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Time course and effective spread of lidocaine and tetrodotoxin delivered via microdialysis: an electrophysiological study in cerebral cortex

Susan E. Boehnke [a], Douglas D. Rasmusson [b,*]

[a] *Department of Psychology, Dalhousie University, Halifax, NS, Canada B3H 4H7*

[b] *Department of Physiology and Biophysics, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, NS, Canada B3H 4H7*

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Abstract

Microdialysis is a useful tool for administering drugs into localized regions of brain tissue, but the diffusion of drugs from the probe has not been systematically examined. Lidocaine (10%) and tetrodotoxin (TTX, 10 μM), drugs typically used in neural inactivation studies, were infused through a microdialysis probe into raccoon somatosensory cortex while evoked responses were recorded at four electrodes equally spaced 0.5–2.0 mm from the probe. The decreases in evoked response amplitude as a function of time and distance from the probe were used as functional measures to describe the time course and spread of the drugs. TTX inactivated distant sites more quickly and to a greater extent than lidocaine. Responses recovered within approximately 40 min after termination of lidocaine, but did not recover for at least 2 h after TTX. Based on these measurements, we estimated that, at the concentrations used, lidocaine has a maximal spread of 2.1 mm, while TTX could spread as far as 4.8 mm from the microdialysis probe. However, in terms of significant inactivation of neuronal activity, lidocaine and TTX have an effective spread of 1 and 2 mm, respectively.

Keywords: Cerebral cortex; Evoked potentials; Inactivation; Lidocaine; Microdialysis; Raccoon; Somatosensory; Tetrodotoxin

1. Introduction

The delivery of drugs, particularly inactivating agents, to the brain is useful for studying the function of particular nuclei or tracts. Inactivation through sodium channel blockers, such as lidocaine and tetrodotoxin (TTX), has the advantage over permanent lesion techniques of being temporary, reversible, and not subject to the compensatory plasticity that may result from permanent lesions. An important variable in using these drugs is how far they are spreading. This can be estimated from diffusion equations (Shippenberg and Thompson, 1997), but this depends on variables such as metabolic turnover and tissue resistance that are difficult to determine. Alternatively spread can be estimated using radiolabeled drugs and autoradiographic detection (Martin, 1991; Dykstra et al., 1992). This, however, shows the maximal diffusion at the limits of autoradiography rather than the distance over which the drug is having a physiological effect. Of more practical use have been the studies that have used a behavioral or a neural index to estimate the effective spread of various drugs delivered by pressure injection (Albert and Madryga, 1980; Sandkühler et al., 1987; Tehovnik and Sommer, 1997; Martin and Ghez, 1999).

Another method for delivering drugs is via microdialysis, a technique that was developed to collect neurochemicals from the brain rather than deliver them (Quan and Blatteis, 1989; Ludvig et al., 1992, 1994). In one sense, microdialysis introduces a complication because the movement of the drug through the microdialysis tubing (the probe efficacy) is an additional variable. However, microdialysis presents a major advantage over injection in that the drugs in the perfusate can be altered during an experiment without changing the pressure or volume surrounding the probe, a major

* Corresponding author. Tel.: +1-902-4946520; fax: +1-902-4941685.

E-mail address: rasmus@is.dal.ca (D.D. Rasmusson).

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worry with injections. Pressure injections are limited to a maximum volume of 12–20 μl because of the likelihood of tissue damage by volume displacement (Demer and Robinson, 1982; Tehovnik and Sommer, 1997; Martin and Ghez, 1999); this in turn limits the volume of tissue that can be inactivated. While insertion of a microdialysis probe also produces tissue damage, this occurs only at the beginning of the experiment without any additional damage associated with drug delivery.

The theoretical characteristics of diffusion into and out of a microdialysis probe have received a great deal of attention (Benveniste, 1989; Kehr, 1993), but little practical information is available about the effective spread of molecules that have been delivered through a microdialysis probe or the time course of that spread. The present study uses the sensory evoked response to describe the effective spread of two inactivating agent, lidocaine and TTX, from a microdialysis probe placed in the cerebral cortex. Both of these chemicals work by inactivating voltage-gated sodium channels, thereby preventing the occurrence of action potentials. The initial deep-negative (surface-positive) response of the sensory evoked potential is the summation of the post-synaptic potentials produced primarily by thalamocortical afferents (Cauller and Kulics, 1991). Blocking voltage-gated sodium channels will reduce this component of the evoked potential, as well as subsequent intracortical events, by blocking action potentials in these afferents.

