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Influences on Water Intake in the Rat after Lesions of the Septal Subareas

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ABSTRACT

It has been suggested that the septum plays an inhibitory role in the behavioral function. Recent work has shown that the septum is heterogeneous from the neuroanatomical perspective. The present study examined the water intake of rats lesioned with kainic acid (0.5 µg/0.5 µl/site) on three septal subregions: anterior medial (MSa), posterior medial (MSp), and lateral (LS) sites. Drinking volume was enhanced mostly in rats with the MSp lesion, and so was locomotor activity. However, these two measures were not significantly correlated. This polydipsia induced by MSp lesion was also found in a chronic domain. Another experiment further determined the dipsogenic effects of polyethylene glycol (PEG; 20%) and hypertonic saline (1 M NaCl) in MSp lesioned rats. Water intake increased significantly after administration of the hypertonic saline treatment, but not after injection of PEG. However, this disparity approached a nonsignificant level 8 hr after thirst challenges were conducted. In addition to revealing septal hyperdipsia, these data suggest that the septal subareas can be functionally heterogeneous in drinking behavior. The dipsogenic response profiles for the cellular and extracellular thirst challenges could be differentially affected by kainate lesion in the MSp.

Key Words: drinking behavior, septum, kainic acid, polyethylene glycol, hypertonic saline

I. Introduction

The septum is a portion of the limbic system which is generally involved in the behavioral function (Nauta and Feirtag, 1986). Like other nuclei of the limbic system, the septum has been the subject of intense investigation in the past. Based on earlier electrophysiological and behavioral observations, McCleary (1966) suggested that the septal function might be associated with response inhibition. Specifically, the septal area has a general inhibitory influence on a variety of physiological reactions as well as behavioral processes. Drinking behavior, as one of these affected functions, can be altered by either stimulation or lesion of the septal area (Blass and Hanson, 1970; Gordon and Johnson, 1981). Massive electrolytic lesions of the septal region enhance daily water consumption (Blass and Hanson, 1970; Montes *et al*., 1986; Stricker, 1984), while electrical stimulation of the same area decreases drinking volume (Gordon and Johnson, 1981; Moran and Blass, 1976; Wishart and Mogenson, 1970). This has led to a hypothesis that the septum is a satiety neural system for thirst that plays an inhibitory role in the control of drinking behavior. If the septum is indeed a satiety system for thirst, then a question relevant to the selectivity of excessive drinking produced by septal lesion can be raised since cellular and extracellular thirst stimuli to induce drinking has been claimed to contain different mechanisms (Ramsay and Booth, 1991). Stricker (1978, 1984) showed that hyperdipsia after septal lesion has a facilitative effect on drinking responses to extracellular thirst stimuli. Conversely, several other studies failed to observe such an effect (Black and Mogenson, 1973; Thorne *et al*., 1983). Besides these contrasting results on the extracellular compartment, little is known about experimental manipulations on the cellular compartment in rats with septal lesions.

Neuroanatomically, the rat's septum can be divided into medial, lateral, posterior, and ventral subregions (Swanson and Cowan, 1976). These major divisions of the septum are now known to contain distinct ontogeneities as well as afferent and efferent connections. Because of their heterogeneity, these subregions of the septum probably play different roles in governing behavior. A growing number of recent studies indicate that lesions on various septal subdivisions produce distinct behavioral profiles in, to name a few, locomotor activity, exploratory behavior, and reward learning (Lee *et al*., 1988; Myhrer, 1989; Sagvolden and Holth,

1986). In terms of drinking behavior, there is little direct evidence indicating that the septum may also be functionally heterogeneous.

Two experiments were designed to investigate the drinking behavior of rats with septal lesions. Experiment 1 examined the drinking effects of rats lesioned with kainic acid on three different subareas of the septum. In contrast to most of the previous studies, this work used kainic acid, which has been shown to be a potent neurotoxin destroying nerve cell bodies in many parts of the brain without damaging fibers of passage (McGeer *et al*., 1978), to produce septal lesions. The purpose for Experiment 2 was to determine the specificity of thirst stimuli which induced drinking in rats with septal lesion.

