Behavioural Brain Research, 59 (1993) 11–17

BBR 01533

Research Reports

Lesions of the central nucleus of the amygdala I: effects on taste reactivity, taste aversion learning and sodium appetite

Olivier G. Galaverna[a], Randy J. Seeley[b,*], Kent C. Berridge[c], Harvey J. Grill[b], Alan N. Epstein[a], Jay Schulkin[d]

Departments of [a] Biology and [b] Psychology, University of Pennsylvania, Philadelphia, PA (USA), [c] Department of Psychology, University of Michigan, Ann Arbor, MI (USA), [d] Behavioural Neuroscience Unit, Neuroendocrinology Branch, NIMH, Bethesda, MD (USA)

(Received 24 June 1992)
(Revised version received 11 November 1992)
(Accepted 12 May 1993)

Key words: Amygdala; Taste reactivity; Taste aversion learning; Sodium appetite

Bilateral damage to the central nucleus of the amygdala (CeAX) in the rat blunts need-induced NaCl intake and abolishes daily need-free NaCl intake when measured with a two-bottle test. Such a deficit could be the result of impaired taste function. To assess the taste function of the CeAX rat various taste stimuli were introduced directly into the oral cavity and taste-elicited oral motor responses were measured. Oral motor responses elicited by 0.62 M and 0.13 M sodium chloride, 0.3 M sucrose and 0.01 M citric acid, were similar in control and CeAX rats. Additionally CeAX and control rats acquired a taste aversion for fructose or maltose when either was paired with LiCl. Finally, in CeAX rats, like in control rats, the pattern of oral motor responses to 0.5 M NaCl was dependent on internal state; sodium depletion dramatically altered taste-elicited oral motor behavior. These results suggest that, in the rat, the deficits in NaCl intake behavior that follow CeAX do not appear to be a result of dramatic changes in gustatory function.

INTRODUCTION

We have recently shown that damage to the central nucleus of the amygdala (CeA) blunts the NaCl intake induced by sodium depletion and abolishes the NaCl intake evoked by either systemic mineralocorticoid treatment or by renin given into the anterior cerebral ventricles in rats given free access to water and a 0.5 M (3%) NaCl solution[7]. The same rats show normal water intake to these challenges, and their food intake and body weight are normal. Nonetheless rats with CeA-lesions reject NaCl solutions at all concentrations above 0.03 M (0.2%). Given that the CeA is a major limbic projection of the central gustatory system[12,13], the effect of damaging the CeA on the rat's ability to evaluate gustatory input needed to be assessed.

The taste reactivity paradigm developed by Grill and Norgren[10] enables one to evaluate aspects of gustatory function. Briefly, when a taste stimulus is introduced directly into the oral cavity via an indwelling cannula, stereotyped ingestive and aversive oral motor responses can be videotaped, analyzed and quantified. Moreover, the rat's evaluation of a taste stimulus, as measured by the amount of aversive and ingestive responses, can change due to modification of the internal state or to learning. For example, if sucrose is paired with LiCl (conditioned taste aversion or CTA), the proportion of ingestive responses decrease and aversive responses increase[2]. Inversely, if a rat is made sodium deficient, the rat's evaluation of NaCl changes such that the proportion of ingestive responses increase and aversive responses decrease[1]. This paradigm is a useful tool for the assessment of gustatory function when taste guided behaviors are impaired following lesions along the ascending gustatory pathway.

The afferent gustatory nerves enter the brain through the nucleus of the solitary tract (NTS)[15]. Bilateral damage to the rostral NTS results in a rat capable of forming taste aversions but not of expressing sodium appetite after body sodium depletion as measured by either intake or taste reactivity tests[7]. Bilateral damage to the second relay, the parabrachial nucleus (PBN), yields a

* Corresponding author. Present address: Department of Psychology, NI-25, University of Washington Seattle, WA 98195, USA. Email: rseeley @ u.washington.edu

rat incapable of forming taste aversions or expressing a sodium appetite after body sodium depletion[7].

The projection from the PBN to the forebrain is bifurcated[14,15]. One is the classical thalamic–cortical gustatory pathway that has been hypothesized to be the anatomical pathway for gustatory discriminations[16]. Bilateral damage confined to the taste-responsive ventral posteromedial thalamic nucleus (VPMpc) does not alter sodium appetite after sodium depletion, nor does it suppress the ability to form a CTA[7]. The other pathway from the PBN projects to a variety of ventral forebrain sites, including the CeA. In fact a greater number of gustatory fibers terminate in the CeA than in the classical thalamic and gustatory insular cortex (see Norgren[13]). This pathway has been hypothesized to be the anatomical substrate for taste-motivated behavior[16].