In the present study, the decrease in evoked potentials was determined using an array of four electrodes located 0.5–2.0 mm from the probe. Raccoons were used for this experiment because they possess extremely large cortical representations of the forepaw digits. The cytoarchitectonics of the raccoon somatosensory cortex is basically the same as area 3b of the cat (Welker and Seidenstein, 1959), but each digit is represented on a separate gyrus approximately 5 mm in diameter (see Fig. 1C). Consequently, a single 'digit gyrus' is sufficiently large that multiple recording sites will respond to a single sensory input. Therefore, a single digit was stimulated electrically before, during and after delivery of inactivating drugs, and the decrease in the size of the evoked response at each electrode was used to estimate the functional spread of the drug and its time course. In addition to a simple description of the effective spread under different delivery conditions, several interesting differences between lidocaine and TTX were observed that are relevant to the practical use of these agents.

2. Materials and method

2.1. Animals

Subjects were eight adult raccoons (five male, three female), captured from the wild. Their ages were unknown, and they ranged in weight from 4.5 to 13 kg. Animals were kept in communal enriched housing from the time of their capture until they were tested. All procedures were in accordance with the ethical guidelines of the Canadian Council on Animal Care.

2.2. Electrophysiological recording

An electrode array (Fig. 1A) was constructed from four Parylene-C coated tungsten extracellular recording electrodes (A.M. Systems; Everett, WA) affixed to an electrode holder with dental impression compound (Kerr, Romulus, MI). While the compound was still malleable, the electrodes were carefully arranged under a microscope so that the tips of neighboring electrodes were approximately 500 mm apart.

2.3. Microdialysis system

The microdialysis probe (CMA-12, Stockholm) had 2 mm of exposed membrane and was 0.5 mm in diameter. The perfusate was initially artificial cerebrospinal fluid (Elliott's solution; Abbott, Montreal). The perfusate was switched to Elliott's solution containing either lidocaine (10%) or TTX (10 mM) using a liquid switch (CMA-110). The flow rate (2 or 5 ml/min) was controlled by a micropump (CMA-100). The time delay between changing the liquid switch and arrival of the fluid at the probe was approximately 1.3 and 3.2 min for flow rates of 5 and 2 ml/min, respectively. The time at which the drug would reach the probe was considered to be time zero.

2.4. Surgery

Raccoons were injected with ketamine (100 mg, i.m.) and then anesthetized with halothane. The radial vein was exposed and catheterized in order to administer further drugs. Initially, α-chloralose (2 ml, 5%, Sigma, Oakville, ON) and a corticosteroid (Solu-Delta-Cortef, 20 mg, UpJohn, Orangeville, ON) were administered as a bolus, after which α-chloralose was administered continuously at 10 ml/min by micropump in order to maintain stable anesthesia. The animal was placed in a Kopf stereotaxic instrument, and a craniotomy was performed over the left somatosensory cortex. A dam of dental impression compound was constructed around the opening, which was then filled with Elliott's solution warmed to 37°C. The dura was carefully opened while viewing with the aid of an operating microscope.

2.5. Experimental procedure

The craniotomy typically exposed the cortical areas representing digits 3–5 and the palm. The digit cortical areas were verified by recording from single neurons or groups of neurons extracellularly with a tungsten electrode (cf. below), while manually mapping their receptive fields using tactile stimulation of the digits. The four-electrode array (dots in Fig. 1C) was then inserted across the center of a digit gyrus to a depth of approximately 1 mm, corresponding to the major termination region of thalamocortical afferents. If the evoked potentials in response to electrical stimulation of the corresponding digit were strong and consistent at all four electrodes, the microdialysis probe (x in Fig. 1C) was inserted into the cortex approximately 0.5 mm from the nearest electrode at a 40–50° angle and perpendicular to the electrode array (Fig. 1B). The probe was inserted so that the entire microdialysis membrane was within the cortex. In order to insert the probe with

