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RELATIONSHIP between prandial drinking behavior and supersensitivity of salivary glands after superior salivatory nucleus lesions in RATS

Juan M.J. Ramos [a,b,*], María Elena Castillo [c], Amadeo Puerto [a,b,1]

[a] Department of Psychobiology, University of Granada, Granada 18071, Spain
[b] Mind, Brain and Behavior Research Center (CIMCYC), University of Granada, Granada 18071, Spain
[c] Laboratory of Psychobiology, Campus Cartuja, University of Granada, Granada 18071, Spain

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ABSTRACT

Prandial drinking, an increase in the number of drinking responses and secondary or non-homeostatic polydipsia in the presence of dry food, is typically associated with a deficit in salivary secretion. This study investigates the degree of salivary gland supersensitivity to pilocarpine administration after lesions to the superior salivatory nucleus (SSN), the site of origin of the parasympathetic preganglionic neurons that innervate the submandibular-sublingual (S-S) salivary glands. The main aim was to determine if there is a relationship between the degree of glandular supersensitivity, as an index of secretory deficit, and the development of prandial drinking in lesioned rats. Results showed that following SSN lesions two subgroups of rats were obtained. One subgroup exhibited prandial drinking but the other was similar to the control group. The SSN-lesioned prandial drinking subgroup presented significantly greater supersensitivity than the SSN-lesioned non-prandial drinking rats; the non-prandial drinking subgroup, in turn, presented significantly more supersensitivity than controls. Additionally, S-S supersensitivity observed in rats that exhibited prandial drinking due to the sectioning of *chorda tympani* efferent axons was compared to that observed in rats exhibiting prandial drinking due to SSN lesions. It was found that both groups presented the same S-S supersensitivity curve. These results indicate that SSN lesions produce a gradation of S-S supersensitivity values that appear to run parallel to the degree of glandular secretory deficit caused by the lesions. Thus, only the rats with greater secretory deficit (greater supersensitivity) develop prandial drinking. These data support the idea that there is in fact a functional link between the lateral reticular formation of the brainstem (the region associated with the SSN) and S-S salivary glands.

1. Introduction

Prandial drinking was first described in rats that had recovered feeding and drinking following lateral hypothalamic lesions [58,60]. This style of drinking is characterized by the intake of small amounts of water immediately after taking a piece of dry food into the mouth, which leads to a marked polydipsia [27,29]. However, the phenomenon disappears when the animals have a diet of wet food [28]. Some studies have suggested that this peculiar drinking pattern is a learned solution to the failure in saliva secretion caused by the lesions. Indeed, a wide range of data have confirmed this hypothesis, showing that total but not partial desalivation causes prandial drinking [3,18,54,57]. However, in rats recovered from lateral hypothalamic lesions the salivary glands themselves are sensitive to pilocarpine injection [20,21], see also [24-26,53].

Using this characteristic prandial pattern of drinking as a behavioral model of a deficit in salivation, previous studies conducted in our lab tried to functionally locate the superior salivatory nucleus (SSN). This nucleus houses the parasympathetic preganglionic cell bodies that innervate the submandibular ganglion, which controls the submandibular-sublingual (S-S) salivary glands. A consensus regarding the precise anatomical localization of this secretory nucleus was reached based on studies using the horseradish peroxidase tracing method [5, 6, 23, 32, 36, 41, 42, 45, 52, 62, 65, 67], retrograde transneuronal labeling by pseudorabies virus [26, 55] and electrophysiological methods [10, 11, 33, 38, 40]. All of the aforementioned studies found the preganglionic salivatory neurons to be scattered in the lateral reticular formation of the brainstem, specifically, dorsolaterally to the facial motor nucleus (for review see [34]. However, as far as we know, only our studies have explored the effects of the SSN lesion on salivary function [47, 49,50]. In previous studies we observed that SSN lesions + parotidec-

* Corresponding author: Department of Psychobiology, Campus Cartuja, University of Granada, Granada 18071, Spain
E-mail address: jmjramos@ugr.es (J.M.J. Ramos)
[1] In memoriam

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tomy, resulting in total desalivation, caused prandial drinking [49,50]. However, there is no direct physiological evidence that lesions to this nucleus result in a deficit in S-S salivary secretion. To address this issue, in the present study we explore whether SSN lesions produce S-S supersensitivity, as an index of secretory deficit, and whether a relationship exists between the degree of supersensitivity observed and the development of prandial drinking behavior. To the best of our knowledge, the glandular supersensitivity phenomenon has only been observed following damage to peripheral secretory nerves, but not following central lesions [14]. For this reason, a further objective of our study is to examine whether this phenomenon can be produced by cerebral lesions, which would allow us to establish a link between the lateral reticular formation of the brainstem (the region, based on anatomic studies, associated with the SSN) and S-S salivary glands.

