An inexpensive drivable cannulated microelectrode array for simultaneous unit recording and drug infusion in the same brain nucleus of behaving rats

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Running head: Cannulated electrode array for use in behaving rats

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ABSTRACT

Neurons are functionally segregated into discrete populations that perform specific computations. These computations, mediated by neuron-neuron electrochemical signaling, form the neural basis of behavior. Thus, fundamental to a brain-based understanding of behavior is the precise determination of the contribution made by specific neurotransmitters to behaviorally relevant neural activity. To facilitate this understanding, we have developed a cannulated microelectrode array for use in behaving rats that enables simultaneous neural ensemble recordings and local infusion of drugs in the same brain nucleus. The system is inexpensive and easy to use, and produces robust and quantitatively reproducible drug effects on recorded neurons.

KEYWORDS

In vivo electrophysiology, pharmacology, intracranial microinjections
INTRODUCTION

Determining how a neural circuit produces behavior requires two key steps: demonstration that neurons in a circuit encode behaviorally relevant information, and demonstration that disruption or activation of this encoding influences behavior. The first step is accomplished by recording the firing of neurons in behaving animals, but the second step is more complicated. Behavioral pharmacology methods (introduction of biologically active substances, such as neurotransmitter blockers, into specific brain areas of behaving animals) provide powerful means for assessing the contribution of a brain nucleus to specific behaviors, but pharmacological studies are typically performed separately from electrophysiological studies. This means that the connections leading from a neurotransmitter’s action to neuronal firing to behavior are usually established with evidence that is, at best, circumstantial. Thus, neural circuit analysis would benefit from a reliable method for directly determining how receptor activation contributes to neuronal encoding. Here, we describe a technique for localized pharmacological treatment of neurons recorded in behaving rodents.

Reverse microdialysis, iontophoresis and pressure injection are the three most widely used methods for acute in vivo delivery of drugs to discrete brain regions. Each approach offers a unique set of advantages and drawbacks with respect to its use with concurrent electrophysiological recording of the treated neurons. Paired recording and iontophoresis probes have been used in awake behaving animals (Cheer et al. 2007; Haidarliu et al. 1999; Kiyatkin and Rebec 1996a, b, 1997, 1998, 1999a, b, 2000; Sawaguchi 1997; Sawaguchi et al. 1990; West and Woodward 1984; Williams and Goldman-Rakic 1995). These systems use electrical current to eject small quantities of drugs from a multi-barreled glass pipette, which is also used to record neuronal activity. An advantage of this technique is that drug effects are highly localized to the area near the recorded neuron. However, iontophoresis in freely moving rats is technically difficult due to the fragility of the glass pipette and the tendency of the small
diameter tip to become clogged by brain tissue. Furthermore, only one neuron can be recorded at a time, in contrast with paired microdialysis or microinjection techniques in which arrays of electrodes or tetrodes can be used. These technical difficulties substantially limit the rate of data collection with this approach.

In contrast to iontophoresis, reverse microdialysis can be combined with in vivo extracellular unit recordings from multiple electrodes (Boehnke and Rasmusson 2001; Brazhnik et al. 2004; Dudkin et al. 1994; Ludvig et al. 2000; Ludvig et al. 1994; Sakai and Crochet 2000, 2001; van Duuren et al. 2007). In these procedures, drug is circulated to a probe containing a dialysis membrane inserted in the brain. An advantage of reverse microdialysis over pressure injection is that drug molecules diffuse across the dialysis barrier into the brain, thus avoiding volume displacement of tissue by the perfusate (which can cause neurons to be “lost” – i.e., moved away from the electrode so that the firing signal can no longer be detected). However, microdialysis is more difficult than local microinjection because both input and output fluid lines are required, and dialysis membranes can become clogged. The complexity of combined reverse dialysis and recording systems may have limited their widespread use, although several studies have taken advantage of this technique to report local pharmacological effects on behaviorally-relevant firing (Brazhnik et al. 2004; Dudkin et al. 1994; Sakai and Crochet 2000, 2001).

Pressure microinjections have also been used to infuse drugs in close proximity to recording electrodes in awake animals. This method has been used less frequently than iontophoresis or microdialysis, perhaps because of fears that pressure injection would disrupt neuronal recordings by displacing brain tissue. However, the technique has been used successfully to observe effects of injected drugs on dopamine signals measured electrochemically in the prefrontal cortex of awake rats (Doherty and Gratton 1999), and to inactivate (using muscimol injections) the firing activity of cortical neurons recorded with arrays of electrodes (Krupa et al.
These results suggest that pressure injection can be used to treat locally recorded neurons with drugs, but complete details regarding the fabrication and testing of these hybrid systems have either not been reported, or have been reported but not tested (Greger et al. 2007).

