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Recovery by Push-Pull Perfusion of Neurochemicals Released Within the Cuneate Nucleus of the Cat by Somatosensory Stimulation[1]

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GOLDFINGER, M. D., C. W. SIMPSON AND G. E. RESCH. *Recovery by push-pull perfusion of neurochemicals released within the cuneate nucleus of the cat by somatosensory stimulation.* PHARMACOL BIOCHEM BEHAV 21(1) 117–123, 1984.—The present work describes a combination of techniques for the identification of neurochemicals released within the cuneate nucleus. During electrical stimulation of the superficial radial nerve, the extracellular fluid of the nucleus is continuously sampled by push-pull perfusion. In addition, the population electrical activity of peripheral nerve as well as the activity of cuneate neurons are recorded. Subsequently, the neurochemical content of the sampled fluid is assessed by HPLC analysis. The comparison of sampled fluid content during control (no stimulation) versus stimulation runs indicates that somatosensory stimulation elicits the release of specific neurochemicals within the cuneate nucleus. The possible sources of released neurochemicals are discussed.

HPLC Cuneate nucleus Superficial radial nerve Electrical stimulation
Catecholamine and indoleamine metabolites Push-pull perfusion

A major goal of research in somatosensory neuroscience is the identification of neurotransmitters associated with the somatosensory relay sites in the CNS. For this objective, three major techniques are required. First, the extracellular fluid of a specific CNS relay site must be locally sampled. Second, the chemical composition of the sample must be determined. The third requirement is the activation of the input neurons, which results in the liberation of neurochemical within, for the present study, the cuneate nucleus. "Push-pull" cannulation accomplishes the first technical requirement [8,19]; small-volume samples are obtained within a small region (1 cubic mm) without interfering with the function of the perfused region [10, 18, 22]. The second technical requirement is met by High Performance Liquid Chromatography (HPLC) which allows a highly sensitive measurement of specific chemicals in very small (e.g., 100 μl) volumes. The third requirement is realized with the electrical stimulation of the superficial radial nerve, which provides a major direct projection of input fibers into the cuneate nucleus.

These techniques have been successfully used in studies of the CNS. For example, Weiner *et al.* [32] and Loullis *et al.* [15] used the push-pull technique to sample from specific brain locations, and were able to identify several endogenous neurochemicals. HPLC was shown to be sensitive enough to detect specific neurochemicals from sampled CSF [25,33]. HPLC analyses of push-pull perfusion samples have been previously reported by McCaleb and Myers [16, 17, 20, 21]; these authors showed that physiological manipulation could change the levels or kinetics of norepinepherine content within the sampled CNS regions. The present work also incorporates both localized push-pull sampling from a discrete site of a known neuronal circuit and HPLC analysis of sampled fluid. It is shown that the use of both techniques—together with appropriate electrophysiological measurements—provides a more thorough description of the function of a major somatosensory relay nucleus.

A variety of neurochemicals have been described in the mammalian caudal brainstem. Catecholamines and indoleamines have been reported to occur in the vicinity of the dorsal column nuclei [14,24]. Therefore, HPLC analysis was directed towards monoamines and their metabolites.

In general, a temporal correlation between electrical neuronal input and neurochemical local output within the nucleus was sought. The first objective was the correlation between somatosensory input with specific intranuclear neu-

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rochemical output. Second, the neurochemical correlates to supraspinal inputs to the relay nucleus were also studied. Such *in vivo* studies provide a new dimension of information about the transmission of somatosensory information through the dorsal column nuclei, a process which mediates discriminative touch [31].

METHOD

Data have been obtained from 5 fully anesthetized adult cats (cats 1–3: Nembutal-50 mg/kg, cats 4–5: alpha-chloralose-70 mg/kg) [11]. Each cat was initially anesthetized by intraperitoneal injection, and placed in a stereotaxic apparatus. Core temperature was maintained at approximately 37°C with a heating lamp. The femoral vein was cannulated for the delivery of intravenous maintenance doses of anesthetic. Electrocardiogram was monitored continuously. A tracheal cannula was inserted; it was determined that artificial ventilation with water-saturated 95 percent oxygen, 5 percent carbon dioxide together with flaxedil (IV) provided necessary stabilization of brainstem pulsations and removal of reflexive muscle movements.

The cuneate nucleus was exposed conventionally [2,9]. Briefly, the brainstem is accessed through the atlanto-occipital membrane; atlas and occipital bone are removed to promote cisternal drainage of CSF and to permit precise placement of the push-pull cannula via micromanipulator and the aid of a dissecting microscope.

The push-pull cannula used has been described by Weiner *et al.* [32]. The assembly consists of two concentric stainless-steel tubes. The inner "push" tube (29 gauge) delivers the vehicle solution (artificial CSF) [19]; the outer "pull" tube (24 gauge) recovers the vehicle solution by negative pressure. The distance between push tube tip and pull tube lumen is 0.5 mm. The push and pull tubes are held together by solder joints. The other ends of the tubes are separated such that each can be individually accessed with polyethylene tubes connected to respective glass syringes (2 ml) placed in a Harvard reciprocal infusion/withdrawal pump (model 935), so that infusion and withdrawal volumes are exactly equal. The push syringe is filled with artificial CSF (pH=7.0) [19] and connected through the polyethylene tubes to the cannula. The pull syringe is flushed with artificial CSF, and backloaded with 100 μl of antioxident (0.1 percent HCl). The polyethylene tubing connecting the pull syringe and the pull cannula tube is also filled with the antioxident, such that there is a continuous volume of antioxident solution between the pull syringe and the back of the pull cannula tube. The cannula is placed onto the brainstem surface such that the push tip just dimples the pial interface. Since the nests of cuneate relay cells lie 0.5 to 2.0 mm below the surface [13,26], this placement promotes push-pull sampling from only the most superficial structures, i.e., the dorsal column afferent axons, their terminals, and the cuneate cells (relay and interneuronal), with their associated synaptic contacts.

