Orexin A exerts more thermogenic than orexinergic functions

In this article we focus on the role of orexin A in the thermoregulatory functions and its link to food intake. This peptide is named orexin A to emphasize the increase in food intake due to this peptide. The influence of eating behavior could be only secondary to change in the thermoregulatory set-point to reach a determined core temperature. Our viewpoint is compared with vision of other authors, finding possible concordance and disagreement. Activity of the sympathetic nerves system, brown adipose tissue and central body temperatures, heart rate and food intake were monitored to measure the modifications induced by an intracerebroventricular injection of orexin A on the thermoregulation and eating behavior in various experimental conditions.

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Orexin A exerts more thermogenic than orexinergic functions

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1. Introduction

The aim of this brief review is to report our studies which demonstrate that orexin A is a neuropeptide that primarily affects body temperature through influences exerted on the sympathetic nervous system. The modification of eating behavior due to orexin A could be only secondary to change in the thermoregulatory set-point. Furthermore, the purpose is to compare this our viewpoint with vision of other authors, so that concordance and disagreement can be analyzed.

An intracerebroventricular (icv) injection of the hypothalamic neuropeptide “orexin A” is able to induce multiform reactions, as expression of generalized activation of sympathetic nervous system. Although this neuropeptide is named for its influence on food intake (Sweet et al., 1999), an icv injection of orexin A does not merely affect eating behavior. Rather it also induces an increase in heart rate (Monda et al., 2005) , blood pressure (Shirasaka et al., 1999) and metabolic rate (Lubkin & Stricker-Krongrad, 1998) ,indicating that this neuropeptide plays a role in the control of vegetative functions. Orexin A also influences body temperature. In fact, an icv administration of orexin A induces an increase in the firing rate of the sympathetic nerves to interscapular brown adipose tissue (IBAT), accompanied with a rise in IBAT and colonic temperatures (Monda, Amaro & De Luca, 1994a; Monda et al., 1996a) . In addition, the presence of orexin receptors in many cerebral areas suggests that additional functions are played by orexin A (Kukkonen et al., 2002). A role for the orexins in sleep regulation has also been demonstrated (Narcos et al., 2001).

The name orexin A is utilized to indicate the above mentioned peptide that has been also named hypocretin-1 in time past. This name has been changed to orexin A to

emphasize the increase in food intake due to this peptide, because an icv injection of orexin A induces an increase in food intake in fasted or satiated rats (Sweet et al., 1999).

2. Experimental evidences

The first evidence reports the experiment where the food intake, firing rate (FR) of the sympathetic nerves to IBAT, IBAT and colonic temperatures (T_{IBAT} and T_C), were monitored in 24h-fasting male Sprague-Dawley rats for 15 h after food presentation during the dark period. Orexin A (1.5 nmol) was injected into the lateral cerebral ventricle 6h before food presentation while FR, T_{IBAT} and T_C were also monitored. The same variables were controlled in rats receiving orexin A at the same time of food presentation. Two other groups of control animals were tested with the same procedure, but orexin A was substituted by saline. The results (see figure 1 and panel A of figure 5) showed that food intake was significantly lower in the group receiving orexin A 6h before food presentation in comparison to all the other groups. FR, T_{IBAT} and T_C were significantly higher in the rats receiving orexin A with respect to rats receiving saline. In this experimental demonstration, the saline groups were tested but not reported in the paper. Food intake of both saline groups was intermediate between orexin 0 group and orexin -6 group. These findings demonstrate that the effects on food intake induced by orexin A depends on the time of food presentation (Monda, Viggiano & De Luca, 2003a). This induces us to revise the role of orexin A in the control of food intake. The name assigned to this peptide was due to the strong increase in food intake after an orexin A administration, assigning a fundamental role in the induction of food intake (Shiraishi et al., 2000; Wolf, 1998). The results of the above mentioned experiment call for a re-discussion of this role, underlining the importance of orexin A in the control of the sympathetic activity and body temperature, which in turn affects food intake. The anorexic effect of substances is better detected in fasted animals. Since this experiment tested a possible anorexic role of orexin A, fasted