Given the gustatory projection from the PBN to the CeA and the deficit in NaCl intake after CeA damage, it was logical to test the oral motor responses to common tastants of rats with bilateral lesions of the CeA (CeAX). Additionally we examined tastes paired with LiCl for evidence of the ability to form a CTA. Finally, we evaluated NaCl-elicited oral motor responses before and after body sodium depletion.

GENERAL METHODS

Rats and housing

Adult male Sprague–Dawley rats, weighing 350–400 g at the beginning of the experiment were housed in individual wiremesh cages in a temperature and humidity-controlled room on a 14:10 light/dark cycle, fed Purina rat chow pellets (Na^+ content approximately 0.5%) and given tap-water ad lib.

Surgery and recovery

Rats received stereotaxically-guided electrolytic lesions of the CeA under intramuscular ketamine hydrochloride (40 mg/kg) and acepromazine maleate (15 mg/kg). A prophylactic dose of gentamicin sulfate was administered intramuscularly immediately prior to surgery as well as for the 3 days that follow surgery. Anodal lesions were made by delivering a current of 1 mA for 20 s through an insulated tungsten electrode with 0.5 mm exposed at the tip. Two lesions were placed within each CeA. The lesion coordinates were 2.2 mm and 2.7 mm caudal to bregma, 4.1 mm lateral from the midsagittal sinus, and 7.4 mm in depth from the dural surface. No current was passed in rats with sham lesions. Rats were also implanted with an intraoral cannulae for taste stimulus delivery. Cannulae consisted of PE-100 tubing that was inserted through the temporalis muscle and opens in the oral cavity just lateral to the first molar. The cannulae was anchored to the skull with skull screws and dental acrylic (for a detailed description se Grill et al.[11]).

The rats recovered for 2 weeks prior to testing. Some rats with CeA damage showed hypophagia and hypodipsia for the first 3 to 5 days following surgery and a weight loss of approximately 15 g. These rats were fed wet mash made from standard Purina rat chow and tap water until normal feeding and drinking resumed.

Taste reactivity tests

The rats were placed in a cylindrical, clear plastic testing chamber, and their oral cannulae were connected via tubing to an infusion pump which delivered the stimulus for 1 min at a constant rate of 1 ml/min. The rat's face and ventral body were videotaped through a mirror below the cage to record the behavioral response to the taste stimuli.

Behavioral analysis

Using slow motion videotape analysis, each occurrence of ingestive and aversive behaviors was scored. These behaviors included: mouth movements, tongue protrusions, lateral tongue protrusions, and paw licking, aversive components were gaping, chin rubbing on the floor of the cage, head shaking, forelimb flailing, face washing and fluid ejection (for a more detailed description of each of these behaviors see Grill et al.[11]). The ingestive and aversive behaviors were scored by counting the 'bins' where these responses occurred[4]. The clock was started at the time the rat began engaging in a typical response. The time the rat spent engaging in that response is divided into equal 'bins' of 2 s or 5 s, depending on the response being scored. The different sized bins are used so that equal weight is given to each of the behaviors independent of their relative frequencies (for a detailed description of how this weighting scheme was devised see Berridge and Grill[3]). Each behavior is scored either for the number of discrete occurrences or for how many bins in which it appears, i.e. several identical responses that occur continuously within the same bin is scored as one occurrence. Ingestive tally was the number of 2-s bins in which tongue protrusions occurred plus the number of 5-s bins in which paw licking occurred plus the number of lateral tongue protrusions. In this scoring method, mouth movements are considered a neutral rather than an ingestive behavior and hence are not included in the ingestive tally. Aversive score is the number of gapes plus the number of chin rubs plus the number of forelimb flails.

Histology

At the conclusion of each experiment the rats were euthanized with 0.5 ml of Socumb (Butler) and perfused with 10% formalin through a cardiac catheter. Brains were removed and immersed for 1 week in a 10% formalin solution, after which it was transferred to a 50% w/v sucrose-formalin for 1 more week, and then sectioned into slices of 16 micron with a cryostat, and stained with Thionin blue to evaluate the anatomical extent of the lesions.