Previous studies have shown that surgical or pharmacological parasympathetic descentralization of the salivary glands produce glandular supersensitivity, that is, an increase in the secreting capacity of the glands in response to the injection of low doses of pharmacological agonists [2,12-17,56,61]. Therefore, we hypothesized that decentralization of the S-S salivary glands following SSN lesions should produce a phenomenon of glandular supersensitivity. That is, the administration of a small dose of a non-selective muscarinic agonist such as pilocarpine should cause the lesioned rats to secrete higher amounts of S-S saliva than the control rats. In our laboratory we have normally obtained two populations of rats after SSN lesions, one that exhibits prandial drinking and one that does not [50]. Based on this, the specific objective of this study was to investigate whether there are differences in supersensitivity not just between lesioned vs. control rats, but, importantly, between prandial drinking SSN-lesioned rats vs. non-prandial drinking SSN-lesioned rats. We hypothesized that prandial drinking rats after salivatory nucleus lesions should have a higher degree of decentralization of the S-S salivary glands than the lesioned rats that do not exhibit prandial drinking. Consequently, a higher degree of supersensitivity should be observed in prandial drinking rats vs. non-prandial drinking rats.

2. Materials and methods

2.1. Subjects

A total of 61 male Wistar rats (280–310 g), bred in the University of Granada Animalarium, were used. In 29 of the rats the SSN was lesioned, with the remaining 32 being assigned randomly to a peripheral decentralization group or to a sham-operated group. After the behavioral procedure it was observed that of the 29 lesioned rats, 14 developed a prandial style of drinking and 15 did not. The animals were thus divided into four groups: SSN-lesioned exhibiting prandial drinking ($n=14$), SSN-lesioned not exhibiting prandial drinking ($n=15$), peripheral decentralization of S-S salivary glands ($n=10$) and sham-operated ($n=22$). A peripheral decentralization group was included in order to compare the values of supersensitivity obtained after SSN lesions with those obtained after complete parasympathetic S-S secretory deficit. The rats were individually housed in single polycarbonate cages ($480\times265\times210$ mm, Tecniplast, Italy) and maintained at a constant temperature of 22 ± 1 °C. Rats were given ad libitum food and water until the experiment started. Experimental procedures were performed in conformity with European and Spanish legislation (2010/63 EEC and BOE 53/2013, respectively) and were approved by the Ethics Committee for Animal Research of the University of Granada.

2.2. Surgery

Stereotaxic surgery. Under sodium pentobarbital anesthesia (65 mg/kg, i.p.; Sigma Chemical, St. Louis, MO, USA), rats were mounted in a David Kopf stereotaxic instrument (model 900, Tujunga, CA, USA). The distance between the horizontal plane passing through the interaural line and the horizontal plane passing through the incisors was 5 mm. The anatomical location of the SSN in the rat, as described in previous studies using horseradish peroxidase and transneuronal labeling based on pseudorabies virus methods [6,26,36,41], was transferred to a stereotaxic atlas of the rat brain [44]. Given the large size of the SSN, two contiguous electrolytic lesions were made on either side of the brainstem, one in the ventral part and one in the dorsal part of the lateral parvocellular reticular formation. This made it possible to affect as large an area of the salivatory center as possible.

The bilateral lesions aimed at the ventral part of the SSN were located 2.6 mm posterior to the auditory meatus, ±2.2 mm lateral to the sagittal sinus and 0.1 mm ventral to the horizontal interaural zero plane [44]. These lesions were made with a lesion-generating device (Grass Instruments, model DCLMS, Quincy, MA, USA), by passing 0.5 mA DC cathodal current for 7 s through a stainless steel electrode (insect pin size 00) insulated with INSL-X except for the cross section of the cut tip. Bilateral lesions aimed at the dorsal part of the SSN were located 2.6 mm posterior to the auditory meatus, ±1.8 mm lateral to the sagittal sinus and 0.3 mm above the horizontal interaural plane (see Fig. 1). The procedure used was the same as above except that 0.5 mA DC cathodal current was passed for 13 s. In the sham-operated group the electrode was lowered to a point 0.5 mm above the horizontal interaural plane, and no current was passed. Nontraumatic ear bars developed in our own laboratory were used to avoid possible damage to the salivatory fibers running through the middle ear. After surgery, each rat was injected with buprenorphine to reduce post-operative pain (0.2 mg/kg, i.p., Bupaq®, Richter Pharma AG, Austria).

Parotidectomy. On experimental day 17 all animals were anesthetized (sodium pentobarbital 65 mg/kg, i.p.) for bilateral transection of the parotid duct. The surgical procedure used involved a midline incision approximately 2 cm long on the ventral throat, through which the parotid ducts were ligated and transected at the level of the lateral region of the masseter muscle. Each rat was injected with buprenorphine to reduce post-operative pain (0.2 mg/kg, i.p., Bupaq®, Richter Pharma AG, Austria).

