Novel Instrument for Electrophysiological Guidance of Nanoliter Injections in the Brain.

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Introduction

Iontophoresis, air pressure, or manual ejection of neuronal tracer and excitotoxin are techniques often used to study connectivity and function of specific brain nuclei. The interpretation of data obtained from such studies depend on two major parameters -- electro-physiological identification of the target site and injection volume.

Although not routine, recording extracellular neural activity can be used to center injections in functionally identified areas. It remains a challenge, however, to place small injections (<100nl) reliably in the brain. This often makes interpretation difficult especially for small subcortical structures where large injections usually extend well beyond the target site.

To more reliably place small injections, we modified a commercialy available device (Nanoject II, Drummond Scientific), to record extracellular neural activity. The Nanoject II uses a direct drive, a stainless steel piston inside a fluid filled pipette, and can inject as little as 2.3nl with each activation. The present experi-ments tested this new instrument for electrophysiological localiza-tion of the gustatory responsive area of the parabrachial nucleus (PBN) and injection of small volumes (<60nl) of the tracer Fluorogold or the excitotoxin ibotenic acid.

General Methods

Subjects: Eighteen male Sprague-Dawley rats weighing between 340-485 grams. Animals had free access to normal rat chow and distilled water unless otherwise noted. In all surgical procedures the rats were anesthetized with a 50 mg/kg injection of Nembutal.

Modified Nanoject II: The Nanoject II uses direct piston displacement. To enable recording of extracellular neural activity, the piston/fluid-filled pipette was electrically isolated from the rest of the instrument.

Gustatory Localization: By recording through the solution in the Nanoject II pipette, gustatory neurons were identified by their responsiveness to stimulation of the anterior tongue with 0.1M NaCl.

Fluorogold and Ibotenic Acid: The retrograde neuronal tracer Fluorogold (FG) was used at a concentration of 2% mixed with 0.15M NaCl. The volume of FG injected was 59.8nl (n=1), 23nl (n=2), or 11.5nl (n=3). The excitotoxin ibotenic acid (IBO) was used at 20mg/ml mixed in phosphate buffered saline. The volume of IBO injected was 23nl (n=5) or 11.5nl (n=4). The control group (n=4) consisted of two non-surgical rats and two rats in which the gustatory PBN was localized, but IBO was not injected.

Immunohistochemistry:

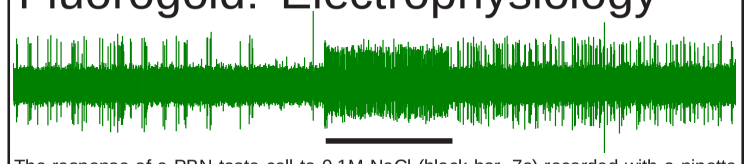
Fluorogold: Five days post injection, rats were euthanized with a lethal dose of Nembutal (100 mg/kg ip) and perfused with 0.9% heparinized saline, followed by buffered 4% paraformaldehyde (pH 7.4). The brain was removed, cut at 50µm, and processed for intensification of Fluorogold.

Ibotenic Acid: The procedures were identical to those described above with 2 exceptions. First, the animals were perfused following behavioral testing, which lasted about 2 months. Second, the tissue sections were stained for the neuronal marker NeuN (Chemicon).

Learned Taste Aversion (LTA): Two weeks after surgery, animals were water restricted with 15min access each morning and 1hr each afternoon. The conditioning procedure consisted of replacing morning water with 0.2M sucrose (CS) followed 30min later with an injection of 0.15M LiCl (US; 1.33ml/100g ip). Water was available for the next two days. This sequence was repeated a second and third time for a total of 3 CS-US pairings. Following two water days, the animals again had 15min access to the CS, but not subsequently injected with LiCl (1-bottle test). Following two more water days, the animals were presented with water and the CS (2-bottle test).