II. Materials and Methods

1. Subjects

The subjects were male Sprague-Dawley rats, averaging approximately 250 g of body weight each upon receipt. Unless the specific procedure is addressed, food and water were continuously available in each home cage. Each rat was housed individually in a vivarium with a 12/12 hr light/dark cycle. The temperature of the colony was maintained at 23 ± 1˚C throughout the experiment.

2. Surgery

General stereotaxic protocol was applied to complete the septal lesion with kainic acid while the rat was under pentobarbital anesthesia (30 mg/kg, IP). Briefly, a 28-gauge injector connected to a 2 µl microsyringe (Hamilton) by means of PE20 tubing attached to a stereotaxic instrument was used to deliver either kainic acid or the control vehicle. Kainic acid (Sigma Chemical Co.) was dissolved in distilled water to a concentration of 1 µg/µl and titrated to a pH of 7.4 with NaOH. In order to enhance diffusion at the injection site, the delivery was conducted for 2 min followed by the injector kept in place for an additional 3 min. 0.5 µl kainate solution was administered at each lesion site.

3. Procedure

Experiment 1 investigated the lesion effects of kainic acid in the septal subregions on water intake and locomotor activity. Each subject adapted to its home cage, including water drinking from a measuring graduate cylinder, for one week prior to receiving surgery. Rats were divided into four groups by matching their daily water consumption. Each group (n = 7) was assigned to one of the following treatments which was characterized by the sites of septal lesion. As determined from Paxinos and Watson (1986), the coordinates were as follows: lateral septum (LS; AP = + 0.5 mm, L = ± 0.6 mm, D = – 4.5 mm), anterior medial septum (MSa; AP = + 1.0 mm, L = 0 mm, D = – 5.5 mm), and posterior medial septum (MSp; AP = + 0.2 mm, L = 0 mm, D = – 5.5 mm). The anterior-posterior and lateral coordinates were determined according to bregma, and the depth was determined relative to the dura. Notice that LS lesions were conducted bilaterally. While the lesioned group received kainic acid, the sham control group was given the vehicle. In the sham control group, the number of subjects which received infusion of the vehicle into the LS, the MSa, and the MSp was 3, 2, and 2, respectively. Each rat was then tested one week after surgical operation. Food pellets were always removed from the home cage just before the commencement of a behavioral test session. Water intake for 8 hr during the light cycle in the home cage was recorded by using a graduate cylinder with a minimum scale of 0.2 ml. Data for locomotor activity in the home cage were simultaneously screened by an infrared motion sensor (Coulbourn Instruments). Briefly, the sensor consisted of two infrared pyroelectric detectors which differentially measured the radiated body heat of the subject's image formed by the lens array. The sensor’s output signals represented the magnitude of the subject’s spatial movement. A movement event was defined as a motion that lasted continuously for 1 sec or more.

Since the findings from Experiment 1 indicated that water intake profoundly increased in rats lesioned with kainic acid in the MSp region, Experiment 2 was designed to test the sensitivity to thirst stimuli in the MSp lesion rats. Firstly, the enhanced water intake in the rats with the MSp lesion in Experiment 1 was revealed from an acute rather than a chronic paradigm in terms of postlesional measures. Experiment 2 first intended to observe the long term effects of kainate lesion in the MSp area on drinking. Daily drinking volumes were cumulatively recorded for rats in a sham control (n = 6) and a MSp lesion (n = 7) group before and after the lesions were made for a total of 31 days. Secondly, four additional groups were assigned to receive different thirst stimuli. Two groups receiving the vehicle administration were assigned as a sham control (n = 6) and a MSp lesion only (n = 7) group. The other two MSp lesion groups were assigned to receive cellular thirst (n = 7) and extracellular thirst (n = 8) challenges, respectively. These dipsogenic agents used to produce cellular and extracellular thirst were injected on the 9th day postlesion. Polyethylene

glycol (PEG) and sodium chloride (NaCl), purchased from Sigma Chemical Co., were dissolved in distilled water and adjusted to specific concentrations. Hypertonic saline (1M NaCl) was injected intraperitoneally with a volume equivalent to 1% body weight as the cellular thirst stimulus. Extracellular thirst was produced by a subcutaneous injection of 20% weight/volume of PEG. Daily water intake was meaured right after these dipsogenic injections, the data for which were divided of two parts: 8 hr during the light cycle and 12 hr during the dark cycle.