Peripheral decentralization of S-S salivary glands. In the peripheral decentralization group, a sectioning of the parasympathetic preganglionic fibers to the submandibular ganglia was performed at the level of the submandibular-sublingual ducts, precisely when these efferent neurons from the *chorda tympani* reach the ducts. Under sodium pentobarbital (65 mg/kg, i.p.) the ducts supplying the submandibular-sublingual glands were exposed and with the aid of a dissecting microscope and fine point surgical tweezers, the preganglionic fibers that run along the ducts were damaged and separated from the ducts' outer walls. Then the ducts were carefully cleaned with cotton swabs to eliminate the transected fibers and prevent reinnervation. After surgery, each rat was injected with buprenorphine to reduce post-operative pain (0.2 mg/kg, i.p., Bupaq®, Richter Pharma AG, Austria).

2.3. Behavioral procedure

After the stereotaxic or peripheral decentralization surgery the animals were given a recovery period of 8–10 days. Over the next five days the rats were habituated to eating their usual daily dry diet (Envigo, Global Diet 2914, Madison, WI, USA) from 10.00 to 12.00 h only, while water remained available 24 h *ad lib*. On the following day (experimental day 16), the feeding and drinking behavior of the rats was videotaped (Sony Handycam, HC1000E, Tokyo, Japan) in order to determine the number of drinking responses emitted by all subjects during the 2-h feeding period. The frequency of drinking, during this and similar phases, was computed by one of the researchers following a blind methodology, viewing the video film a few days later. An animal was considered to emit a drinking response each time it licked the drinking spout and at the same time air bubbles rising from the spout were observed. On the next day (experimental day 17) all animals were anesthetized for bilateral transection of the parotid ducts (see Surgery). After parotidectomy, food and water were available *ad lib* for 7