The superficial radial nerve is exposed between the wrist and elbow of the ipsilateral forelimb, and placed across a pair of stainless-steel wires coupled to an isolated Grass stimulator for stimulation. To assess sensory nerve activity, evoked potentials of the superficial radial nerve are recorded through fine stainless steel needles inserted some 30 mm central to the point of stimulation. The push-pull cannula is coupled to an amplifier to record cuneate surface responses. The peripheral nerve is stimulated with periodic trains of 0.1 msec duration rectangular pulses at 1/sec to elicit cuneate evoked potentials. In some experiments, stimulation at 1/sec is preceded by a burst of the same pulses delivered at 10/sec. Based on these responses, the push-pull cannula is repositioned if necessary to a location of maximal cuneate surface response (see Results).

Push-Pull Sampling

Samples are obtained from the push-pull system under control conditions or during electrical stimulation of the peripheral nerve. For any sample, the pump rate is set to exchange 20 μl/min; sampling periods used are between 5 to 10 minutes for a given set of control vs. stimulation runs. After the end of each sampling run, the pull polyethylene tube is disconnected from the pull cannula tube, and the contents are immediately evacuated into a 1.5 ml conical collection vial and placed on ice. Control samples were collected before and after samples obtained during stimulation by the following sequence: (a) 5 minutes sampling: control (no stimulation); (b) 5 minutes no sampling, no stimulation; (c) 5 minutes of continuous nerve stimulation with sampling; (d) 5 minutes no sampling, no stimulation; (e) 5 minutes sampling: control (no stimulation).

After this sequence, the sample vials in ice are taken to the HPLC for analysis.

In addition, during push-pull sampling with stimulation, the electrical activity from the surface of the cuneate nucleus is recorded from the push cannula. Signals were amplified conventionally, monitored continuously on an oscilloscope, and stored on magnetic tape. In the most recent experiments, the evoked potential activity from the driven peripheral nerve is recorded as described above; also, the surface evoked potential from contralateral somatosensory cerebral cortex (SI) is recorded with silver wire electrodes.

HPLC Analysis

Sample analysis for neurochemicals was performed with High Performance Liquid Chromatography using an LC-EC (BioAnalytic Systems, W. Lafayette, IN) with a 5 micron C18 ODS reverse-phase, stainless-steel column (4.6×250 mm). The other components of the system included an LC4B-BAS amperometric detector with a glassy carbon working electrode and a calomel reference electrode, a Waters M45 pump, a Rheodyne injection port with a 200 μl-capacity injection loop, and a RYT single-channel strip chart recorder (speed=0.5 cm/min; 1 V full-scale deflection). Flow rates were set at 1 ml/min at a pressure of 1500–2000 PSI. The mobile phase used in detecting the presence of catecholamines and/or indoleamines consist of monochloroacetic acid (14.15 g/l), EDTA (0.75 g/l), sodium hydroxide (4.675 g/l), and 10 percent methanol in steam-distilled water (pH=3.8–4.0) [5]. The electrochemical detector is set in oxidation mode with a time constant of 5 seconds, a working voltage of $\pm$0.85 volt and a sensitivity of 1 nA. With these parameters, the following elution times have been measured: norepinepherine—4 min; epinepherine—5 min; dopamine—6 min; 5HT (serotonin)—13 min; 5HIAA—23.6 min; Homovanillic acid—30 min;

Each day's samples from an experiment were run with a standard curve.

RESULTS

Preliminary data have revealed that electrical stimulation of the superficial radial nerve elicits a change in the neuro-