rats were chosen. In this experiment, an icv injection of orexin A induces an increase in the sympathetic activity and in the T_{IBAT} independently of food ingestion, that is reduced in the rats with a delayed presentation of food. This suggests that the effects on body temperature are prevalent with respect to eating behavior. Then, orexin A can induce hyperphagia, but also hypophagia, contradicting the significance of this name that assign a primary hyperphagic effect to this peptide. Other substances with primary hyperphagic effect, as neuropeptide Y or galanin, induce a reduction of the sympathetic discharge and a decrease in body temperature (Szekely, 2005; Egawa, Yoshimatsu & Bray 1991; Nagase, Bray & York, 1996; Patel & Hutson, 1996; Monda et al., 2004b; Monda et al., 2006a; Monda et al., 2008b). Conversely, substances with a primary hypophagic effect cause an increase in the sympathetic activity. For example, leptin induces reduction of food intake (Okamoto, Kimura & Saito, 2001; Messina et al., 2013b; Ukropec, Sebokova & Klimes, 2001), along with an increase in the firing rate of the sympathetic nerves to IBAT and a rise in T_{IBAT} (Haque et al., 1999, Haynes et al., 1999). For this reason, orexin A cannot be considered a substance with a primary hyperphagic effect. The orexin A can induce hypophagia, as in above described experiment, or hyperphagia (Shiraishi et al., 2000), but it always induces an activation of thermogenesis. We can suppose that this peptide elevates the thermoregulatory set-point, inducing the reactions to reach the new level of body temperature. The increase in food intake, obtained in the rats with a non-delayed presentation of food, could be a reaction aimed to reach an elevated body temperature. Indeed, food ingestion induces a rise in body temperature due to post-prandial thermogenesis (Tentolouris, Liatis & Katsilambros, 2006; Monda et al., 2008a; Messina et al., 2012, Messina et al., 2013a; De Luca et al., 1987). The hyperphagic effect of orexin A disappears when the body temperature is already increased, so that a reduction in food intake can happen in this condition.

The second evidence reports the experiment where the firing rate of the sympathetic nerves to IBAT, along with IBAT and T_C were monitored in urethane-anesthetized male Sprague-Dawley rats before and 6h after an injection of orexin A (1.5 nmol) into the lateral cerebral ventricle. The same variables were monitored in rats with an intraperitoneal administration of lysine acetylsalicylate (100 mg/kg bw), an inhibitor of prostaglandins synthesis. The results (see figure 2 and panel B of figure 5) show that orexin A increases the sympathetic firing rate, IBAT and T_C. This increase is reduced by lysine acetylsalicylate. ASA reduces the sympathetic activation induced by orexin A (Monda, Amaro & De Luca, 1994a), suggesting that PGs have an implication in the mediation of this phenomenon. A possible explanation is that orexin A could induce a cerebral synthesis of PGsE, which act on the preoptic area/anterior hypothalamus (Simpson, Ruwe & Myers, 1994; Stitt, 1991), a very responsive structure to PGsE. On the other hand, we cannot exclude that PGsE could stimulate other hypothalamic areas (Monda et al., 1996b), including the ventromedial hypothalamus (Simpson, Ruwe & Myers, 1994), which directly controls the activity of nerves to IBAT (Thornhill & Halvorson, 1994). The icv injection stimulates thermogenesis and increases body temperature in anesthetized rats, showing that orexin A is involved in thermoregulation independently on eating behavior (Monda et al., 1996b; Monda et al., 2004a). Because food intake activates thermogenesis (De Luca et al., 1987; Bray, 2000), substances affecting food consumption induce a secondary influence on body temperature (Bray, 2011). Since our experiment is carried out in anesthetized animals, the rise in body temperature induced by orexin A is a primary effect of this neurotransmitter. The orexin A affects the temperature of IBAT, which is the most important effector of non-shivering thermogenesis in the rat, illustrating that the rise in heat production is also due to the activation of thermogenesis unrelated to muscle activity. The increase in colonic temperature emphasizes the effect of orexin A on “core” temperature suggesting the

inclusion of orexin A among the peptides controlling body temperature. ASA injection reduced both temperatures, indicating that these thermic reactions are under the control of PGs, which are classic pyrogens. Further experiments should be carried out to demonstrate definitively an elevation of set-point induced by orexin A. Since a fever-like hyperthermia is associated with suppression of heat-loss mechanisms, thermocouples fixed on the surface of the tail skin of rats recording tail skin temperature (indicating the presence of vasoconstriction or dilation occurring parallel with an increase in the metabolic rate as indicated by increased brown adipose tissue temperature) could provide proof for a coordinated rise in core temperature that usually characterizes such an elevation of set-point.

The third evidence reports the experiment where the firing rate and cytochrome oxidase activity of the ventromedial hypothalamic neurons, and T_C were monitored in 12 urethane-anesthetized male Sprague-Dawley before and over a period of 2h after an injection of orexin A (1.5 nmol) into the lateral cerebral ventricle. The results showed an increase of firing rate in 9 rats, a decrease in 2 rats and no modification of firing rate in 1 rat. In all rats, orexin A induced rise in T_C and cytochrome oxidase activity. A group of 12 rats was used as control and saline, but not orexin A, was injected into the cerebral ventricle. No modifications in firing rate, cytochrome oxidase reactivity and T_C were noted, as reported in figure 3 and panel C of figure 5. Furthermore, 12 male rats were anesthetized and lesioned bilaterally in the ventromedial hypothalamus (VMH) with an injection of ibotenic acid (30 nmol into each side), which destroys cell bodies. Sham-lesions were carried out in 12 control rats. After 48 hours, all animals were anesthetized with ethyl-urethane. The firing rates of the sympathetic nerves to IBAT, along with IBAT and T_C were monitored before and over a period of 2h after an injection of orexin A (1.5 nmol) or saline into the lateral cerebral ventricle in the lesioned and sham lesioned rats.