Data analysis

Data were analyzed using ANOVAs and post-hoc comparisons were done using Tukey's HSD test with a rejection criterion of $P<0.05$.

Histological analysis of the lesions

Fig. 1 gives, on the right panel, the extended area drawn from the extreme border of all the lesions used for this paper and for the companion paper, and on the left panel an example of a lesioned rat brain taken from a rat that was used in the present study. The charts use Paxinos and Watson rat brain atlas drawings (2nd edn., 1986) at 3 different rostro-caudal levels of the amygdaloid complex, and the example of the brain lesion is given at similar levels. The lesions extended from Bregma −2.0 to Bregma −3.4. Each lesion destroyed most of the CeA with some damage to adjacent structures. According to the Paxinos and Watson rat brain atlas nomenclature, the adjacent structures involved in the lesions were: the intercalated amygdaloid nucleus, the dorsal portion of the medial amygdala, the medial portion of the basolateral amygdala, the rostral portion of the bed nucleus of the stria terminalis, the caudal portion of the globus pallidus and the caudate putamen. Finally, in the caudal part of the lesions, the optic tract and the internal capsule were damaged in some of the rats.

Experiment I: immediate responses to taste stimulation

Subjects. Ten naive CeAX rats and 6 sham-operated rats were used in Expt. I.

Procedure. Rats were intraorally infused as described above. They were either infused with 0.5 M NaCl, 0.13 M NaCl, 0.3 M sucrose, or 0.01 M citric acid in random order with water infused between each gustatory stimulus.

Results and Discussion

Overall, CeAX rats showed a pattern of responses across all taste stimuli that was very similar to that of control rats. The discriminative response pattern of both groups was similar for all tastes except 0.5 M