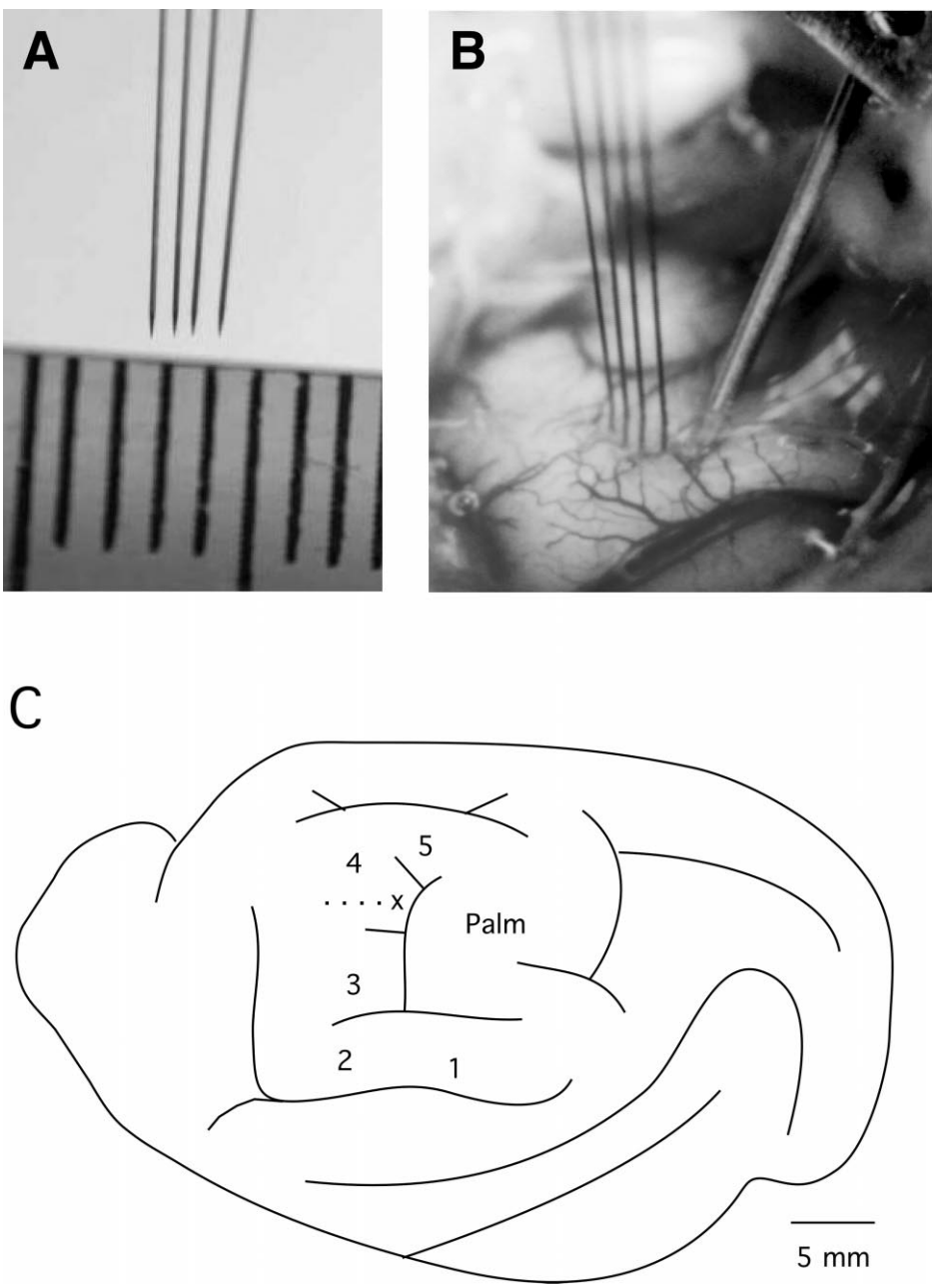

Fig. 1. (A) Electrode array used to record evoked potentials at approximately 0.5 mm increments from the probe. (B) Experimental set-up showing the electrode array and the microdialysis probe in the raccoon somatosensory cortex. Although a portion of the microdialysis tubing is visible in this photograph, during experiments, it was inserted until it was entirely within the cortex. (C) Organization of the left raccoon cerebral cortex, showing the representational areas of different body parts. 1–5 refer to digits 1–5 of the forepaw. An example of the probe location (x) and recording sites (dots) is shown in the fourth digit region. The distance between dots is exaggerated for clarity.

Table 1
Description of the individual experiments

Experiment	Cortical location	Drug	Concentration	Flow rate (μl/min)	Duration of drug infusion (min)
1a	D5	Lidocaine	10%	2	20
1b	D5	Lidocaine	10%	5	20
2a	D4	Lidocaine	10%	5	22
2b	D4	Lidocaine	10%	2	21
3	D4	TTX	10 μM	5	10
4a	D5	TTX	10 μM	5	10
4b	D3	TTX	10 μM	5	5
5	D4	TTX	10 μM	5	14
6	D3	TTX	10 μM	5	11
7a	D5	TTX	10 μM	5	5.25
7b	D4	TTX	10 μM	2	5
8	D5	TTX	10 μM	5	3

minimal disturbance to the electrode array the pia was first pierced with a 25-gauge needle to prevent dimpling of the cortex. The digit corresponding to that cortical area was then stimulated every 3 s via intradermal stimulating electrodes attached to a Master 8 stimulator (A.M.P.I., Jerusalem). The stimulus was a 0.2 ms constant current pulse, with an amplitude of 300–800 μA in different experiments.