The results (see figure 4 and panel D of figure 5) showed that orexin A increased the sympathetic firing rate, IBAT and T_C in the sham-lesioned rats. These increases were reduced by lesion of the VMH. Saline did not induce any modification. These results strongly indicate that the VMH is involved in the sympathetic and hyperthermic reactions induced by this hypothalamic neuropeptide. The relationship between activation of the ventromedial hypothalamic neurons and thermogenic reaction due to orexin A is demonstrated by the reduction of hyperthermia in the rats with ibotenate lesion (Monda et al., 2005). The findings of the above mentioned experiment indicate that the VMH regulates the discharge of nerves to IBAT in this experimental model, thus demonstrating the agreement of this model to other evidences showing that VMH controls the IBAT activity (Monda et al., 2002). Indeed, a lesion of the VMH reduces the IBAT temperature and related metabolic rate in sedentary (De Luca et al., 1987) or trained rats (Monda, Amaro & De Luca, 1993). This experiment emphasizes the influences exerted by orexin A on the VMH that is named “center of satiety”. Orexin A increases the activity of ventromedial hypothalamic neurons (as demonstrated by rise in cytochrome oxidase reactivity) with a parallel increase in the sympathetic activity. This demonstration indicates that orexin A exerts a stimulation of the “center of satiety” with a role similar to other substances, as cholecystokinin. This peptide is able to induce a reduction in food intake and an increase in firing rate of sympathetic nerves to IBAT after injection into the third ventricle or VMH (Yoshimatsu, Egawa & Bray, 1992) . The above mentioned study supports the hypothesis of a reciprocal relationship between the effects of anorexigenic substances on the thermogenic component of the sympathetic nervous system and food intake.

These experiments were approved by the Ethics Committee of the Second University of Naples with no.12.1.61.64.72

159 3. Discussion

160 Several experiments carried out by various authors demonstrate that orexin A is
161 able to increase the sympathetic discharge and body temperature. For example
162 (Berthoud et al., 2005) have demonstrated that the caudal raphe nuclei in the medulla
163 (known to harbor sympathetic preganglionic motor neurons involved in thermal and
164 cardiovascular regulation) are innervated by orexin A fibers. Since the acute rise in
165 sympathetic activity plays a role in the onset of satiety (Bray, 2000; Viggiano et al.,
166 2006), the orexin A cannot be included among the classic orexigenic peptides (Szekely,
167 Petervari & Szelenyi, 2004).

168 Girault et al. (Girault et al., 2012) showed that through the autonomic nervous system,
169 the orexin system plays a key role in the control of glucose metabolism, but it has also
170 been shown to stimulate sympathetic outflow, to increase body temperature. For these
171 authors, the well-known effects of orexin on the control of food intake appear to be more
172 extensive than originally thought, with additional effects on the autonomic nervous
173 system, that is, to increase body temperature and energy metabolism. These authors
174 indicate increase in body temperature as a “crucial effect” of orexin A. Teske et al.
175 (Teske, Billington & Kotz, 2010) emphasized the role of orexins in modulating non-
176 sleep-related energy expenditure with specific focus on the augmentation of whole body
177 energy expenditure as well as hypocretin-induced sympathetic activity, showing a
178 predominant role of hypocretin-1 receptors in the influence on energy expenditure and
179 body temperature.

180 On the other hand, (Jászberényi et al., 2002) reported that orexin A induces hypothermia
181 and they argue that this appetite-regulating peptide might also play a role in
182 thermoregulation.

This orexin-induced hypothermia has not been found by other authors, who instead found that orexin A functions as a key driver of brown adipocyte differentiation through direct actions on brown adipose precursors (Sellayah, Bharaj & Sikder, 2011) and orexin A turns up the heat on obesity (Seale, 2011).

In general, the effects of orexin A on the firing rate to IBAT corroborate recent evidences demonstrating the role played by this novel neuropeptide in the control of the autonomic nervous system (Monda, Amaro & De Luca,1994a. Shirasaka et al.(Shirasaka et al., 1999) illustrated that icv injections of orexins increase the activity of the renal sympathetic nerves, which play an important role in the homeostasis of body fluids and the circulatory system.