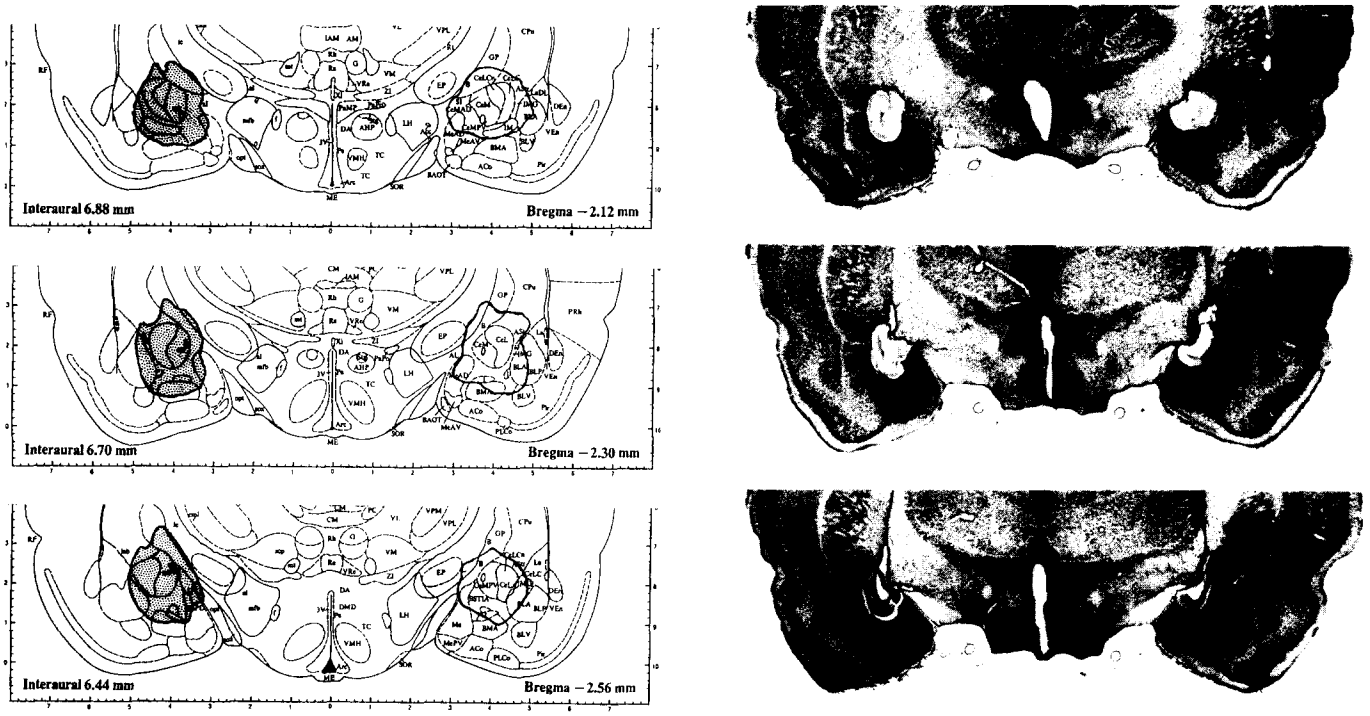

Fig. 1. **Left panel:** representation of the extent of the electrolytic damage for all the CeAX rats in the experiments at three different rostro-caudal levels of the amygdaloid complex. The chart uses drawings from Paxinos and Watson (2nd edn., 1986). The outermost line represents the area around the extreme border of the lesions from all the rats and shaded portion represents the area damaged in every rat. **Right panel:** representative sections from a CeAX rat.

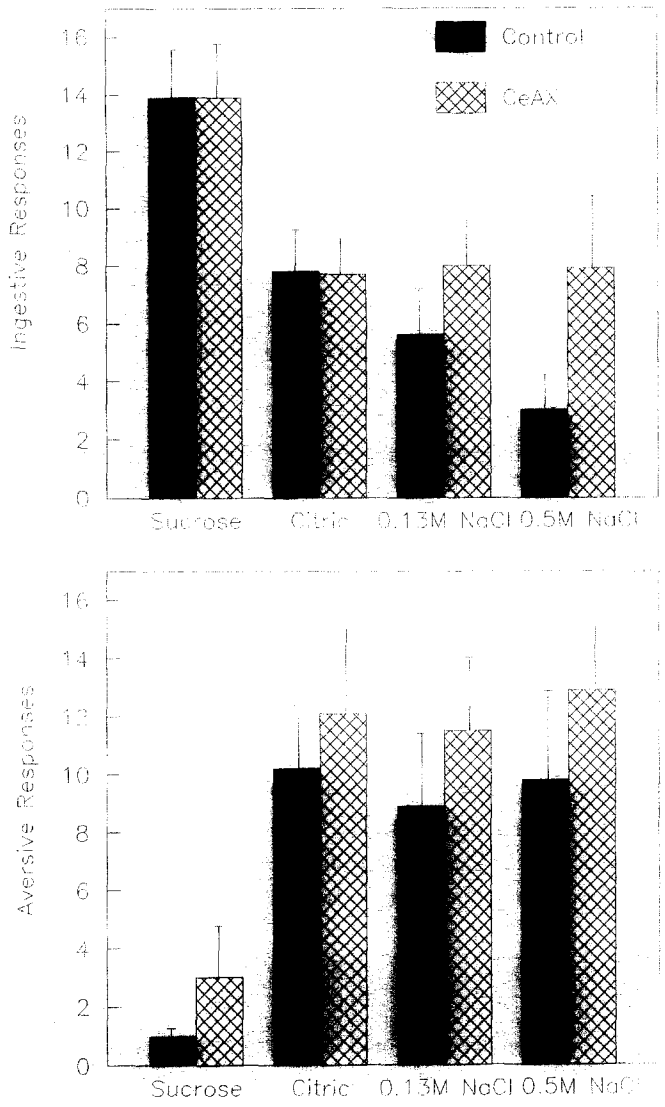

Fig. 2. Ingestive tally and aversive score of oral motor behaviors for both CeAX and control rats elicited by intraoral infusions of sucrose, citric acid, 0.13 M NaCl, and 0.5 M NaCl.

NaCl. Clearly, the oral motor behavior of both groups was similarly influenced by taste stimulation.

For ingestive oral motor responses, we found a significant effect of surgical condition (between groups: $F_{1,14} = 5.05, P < 0.05$). A post-hoc analysis revealed that the difference between groups was due to the higher ingestive tally of the CeAX rats to the 0.5 M NaCl solution (see Fig. 2). The responses to the different taste stimuli were also significantly different (between treatments: $F_{3,42} = 9.02$, $P < 0.01$). There was no between groups effect for aversive score. For aversive responses, the only effect was due to taste stimuli (between treatment: $F_{3,42} = 11.11$, $P < 0.01$).