4. Histology

After behavioral testing, the subjects were administered an overdose of sodium pentobarbital and then perfused intracardially with normal saline, followed by 10% formalin. The brain was removed and placed in a sucrose/formalin mixture for at least 24 hr. Next, the brain was sectioned at 40 µm with a freezing microtome. These sections were stained with cresyl violet to verify the location and extent of cellular depletion under a light microscope. Only the subjects which sustained significant loss of neurons from the specific site without damage to circumambient areas were included for data analyses. Histological verification is summarized in Fig. 1; each schematic diagram, respectively, shows the location with the extent of a typical lesion in the LS, MSa, and MSp.

III. Results and Discussion

1. Experiment 1

The effects of kainic acid lesion in the septum on water intake and activity are shown in Fig. 2. Data for these two variables were analyzed using the one-way analysis of variance (ANOVA). As illustrated in the top panel in Fig. 2, the water intake in the four groups was significantly different, $F(3,24) = 4.187$, $p < 0.05$. On average, rats lesioned with kainic acid in any of the septal subareas drank more than the sham control subjects did. Further Dunnett t-test indicated that only the rats lesioned in the MSp drank significantly more than the control rats, $t(4,24) = 3.343$, $p < 0.05$. As shown in the bottom panel in Fig. 2, the movement events of the groups were significantly different, $F(3,24) = 3.473$, $p < 0.05$. Subsequent comparisons using Dunnett's method revealed that the MSp lesion group produced significantly more movement events than did the control group, $t(4,24) = 2.46$, $p < 0.05$.

Polydipsia produced in this experiment is consistent in terms of the increase of water intake after septal damage with that reported in other studies (Blass and Hanson, 1970; Stricker, 1978). Results of this experiment further demonstrated that the drinking volume and the number of movement events of the MSp lesion group were significantly higher than those of the LS and MSa lesion groups. This raised a question as to whether the polydipsia resulted from the increase in the number of movement events. Correlation coefficients were computed, which suggested no significant correlation between the ploydipsic and hyperactive effects, $r = 0.007$, $p > 0.1$. Since these data for drinking and movement events were collected simultaneously, it is conceivable that increased water intake is not necessary for hyperactive effects after septal damage to exist in the rat.

2. Experiment 2

Although water intake was recorded during both the light and dark portions of the day, no significant

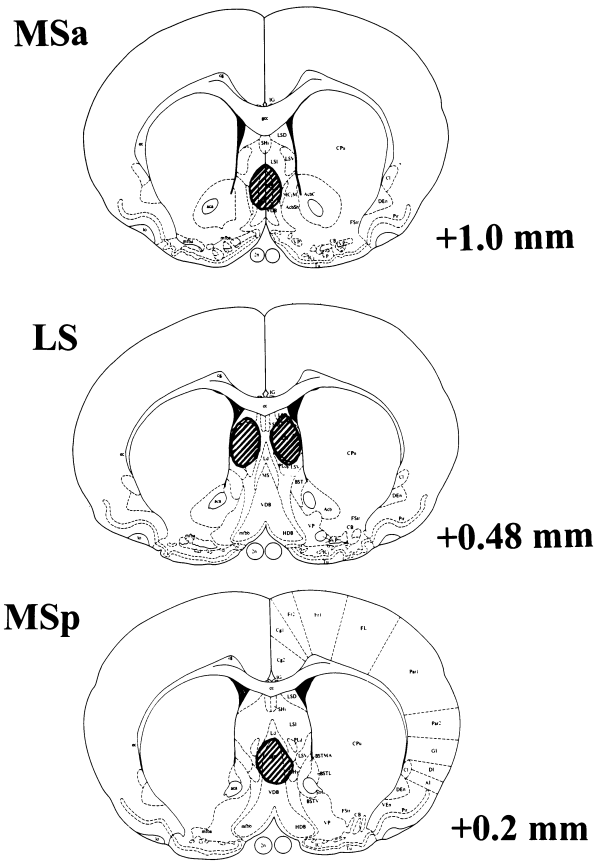

Fig. 1. Reconstruction of the coronal sections illustrating the sites and dye distribution of the anterior medial septum (MSa, top), the lateral septum (LS, middle), and the posterior medial septum (MSp, bottom). The numbers refer to the anterior-posterior coordinates from bregma according to the atlas of Paxinos and Watson (1986).