If the problem of displacement of neural tissue can be overcome, the pressure injection technique has a major advantage over iontophoresis and microdialysis: microinjections are easy to perform. Here, we report that when appropriate injection rates are used, drug effects are extremely consistent and loss of recorded neurons is minimal. We provide complete methods for the construction of a versatile, inexpensive, light-weight, and easy-to-use microwire electrode array surrounding a central microinjection cannula, including a microdrive that allows chronic implantation in rat or mouse brain. The microdrive provides a stable, simple and inexpensive ($8 per drive) platform for microwire array recordings with or without inclusion of an injection cannula. Construction procedures for the entire system are extremely simple, and yet result in cannulated arrays and microdrives with physical parameters that are highly replicable. We demonstrate that this system results in highly reproducible effects of lidocaine on the firing of neurons in the nucleus accumbens (NAc) of awake, unrestrained rats.

**MATERIALS AND METHODS**

**Microdrive:** We developed a simple microdrive (Fig. 1) that enables movement of chronically implanted electrodes and cannulae in increments of ~80 μm. In addition to off-the-shelf components (Table 1), the drive consists of two acrylonitrile butadiene styrene (ABS) plastic components: the drive stage and drive hub (Fig. 1). These are custom-designed with computer assisted design (CAD) software and manufactured with low-cost, high-precision stereolithography. CAD designs (available in the online supplement) are uploaded to the
manufacturing company (American Precision Prototyping), which produces the components. The hub consists of a block of ABS plastic (11.2 mm in height, 3 mm thick, and 6.95 mm wide) containing two holes for 17 ga hypodermic tubes and a central hole for the drive mechanism (a brass press insert with internal threads, and a stainless steel 0-80 socket-head screw) (Fig. 1). The hub will be secured to the animal’s skull, and one of the 17 ga tubes (the drive tube) will contain the electrodes and microinjection guide cannula. The plastic hub also includes a ring-shaped protrusion (protective collar) surrounding the drive tube. During electrode/cannula implantation, the protective collar will extend just below the level of the dura before the drive is cemented in place; the protective collar thus serves to protect the drive mechanism from the cement. The drive stage (3.175 mm height, 3 mm thick and 6.95 mm wide) is secured to the electrodes and microinjection cannula and can be driven downwards relative to the base and skull (to move the cannula and electrodes through the brain tissue). Both the drive hub and stage are rounded at one end to reduce their bulk, facilitating dual placements in nearby brain regions (e.g., left and right NAc core).

To construct the microdrive, the hole at the bottom of the protective collar (Fig. 1A) is drilled out using a 1.5 mm diameter drill bit. This step is necessary because there is a minimum wall size requirement in the plastic component manufacturing process. Next, a brass insert is pushed into the drive hub where shown in Fig. 1, and a 1.585 cm long 0-80 stainless steel socket cap screw is repeatedly threaded through the brass insert into the drive hub, to ensure that the screw turns smoothly. Next, two 2.5 cm 17 ga hypodermic tubes are inserted into the outer holes of the drive stage and hub. One of these is the drive tube that will contain the electrodes and microinjection guide cannula. The other is the mount tube, which provides additional stability and will be used to hold the array in the stereotaxic instrument during surgical implantation. Finally, the 0-80 screw is threaded through the stage, and a flat washer and two retaining rings are placed around the screw just under the drive hub. The screw is inserted into the hub, and two-ton
epoxy (Devcon, 5 Minute Epoxy) is applied liberally to attach the drive tube, mount tube, flat washer and retaining rings to the drive stage, and to attach the brass insert to the hub (blue stars in Fig. 1). Finally, the drive and mount tubes are cut so that when the drive stage is fully driven towards the hub, the drive tube extends 3-4 mm below the protective collar, and the mount tube is flush with the bottom of the hub.

Jig: To precisely mount the cannula and electrodes in the microdrive, we first construct a jig (a device that holds components in place so that they can be mounted in the microdrive) (Fig.1B). The jig is made from stainless steel hypodermic tubes and can be reused to ensure that the positioning of the probe components is reproduced accurately. To construct the jig, one cannula guide (23 ga hypo tube) and eight electrode guide tubes (31 ga hypo tubes) are cut to a length of 8 mm with a diamond tipped cutting wheel mounted on a rotary tool (e.g., Dremel®). To prevent clogs, a tungsten rod is inserted into each of the electrode guide tubes before they are trimmed to 8 mm. The jig components are placed on a piece of flat wax paper and a thin layer of Krazy glue is applied to the cannula guide. Two electrode guide tubes are then quickly pushed into the glue so that they are positioned flush against the cannula guide. Small amounts of modeling clay can be used as spacers between the remaining electrode guides, which are sequentially arrayed around the central cannula guide. Finally, the jig is embedded in the jig sleeve (17 ga hypodermic tube) using two-ton epoxy.