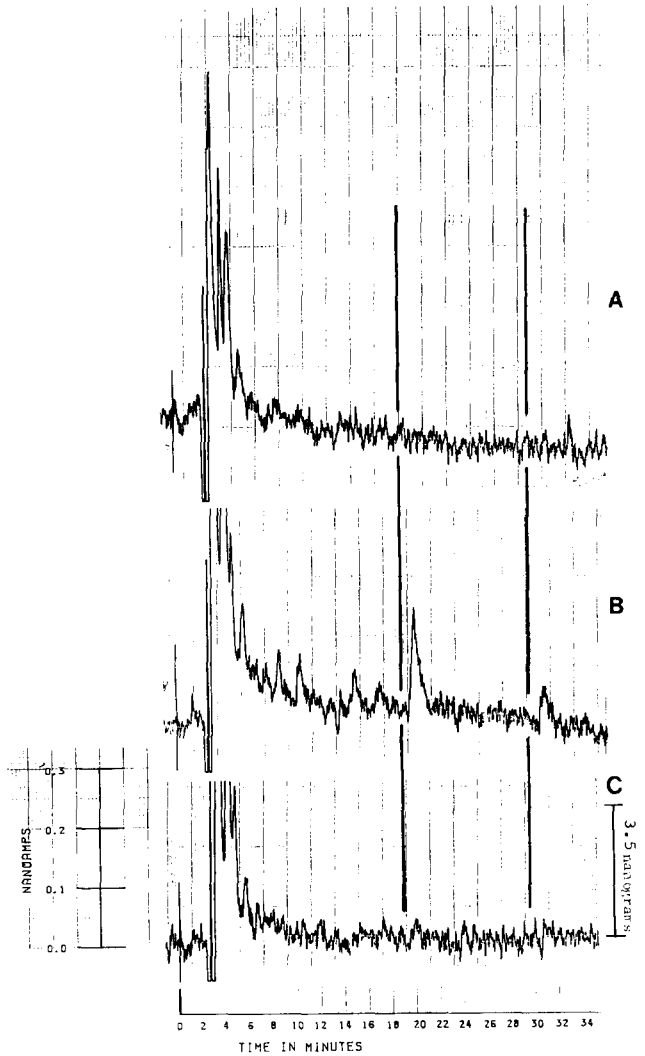

FIG. 1. Effect of peripheral nerve electrical stimulation. HPLC chromatograms are shown in this and subsequent Figs. (2–4; 6). For each record, 100 μl of sample were injected into the HPLC at Time=0 (abscissa origin), also indicated on each chromatogram as a small thin vertical line. In each Figure, abscissa and ordinate scales of the bottom record apply to all records. Vertical calibration scales are given in nanoamps (left) or nanograms (right, as determined with homovanillic acid standard solutions). The heavy vertical lines are drawn for reference to the time scale, and precede the elution times under study by 20–40 seconds. Off scale peaks (including the 'solvent front' artifact) are shown truncated. All push-pull samples were obtained from the left cuneate nucleus (see the Method section). A. Control: no stimulation. B. Stimulation: maximal amplitude, 0.1 msec duration pulses, 1/sec, applied to the Left Superficial Radial Nerve (LSRN) for the entire push-pull sampling period. C. Control: no stimulation. Push-pull sampling period was 5 minutes. LSRN stimulation. Cat 2.

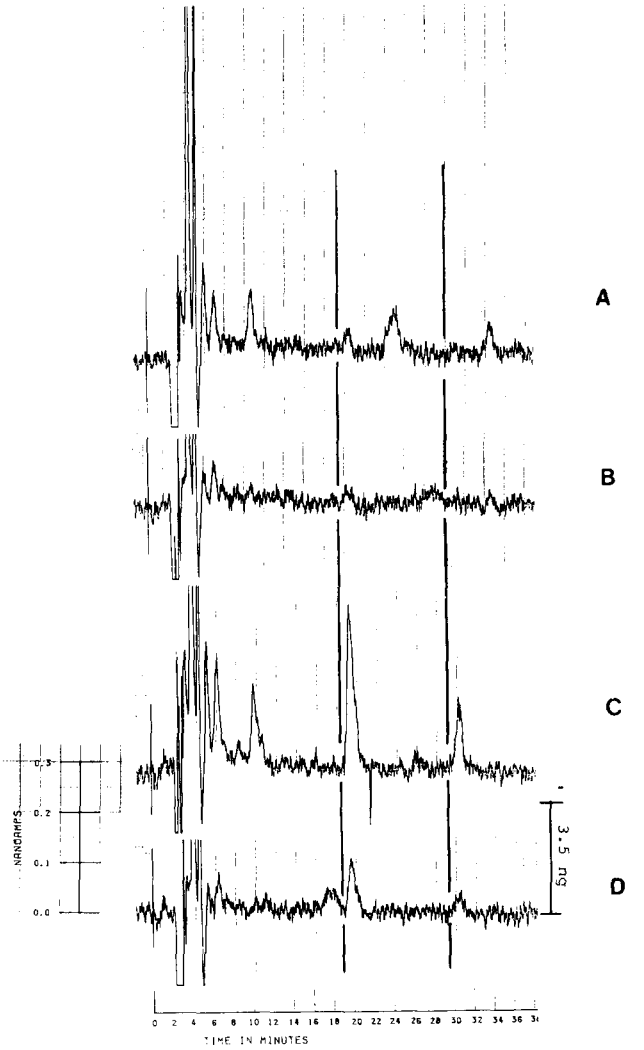

FIG. 2. Effect of stimulus amplitude. A. Control: no stimulation. B. Stimulation: submaximal amplitude, 0.1 msec duration pulses. Conditioning burst at 10/sec for 30 sec was followed by 1/sec stimulation for the remaining sampling period (9.5 min). C.,D. Stimulation: same as B. except maximal amplitude pulses were used. Push-pull sampling period was 10 minutes. LSRN stimulation. Cat 3.

chemical content of the extracellular fluid sampled within the cuneate nucleus, as reflected by the HPLC records. Figure 1 shows a sample set of data. The three chromatograms were obtained from a control run (no stimulation: A), a stimulation run (B), and a subsequent additional control (C). Prominent peaks present in the stimulation data chromatograms (elution times=19.5 and 30 minutes: Fig. 1B) are not present in either control runs (Fig. 1A, C). Two observations can be made from this finding: one, that released neurochemicals can be measured at the concentrations obtained by push-pull sampling, and two, that the presence of an HPLC signal peak can be associated with peripheral nerve stimulation. In this case, other (smaller) peaks are also seen in the stimulation data but not in control records.

