nogueiras, R., Andermann, M.L., Andrews, Z.B., Anker, S.D., Argente, J., Batterham, R.L., Benoit, S.C., Bowers, C.Y., Broglio, F., Casanueva, F.F., D'Alessio, D., Depoortere, I., Geliebter, A., Ghigo, E., Cole, P.A., Cowley, M., Cummings, D.E., Dagher, A., Diano, S., Dickson, S.L., Dieguez, C., Granata, R., Grill, H.J., Grove, K., Habegger, K.M., Heppner, K., Heiman, M.L., Holsen, L., Holst, B., Inui, A., Jansson, J.O., Kirchner, H., Korbonits, M., Laferrere, B., LeRoux, C.W., Lopez, M., Morin, S., Nakazato, M., Nass, R., Perez-Tilve, D., Pfluger, P.T., Schwartz, T.W., Seeley, R.J., Sleeman, M., Sun, Y., Sussel, L., Tong, J., Thorner, M.O., van der Lely, A.J., van der Ploeg, L.H., Zigman, J.M., Kojima, M., Kangawa, K., Smith, R.G., Horvath, T., Tschop, M.H., 2015. Ghrelin. Mol. Metab. 4, 437–460.

Naleid, A.M., Grace, M.K., Cummings, D.E., Levine, A.S., 2005. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides 26, 2274–2279.

Newman, S., Pascal, L., Sadeghian, K., Baldo, B.A., 2013. Sweetened-fat intake sensitizes gamma-aminobutyric acid-mediated feeding responses elicited from the nucleus accumbens shell. Biol. Psychiatry 73, 843–850.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, 4th ed. Academic Press, San Diego.

Prieto-Garcia, L., Egecioglu, E., Studer, E., Westberg, L., Jerlhag, E., 2015. Ghrelin and GHS-R1A signaling within the ventral and laterodorsal tegmental area regulate sexual behavior in sexually naive male mice. Psychoneuroendocrinology 62, 392–402.

Quarta, D., Di Francesco, C., Melotto, S., Mangiarini, L., Heidbreder, C., Hedou, G., 2009. Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. Neurochem. Int. 54, 89–94.

Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Brain Res. Rev. 18, 247–291.

Schultz, W., Dickinson, A., 2000. Neuronal coding of prediction errors. Annu. Rev. Neurosci. 23, 473–500.

Skibicka, K.P., Hansson, C., Alvarez-Crespo, M., Friberg, P.A., Dickson, S.L., 2011. Ghrelin directly targets the ventral tegmental area to increase food motivation. Neuroscience 180, 129–137.

Skibicka, K.P., Hansson, C., Egecioglu, E., Dickson, S.L., 2012. Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. Addict. Biol. 17, 95–107.

Soderlund, G.B., Eckernas, D., Holmblad, O., Bergquist, F., 2015. Acoustic noise improves motor learning in spontaneously hypertensive rats, a rat model of attention deficit hyperactivity disorder. Behav. Brain Res. 280, 84–91.

Sommer, S., Hauber, W., 2016. Ghrelin receptor activation in the ventral tegmental area amplified instrumental responding but not the excitatory influence of Pavlovian stimuli on instrumental responding. Neurobiol. Learn. Mem. 134 (Pt B), 210–215.

Stratford, T.R., Kelley, A.E., 1997. GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. J. Neurosci. 17, 4434–4440.

van der Plasse, G., van Zessen, R., Luijendijk, M.C., Erkan, H., Stuber, G.D., Ramakers,

G.M., Adan, R.A., 2015. Modulation of cue-induced firing of ventral tegmental area dopamine neurons by leptin and ghrelin. Int. J. Obes. 2005 (39), 1742–1749.
Villalba, R.M., Smith, Y., 2013. Differential striatal spine pathology in Parkinson's disease and cocaine addiction: a key role of dopamine? Neuroscience 251, 2–20.
Wellman, P.J., Clifford, P.S., Rodriguez, J.A., Hughes, S., Di Francesco, C., Melotto, S., Tessari, M., Corsi, M., Bifone, A., Gozzi, A., 2012. Brain reinforcement system function is ghrelin dependent: studies in the rat using pharmacological fMRI and intracranial self-stimulation. Addict. Biol. 17, 908–919.
Yin, H.H., Knowlton, B.J., Balleine, B.W., 2006. Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. Behav. Brain Res. 166, 189–196.