Bioelectric signals recorded at each electrode were amplified (A.M. Systems, model 1800) with filters set at 1 and 500 Hz, observed on a Tektronix oscilloscope and digitally converted by a DataWave interface (Thornton, CO). Each channel was sampled at 10 kHz for 10 ms before and 90 ms after delivery of the stimulus. The average of 10 responses was computed and saved on a microcomputer for each of the four channels. Ten to 20 min of baseline responses were recorded with ACSF perfused through the microdialysis probe, before the drug (lidocaine or TTX) was administered. The concentration, flow rate and duration of administration of the drug for each experiment are presented in Table 1. After drug administration was terminated, Elliott's solution was administered continuously through the probe for up to 2 h, or until the response began to return to baseline.

2.6. Data analysis

The amplitude and latency of the first negative peak (N1) of each of the averaged evoked potentials was measured using DataWave software.

2.7. Analysis of the spread

The N1 amplitude for each channel was normalized to a percentage of its mean baseline amplitude. The maximum effect of the drug at each electrode was considered to be the 10 min period with the smallest response. The mean amplitude during this period was expressed as the percentage decrease from baseline for each electrode. This maximum decrease was then plotted against distance from the probe to estimate the maximum effective spread of the drug.

2.8. Time to inactivation

The response curves were normalized for both the maximum and minimum values at each distance from the probe by subtracting the mean value at the lowest point after inactivation from each value and then expressing this value as a percentage of the baseline amplitude. This aligned the curves and enabled measurement of the time required for the response to decrease to 37% of baseline at each distance from the probe, a value that is similar to a time constant for each electrode.

3. Results

In two preliminary experiments, 2% lidocaine was found to be ineffective in decreasing the evoked potentials recorded at even the nearest electrode, and 5% lidocaine had only a minimal effect. Thus, a 10% solution of lidocaine was used for subsequent experiments. Lidocaine was applied in four experiments in two animals using two different flow rates (Table 1). The times between lidocaine offset and its second application were 51 and 56 min, by which time the response had recovered completely. Although not studied systematically, there were no obvious differences in the effects of flow rates of 2 and 5 μl/min. Examples of the evoked potentials and the changes produced in one animal by a 20 min perfusion of lidocaine (2 μl/min) are shown in Fig. 2. Lidocaine produced suppression of evoked responses at 0.5 and 1.0 mm from the probe (maximum of 82 and 44% decrease from baseline, respectively), but had little or no effect on evoked responses recorded at 1.5 or 2.0 mm (25 and 4% decreases, respectively). This figure also clearly illus-

trates that the time to the onset of suppression and the time to maximal suppression increased as the distance from the probe increased, reflecting the diffusion to these electrodes. Recovery was also a function of distance, occurring within 15 min for the electrodes that were 1 and 1.5 mm from the probe, but not until 35 min for the closest electrode.

TTX produced an even stronger suppression of the evoked response, even when applied for periods as short as 3 min. An example of the effect of TTX is shown in Fig. 3. The almost complete suppression of the evoked potentials with TTX can be seen in the amplitude measurements at 0.5 and 1 mm from the probe as well as from the waveforms during application of the drug (insets). It is also apparent that the effective spread was greater with TTX than with lidocaine. TTX produced a 60% decrease in the response 2 mm from the probe, whereas a longer application of lidocaine had no effect at this distance. While the responses recovered quickly following lidocaine, this did not occur after TTX administration. In fact, the responses continued to decrease for approximately 20 min after TTX was turned off. In the experiment shown in Fig. 3, there was no recovery within 70 min after TTX application, but in other experiments where recovery was monitored for 2 h (not shown), the responses returned towards baseline only at the furthest electrodes with little recovery at the nearer electrodes. Due to this prolonged recovery time, in the two animals in which repeated TTX experiments were performed, the probe and electrodes were moved to a different cortical gyrus. The intervals between successive TTX applications in these two experiments were 2 and 5 h.