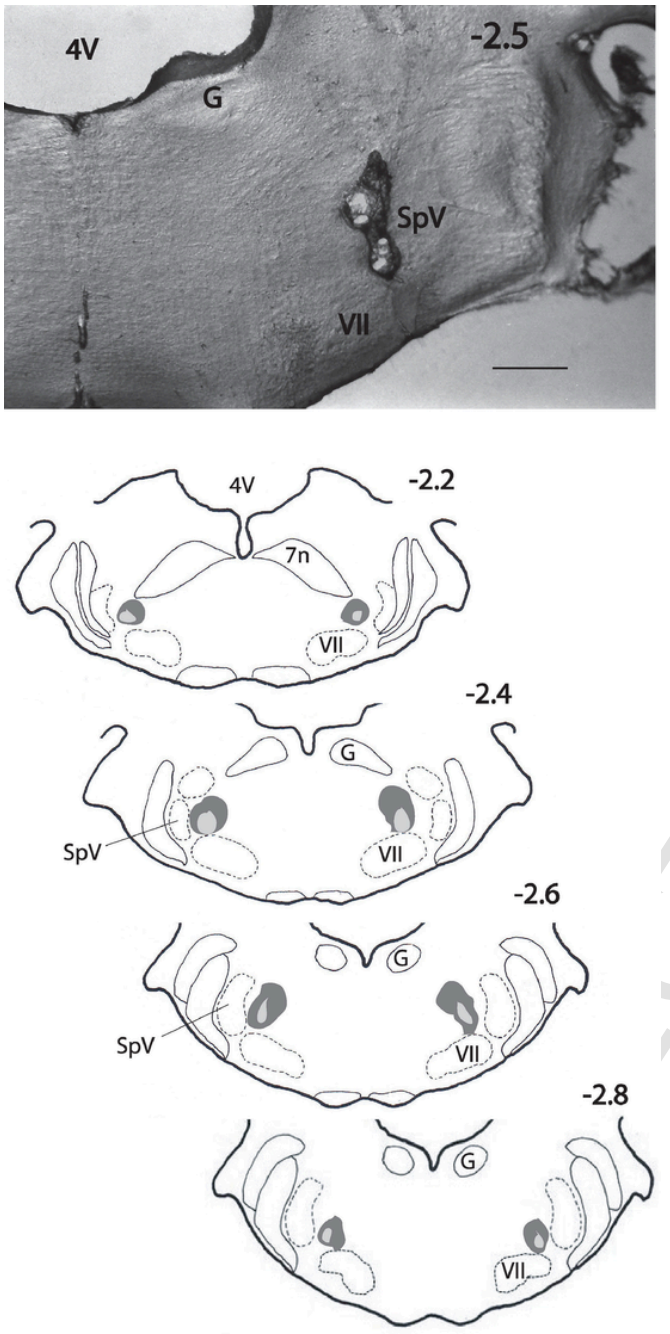

Fig. 1. Top: Micrograph showing two contiguous electrolytic lesions to the lateral reticular formation in a representative rat. Abbreviations: G, genu of the facial nerve; 4 V, ventricle; SpV, spinal nucleus of the trigeminal nerve; VII, nucleus of the facial nerve; 7n, descending root of the facial nerve. The horizontal bar equals 1 mm. AP coordinate with reference to the auditory meatus according to the atlas of Pellegrino et al., [44]. Bottom: Coronal sections showing the largest (gray) and smallest (clearer central area) superior salivatory nucleus lesions. Abbreviations as indicated above. AP coordinates are shown in relation to the auditory meatus.

days, after which the animals were once again subjected to a food restriction schedule like the one described above. On experimental day 30 the number of drinking responses emitted by all subjects was recorded during the 2-h period when dry food was available. On day 31 the dry food was replaced by a wet mash consisting of 3 parts isotonic saline to 2 parts dry food, and the number of drinking responses was recorded throughout the 2-h feeding period. After that, the rats received dry food and water *ad lib* for four days. Finally, on experimental day 35, all rats were tested to determine S-S salivary gland supersensitivity. Specifically, animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p) and 15 min later were injected with pilocarpine nitrate (0.3 mg/kg, i.p.; Sigma Chemical) in an amount that previous pilot studies in our lab had shown to be sensitive to detecting differences in the supersensitivity of salivary glands (see also 49, expt. 4). S-S saliva secretion was measured in each rat 10, 20, 30, 40, 50 and 60 min after pilocarpine injection. Saliva secreted was collected directly from the oral cavity, using a slight modification of a technique developed by others (8,19,53). Briefly, 3 pieces of absorbent cotton weighing approximately 10 mg each were used. Two pieces were placed in the lateral zones of the oral cavity while the third was placed under the tongue. After 2 min the pieces of cotton were removed and weighed to the nearest 0.1 mg on a precision balance (Cobos, Barcelona, Spain), the amount of saliva secreted being taken as the difference between the initial weight of the cotton and its weight after 2 min in the rat's mouth.

The amount of food ingested and the amount of water consumed by all subjects were also recorded during the 2-h feeding period on all test days. The water consumed during the 22-h period of food restriction on experimental day 30 was also measured.

2.4. Histology

When the behavioral testing was completed, the rats were deeply anesthetized with sodium pentobarbital (90 mg/kg, i.p.) and perfused intercardially with 0.9% saline, followed by 10% formalin. After extraction from the skull the brains were post-fixed in 10% formalin for several days and subsequently in 10% formalin-30% sucrose until sectioning. Coronal sections (40 μm) were cut on a cryostat (Leica CM 1850, Leica Microsystems, Germany) and stained with cresyl violet, a Nissl stain.

2.5. Data analyses

ANOVAs and post-hoc Newman-Keuls tests were used. All analyses were conducted with the Statistica software 10.0 (StatSoft, Tulsa, OK, USA).

3. Results

3.1. Histological results

Tissue damage was microscopically identified by the presence of pronounced necrosis or missing tissue (Fig. 1). In all experimental rats lesions affected an extensive area located just dorsal to the lateral half of the facial nucleus and just medial to the spinal nucleus of the trigeminal nerve. In some animals a small portion of the most dorsal area of the facial nucleus was also slightly affected. In addition, in a small number of animals minimal unilateral damage in the most medial region of the spinal trigeminal nucleus was observed. In the lateral plane the size of the lesion was 0.4–0.6 mm. In the dorsoventral plane, the lesions extended to the ventrolateral edge of the genu of the facial nerve, presenting a length of 0.5–0.9 mm. In the majority of the brains the center of the lesions was located 2.4/2.6 mm posterior to the interaural zero point [44]. At the most rostral level, lesions were observed to be dorsolateral to the facial nucleus and medial to the descending root of the facial nerve, within the lateral reticular formation, between 2.0 and 2.2 mm posterior to the interaural coronal plane. Caudally, damage extended in the majority of animals up to 2.8/3.0 mm posterior to the auditory meatus, always within the lateral reticular formation. Therefore, the entire damaged area corresponded very precisely with the parvocellularis lateral reticular formation, which has been identified as the SSN (see, for example, 6, 26).