The peaks associated with stimulation (Fig. 1) were also observed in a different cat; it was found that the peak heights were a function of stimulus amplitude, as shown in Fig. 2. While the control chromatogram (Fig. 2A) does show small peaks, these do not correspond to the stimulus-elicited peaks of Fig. 1 (i.e., elution times=19.5 and 30 minutes, respectively). The heights of these peaks are modulated by stimulus amplitude: they were only elicited by maximal stimulus amplitudes (Fig. 2C, D) but were not elicited by submaximal

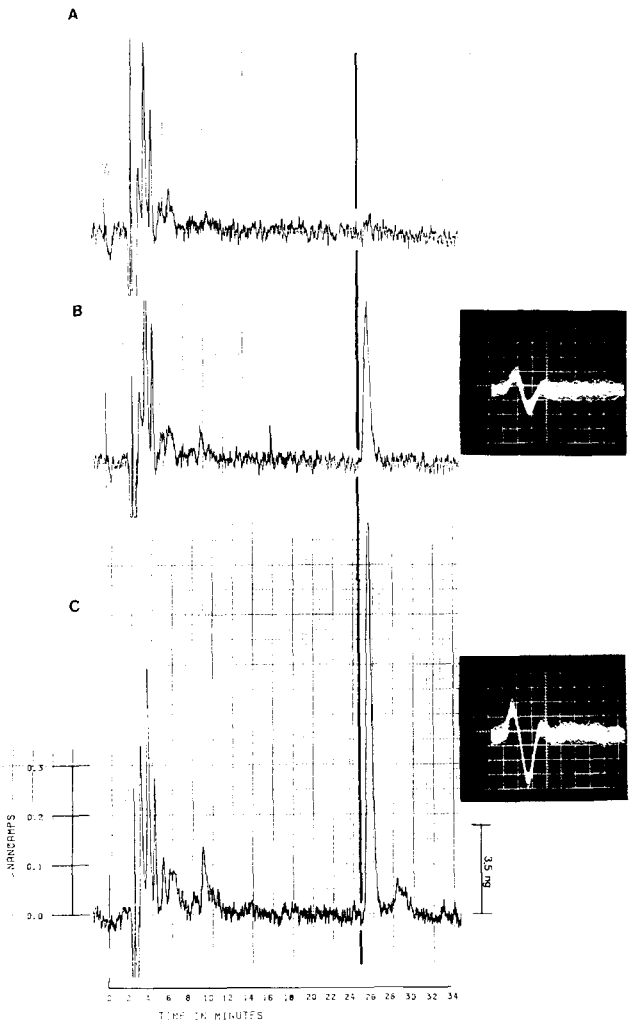

FIG. 3. Relation between driven axonal input and neurochemical output. A. Control: no stimulation. B. LSRN electrical stimulation: 0.4 V-amplitude, 0.1 msec duration, 10/sec. C. Same as B except stimulus amplitude was 6.5 V. In B and C, LSRN evoked potential recorded (at the same gain) during respective push-pull sampling period is shown to the right of the chromatogram of 100 μl of the given sample. Each record consists of 70 superimposed sweeps. The common scaling is: 2 mV/ordinate division and 100 μsec/abscissa division. Responding axons conducted mainly in the A-alpha-beta ranges in both B and C, as determined from the total waveform latencies (not shown) and the measured conduction distance. Push-pull sampling periods were 6 minutes. Cat 5.

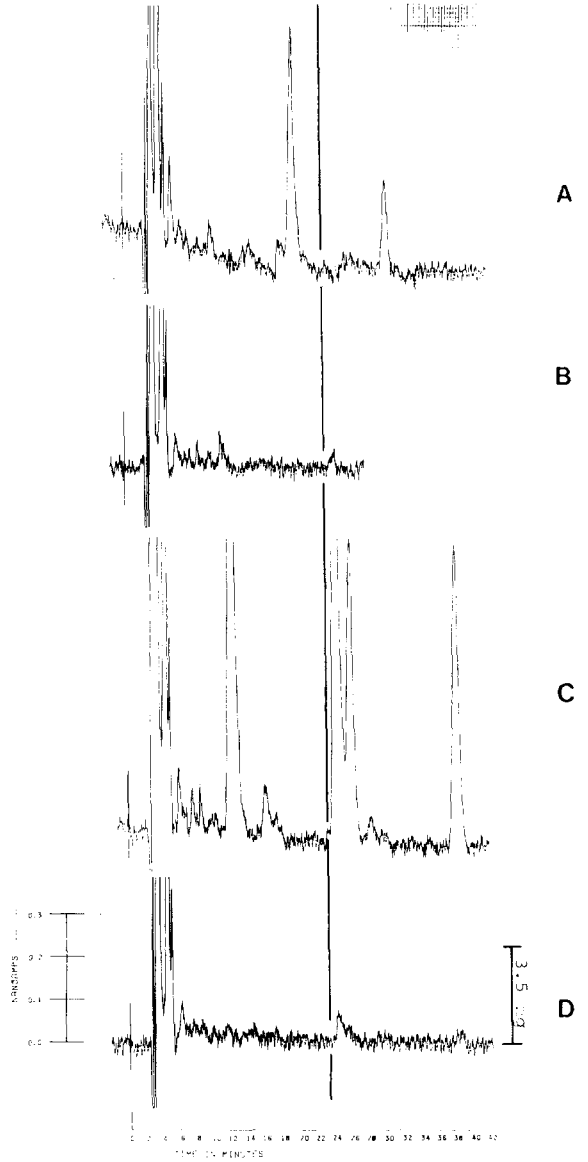

FIG. 4. Effect of higher repetition rate conditioning burst. A. Control: no stimulation. B. Stimulation: maximal amplitude, 0.1 msec duration pulses, 1/sec throughout. C. Stimulation: pulse duration and amplitude as in B; 3 sec of stimulation at 10/sec was followed by 1/sec stimulation for the remainder of the sampling period. D. Stimulation: same as B. Push-pull sampling period was 5 minutes. LSRN stimulation. Cat 1.