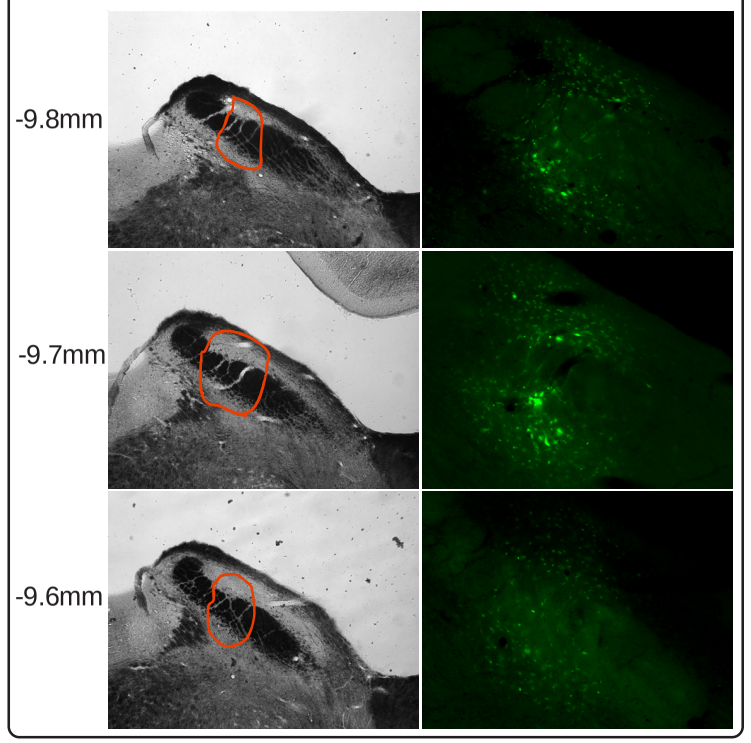
Sodium Appetite: One week after the LTA experiments, animals were housed in wire-mesh metabolic cages. For 7 days, the animals had access to water and 0.51M NaCl attached to the front of the cages. On day 8, animals were made sodium deficient by injection of furosemide (6mg/kg sc). Only water and sodium deficient chow (Teklad) were available overnight. The next day water and NaCl solution were returned to the cages and intake was measured at .25, .5, 1, 2, and 24hr intervals. Water and NaCl remained on the cages for 6 more days. This sequence, an injection day with furosemide followed by a test day and a subse-quent 6 day baseline period, was repeated a second and third time. Finally, this sequence was repeated a fourth time except that the injection day was with an equivalent volume of 0.9% saline instead of furosemide.