3.2. Prandial drinking and water intake

Prandial drinking behavior, measured by the frequency of drinking, and the water intake recorded on the different test days are shown in Fig. 2. After recovery from the stereotaxic or peripheral decentralization surgery, on experimental day 16, groups did not differ significantly in the number of drinking responses in the presence of dry food ($F_{3, 57} = 0.14$, $p = 0.93$. Fig. 2A) nor in the total amount of water consumed during the 2-h period ($F_{3, 57} = 0.88$, $p = 0.45$. Fig. 2B). However, after all the animals had been parotidectomized, significant differences in the frequency of drinking became evident. On experimental days 30 and 31, a 2-way mixed ANOVA (4 group x 2 type of diet), with group as the between-subject variable and type of diet as within-subject variable, showed a significant effect of group ($F_{3, 57} = 121.27$, $p < 0.0001$, $\eta^2_p = 0.86$. Fig. 2C and 2E), type of diet ($F_{1, 57} = 460.94$, $p < 0.0001$,

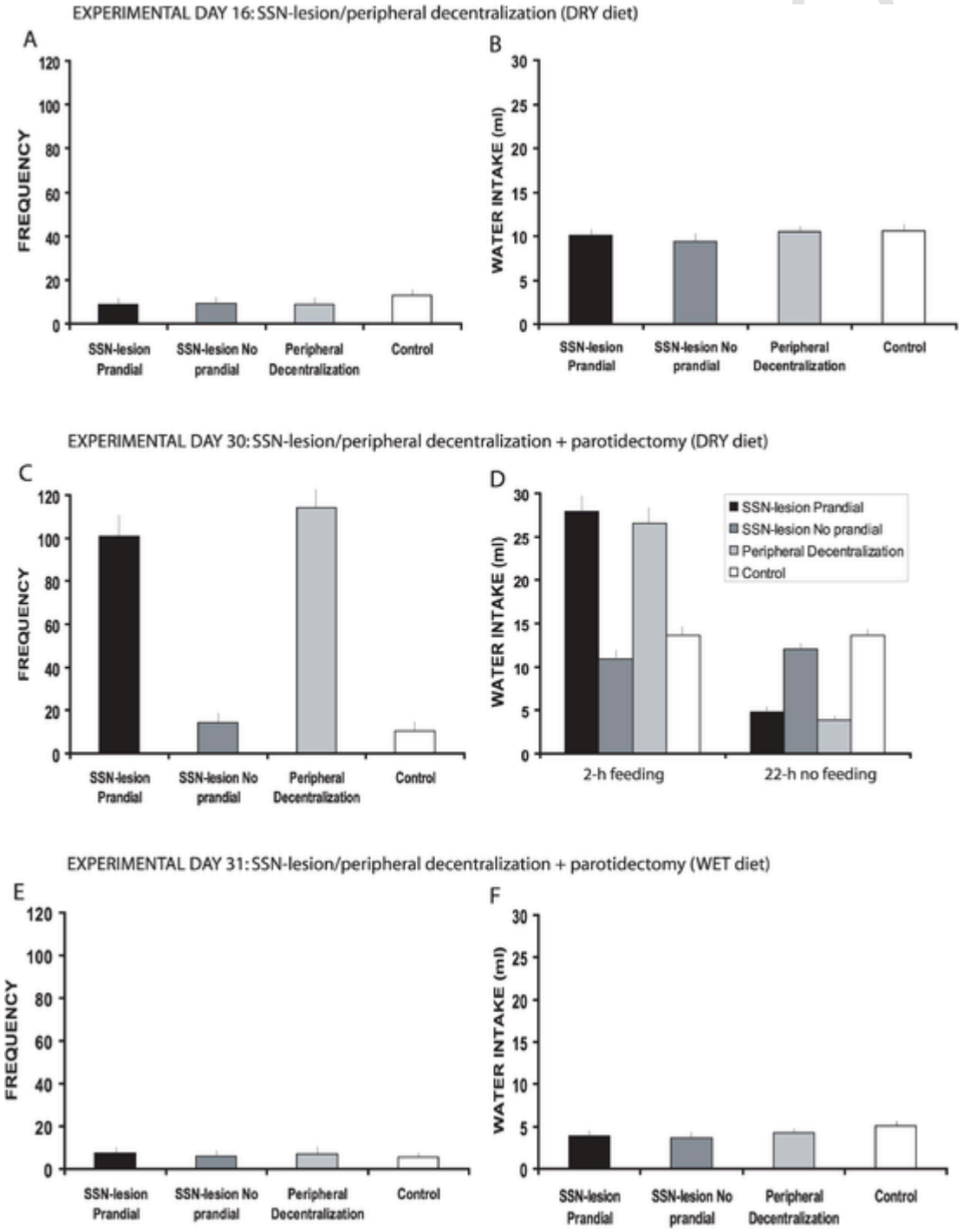

Fig. 2. Prandial drinking and water intake. A) Mean frequency of drinking (±SEM) during the 2-h period of feeding of experimental day 16. B) Mean amount of water consumed (±SEM) during the 2-h period of feeding of experimental day 16. C) Mean frequency of drinking (±SEM) during the 2-h period of feeding (dry food) of experimental day 30. D) Mean amount of water consumed (±SEM) during the 2-h period of feeding (dry food) and during the 22-h period of privation of food, of experimental day 30. E) Mean frequency of drinking (±SEM) during the 2-h period of feeding (wet diet) of experimental day 31. F) Mean amount of water consumed (±SEM) during the 2-h period of feeding (wet diet) of experimental day 31.