between-group differences in lesion effects were revealed by analysis of the drinking data collected during the dark cycles. Chronic effects of kainate lesion in the MSp on drinking during the 8 hr of light cycle are shown in Fig. 3, where the data were cumulated in the first, the fourth, and the eighth hour of test. A two-way ANOVA was conducted to analyze the data for each panel shown in Fig. 3. As shown in the top panel in Fig. 3, water intake for the MSp lesion group was significantly higher than that of the control group during the first hour of the test, $F(1,11) = 9.91$, $p < 0.05$. Even though the ANOVA yielded no significant day effect, the group-by-day interaction reached a significant level, $F(7,77) = 2.368$, $p < 0.05$. As indicated by the middle panel of Fig. 3, neither the group nor the day effect was significantly confirmed for the 4th hour test. Only significant group-by-day interaction was observed, $F(7,77) = 2.235$, $p < 0.05$. From the bottom panel in Fig. 3, water intake recorded in the lesioned rats over a period of 8 hours was significantly higher than that of the control rats, $F(1,11) = 5.264$, $p < 0.05$. These drinking effects significantly shifted

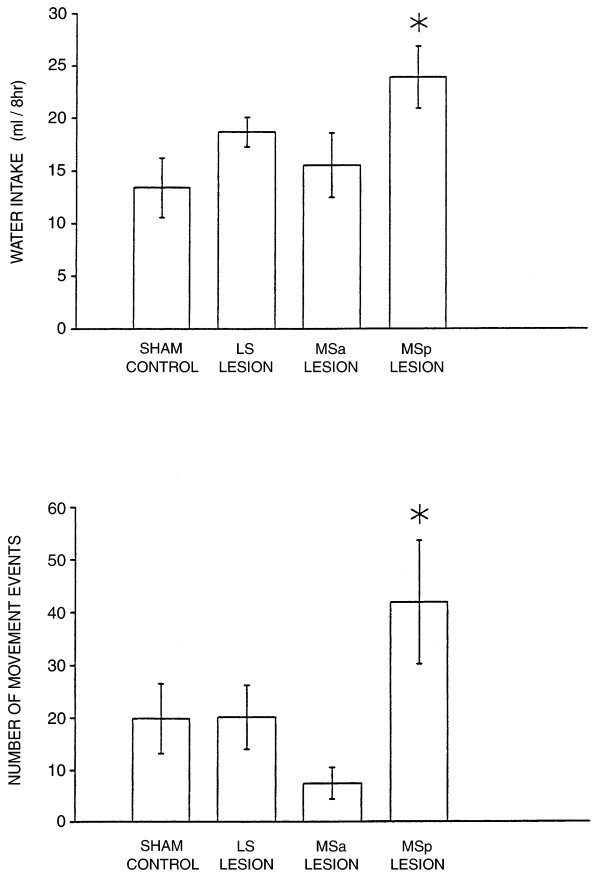

Fig. 2. Water intake (top) and movement events (bottom) of rats with kainate lesion in the septal subregions: the anterior medial (MSa), posterior medial (MSp), and lateral (LS) sites, and the sham control operation ($n = 7$ for each group). Each bar represents a group mean ± 1 standard error of means (SEM). * $p < 0.05$ when compared with the control group.

