Neural substrates of the orexigenic hormone ghrelin: a pharmacological MRI study in the rat

A. Gozzi[1], A. Schwarz[1], V. Crestan[2], C. Di Francesco[3], F. Marini[3], M. Massagrande[3], T. Reese[1], P. Gerrard[3], C. Heidbreder[4], S. Melotto[4], and A. Bifone[1]

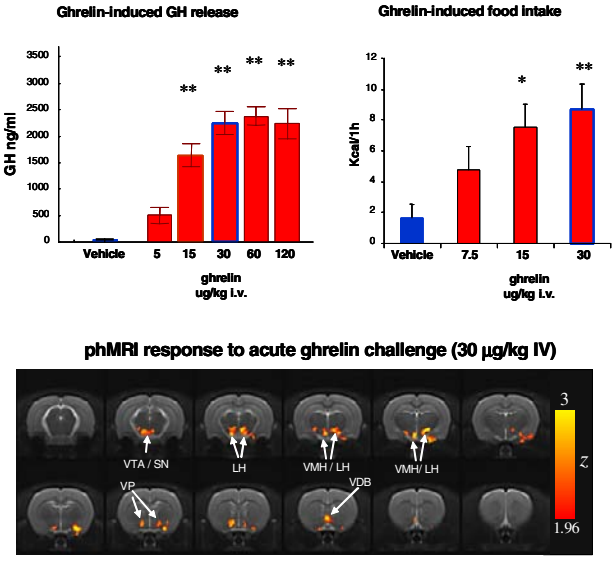
[1]Neuroimaging, Centre of Excellence for Drug Discovery, Psychiatry, GlaxoSmithKline Medicines Research Centre, Verona, Italy, [2]Laboratory animal sciences, Centre of Excellence for Drug Discovery, Psychiatry, GlaxoSmithKline Medicines Research Centre, Verona, Italy, [3]Behavioural Neuroscience, Centre of Excellence for Drug Discovery, Psychiatry, GlaxoSmithKline Medicines Research Centre, Verona, Italy, [4]Neuropsychopharmacology, Centre of Excellence for Drug Discovery, Psychiatry, GlaxoSmithKline Medicines Research Centre, Verona, Italy

Introduction Ghrelin is a peptide secreted by gastric endocrine cells that stimulates appetite and food-intake, and promotes the release of growth hormone (GH) by the pituitary gland [1]. Ghrelin receptors (GH secretagogue receptors, GHS-Rs) are located both in the CNS and the periphery. In the brain, high-density of GSH-Rs has been observed in circumventricular hypothalamic nuclei, a finding that is consistent with the established role of ghrelin as a mediator of appetite and energy homeostasis. Recently, the presence of focal nuclei expressing the GSH-R has also been reported in extrahypothalamic areas without immediate access to peripheral circulating ghrelin [2]. However, it is as yet unclear if and how circulating ghrelin would gain access and influence the activity of these nuclei, and therefore the exact physiological role of peripheral ghrelin in the brain remains to be determined. Here we combined biochemical and behavioural readouts with pharmacological MRI (phMRI) to assess the brain substrates of a peripheral challenge of ghrelin at a dose that produces significant effects on GH-release and food intake in the rat.

Methods All experiments were carried out in accordance with Italian regulations governing animal welfare and protection. Protocols were also reviewed and consented to by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985).
Biochemistry: Male Sprague-Dawley (SD) rats were intravenously (iv) injected with vehicle (saline, n=9) or ghrelin (5, 15, 30, 60 or 120µg/kg, n=9 each group). GH, ACTH or corticosterone serum or plasma levels were measured in blood samples collected 15' after ghrelin injection.
Behaviour: In a second group of SD rats the effect of vehicle (n=10) or ghrelin (7.5, 15 or 30 µg/kg, n=10 each group) on spontaneous food intake (chow) was recorded over a 1 hr post-challenge time-window during which rats were kept under physiologically resting conditions (light).
phMRI: N=16 male SD rats were surgically prepared as detailed previously [3] and imaged under 0.8% halothane maintenance anaesthesia, neuromuscular blockade and artificial ventilation. PhMRI time series data were acquired on a Bruker Biospec 4.7T system using a T_2-weighted RARE sequence (RARE factor 32, matrix 128x128, FOV 40mm, slice thickness 1mm, 16 contiguous coronal slices, TR_{eff} = 2700ms, TE_{eff} = 100ms) in the presence of a blood-pool contrast agent (Endorem, Guerbet) in order to sensitise signal changes to alterations in relative cerebral blood volume (rCBV), as described in detail elsewhere [4;5]. After 20 min, animals were challenged with ghrelin (30 µg/kg, i.v., n=7) or its vehicle (saline, n=9). RCBV time series data were spatially normalised to a stereotactic rat brain template[6]. Individual subject response amplitude maps were calculated within the framework of the general linear model [7]. Blood pressure response to ghrelin was modest, transient, not correlated with the time-profile of the rCBV changes, and well within the range of cerebral blood flow autoregulation under halothane anaesthesia [8].