The orexin A affects the temperature of IBAT, which is the most important effector of non shivering thermogenesis in the rat (Cannon, Houstek & Nedergaard, 1998), illustrating that the rise in heat production is also due to the activation of thermogenesis unrelated to muscle activity. IBAT is the organ responsible for evoking 35-65% of the total increase in metabolic heat production during various experimental manipulations in rodents (Monda et al., 1994b; Richard & Picard, 2011). IBAT activity is controlled by the sympathetic nervous system, and factors, which influence thermogenesis, appear to act centrally to modify the sympathetic outflow to IBAT (Monda et al., 1995; Silva, 2011). The significant role of IBAT in the hyperthermia induced by orexin A (Monda, Amaro & De Luca, 1994b; Monda, Viggiano & De Luca, 2003b; Monda et al., 2006b) is confirmed by these findings. Throughout our experiment, we report direct evidence of increased sympathetic tone in nerves innervating IBAT after an orexin A injection. This confirms the role of the sympathetic nervous system on IBAT activity.

The strong influence of orexin A on body temperature, independently on eating behavior, is emphasized by the above-mentioned demonstrations, suggesting that the effects on body temperature are prevalent in comparison to eating behavior. Orexin A can induce both hyperphagia or hypophagia, but it always induces an activation of thermogenesis, contradicting the significance of its name that assign a primary hyperphagic effect to this peptide. We can suppose that this peptide elevates the thermoregulatory set-point, inducing the reactions to reach the new level of body temperature. The increase in food intake, obtained in various experiments, could be a reaction aimed to reach an elevated body temperature. On the other hand, these reactions are different from those observations of the literature that describe the fever-like elevation of core temperature as part of “sickness behavior” regularly associated with anorexia (Elmquist, Scammell & Saper, 1997). Probably, a different mechanism is involved in the association between orexin-hyperthermia and food intake. Since it has been recently demonstrated (Kotz et al., 2012; Perez-Leighton, 2012) that brain orexin promotes obesity resistance, the orexin A should be not counted among the anabolic neuropeptides (Szekely, Petervari & Balasko, 2010), but among catabolic peptides (Girault et al., 2012 ; Teske & Mavanji, 2012). A possible thermoregulatory role for orexin has been proposed by other authors. Cold exposure increased orexin mRNA in the hypothalamus (Ida et al., 2000). Transneuronal retrograde transport of pseudorabies virus from the BAT identified the caudal raphe neurons with orexinergic innervation (Berthoud et al., 2005) and orexin-containing neurons in the hypothalamus (Oldfield et al., 2002). Orexin knockout mice showed elevated body temperature during sleep (Mochizuki et al., 2006) and orexin neuron-ablated mice had an attenuated body temperature fluctuation (Zhang et al., 2007). Also, orexin neurons are indispensable for stress-induced thermogenesis in mice. Indeed, these authors pointed out, for the first time, the possible importance of co-existent

neurotransmitter/modulators in the orexin neurons for stress-induced hyperthermia and the importance of integrity of the orexin neurons for full expression of multiple facets of the fight-or-flight response (Zhang et al., 2010). Furthermore, the importance of orexin A in the thermoregulation is corroborated by recent studies, showing that the thermosensitivity of orexin neurons may be an important part of maintaining energy homeostasis during fever (Parsons et al., 2012; Rusyniak et al., 2011).

In conclusion, the above evidences suggest that orexin A exerts a key role in the thermoregulation.

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409 Captions

410 Figure 1: Means ± SE of cumulative change in food intake (FI), firing rate of sympathetic
411 nerves (FR), temperature of brown fat (IBAT) and core temperature (TC). Food
412 presentation at time 0. Intracerebroventricular injection of orexin A was made 6 h before
413 food presentation (OREXIN -6) or contemporaneously to food presentation (OREXIN 0).
414 The asterisk indicates a significant difference ($p<0.05$).

415 Figure 2: Means ± SE of changes in sympathetic firing rate (FR), in brown fat temperature
416 (TIBAT) and in core temperature (TC) of rats with intraperitoneal injection of saline or
417 lysine acetylsalicylate (ASA) plus intracerebroventricular injection of orexin A. The asterisk
418 indicates a significant difference ($p<0.05$).

419 Figure 3: Means ± SE of changes in unit activity of VMH neurons (FR) and in core
420 temperature (TC). The orexin A or saline was injected in a lateral cerebral ventricle (icv)
421 at time 0. In lower panel, means ± SE of values of cytochrome oxidase reactivity (CYT-
422 OX) of VMH, expressed as relative optical density (ROD) units. The asterisk indicates a
423 significant difference ($p<0.05$).