η^2_p = 0.88) and group x type of diet interaction ($F_{3, 57}$ = 119.08, p < 0.0001, η^2_p = 0.86). Newman-Keuls tests performed to analyze the interaction revealed that on day 30, when dry food was presented, SSN-lesioned prandial drinkers showed a significantly higher frequency of drinking than SSN-lesioned non-prandial drinkers (p < 0.0001) and than sham-operated rats (p < 0.0001); however, SSN-lesioned non-prandial drinkers and sham rats did not differ significantly (p = 0.45). Additionally, peripheral decentralized rats did not differ significantly from the SSN-lesioned prandial drinking group (p = 0.15), however, a significant effect was found upon comparing peripheral decentralized rats with SSN-lesioned non-prandial drinking (p < 0.0001) and with the sham group (p < 0.0001). In contrast, on day 31, the day on which all animals were presented with a wet diet, these differences disappeared completely and no significant differences between groups were detected (SSN prandial drinking vs. SSN non-prandial drinking, p = 0.92; SSN prandial drinking vs. sham, p = 0.97; peripheral decentralization vs. sham, p = 0.93).

As a consequence of the high frequency of drinking in the presence of dry food, on experimental day 30, SSN-lesioned prandial drinking rats developed secondary polydipsia. A 2-way mixed ANOVA (4 group x 2 type of diet) indicated a significant effect of group ($F_{3, 57}$ = 21.83, p < 0.0001, η^2_p = 0.53; Fig. 2D and 2F), type of diet ($F_{1, 57}$ = 537.83, p < 0.0001, η^2_p = 0.90) and group x type of diet interaction ($F_{3, 57}$ = 45.17, p < 0.0001, η^2_p = 0.70). Newman-Keuls tests showed that on day 30 (dry food) SSN-lesioned prandial drinking rats consumed over twice the volume of water as did the SSN-lesioned non-prandial drinking (p < 0.0001) and the control rats (p < 0.0001), however, SSN-lesioned prandial drinking and peripheral decentralized animals consumed a similar amount of water (p = 0.37). In contrast, during the 2-h period of exposure to wet food on day 31, these differences were completely abolished and the 4 groups showed an equivalent intake of water (SSN prandial drinking vs. SSN non-prandial drinking, p = 0.88; SSN prandial drinking vs. sham, p = 0.74; peripheral decentralization vs. sham, p = 0.61). Interestingly, despite the polydipsia observed in the prandial drinking and peripheral decentralization groups during the 2-h period when dry food was offered on day 30, the rats in these groups showed hypodipsia throughout the remaining 22-h of daily food deprivation ($F_{3, 57}$ = 48.77, p < 0.0001, η^2_p = 0.71. Fig. 2D). Newman-Keuls indicated that SSN prandial drinking and peripheral decentralized rats drank significantly less water than the SSN non-prandial drinking (p < 0.0001) and sham rats (p < 0.0001). However, upon comparing the last two groups (p = 0.14) or SSN-lesioned prandial drinking vs. decentralized rats (p = 0.35), an equivalent intake was evident during the 22-h of food deprivation. Finally, the 4 groups consumed similar amounts of food on experimental days 16, 30 and 31 ($F_{3, 57}$ = 0.84, p = 0.47; $F_{3, 57}$ = 0.43, p = 0.72; $F_{3, 57}$ = 0.49, p = 0.68, respectively).