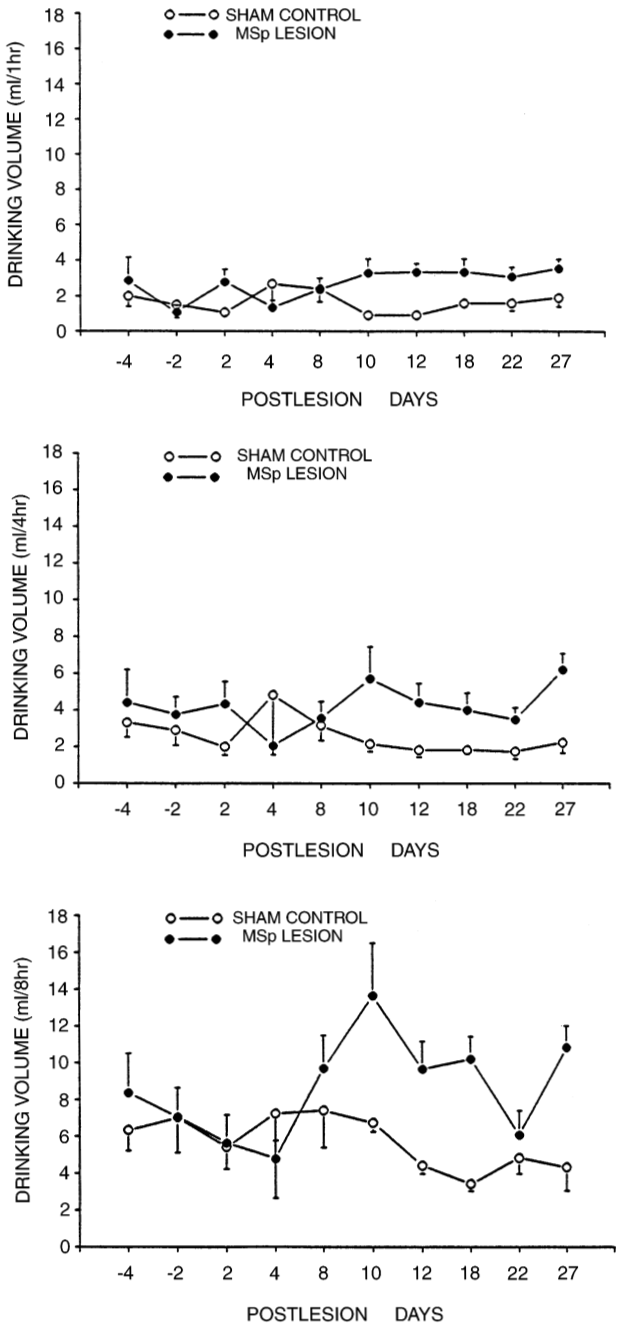

Fig. 3. Water consumption during the light cycle in vivarium of rats with MSp lesions (filled circles; $n = 7$) and the sham control operation (open circles; $n = 6$) from the prelesion to postlesion. Data presented as group means ± SEM's of drinking volume were collected during the 1st (top), 4th (middle), and 8th (bottom) hours of each daily session.

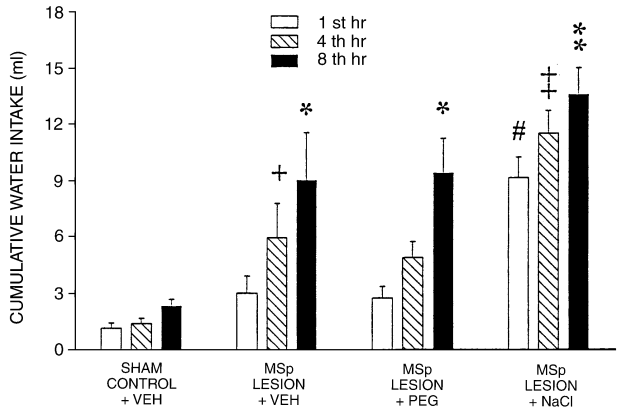

Fig. 4. 8-hr water intake cumulatively recorded after injection of polyethylene glycol (PEG; $n = 8$), hypertonic saline (NaCl; $n = 7$) or vehicle (VEH; $n = 7$) in the MSp lesion rats and in the sham control group ($n = 6$). Each bar represents a group mean + 1 standard error of means (SEM). # $p < 0.01$ when compared with the sham control group during the 1st hr; + $p < 0.05$, ++ $p < 0.01$ when compared with the sham control group during the 4th hr; * $p < 0.05$, ** $p < 0.01$ when compared with the sham control group during the 8th hr.

across days, $F(7,77) = 2.184$, $p < 0.05$. The group-by-day interaction was also significant, $F(7,77) = 2.587$, $p < 0.05$.