424 Figure 4: Means ± SE of changes in sympathetic firing rate (FR), in brown fat temperature
425 (TBAT) and in core temperature (TC) of sham-lesioned or lesioned rats with
426 intracebroventricular injection of orexin A at time 0. The asterisk indicates a significant
427 difference ($p<0.05$).

428 Figure 5: Scheme of the experimental demonstrations (1st: panel A; 2nd:panel B; 3rd:
429 panel C; 4th: panel D)

Figure 1

Orexin A and eating behavior

Figure 1: Means ± SE of cumulative change in food intake (FI), firing rate of sympathetic nerves (FR), temperature of brown fat (IBAT) and core temperature (TC). Food presentation at time 0. Intracerebroventricular injection of orexin A was made 6 h before food presentation (OREXIN -6) or contemporaneously to food presentation (OREXIN 0). The asterisk indicates a significant difference ($p<0.05$).

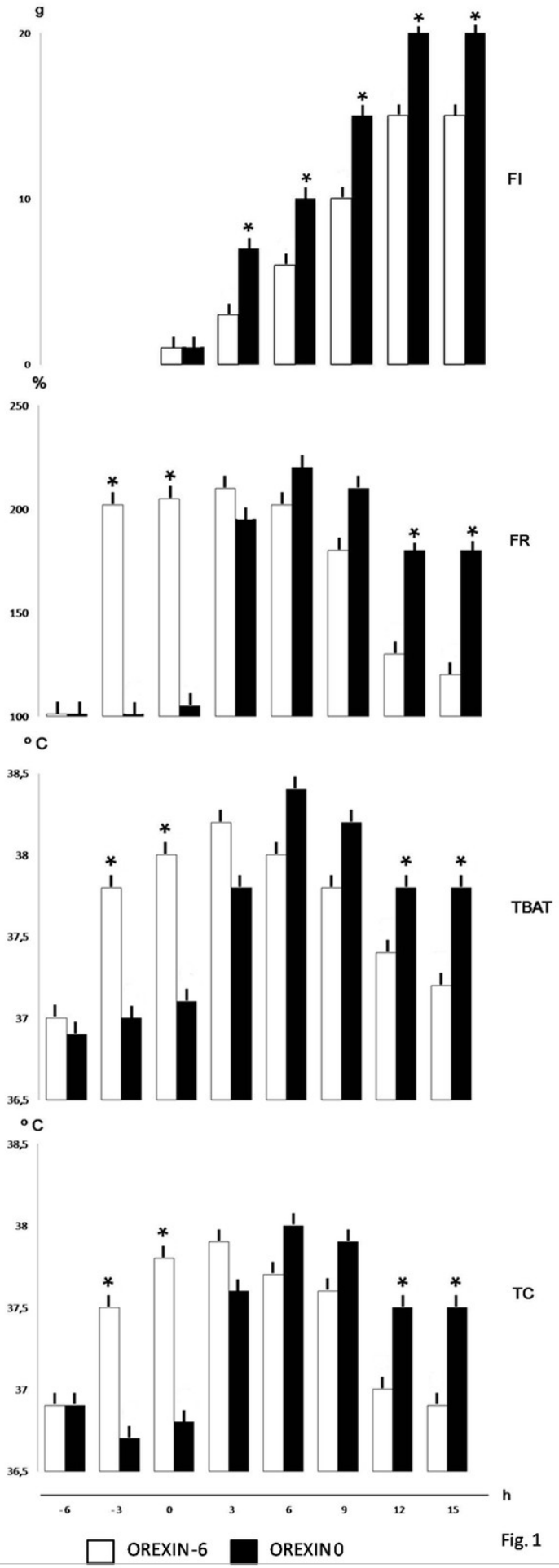

Fig. 1

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Figure 2

Orexin A and lysine acetylsalicylate

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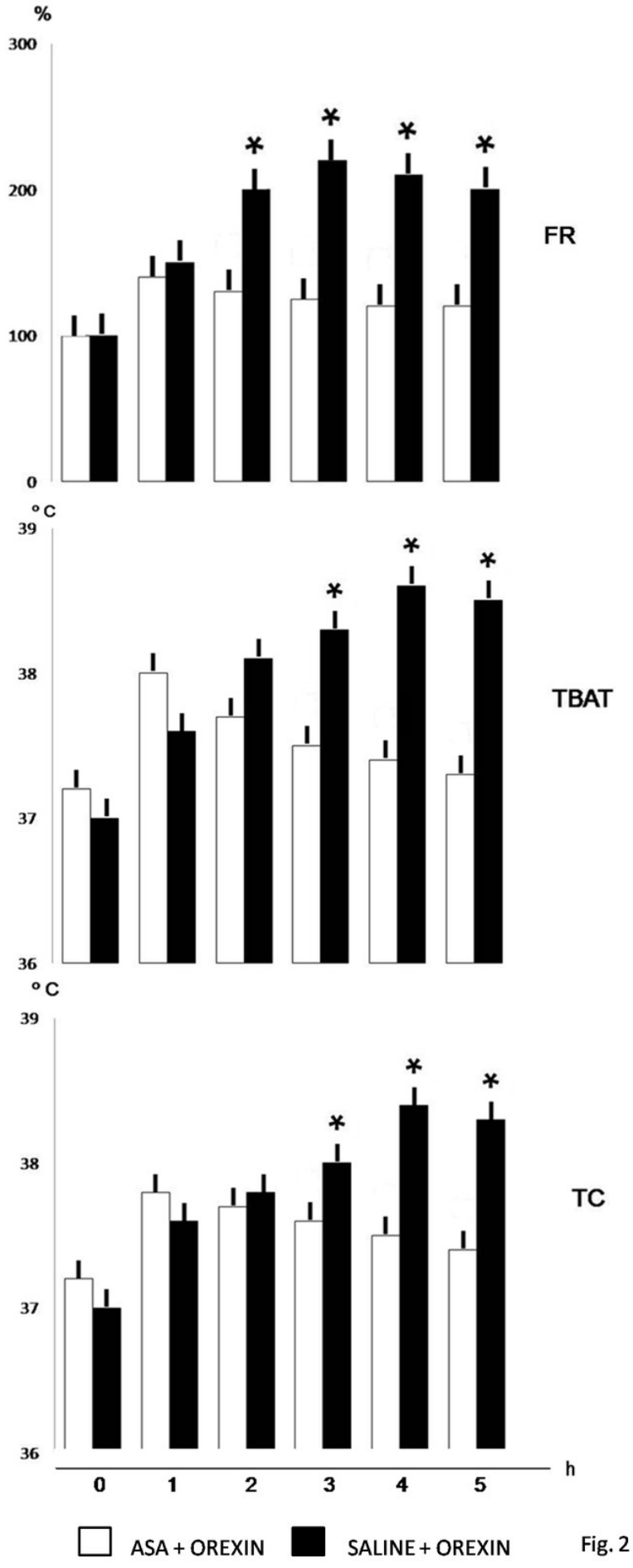

Fig. 2

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Figure 3

Orexin A and activity of the ventromedial hypothalamus

Figure 3: Means ± SE of changes in unit activity of VMH neurons (FR) and in core temperature (TC). The orexin A or saline was injected in a lateral cerebral ventricle (icv) at time 0. In lower panel, means ± SE of values of cytochrome oxidase reactivity (CYT-OX) of VMH, expressed as relative optical density (ROD) units. The asterisk indicates a significant difference ($p<0.05$).