stimulus amplitude (Fig. 2B). In preliminary measurements of the superficial radial nerve evoked potential, it was observed (with 0.1 msec duration pulses delivered at 1/sec) that this submaximal intensity stimulation amplitude activated mainly A-alpha and A-beta fibers, while the maximal amplitude stimulation activated A-alpha, A-beta and A-delta fibers. This was assessed from the relative latency of evoked potential peaks with respect to the conduction velocities of known fiber groups [6].

In another cat, the relative amplitude of the peripheral nerve stimulus caused a proportionate release of neurochemical, as shown in Fig. 3. Specifically, the larger of two different submaximal stimulus intensities caused the release of a greater amount of neurochemical, as evidenced by the relative height of the HPLC peak. Figure 3 also shows that the larger stimulus elicited a greater amount of peripheral nerve activity, as indicated by the larger evoked potentials (Fig. 3—right: oscilloscope records).

In another experiment, a different peak (elution time=23.6 min.) was modulated by stimulus repetition rate. In the control (Fig. 4A), the 23.6 minute peak did not occur, although other peaks are present. During stimulation at a fixed stimulus amplitude, the 23.6 peak was elicited by stimulation at 1/sec with a 3-second 10/sec conditioning

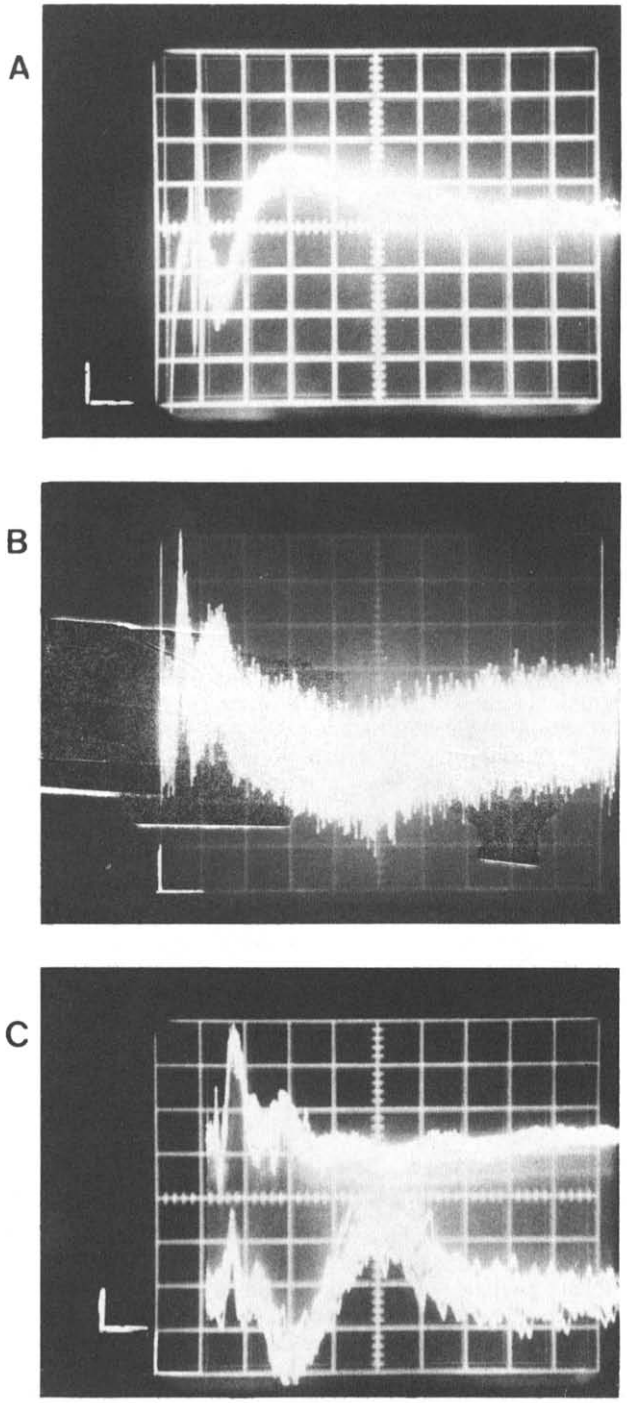

FIG. 5. Electrical responses to superficial radial nerve stimulation. For all traces, positivity is upward with respect to the baseline. Each trace is triggered by successive 0.1 msec rectangular stimuli. A. Cuneate surface evoked potential. Stim. rate: 10/sec, scales: 1 V; 5 msec, 25 superimposed sweeps. Responses recorded during push-pull run 92. (HPLC record not shown). B. Cuneate surface evoked potential. Stim. rate: 10/sec, scales: 1 V; 10 msec, 60 superimposed sweeps. Responses recorded during push-pull run 102. (HPLC record not shown). C. Top: cuneate surface evoked potential. Bottom: Contralateral SI surface evoked potential. Stim. rate: 1/sec, scales: 1 V (top), 0.2 V (bottom); 20 msec. 10 superimposed sweeps. (No push-pull sampling during recordings). Cat 5.