The spatial extent of block by lidocaine and TTX, averaged across all experiments, is illustrated in Fig. 4A. It is evident that lidocaine had no effect at 2 mm or further from the probe; however, TTX produced an average decrease of 60% at that distance. Linear extrapolation of this curve suggests that TTX might affect neurons within a 4.8 mm radius.

The relationship between the drug and the distance from the probe was tested using a mixed 4×2 analysis of variance (ANOVA), with the four electrode locations as a repeated measure within an experiment and drug as a between groups factor. Two missing cells in the array

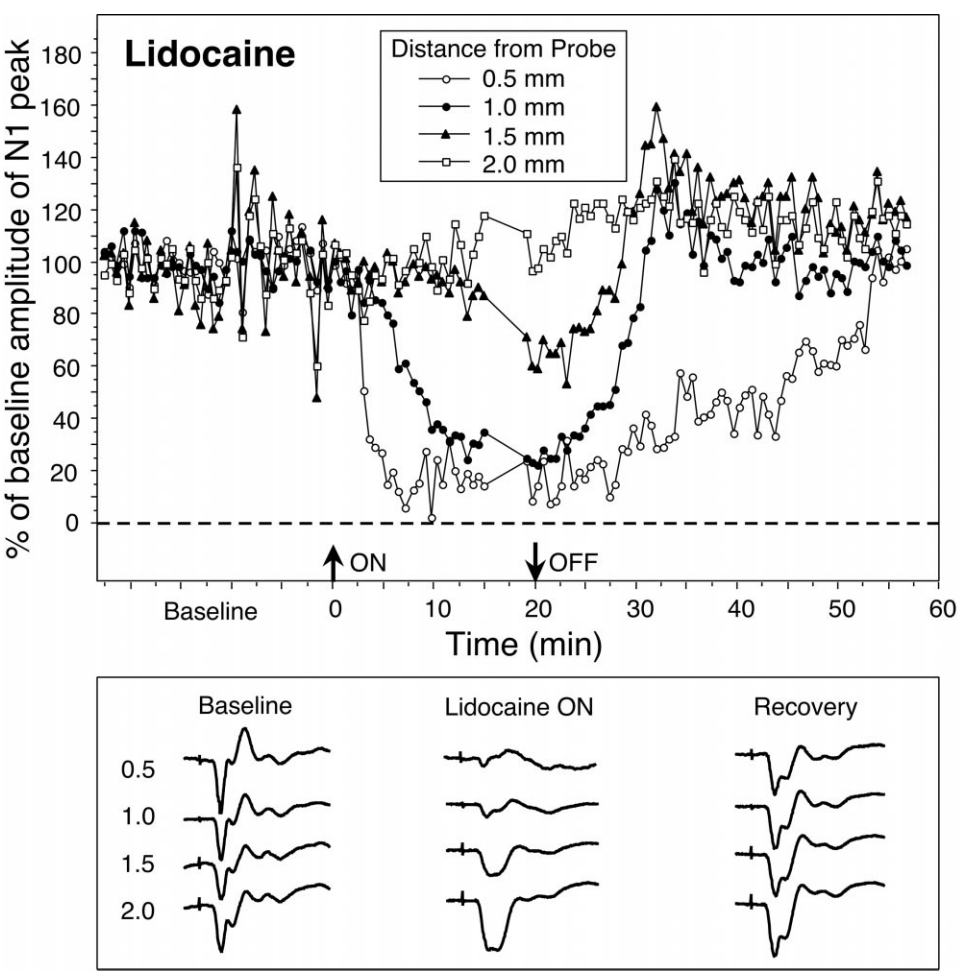

Fig. 2. Example of the time course of inactivation by a 20 min application of lidocaine at each of four electrodes at different distances from the probe (2 μl/min). Below are averaged waveforms from 10 min segments during the baseline, inactivation, and recovery periods.