Results - Biochemistry: Peripheral ghrelin administration stimulated the release of GH in a dose-dependent manner, reaching a plateau at doses comprised between 30 and 60 µg/kg iv (Fig. 1 top). A significant release of ACTH was apparent at the highest doses of the peptide, while no alterations in corticosterone serum levels were observed at any of the doses tested. **Behaviour:** Intravenous administration of ghrelin induced a dose-dependent increase in food intake in pre-treated animals exposed to normal diet during their resting period, an effect that reached statistical significance at 15 and 30 µg/kg (Fig. 1) Based on these findings, 30 µg/kg was selected as a dose to be subsequently tested in the phMRI protocol. **phMRI:** Acute ghrelin challenge (30/µg kg) induced focal rCBV increases in several nuclei of the ventromedial and ventrolateral hypothalamus (Fig. 1 bottom). Significant rCBV increases were also observed in the diagonal band of the septum, and in dopaminergic structures such as the VTA and the substantia nigra. The time-course of the rCBV increase in treated animals showed a slow and constant increase over time which plateaued approximately 10 min after the injection.

Discussion Our study shows *in vivo* functional activation of focal hypothalamic and extra-hypothalamic structures following a peripheral injection of a dose of ghrelin that significantly stimulates food-intake and GH release. All the structures activated have been shown to have a high density of GHSR mRNA [2]. The hypothalamic structures involved have long been recognised as playing roles in the regulation of body weight and food intake, a finding that corroborates the key role played by ghrelin in mediating energy homeostasis. Notably, our data also provide for the first time evidence of focal activation *in vivo* of extra-hypothalamic dopaminergic regions such as the VTA and the substantia nigra, a finding consistent with a role of ghrelin in mediating aspects of reward behaviours and locomotor activity, respectively [2, 9, 10]. The presence of discrete foci of activation in the diagonal band of the septum, a structure that belongs to the cholinergic septal-hippocampal pathway [11], supports a role of ghrelin in higher brain functions such as learning and memory processes, an observation that is consistent with the findings of recent behavioural studies in which ghrelin has been shown to affect hippocampal spine synapse density and memory performance [12].



Conclusions We have used phMRI to assess the neural correlates in the brain of a peripheral challenge with a dose of ghrelin that produces significant effects on GH-release and food intake in the rat. The activation maps showed a focal pattern of activation that includes hypothalamic areas, along with extrahypothalamic dopaminergic and cholinergic nuclei. Our results corroborate a key modulatory role of ghrelin in the brain, which involves structures mediating aspects of reward, locomotion, and possibly higher brain functions such as memory and learning.

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Figure 1: (top) *GH response and induced food intake following ghrelin challenge in freely-moving rats. The dose selected for the phMRI study has been highlighted with a blue line. Values expressed as means ± SEM. (*p<0.05, **p<0.01 vs. control vehicle; data analysed using ANOVA followed by Dunnett's post-hoc test).* ***(bottom)*** *Statistical parametric maps of the rCBV changes induced by an acute ghrelin challenge (30 µg/kg i.v., N=6) vs. vehicle (saline, N=9) (Z<1.96, cluster threshold p_{corr}<0.05).VTA:ventral tegmental area,SN: substantia nigra, LH, Lateral hypothalamus, VMH, ventromedial hypothalamus, VP, ventral pallidum, VDB vertical diagonal band.*