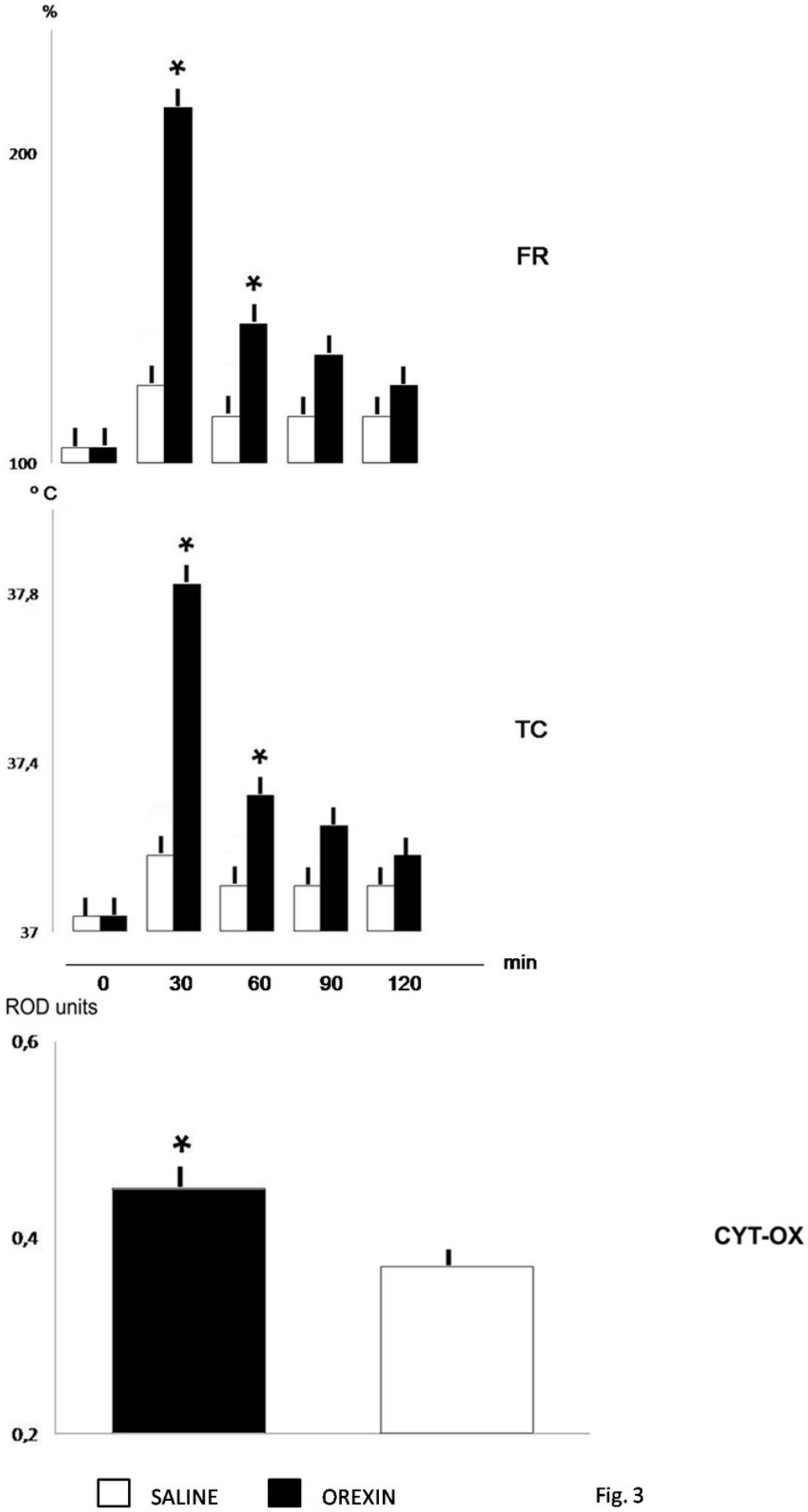

Fig. 3

Figure 4

Orexin A and lesion of the ventromedial hypothalamus

Figure 4: Means ± SE of changes in sympathetic firing rate (FR), in brown fat temperature (TBAT) and in core temperature (TC) of sham-lesioned or lesioned rats with intracebroventricular injection of orexin A at time 0. The asterisk indicates a significant difference ($p<0.05$).