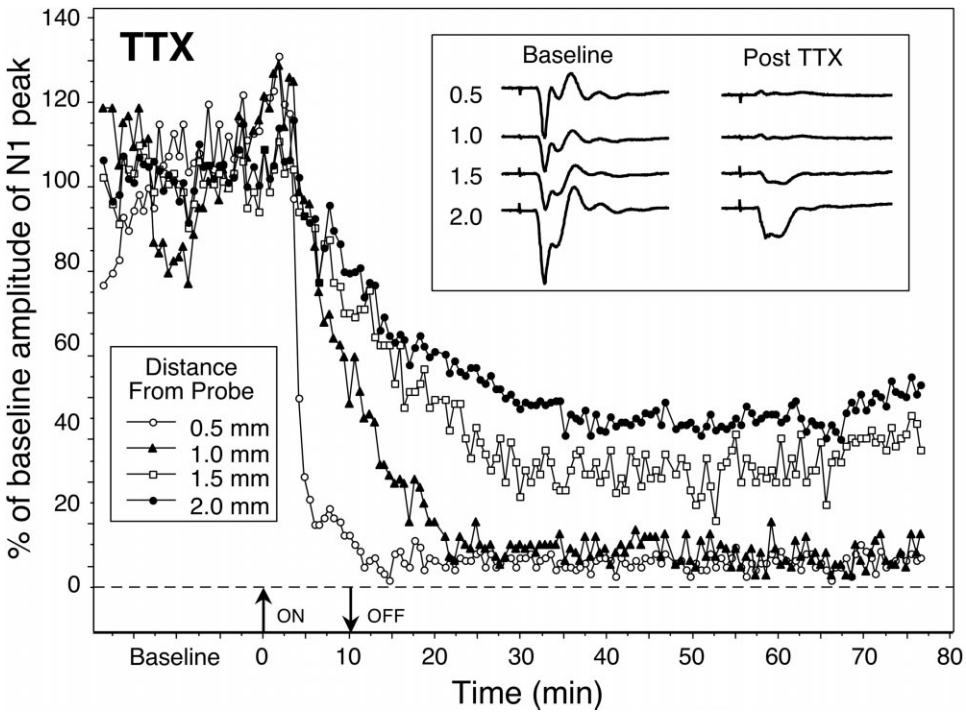

Fig. 3. Example of the time course of inactivation by a 10 min application of TTX at each of four electrodes at different distances from the probe (5 μl/min). Insets are averaged waveforms from 10 min segments during the baseline and the inactivation periods.

(caused by amplifier failure for one electrode during two TTX experiments) were corrected by substitution with the group mean for all ANOVAs completed. There was a significantly greater overall decrease in response with TTX than with lidocaine [$F(1,10) = 9.132$, $P < 0.05$] and with proximity to the probe [$F(3,10) = 35.80$, $P < 0.01$]. Further, there was a significant interaction between the type of drug and the distance from the probe [$F(3,30) = 8.829$, $P < 0.001$], reflecting the difference in the effect of lidocaine and TTX at the furthest electrodes.

The time constants for the suppression were calculated as the time to reach 37% of the maximum decrease. Mean values for the two drugs are shown in Fig. 4B. Since there was little or no decrease in response in the lidocaine experiments at the furthest electrode, no values could be calculated for that data point. Consequently, separate one-way repeated measures ANOVAs were conducted for each drug. The time to reach 37% of maximum suppression increased with distance from the probe for both lidocaine [$F(2,6) = 14.57$, $P < 0.01$] and TTX [$F(3,21) = 10.67$, $P < 0.001$]. To compare the two drugs in terms of the change in the time course with distance, the slope of the line of best fit was measured for each animal using the first three channels in the lidocaine experiments and all four channels in the TTX experiments. The slope of the curves for lidocaine was 7.9 ± 1.7 (mean $\pm$ S.E.M.), and for TTX, it was 3.7 ± 0.8. The difference between the two groups was statistically significant, as determined by an unpaired t-test [$t(10) = 2.496$, $P < 0.05$]. This difference in slopes reflects the more rapid effect of TTX at distances of 1 mm or more from the probe.

4. Discussion

4.1. Concentration and flow rate

Several different concentrations (2–10%) and flow rates (2 and 5 μl/min) were tested with lidocaine. Preliminary studies found that 2 and 5% lidocaine were ineffective in reducing the evoked potentials, even within 1 mm from the probe. Consequently, the data presented here were taken only from experiments using 10% lidocaine. Ludvig et al. (1994) found that 1% lidocaine delivered via microdialysis at a flow rate of 10 μl/min was effective in stopping theta cell firing within 0.5 mm of the probe, whereas we found a decrease in evoked responses only with 10% lidocaine. This difference may reflect the fact that evoked potentials are the summation of the responses in many thousands of neurons, so that higher levels of lidocaine are necessary to block a significant proportion of these neurons. In microinjection studies, it has been found that lidocaine concentrations of 1–4% are necessary to inactivate tissue (Tehovnik and Sommer, 1997); this value is consistent with our results since it is commonly found that microdialysis probe efficacy is around 10% (Morrison et

al., 1991). Theoretical models of microdialysis collection of chemicals that are present in small concentrations in the brain indicate that the extraction ratio across the membrane is inversely proportional to the flow rate (Benveniste and Jansen, 1991). A low flow rate (e.g. 1 μl/min) may therefore be necessary to collect measurable levels in the dialysate. However, with reverse dialysis, the drug concentrations in the probe can be higher so that low flow rates are not necessary. Although we did not study this systematically, flow rates of 2 and 5 μl/min appeared to be equally effective in blocking the evoked potentials; other studies using reverse dialysis have used even higher flow rates (7–10 μl/min; Ludvig et al., 1994; de Lange et al., 1995).