Fluorogold: Electrophysiology

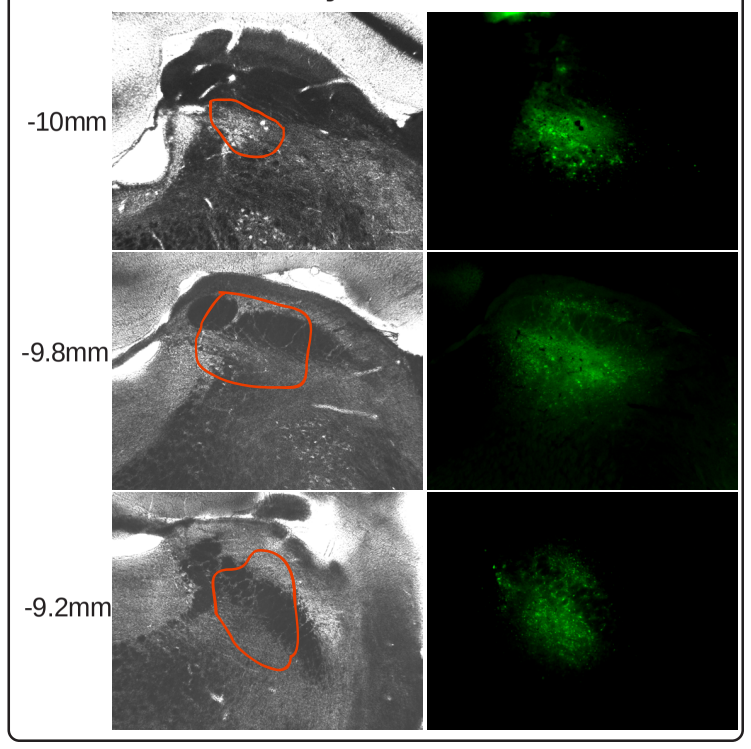


The response of a PBN taste cell to 0.1M NaCl (black bar, 7s) recorded with a pipette attached to the new Nanoject II. Once gustatory responsive cells were localized, the retrograde neuronal tracer Fluorogold was ejected from the pipette at a rate of 2.3nl/step.