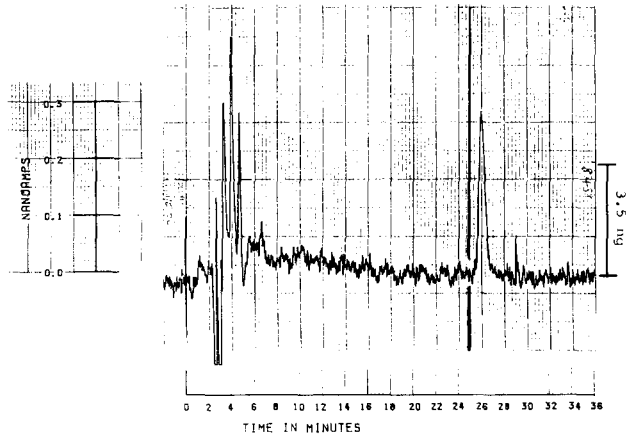

FIG. 6. Effect of contralateral SI electrical stimulation. Stimulation: maximal amplitude, 0.1 msec duration pulses, 10/sec; bipolar stimulating electrodes (0.5 mm separation) dimpling pial surface. Data was obtained 6 minutes after LSRN stimulation. Cat 5. Push-pull sampling period was 6 minutes.

burst, but was not elicited by 1/sec stimulation without the burst (Fig. 4C vs. D and B).

An important correlate of neurochemical measurement in these experiments is the establishment that the site being sampled is also generating electrical activity previously described to occur under similar stimulus conditions. For this purpose, the cuneate nucleus surface potentials elicited by superficial radial nerve stimulation during push-pull sampling were recorded. Figure 5 shows that the expected PNP configuration cuneate surface potential [4] does obtain. The latency of the first P wave components (3–6 msec) corresponds to that measured for single units activated peripherally and recorded at the cuneate nucleus [1]. This response is seen during successive push-pull samplings; that is, the push-pull cannula and fluid exchange do not appear to adversely affect the electrical activity (pre- and post-synaptic) of the neurons at the push-pull site. We have also observed the more complex PNPN wave, where the second P wave is shortened and the late N wave is long (Fig. 5B) [29].

To test the functional state of somatosensory pathways rostral to the cuneate nucleus, the evoked potential was recorded from contralateral SI in response to superficial radial nerve stimulation (Fig. 5C). These data were obtained between push-pull sampling episodes. The SI response latency and duration imply normal transmission of somatosensory electrical information from cuneate to ML, VPL, and to SI.

In addition, to assess a possible role of corticofugal projections to the cuneate nucleus, in a separate experiment SI was directly stimulated during push-pull sampling from the cuneate nucleus. Figure 6 shows that SI stimulation appears to elicit a peak (25-minute elution time) not occurring in the most recent control run. This peak coincides with that elicited by peripheral nerve stimulation in the same preparation at the same sampling site (cf. Fig. 3).

The present experiments show four separate peaks to have been elicited by superficial radial nerve stimulation. In comparing their respective elution times (19.4, 23.6, 25, and 30 minutes) with those of known chemicals tested in artificial CSF standard solutions, these peaks do not have the same elution times of norepinepherine, epinepherine, dopamine, or 5-HT. However, it does appear that the 30 minute elution

time peak (Fig. 2) corresponds to the elution time of HVA, while the 23.6 minute elution time (Fig. 4) corresponds to the elution time of 5HIAA. These data suggest that the two other unidentified sample peaks may be due to other metabolites of catecholamines and/or indoleamines. However, other catecholamine metabolites (3,4-dihydroxy-mandelic acid, 3,4-dihydroxyphenylglycol, VMA and DOPAC) did not have the same elution times as any of the stimulus-elicited peaks described above. The identification of metabolites suggests that either of the metabolites themselves are released or that their precursors (i.e., dopamine and/or 5-HT) [28] are released and subsequently metabolized.

DISCUSSION

Our preliminary data indicate that electrical stimulation of the superficial radial nerve elicits a release of neurochemicals into the extracellular fluid of the cuneate nucleus. The occurrence of a given HPLC peak appears in our data to be independent of anesthetic used. These neurochemicals may have several sources.

The neurochemicals observed may be associated with local injury due to push-pull cannulation; however, this seems unlikely. First, their presence can be specifically elicited by stimulation. Also, the amount of released neurochemical could be changed by either stimulus amplitude, repetition rate, or both (Figs. 2–4). In non-stimulated control samples, peaks are attenuated or absent, as would be expected from variable spontaneous synaptic activity [26,27]. Second, blood in the pull line has only rarely been seen and is due to the rupture of cuneate vasculature out of view in the microscope; when this occurs, no data are taken and the cannula is repositioned. Based on the ability to modulate peak heights with stimulus amplitude or rate as well as from cuneate surface potential recordings, it appears that the push-pull cannula does little or no damage to the sampled region.