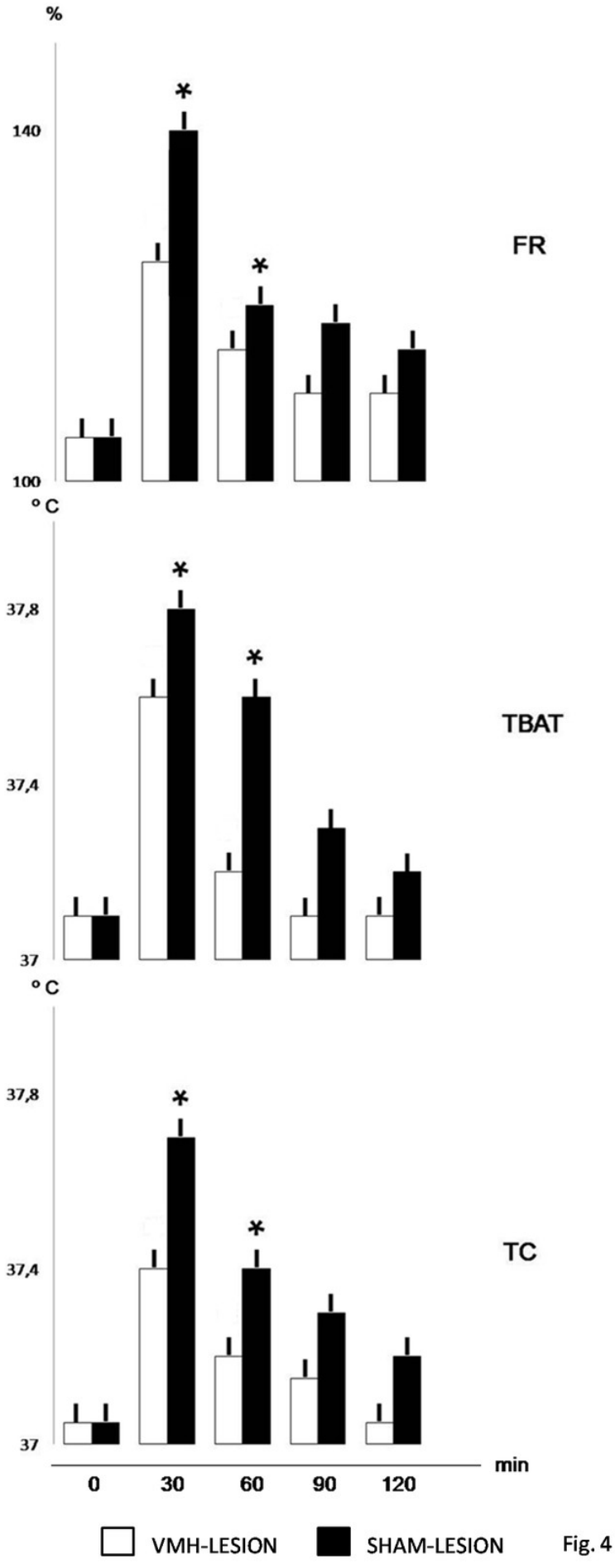

Fig. 4

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Figure 5

Summary diagrams

Figure 5: Scheme of the experimental demonstrations (1st: panel A; 2nd:panel B; 3rd: panel C; 4th: panel D)

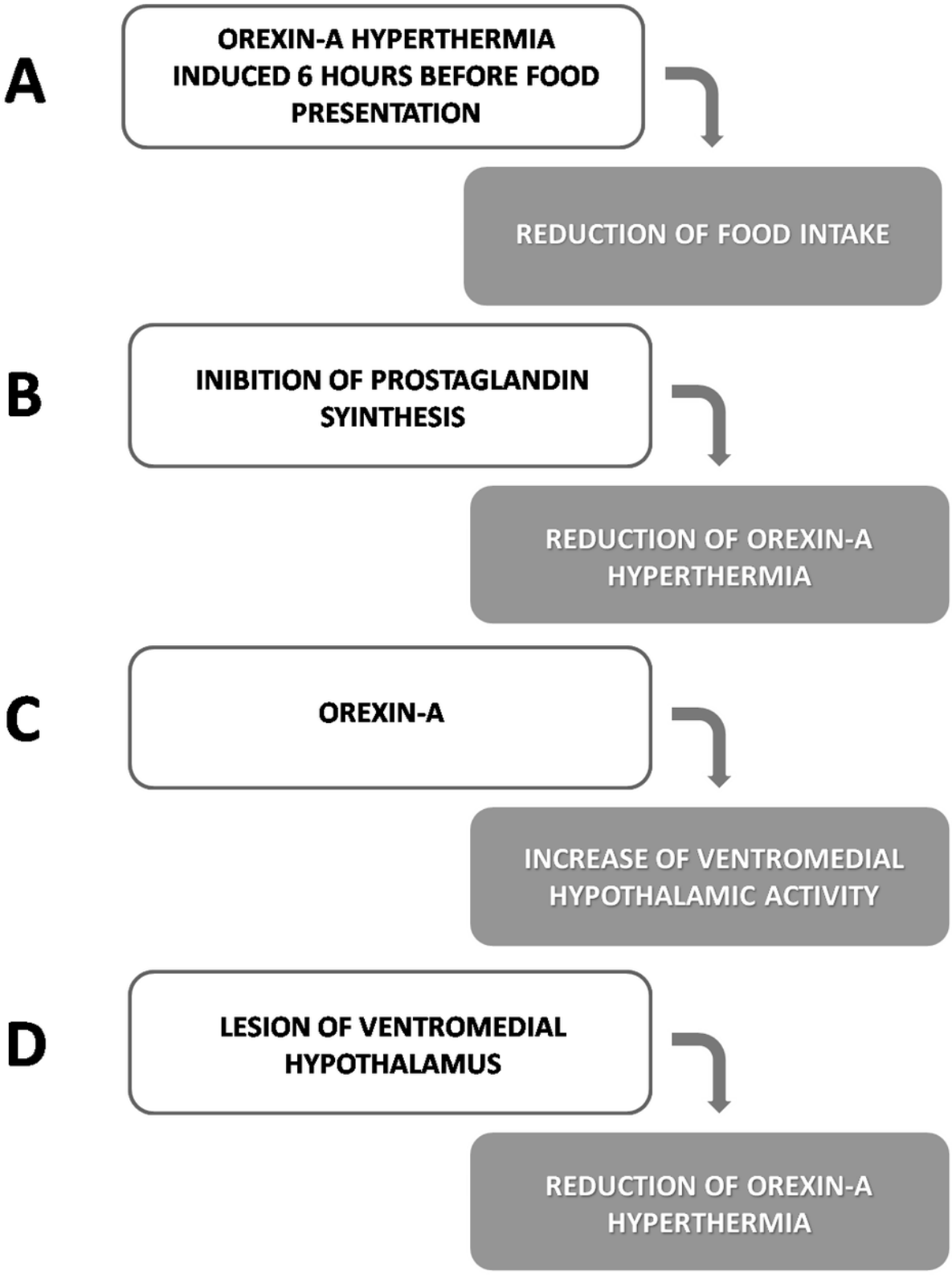

Fig. 5

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