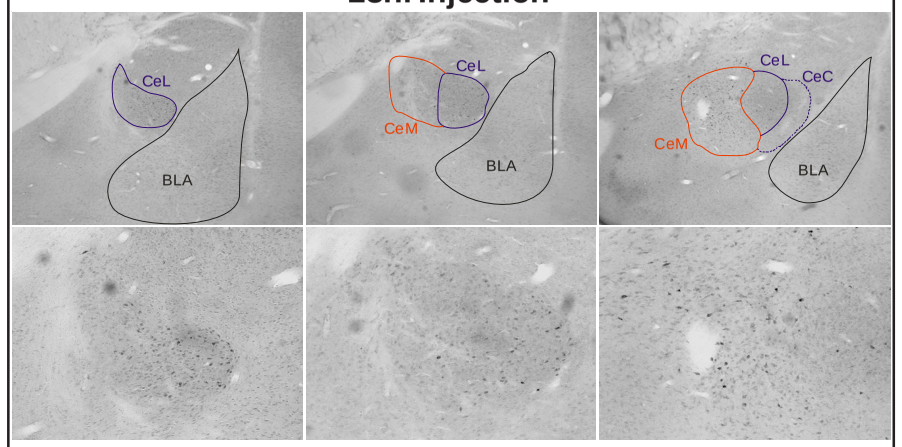
PBN: 23nl Injection



PBN: 59.8nl Injection

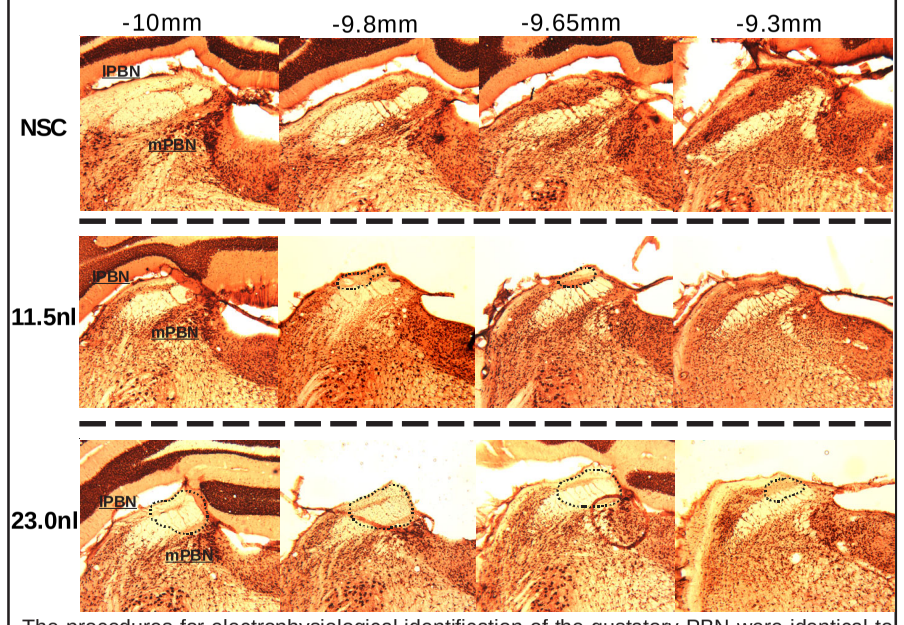


Retrograde Transport: Amygdala



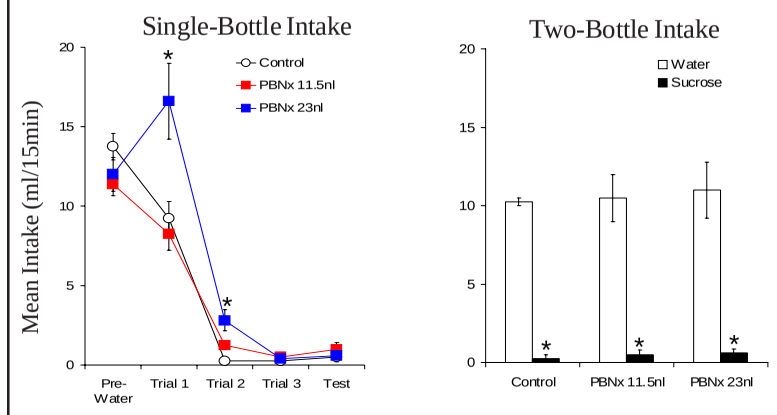
Ce, central nucleus of the amygdala (M, medial; L, lateral; C, capsular); BLA, basolateral nucleus of the amygdala. Column 1, approx. -3.14mm posterior to bregma; Column 2 approx.-2.8mm; Column 3 approx.-2.3mm.

Ibotenic Acid Lesions: PBN



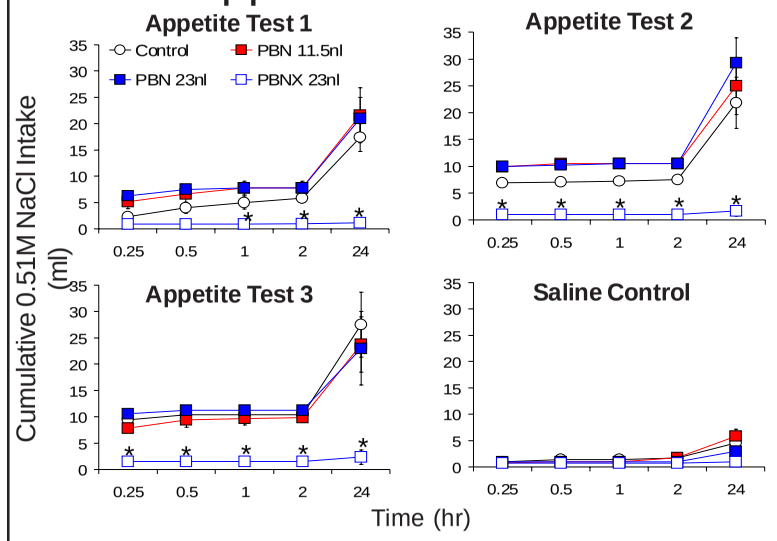
The procedures for electrophysiological identification of the gustatory PBN were identical to that for Fluorogold. Dotted area for 11.5 and 23.0nl is the approximate size of excitotoxin lesion.

Learned Taste Aversion



All animals learned to avoid drinking 0.2M sucrose following Trial 1 pairing with LiCl.

Sodium Appetite



Sodium appetite tests 1, 2, & 3 occured 24hr following furosemide-induced sodium loss. Three of the 5 rats in the 23nl PBNx group failed to express a sodium appetite. The remaining 2 animals in this group and all 4 of those with 11.5nl IBO injections displayed a normal sodium appetite.

Summary

The Nanoject II was successfully modified to record extracellular neural activity.

Small nanoliter injections were consistently placed in the gustatory parabrachial nucleus.

The effects of small excitotoxic lesions suggest a functional topography within the gustatory parabrachial nucleus.

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