Experiment II: conditioned taste aversion

Subjects. Nine of the 10 CeAX rats and the 6 sham operated rats of Expt. I were used in this experiment.

Procedure. Two taste stimuli, a 15% fructose and a 15% maltose solution, were used for this experiment. For each rat, one taste was paired with LiCl injections (CS +) while the other was not (CS −). Which taste was the CS + and which was the CS − was counterbalanced across subjects. Each rat received five 0.5-ml infusions at 1 ml/min at approximately 1 min intervals of the CS + through the intraoral cannula; an i.p. injection of isotonic LiCl (1.5 mEq/kg) was then administered. This procedure was repeated the following day. On the third day each rat received a 0.5 ml infusion and their oral motor responses were videotaped for both the CS + and CS − which were counterbalanced in their order of presentation across animals. Each rat was then given access to the CS + and the CS − in their home cages for the next 24 h where their intake was measured. The videotapes were analyzed using the scoring method from Expt. I.

Results and Discussion

Both groups, lesioned or controls, showed similar responses to the CS + and CS − . Both groups acquired the aversion for the CS + as demonstrated by the differential pattern of oral motor responses toward the CS + and the CS − . The aversive responses were significantly higher for the CS + ($F_{1,13} = 8{,}81$, $P < 0.02$). The ingestive responses were lower for the CS + even though this tendency was not statistically significant. There was not a significant interaction between the within and between subject variables ($F_{1,13} = 2.29$, $P < 0.15$). In addition, the 24 h intake of the CS + was significantly reduced ($F_{1,13} = 42.53$, $P < 0.001$).

Experiment III: sodium appetite

Subjects. For this experiment, 15 male Sprague–Dawley rats (Charles River Co.) were used. Eleven of the rats (6 CeAX and 5 sham rats) were sodium depleted 3 times before this experiment for experiments presented in the companion paper (Seeley et al. this issue) while the remaining 4 CeAX rats were depleted only once.

Procedure. The oral motor responses to 0.5 M NaCl was first assessed while the rats were sodium replete, i.e., the rats were fed standard chow. A week later, each rat was sodium depleted by two injections (s.c.) of 5 mg of furosemide (Lasix) separated by 2 h. At the time of the first injection, the cage was washed in order to remove adherent NaCl, and the standard rat chow was replaced by a sodium-deficient diet (Teklad #81263, Na^+ content: 0.005–0.01%) for the next 24 h. The following day the rats were again videotaped while a 0.5 M NaCl solution was orally infused. For the rats