The problem of local tissue damage caused by push-pull cannulation has recently been addressed by Errington *et al.* [7]. Push-pull cannulation was performed in rat hippocampus; minimal local damage was observed in examination of histological sections of the tissue area (CA I) cannulated and perfused for 5–6 hours. In comparison with our push-pull assembly, that of Errington *et al.* was wider by 16 and 5 percent for pull and push tubes, respectively, while the two tube tips were also separated by 0.5 mm. Given our similar cannulation-perfusion times together with the non-invasive cannula position used, we anticipate no tissue damage at the cuneate perfusion site.

The neurochemicals observed may be residual in the local CSF. There could be endogenous circulating levels of catecholamines or indoleamines which are part of the normal CSF composition. However, such levels would presumably be constant and would not be influenced by afferent nerve input. If endogenous circulating neurochemicals happened to be the same as the elicited neurochemicals, they would appear in the control record at the same elution time as the elicited peaks, but would be distinguished from the elicited peaks by their smaller amplitude in the control records.

Neurochemicals measured may be released by other CNS regions also affected by peripheral nerve activity (e.g., reticular formation, thalamus, cortex, cerebellum, substantia nigra, caudate nucleus, etc). These released neurochemicals and/or their metabolites would then diffuse through the extracellular space to the cuneate nucleus and be collected by the cuneate push-pull cannula. Regarding the present experimental data, this possibility seems remote. Diffusion of stimulus-released neurochemicals from other regions is minimized by the brainstem exposure methods. The CSF is initially and continuously drained through the 4th ventricle; dura and much of the arachnoid are severed between the top of the occipital bone and the C1 posterior margin, thus minimizing the contact between flowing CSF and the push-pull site. Further, we have found that if a 100 μl sample which yields a finite HPLC peak (under the measurement conditions described in Method section) is diluted by another 100 μl of vehicle solution, the signal is largely or entirely abolished. Cat wholebrain extracellular space of course exceeds 100 μl. Thus, it is presumed that neurochemical released by peripheral nerve stimulation in CNS regions outside of the cuneate nucleus is most likely diluted to undetectable levels by the time it reaches the cuneate sampling site.

The most likely interpretation of the data is that they show the presence of neurochemical released into the cuneate nucleus in response to afferent peripheral nerve activity. Given the known cuneate circuitry [4], there are three sources of synaptic contacts which could release neurochemicals under the experimental conditions. The first source is primary afferent dorsal column axons terminating on cuneate relay cells and/or interneurons. Price and Mudge [23] recently reported that in rat: ". . . a subpopulation of DRG neurones is catecholaminergic and that the neurotransmitter they make is probably dopamine." Assuming the presence of such neurons in cat DRG, our detection of HVA would be consistent with their activation by LSRN stimulation, central projection to the dorsal column nuclei, and the breakdown of released dopamine by COMT and MAO [28]. The second source is interneurons activated by primary axons and/or other circuits. The third source is "efferent" axons—including dorsal column post-synaptic axons [30]—terminating onto cuneate relay neurons and/or interneurons. One site of origin of these efferent fibers is those regions of the cortex giving rise to corticofugal (i.e., corticocuneate) projections [3]; this projection could account for the release of neurochemical into the cuneate nucleus elicited by contralateral SI electrical stimulation (Fig. 6). However, non-cortical supraspinal regions may also send projections to the cuneate; such regions may include thalamic VPL/VPM, periaquaductal gray (PAG) and the raphe nuclei. The raphe and PAG have recently been implicated as having a role in the transmission of somatosensory information through the cuneate nucleus [12]. Whatever the source of the observed neurochemicals, their mechanistic role in synaptic transmission within the cuneate nucleus is yet to be determined.

In summary, the present work demonstrates that through a combination of push-pull cannulation and HPLC analysis together with appropriate electrophysiological measurements, functional parameters of a given central synaptic region may be described. Specifically, for the somatosensory relay cuneate nucleus, neurochemicals released locally in response to peripheral nerve stimulation can be sampled, measured, and identified. This combination of techniques represents a new approach to the discovery of the role of the observed neurochemicals in the transmission of somatosensory information by the cuneate nucleus.