3.3. Supersensitivity

S-S salivary secretion recorded on experimental day 35 is shown in Fig. 3. A 2-way mixed ANOVA (4 group x 6 time) found a significant effect in the group factor ($F_{3, 57}$ = 8.81, p < 0.0001, η^2_p = 0.31), time factor ($F_{5, 285}$ = 58.16, p < 0.0001, η^2_p = 0.50) and group x time interaction ($F_{15, 285}$ = 6.84, p < 0.0001, η^2_p = 0.26). Ten minutes after pilocarpine injection, Newman-Keuls tests for the analysis of the interaction revealed significant differences between SSN prandial drinking vs. sham (p < 0.0001) and a greater but still marginal S-S saliva secretion in SSN non-prandial drinking vs. sham (p = 0.06), suggesting in this way a phenomenon of "true" supersensitivity following SSN lesions. Importantly, SSN-lesioned rats that had developed prandial drinking secreted a significantly higher amount of saliva than the SSN-lesioned rats that were not prandial drinkers (p < 0.04). In addition, as could be expected, the peripheral decentralization group secreted significantly higher amounts of saliva than SSN non-prandial drinking (p < 0.001) and sham groups (p < 0.0001), but an amount similar to that of the SSN prandial drinking group (p = 0.28). Twenty minutes after pilocarpine injection differences similar to those described above were still detected (SSN prandial drinking vs. sham, p < 0.0001; SSN non-prandial drinking vs. sham, p < 0.05). However, at twenty minutes no significant differences were found between SSN prandial drinking vs. SSN non-prandial drinking (p = 0.15). Likewise, the amount of saliva secreted by the peripheral decentralization group was significantly greater than that observed in the sham group (p < 0.001) but similar to that registered in the SSN prandial drinking (p = 0.74) and SSN non-prandial drinking groups (p = 0.26). Thirty minutes after pilocarpine administration significant differences were observed only when comparing SSN prandial drinking vs. sham rats (p < 0.01). Finally, at minute 40 and in successive measurements, the differences disappeared and equivalent amounts of saliva were recorded in the four groups, with no significant difference being detected.

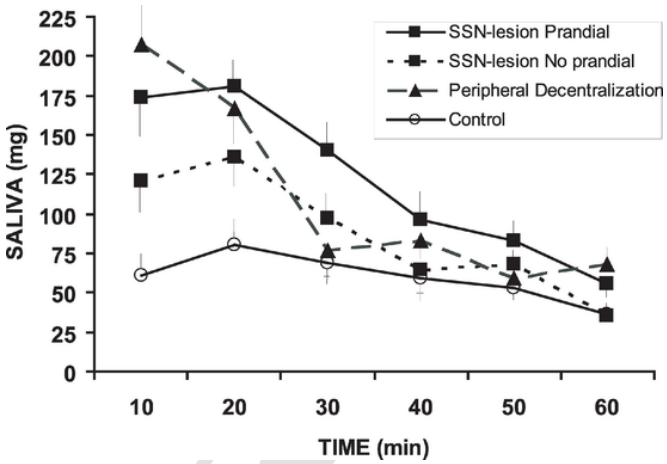

Fig. 3. Supersensitivity. Mean amount of S-S saliva (±SEM) secreted in response to pilocarpine injection (0.3 mg/kg, i.p.), during the 60 min immediately after.

This data suggest that SSN lesions produce a profound deficit in S-S saliva secretion. This deficit is manifested in a significant glandular supersensitivity during the first 20–30 min after pilocarpine injection. An important finding is that only some rats develop a prandial style of drinking after central lesions + parotidectomy. Upon comparing these two groups, the results of the present study show that the rats exhibiting prandial drinking have greater supersensitivity than SSN-lesioned non-prandial drinking rats. On the other hand, the supersensitivity observed in SSN prandial drinking is similar to that observed after parasympathetic peripheral decentralization of the S-S salivary glands, suggesting that salivatory nucleus lesions are capable of producing the maximum supersensitivity. These results suggest, therefore, a greater glandular secretory deficit in lesioned prandial drinking rats as compared to lesioned non-prandial drinking rats.

4. Discussion

In the present study we used glandular supersensitivity, as an index of salivary secretion deficit, to examine whether there is a relationship between the degree of S-S secretory deficit and the appearance of prandial drinking behavior in rats with SSN lesions. Following SSN lesions two subgroups of rats were obtained: one had developed prandial drinking and polydipsia but another was similar to the controls. Importantly, a gradation in the supersensitivity values were obtained in all three groups, with the prandial drinking rats showing significantly more supersensitivity than the non-prandial drinking subjects and the latter, in turn, showing greater supersensitivity than the control group. When the SSN-lesioned prandial drinking rats were compared to a group in which the efferent axons of the *chorda tympani* had been sectioned, causing a total decentralization of the S-S salivary glands, no differences in supersensitivity were found.

The present results suggest that the greater the supersensitivity of the S-S glands the higher the degree of secretory deficit in the rats.

In this respect, the SSN-lesioned prandial drinking rats present a maximum S-S secretory deficit, similar to the one observed in the rats with peripheral decentralization. In line with the foregoing, the complete deficit of saliva secretion leads the SSN-lesioned prandial drinking rats to develop a prandial pattern of drinking to alleviate their dry mouth and swallow food properly. However, the SSN-lesioned non-prandial drinking rats present less supersensitivity, which implies they have a smaller secretory deficit and maintain a certain saliva flow, which is sufficient to swallow food efficiently. Consequently, in this last group of animals, with partial affectation of the S-S glands, there is no need to develop prandial drinking [54, 57].