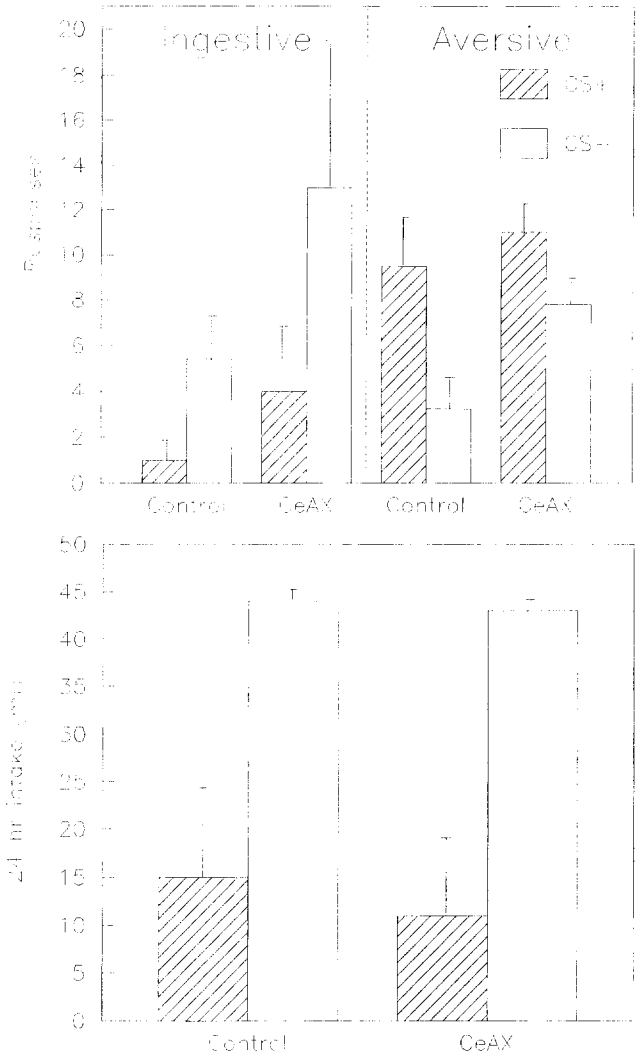

Fig. 3. **Top panel:** ingestive tally and aversive score of oral motor behaviors for both CeAX and control rats elicited by either a maltose or fructose solution paired with LiCl injection (CS +) or saline injection (CS −). **Bottom panel:** the amount consumed of the CS + and CS − solutions in a 24-h two-bottle intake test.

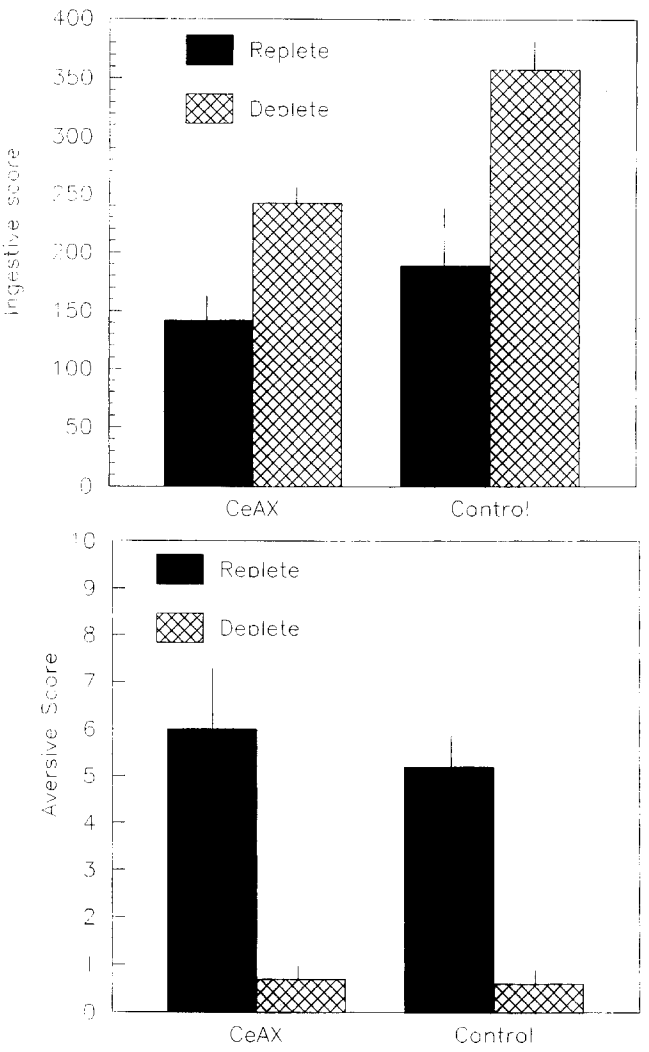

Fig. 4. **Top panel:** ingestive score of oral motor behaviors for both CeAX and control rats elicited by 0.5 M NaCl when either sodium replete or sodium deplete. **Bottom panel:** aversive score of oral motor behaviors for both CeAX and control rats elicited by 0.5 M NaCl when either sodium replete or sodium deplete.

that were depleted 3 times, a period of 1 week was given between each sodium depletion.

Videotape analysis. This work was performed in a different laboratory and consequently the videotape analysis was slightly different. Rather than count the number of bins in which a response occurred, the actual number of mouth movements, tongue protrusions, and lateral tongue protrusions were counted and summed for the entire minute of the infusion. The total number off these behaviors was defined as the ingestive score.

Results and Discussion

Both CeAX and intact rats changed their oral motor responses appropriately to NaCl when made sodium deplete. The number of sodium depletions did not produce a significant difference in the oral motor responses to 0.5 M NaCl, so the data were pooled for the analysis. As can be seen in Fig. 4, both intact and CeAX rats significantly increased the number of ingestive responses after sodium depletion ($F_{1,13} = 22.58$, $P < 0.001$). Additionally, both intact and CeAX rats decreased their aversive behaviors to NaCl while sodium deplete ($F_{1,13} = 14.003$, $P < 0.01$).