Figure 4 shows the 8-hour cumulative water intake following colloid and hypertonic treatments in the MSp lesion rats. One-way ANOVAs were conducted for the drinking measures of all four groups collected in the 1st, 4th, and 8th hour, respectively. Significant between-group differences were obtained: $F(3,24) = 18.87$ for the 1st hour, $F(3,24) = 11.276$ for the 4th hour, and $F(3,24) = 5.914$ for the 8th hour, all $p < 0.001$. Group means were further compared by using Dunnet's t-test for each ANOVA outcome. For the 1st hour, only a significant difference appeared when we compared the sham control and the MSp lesion plus NaCl group, $t(4, 24) = 6.703$, $p < 0.01$. For the 4th hour, the drinking volumes of the groups with MSp lesion alone and with MSp lesion with hypertonic saline were significantly larger than that of the sham control, $t(4,24) = 2.529$, $p < 0.05$ and $t(4,24) = 5.652$, $p < 0.01$, respectively. For the 8th hour, the drinking volumes of the MSp lesion, the MSp lesion plus PEG, and the MSp lesion plus NaCl groups were significantly larger than that of the sham control, $t(4,24) = 2.483$, $p < 0.05$, $t(4,24) = 2.702$, $p < 0.05$ and $t(4,24) = 4.185$, $p < 0.01$, respectively. These data indicate that the MSp lesion rats treated with 1M NaCl drank significantly more than did the other three groups during both the 1st and 4th hour measurements. However, the differences in water intake among the three MSp lesion groups approached a nonsignificant level for the 8th hour test.

The first part of the results from this experiment indicate that the polydipsia induced by kainate lesion in the MSp area could be a long term effect. This chronic increase in water intake appeared to last at least for 27 days postlesion, based on the experimental conditions of this study. Consistent with previous findings, chronic polydipsia was observed in rats with electrolytic lesion in the medioventral septum (Iovino *et al.*, 1983; Montes *et al.*, 1986). After the lesions were made, there was a delay of 3 to 5 days before an increase in water intake occurred as reported in those studies. This observation is similar to the present finding that a marked increase in water consumption occurred 8 days after the kainate lesion was made. This delay may be essential for the degeneration processes induced by septal lesion. A slight difference on the day numbering for this delay may be due to the different lesioning techniques used between the present work and the two previous studies.

From the second part of results of this experiment, the MSp lesion rats demonstrated different levels of sensitivity in their response to the hypovolemic and hypertonic thirst challenges. Especially measured during the first 4 hours, the MSp lesion subjects injected with hypertonic saline significantly drank more water than did those injected with PEG. This slow commencement of the subject's response to PEG treatment has been reported previously (Stricker, 1978). It was noted that water intake was not significantly enhanced for 7 hr after PEG administration. A similar observation was also made in the present work. After the MSp lesion was made, the water intake during 8-hr test in the PEG treatment group increased. However, such effect was not significantly different from that of the hypertonic saline treated group. In this experiment, these animals which received PEG treatment ultimately did consume excessive amounts of water. It is likely that an initial period in which angiotensin level induced by PEG could be insufficient to provoke hyperdipsia.

IV. General Discussion

In contrast with previous works that used electrolytic lesion, the present study applied a kainate lesion to re-examine "septal hyperdipsia" (Blass and Hanson, 1970). Basically, the results of this study were consistent with those of other studies on the increases of water intake after the septal destruction (Iovino *et al.*, 1983; Montes *et al.*, 1986; Stricker, 1978, 1984). However, the lesion areas located in the septum were not specifically delineated in those studies. An interesting aspect of the current results is the different