Although SSN lesions produce prandial drinking and polydipsia in an intermediate percentage of experimental rats (50% approximately), this nucleus, apparently, has no relationship with the brain circuits involved in thirst. In effect, in our study the high intake of water is associated with the intake of dry food and it disappears completely in the presence of wet food, thus suggesting that the polydipsia observed in these animals arises from the use of water to facilitate the swallowing of dry food in the absence of saliva [27-29,48]. In support of the foregoing it must be noted that the high intake of water during the 2-h period of dry food presentation was completely reversed during the following 22-h period of food deprivation on experimental day 30, when prandial drinking rats showed hypodipsia. This hypodipsia suggests that the excessive drinking seen in these animals during the 2-h period is of a non-homeostatic nature and is thus secondary to the consumption of dry food. For this reason, the fact that the prandial drinking rats significantly reduce their intake of water during the 22-h of food deprivation to compensate the excessive consumption observed during the 2-h period of feeding with dry food, suggests that homeostatic mechanisms of water regulation are intact in these animals.

The present findings provide physiological and behavioral evidence that supports a close correspondence between the brainstem lateral reticular formation and the SSN. Since prandial drinking appears exclusively when salivary secretion is completely blocked [57], the present data suggest that lesions to the lateral reticular formation must have caused severe damage to a region directly involved in the neural control of S-S saliva. The development of prandial drinking in SSN-lesioned rats could thus be explained by centrally induced impairment of S-S salivary secretion in conjunction with peripheral parotidectomy, with these two treatments together causing complete blockage of salivary secretion. Indeed, prandial drinking did not appear in control rats in which only a bilateral parotidectomy was performed, achieving only a partial desalivation. Complementarily, in previous studies we have shown that SSN-lesion + extirpation of S-S salivary glands did not lead to prandial drinking, since only partial desalivation resulted, with the parotid salivary glands remaining intact in this case [50]. Also supporting the existence of a close link between the lateral reticular formation and the SSN, previous data from our lab and others have shown that activation of the lateral reticular formation causes hypersecretion of S-S saliva, with only a minimal magnitude of parotid saliva being obtained [9,32,47,50]. Importantly, the S-S hypersecretion following lateral reticular formation activation was completely abolished by the section of the preganglionic efferent nerves of the *chorda tympani* or after atropine injection, but neither alfa- nor beta-adrenergic antagonists had an effect on the salivary secretion caused by lateral reticular formation activation [47, 51].

The aforementioned studies contrast with classic physiological investigations that had not been able to identify the precise location of the salivatory centers [4,31,64]. These studies observed salivation responses after electrical stimulation of numerous points within the brainstem of cats and monkeys. However, the points that produced salivation were distributed extensively throughout the medulla and pons. Although some points were located within the lateral reticular formation, most were situated along the course of efferent axons from the salivatory nucleus. Other regions were identified around the nucleus of the solitary tract or within the spinal trigeminal nucleus. With respect to the last two structures, it is well known that the stimulation of these regions can activate salivatory neurons, and the activation of regions afferent to a salivatory center can be confused with the stimulation of the salivatory nucleus itself [38-40]; see also, [35].

A final question is the mechanism underlying supersensitivity. With respect to this matter, muscarinic receptors are present in a great number of cerebral structures [30, 43], including the SSN [37, 63]. Thus, pilocarpine injection could produce salivation by acting centrally or by acting directly on the muscarinic receptors of the glands themselves [1,37,46]. However, in SSN-lesioned prandial drinking rats, central cholinergic mechanisms apparently do not contribute to the development of supersensitivity. If, following electrolytic lesions, the surviving neurons of the SSN had developed adaptive changes, increasing their sensitivity in response to the pilocarpine, then SSN-lesioned prandial drinking rats should have secreted more saliva than S-S peripheral decentralized rats during the supersensitivity test. In effect, in this case the saliva secreted by the action of the pilocarpine on the surviving SSN neurons would be added to that secreted by the action of the pilocarpine on the salivary glands themselves. Yet this did not occur and the two groups secreted equivalent amounts of saliva during the supersensitivity test. In consequence, a glandular mechanism is likely responsible for the increase in sensitivity to pilocarpine after salivatory nucleus lesions [7,14,22,59,66].

In summary, following SSN lesions two subgroups of rats were obtained, with some animals becoming prandial drinkers and others not. The main difference between the two subgroups of rats is the magnitude of S-S salivary gland supersensitivity. Prandial drinkers show greater glandular supersensitivity than non-prandial drinkers. These data support a greater S-S secretory deficit in prandial drinking vs. non-prandial drinking rats. In fact, our data indicate that the S-S deficit observed in SSN-lesioned prandial drinking rats is similar to that observed after sectioning *chorda tympani* preganglionic fibers that control S-S secretory activity.

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