GENERAL DISCUSSION

The results of the present experiments indicate that bilateral lesions of the CeA do not dramatically alter gustatory function when assessed using taste reactivity. CeAX rats were not significantly different from control

rats in their ingestive or aversive responses to either sucrose or citric acid. For NaCl, only the ingestive tally to the higher concentration differed from controls. Such results indicate that the CeAX rat: (1) can display appropriate numbers and categories of oral motor responses to common tastants and (2) can make discriminative responses to different stimuli.

In Expt. II, both CeAX and intact rats showed a statistically significant increase in aversive responses and a not statistically significant trend towards a decrease in ingestive responses to a taste stimulus paired with LiCl. Additionally, in the 24-h intake test, both CeAX and intact rats show a marked avoidance for the taste stimulus paired with LiCl. The CeAX rat clearly demonstrates an ability to associate a taste with the effects of LiCl injection and learn a taste-avoidance.

The exact role of various amygdala nuclei in taste aversion learning remains unclear. In agreement with the current study, Kemble et al. found no effect of CeA damage on the ability to form a conditioned taste aversion[12]. Simbayi et al. showed that electrolytic lesions of the basolateral nucleus produce significant deficits in taste aversion learning when assessed with both taste reactivity and standard intake tests[16]. Dunn and Everitt have shown that electrolytic lesions of the amygdala that included the basolateral and central nucleus produced deficits in taste aversion learning[6]. They also found, however, that fiber sparing ibotenic acid lesions at the same level do not produce any deficit. More recently, Yamamoto and Fujimoto showed that fiber sparing ibotenic acid lesions of the amygdala that included the central but not the basolateral nucleus produced no deficit in taste aversion learning while lesions that included the basolateral produced a significant deficit compared to controls[20]. In conflict with the current study, Lasiter and Glanzman[13] using electrolytic lesions, found a significant deficit in the ability of CeAX rats to reduce their intake of a LiCl solution on subsequent trials when compared to controls while rats with lesions of the basolateral nucleus showed no deficit[13].

All of the above studies, including those that agree with the present results, suffer from concentrating solely on between subject comparisons rather than within subject comparisons. In the data of Lasiter and Glanzman[13], both CeAX and control rats reduced their LiCl intake over days but the magnitude of the reduction was different for the 2 groups. Thus a 'deficit' emerges only when CeAX rats were compared to controls. Yet it would seem at least as important to note the 'competence' of the CeAX rats to change their ingestive behavior on the basis off a taste-visceral relationship. This interpretational issue aside, the present study adds weight to the evidence that the cells located within and the fibers running through the CeA area are not critical for an association between a taste and the effects of LiCl.

In expt. III, the CeAX rat shifted its profile of ingestive and aversive oral motor responses to 0.5 M NaCl as a function of its internal sodium state. The three experiments presented here suggest that the deficit in NaCl intake observed in CeAX rats is not a result of gustatory impairment. The taste guided oral motor behaviors of the CeAX rat are very similar to those of intact rats and would not predict any deficit in sodium intake behaviors. Nevertheless, detailed analysis of gustatory sensitivity would require more pointed psychophysical experiments.

The fact that CeAX rats show an appropriate change in oral motor responses to NaCl when made sodium deplete contrasts strongly with the impairment in sodium intake measured by the two bottle test. It appears that the CeAX rat will not consume NaCl from a bottle when made sodium deplete despite having a more positive evaluation of the NaCl solution. To understand these apparently contradictory results one could measure the amount of NaCl solution a CeAX rat would consume when it is presented directly into the mouth, e.g. in the same manner as the NaCl is presented in the taste reactivity test. It is this issue which is addressed in the companion paper (Seeley et al.).

Finally, these results should be placed in the context of earlier studies discerning the effects of electrolytic lesions of the gustatory system. Like NTS or VPMpc damaged rats, CeAX rats demonstrate taste aversion learning while PBN damaged rats do not[5,7,18]. Like NTS and PBN damaged rats do not increase their sodium intake during bottle tests but unlike NTS or PBN lesioned rats CeAX rats dramatically alter their oral motor responses to intraoral infusions of NaCl depending on internal sodium state[7–9]. Such systematic experiments on rats with damage to the gustatory system continue to shed light on the role of these structures in taste-guided behaviors.

ACKNOWLEDGEMENT

This manuscript is dedicated to Dr. Alan N. Epstein who was tragically killed last year in a car accident. We miss him. This study was supported by a NIMH program project grant (MH 455787).