drinking effects after kainate lesions were administered in the septal subareas. Among the three subareas defined in Experiment 1, kainate lesion of the posterior part of the medial septum (MSp) produced profound polydipsia as compared to those parts of the MSa and the LS. Excessive drinking by rats with MSp lesions was observed in both acute and chronic tests. These results imply that the neural control of drinking involved in the septum may be dependent upon different septal subregions. Apart from the drinking paradigm, other studies showed different degrees of behavioral change after selective lesions in the medial and lateral septum. For example, Poplawsky and Hoffman (1979) found that a lesion in the medial septum, but not in the lateral septum, suppressed response under an aversive setting. Thus, the subareas of the septum may be heterogeneous with regard to the behavioral function as well as the anatomical construction. A growing body of evidence suggests that the septum receives afferents and reciprocally sends efferents to a variety of structures, including the amygdala, the hippocampal formation, and the midbrain areas (Swanson and Cowan, 1979; Risold and Swanson, 1997). All these structures may then be involved in regulation of distinct behavioral functions for different septal subareas. Although polydipsia did not appear in rats with LS lesions in the present work, destruction of this septal subarea was found to produce a schedule induced polydipsia (Taghzouti *et al*., 1985). Since this type of polydipsia can be related to the reinforcement schedule of operant conditioning, it is then possible that the medial and lateral septum, respectively, are involved in modulating the distinctive types of drinking behavior based on the homeostatic and learning aspects. Further investigation is needed to confirm whether or not this is the case.

It is well known that cellular and extracellular thirsts are resultant from different types of body fluid imbalance (Ramsay and Booth, 1991). It has been claimed that these two distinctive alternations in body water economy have separate neurological mediators. The present study utilized colloid and hypertonic treatments, which have been traditional administrations, to induce cellular and extracellular dehydration in MSp lesion subjects. The results of Experiment 2 show that the MSp lesioned rats were more sensitive to hypertonic saline than colloid treatment of PEG. This septal hyperdipsia probably stems from the removal of an inhibition which normally attenuates the amount of water consumed after cellular dehydration. Our results were in partial agreement with those of previous works which suggested that rats with septal lesions drank more water than did the controls after PEG treatment (Blass and Hanson, 1970; Stricker, 1978, 1984). A major factor contributing to this disparity in results between this study and previous studies is the lesion sites. The lateral septum, if not a complete septal nuclei, was bilaterally destroyed in the previous studies, whereas the lesion sites in the present study were restricted in the posterior part of the medial septum. Together, it is possible that rats lesioned in the lateral and posterior medial septum were sensitized to extracellular and cellular thirst stimuli, respectively.

In conclusion, the current data support the hypothesis of heterogeneous functioning in the septal nucleus from a behavioral perspective which can be a complement to those from the neuroanatomical viewpoint (Swanson and Cowan, 1976). Compared with the lesions in the LS and the MSa, the kainate lesion in the MSp led to the most pronounced increase in water intake. The profiles of dipsogenic response to the cellular and extracellular thirst challenges could be differentially affected by kainate lesions in the MSp.

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大鼠喝水行為受中膈神經核破壞的影響

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摘　要

大腦中膈神經核被認為對喝水行為扮演抑制性功能，神經解剖的研究證據指出該組織內部具有異質性結構，本研究探討這種異質性結構是否對喝水行為有不等的影響。本研究第一個實驗中利用海人草酸(0.5微克/0.5微升/注射點)之神經破壞法，來檢視破壞大鼠中膈內三個不同次分區後對於飲水行為的影響，其包括腹中膈前區、腹中膈後區，及側中膈。實驗結果顯示腹中膈後區遭破壞之受試對於飲水行為及活動量顯著增加大過於其他者，但兩種測試並無顯著相關。這種破壞腹中膈後區引發之飲水量顯著增加，亦被證實屬長效反應。另一實驗則探討腹中膈後區遭破壞後，其對於引發飲水刺激之專屬性與敏感度。針對此區破壞後之受試發現其對於高張鹽水(1莫耳濃度)之刺激會產生迅速的飲水量增加反應，但對於多乙烯乙二醇(20百分比)的反應則較緩慢，這兩者的差異於引發口渴刺激給予後的八小時趨近無統計顯著性。本研究結果較先前證據發現中膈神經核破壞所引發大量飲水的效果，進一步指出中膈可因不同次級區域有異質性之功能。另外，腹中膈後區受海人草酸破壞之後對不同性質之口渴刺激所引發之喝水行為有不同的反應型態。