4.2. Time course and effective spread

Following pressure injection of lidocaine, the effective spread has been estimated using a mydriatic response (pupil dilation) to injections near the oculomotor nucleus (Albert and Madryga, 1980) and using electrophysiological measurements of neural activity (Sandkühler et al., 1987; Tehovnik and Sommer, 1997). In general, these studies showed that lidocaine

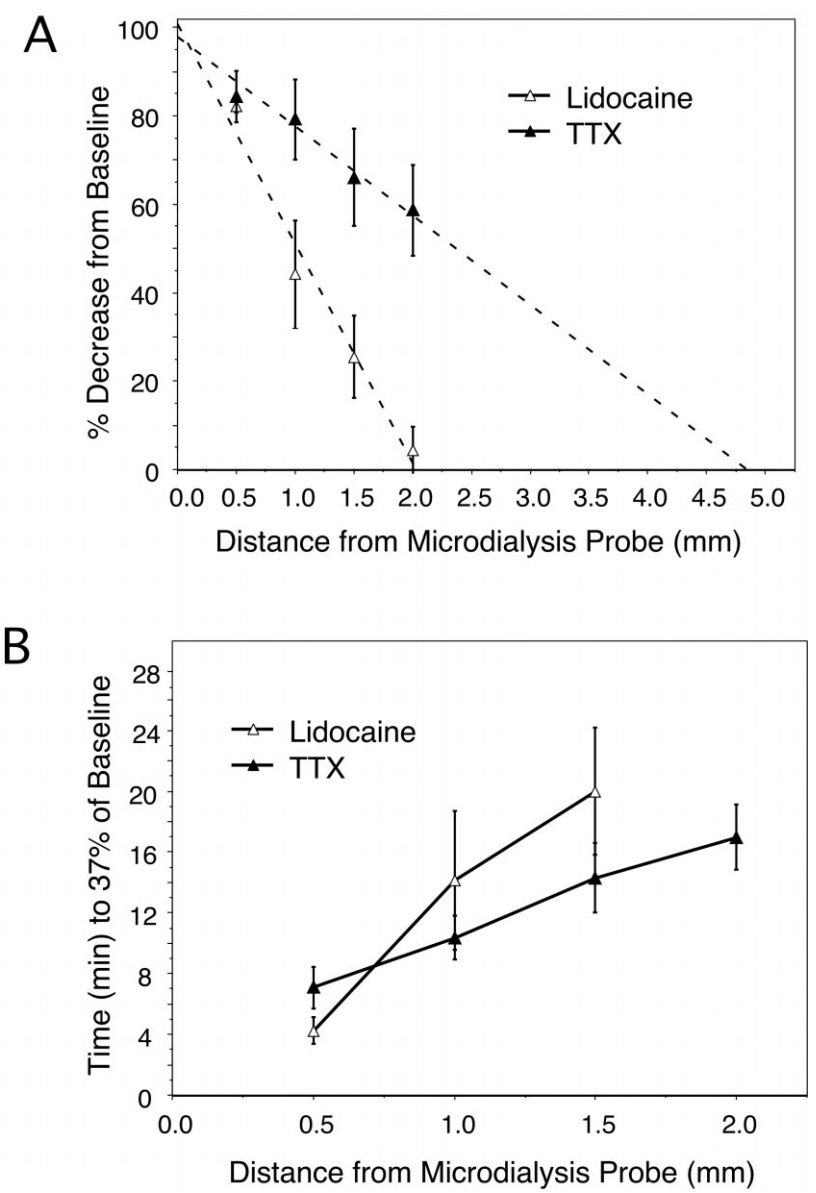

Fig. 4. (A) Mean percentage decrease from baseline of the N1 peak as a function of distance from the probe for the lidocaine ($n = 4$) and TTX ($n = 8$) experiments. Error bars represent the standard error from the mean across experiments. The dotted line is a linear function extrapolated through 100% to estimate the maximum spread (lidocaine, 2.1 mm; TTX, 4.8 mm). (B) Mean time for the N1 response to decrease to 37% of its baseline value as a function of distance from the probe for the lidocaine ($n = 4$) and TTX ($n = 8$) experiments. Error bars represent the standard error of the mean across experiments.