REFERENCES

1 Berridge, K.C., Flynn, F.W., Schulkin, J. and Grill, H.J., Sodium depletion enhances salt palatability in rats, *Behav. Neurosci.*, 98 (1984) 652–660.

2 Berridge, K.C. and Grill, H.J., Isohedonic tastes support a two-dimensional hypothesis of palatability, *Appetite*, 5 (1984) 221–231.
3 Berridge, K.C., Grill, H.J. and Norgren, R., Relation of consummatory responses and preabsortive insulin release to palatability and learned taste aversion, *J. Comp. Physiol. Psychol.*, 95 (1981) 363–382.
4 Berridge, K.C. and Schulkin, J., Palatability shift of a salt-associated incentive during sodium depletion, *Qtr. J. Exp. Psychol.*, 41B (1989) 121–138.
5 Dilorenzo, P.M., Long-delay learning in rats with parabrachial potine lesions, *Chem. Senses*, 13 (1988) 219–229.
6 Dunn, L.T. and Everitt, B.J., Double dissociations of the effects of the amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid, *Behav. Neurosci.*, 102 (1988) 3–23.
7 Flynn, F.W., Grill, H.J., Schulkin, J. and Norgren, R., Central gustatory lesions II: effects on sodium appetite, taste aversion learning, and feeding behaviors, *Behav. Neurosci.*, 105 (1991) 944–954.
8 Flynn, F.W., Grill, H.J., Schwartz, G.J. and Norgren, R., Central gustatory lesions: I. Preference and test reactivity tests, *Behav. Neurosci.*, 105 (1991) 933–943.
9 Galaverna, O., De Luca Jr., L.A., Schulkin, J., Yao, S.Z. and Epstein, A.N., Deficits in Nacl ingestion after damage to the central nucleus of the amygdala in the rat, *Brain Res. Bull.*, 26 (1992).
10 Grill, H.J. and Norgren, R., The taste reactivity test: mimetic response to gustatory stimuli in neurologically normal rats, *Brain Res.*, 143 (1978) 263–279.
11 Grill, H.J., Spector, A.C., Schwartz, G.J., Kaplan, J.M. and Flynn, F.W., Evaluating taste effects on ingestive behaviors. In F.M. Toates and N.E. Rowland (Eds.), *Feeding and Drinking*, Elservier, New York, 1987, pp. 151–188.
12 Kemble, E.D., Studelska, D.R. and Schmidt, M.K., Effects of central amygdaloid nucleus lesions on ingestion, taste reactivity, exploration and taste aversion, *Physiol. Behav.*, 22 (1979) 798–793.
13 Lasiter, P.S. and Glanzman, D.L., Cortical substrates of taste aversion learning: involvement of dorsolateral amygdaloid nuclei and temporal neocortex in taste aversion learning, *Behav. Neurosci.*, 99 (1985) 257–276.
14 Norgren, R., Taste pathways to hypothalamus and amygdala, *J. Comp. Neurol.*, 166 (1976) 17–30.
15 Norgren, R., Central neural mechanisms of taste. In J.M. Brookhart and V.B. Mountcastle (Eds.), *Handbook of Physiology. The Nervous System III: Sensory Processes*, American Physiological Society, Bethesda, MD, 1984, pp. 1087–1128.
16 Pfaffmann, C., Norgren, R. and Grill, H.J., Sensory affect and motivation. In B.M. Wenzel and H.P. Zeigler (Eds.), *Tonic Functions of Sensory Systems*, New York Academy of Sciences, Vol. 290, New York, 1977.
17 Simbayi, L.C., Boakes, R.A. and Burton, M.J., Effects of basolateral amygdala lesions on taste aversions produced by lactose and lithium chloride in the rat, *Behav. Neurosci.*, 100 (1986) 455–465.
18 Spector, A.C., Norgren, R. and Grill, H.J., Parabrachial gustatory lesions impair taste aversion learning in rats, *Behav. Neurosci.*, 106 (1992) 147–161.
19 Wolf, G., Effect of deoxycorticosterone on sodium appetite of intact and adrenalectomized rats, *Am. J. Physiol.*, 208 (1965) 1281–1285.
20 Yamamoto, T. and Fujimoto, Y., Brain mechanisms of taste aversion learning in the rat, *Brain Res. Bull.*, 27 (1991) 403–406.