REFERENCES

1. Amassian, V. E. and J. L. Devito. Transmission through the cuneate nucleus. *Colloques Int Cent Natl Res Sci* **67**:353–393, 1957.
2. Amassian, V. E., J. Macy, H. J. Waller, H. S. Leader and M. Swift. Transformation of afferent activity at the cuneate nucleus. *Proc Int Union Phsyiol Sci* **3**: 235–255, 1964.
3. Anderson, P., J. C. Eccles and R. F. Schmidt. Presynaptic inhibition in the cuneate nucleus. *Nature* **194**: 741–743, 1962.
4. Anderson, P., J. C. Eccles, R. F. Schmidt and T. Yokota. Slow potential waves produced in the cuneate nucleus by cutaneous volleys and by cortical stimulation. *J Neurophysiol* **27**:87–91, 1964.
5. Bioanalytical Systems. Plasma catatecholamines. Application note no. 14 in BAS technical manual. W. Lafayette, IN, 1981.
6. Burgess, P. R. and E. R. Perl. Cutaneous mechanoreceptors and nociceptors. Chapter 2. *Handbook of Sensory Physiology; vol 2: Somatosensory System*, edited by A. Iggo. New York: Springer-Verlag, 1973, pp. 29–78.
7. Errington, M. L., A. C. Dolphin and T. V. P. Bliss. A method for combining field potential recording with local perfusion in the hippocampus of the anaesthetized rat. *J Neurosci Methods* **7**: 352–357, 1983.
8. Gaddum, J. H. Push-pull cannulae. *J Physiol (London)* **155**: 1–2P, 1961.
9. Goldfinger, M. D. and V. E. Amassian. Response of forelimb guard hair afferent units to airjet stimulation of the entire receptive field. *J Neurophysiol* **44**: 961–978, 1980.
10. Greenfield, S. A., B. P. A. Howse and J. F. Stein. Concurrent extracellular recording and local perfusion using push-pull cannulae in the nigral-striatal system of awake cats. *J Physiol (London)* **320**: 10–11P, 1981.
11. Honda, C. N., S. Mense and E. R. Perl. Neurons in ventrobasal region of cat thalamus selectively responsive to noxious mechanical stimulation. *J Neurophysiol* **49**: 662–673, 1983.
12. Jundi, A. D., N. E. Saade, N. R. Banna and S. J. Jabbur. Modification of transmission in the cuneate nucleus by raphe and periaqueductal gray stimulation. *Brain Res* **250**: 349–352, 1982.
13. Keller, J. H. and P. J. Hand. Dorsal root projections to nucleus cuneatus of the cat. *Brain Res* **20**: 1–17, 1970.
14. Leger, L. and L. Wiklund. Distribution and numbers of indoleamine cell bodies in the cat brainstem determined with Falck-Hillarp fluorescence histochemistry. *Brain Res Bull* **9**: 245–251, 1982.
15. Loullis, C. C., J. N. Hingtgen, P. A. Shea and M. H. Aprison. In vivo determination of endogenous biogenic amines in rat brain using HPLC and push-pull cannula. *Pharmacol Biochem Behav* **122**: 959–963, 1980.
16. McCaleb, M. L. and R. D. Myers. Hypothalamic microinjection of norepinepherine (NE) elevates and prolongs basal efflux of unmetabolized NE from injection site. *Brain Res Bull* **8**: 325–327, 1982.
17. McCaleb, M. L. and R. D. Myers. 2-Deoxy-D-glucose and insulin modify release of norepinepherine from rat hypothalamus. *Am J Physiol* **242**: R596–R601, 1982.
18. McGinty, D. J., R. Drucker-Colin, R. and S. S. Bowersox. Reticular formation unit discharge modulation by local perfusion in behaving cats. *Exp Neurol* **75**: 407–419, 1982.
19. Myers, R. D. Methods for perfusing different structures of the brain. In: *Methods in Psychobiology*, edited by R. D. Myers. New York: Academic Press, 1972, pp. 169–211.
20. Myers, R. D. and M. L. McCaleb. Feeding: Satiety signal from intestine triggers brain's noradrenergic mechanism. *Science* **209**: 1035–1037, 1980.
21. Myers, R. D. and M. L. McCaleb. Peripheral and hypothalamic cholecystokinin acts on the noradrenergic "feeding circuit" in the rat's diencephalon. *Neuroscience* **6**: 645–655, 1981.
22. Nieoullon, A. and N. Dusticier. Effect of superficial radial nerve stimulation on the activity of nigro-striatal dopaminergic neurons in the cats: role of cutaneous sensory input. *J Neural Transm* **53**:133–146, 1982.
23. Price, J. and A. W. Mudge. A subpopulation of rat dorsal root ganglion neurons is catecholaminergic. *Nature* **301**: 241–243, 1983.
24. Rea, M. A., M. H. Aprison and D. L. Felten. Catecholamines and serotonin in the caudal medulla of the rat: combined neurochemical-histofluorescence study. *Brain Res Bull* **9**: 227–236, 1982.
25. Sarna, G. S., P. H. Hutson, M. D. Tricklebank and G. Curzon. Determination of brain 5-hydroxytryptamine turnover in freely moving rats using repeated sampling of cerebrospinal fluid. *J Neurochem* **40**: 383–388, 1983.
26. Schwartz, S. An Analysis of Resting Activity in the Cuneate Nucleus. Doctoral Thesis. Albert Einstein College of Medicine. Yeshiva University, New York, NY, 1965.
27. Schwartz, S., D. Giblin and V. E. Amassian. Resting activity of cuneate neurons. *Fed Proc* **23**: 466, 1964.
28. Siegel, G. J., R. W. Albers, B. W. Agranoff and R. Katzman, Editors. *Basic Neurochemistry*. 3rd Ed., Boston: Little Brown, 1981.
29. Towe, A. L. and I. D. Zimmerman. Peripherally evoked cortical reflex in the cuneate nucleus. *Nature* **194**: 1250–1251, 1962.
30. Uddenberg, N. Functional organization of long, second-order afferents in the dorsal funiculus. *Exp Brain Res* **4**: 377–382, 1968.
31. Vierck, C. Somatosensory System. In: *Handbook of Behavioral Neurobiology*, vol 1, Chapter 8, edited by R. B. Masterson. New York: Plenum, 1978.
32. Weiner, H., C. W. Simpson, J. A. Thurman and R. D. Myers. Disulfiram alters dopamine metabolism at sites in rat's forebrain as detected by push-pull perfusions. *Brain Res Bull* **3**: 541–548, 1978.
33. Yaksh, T. L., D. L. Hammond and G. M. Tyce. Functional aspects of bulbospinal monoaminergic projections in modulating processing of somatosensory information. *Fed Proc* **40**: 2786–2794, 1981.