has a fast onset (2–3 min) and recovery (30 min). The spatial extent of the inactivation is dependent on the volume injected; generally, a small injection (4–10 μl) will inactivate a radius of 1 mm, while larger injections (20–30 μl) will inactivate a radius of up to 2 mm (Tehovnik and Sommer, 1997). The effective spread of TTX has received less study. One examination using injections near the Edinger–Westphal nucleus and measuring mydriasis estimated a spherical inactivation of tissue 1.5 mm in radius (Zhuravin and Bures, 1991). Spread of molecules delivered through a microdialysis probe has also been examined using autoradiography of radiolabeled sucrose delivered through a probe. Dykstra et al. (1992) found that spread increased as the duration of infusion increased, with the maximal diffusion being 1.5 mm from the probe. In another study, two probes separated by 1 or 2 mm were used to infuse and detect acetaminophen or atenolol (de Lange et al., 1995). They found that the concentration gradient of the drugs was a linear function of distance from the infusion probe. The applicability of these findings to other drugs is problematic because of differences between molecules in factors such as lipid solubility, capillary permeability and metabolism.

In the present experiment, we used sensory evoked potentials as a measure of function. Since the evoked potential is a summated response from a large population of neurons, decrements in the amplitude of the large initial wave are a good indication of the percentage of neurons within the population that are no longer activated. The definition of 'effective spread' is entirely arbitrary, but the curves presented in Fig. 4A provide a useful indication of the spread. For example, it is apparent that even within 0.5 mm of the probe, neither TTX nor lidocaine produces 100% inactivation. However, lidocaine is much less likely to spread beyond 2 mm, even when it is applied for much longer times than TTX. This property would make lidocaine useful if one wants to inactivate one structure without affecting other nearby structures.

Lidocaine and TTX are both relatively small molecules (mol. wt. 234 and 319, respectively) that bind to different sites on the sodium channel. TTX is noted for its high affinity to the receptor and extremely long 'residency time', estimated to be on the order of 70 s (Hille, 1992). Consistent with this, TTX was able to inactivate a much larger area of the cortex. The fast onset time and rapid recovery with lidocaine are consistent with previous reports using either microdialysis (Ludvig et al., 1994) or microinjection (Tehovnik and Sommer, 1997). Tehovnik and Sommer (1997), for example, found complete recovery of single neuron responses within 30 min. Our data show that this is a function of distance, with recovery within 10 min at 1 mm or further from the probe, but requiring more than 30 min at 0.5 mm (Fig. 2). It is interesting that the effect of lidocaine reached an asymptote while the drug was still being applied (Fig. 2), indicating that an equilibrium was reached with diffusion from the probe equaling removal and/or inactivation of the drug. In contrast, the effect of TTX continued to spread even after the end of perfusion (Fig. 3), indicative of much slower metabolism or removal of TTX compared with lidocaine. Slower removal could also contribute to the long recovery time from TTX, which was just beginning to become obvious at 2 h, in comparison to the almost complete recovery from lidocaine within 40 min. TTX also caused a more complete reduction in the evoked potentials than lidocaine at all distances, even though TTX was applied for much shorter intervals.

In addition to the differences in metabolism and removal, some of the differences between TTX and lidocaine may be due to the relative binding strengths of the two drugs, as TTX binding to the sodium channel is much stronger and longer-lasting than lidocaine binding (Hille, 1992). Sodium channels near the probe would likely be occupied more effectively by TTX than by lidocaine (with less turnover), so that additional TTX molecules diffusing from the probe would be able to diffuse further before finding an unoccupied channel. The short binding time of lidocaine, however, would make the sodium channels near the probe available for occupation by new lidocaine molecules, thereby decreasing the distance over which they would be able to diffuse.

5. Conclusions

1. Lidocaine (10%) produces major (40%) block of neural activity within a 1 mm radius of the probe but may have smaller effects up to 2 mm. TTX (10 μM), in contrast, produces 60% block of neural activity within 2 mm and may affect neurons up to 5 mm from the probe.
2. Lidocaine offers the advantage of rapid reversibility, at the expense of a smaller effective radius. TTX has the advantage of more complete neural blockade over a wider radius than lidocaine, but with very slow recovery.

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