Microwire electrodes and guide cannulae: We use spooled Teflon-coated tungsten microwire for our neural recordings because of its low cost (Table 2), rigidity and high tensile strength. We tested several types of spooled wire and found that tungsten reliably yields robust, well-isolated single unit recordings. Electrodes are prepared by cutting 8 cm segments of the tungsten wire. Approximately 1 mm of insulation is then stripped from one end of each wire using fine-tipped forceps. The stripped end is clipped to the terminal of an impedance meter using a micro-plunger test lead. The wire is held so it is parallel to the table and cut at a 90° angle with
serrated electrode scissors (Fine Science Tools, 14058-09). The wires yielding the best recordings are cut as flat and quickly as possible. The recording tip is then submerged in 0.9% normal saline and the impedance is measured (1000 Hz, 10 nA) several times to ensure a stable impedance measurement of ~100 kΩ. If necessary, the recording tip is repeatedly cut until the target impedance is achieved.

To prepare the guide cannula, a 27 ga hypodermic tube is cut to a length of 30 mm using a diamond-tipped cutting wheel mounted on a rotary tool and then deburred by twisting a syringe needle inside the tube. During experiments, a 33 ga injector cannula will be inserted into the guide cannula. Preliminary experiments found that a 0.5 mm extension of the injector beyond the tip of the guide cannula (extending into the brain) greatly attenuates neuron loss caused by tissue displacement compared with longer (1-2 mm) extensions. This arrangement results in a ~670 μm distance between the center of the microinjector tip and center of each recording electrode. To achieve a precise and consistent difference in lengths of the injector and guide cannula, injectors are cut by placing the microinjector into a pre-cut 30 mm 27 ga hypodermic tube and grinding it to a length of 30.5 mm with a diamond-tipped cutting wheel. A metal precision ruler is useful to assure the accuracy of the cut. Each injector is deburred by repeated twisting of a 30 ga syringe needle in the injector tube.

Mounting the array in the microdrive: We use two linear translators and a rotation mount to install the array into the microdrive (Fig. 2A), although a stereotaxic instruments with two positioning arms can also be used. The XYZ translator (THORLABS model PT3) holds the microdrive and allows small adjustments to be made in all three dimensions, facilitating precise alignment of the microdrive with the jig. The Z translator (THORLABS model LT1) holds a platform on which the electrodes and microinjection guide cannula rest; this moves only in the z dimension. Finally, the rotation mount (THORLABS model RSP05) holds the jig and facilitates the rotation step (described below).
The screw in the drive is first turned to move the drive stage down towards the drive hub as far as possible. The microdrive is then mounted on the arm of the XYZ translator, and the jig is mounted on the rotation mount and positioned above the platform held by the Z translator (Fig. 2A). Next, a 27 ga cannula, pre-cut to a length of 30 mm, is placed into the cannula guide of the jig, and 8 tungsten microwires (prepared as described above), with recording tips up (away from the platform), are inserted into the jig’s electrode guide tubes (Fig. 2B). The jig is then slowly and carefully rotated 180 degrees, so that the recording tips rest gently on the moveable platform. Next, the electrodes are carefully pushed through the microdrive drive tube (Fig. 2C), and, by raising the platform and lowering the microdrive, the microdrive and platform are brought flush with the top and bottom of the jig, respectively (Fig. 2D). Fine adjustment of the drive’s position using the XYZ translator (precision: 10 μm per gradation) allows the jig to be precisely aligned with the drive tube in the microdrive. To fix the drive tube, cannula and electrodes together, epoxy is liberally applied to the top of the microdrive where indicated by the blue star (Fig. 2D) and allowed to harden for several minutes.

The microdrive is then raised several millimeters, taking care that the microwires and cannula remain in their respective jig guide tubes. A syringe needle is then used to apply several drops of epoxy at the point where the electrodes protrude from the bottom of the microdrive drive tube (Fig. 2E), being careful not to spill epoxy onto the jig or the outside of the drive tube. Typically, the epoxy will be drawn upwards into the drive tube by capillary action; several applications of epoxy assure that the electrodes and microinjection cannula are securely embedded in the drive tube. After the epoxy hardens for ~15 min, the array is removed from the jig and the epoxy is allowed to completely harden overnight. This procedure results in the microwires and cannula held securely in the configuration determined by the jig. The length of the microwires below the protective collar on the drive hub is identical to the length of the jig (8 mm); this length is appropriate to reach targets ranging from the most dorsal (cortex) to ventral (e.g., ventral...
pallidum). Finally, the microwires and a bare silver wire ground electrode are soldered to a connector (e.g., Omnetics) appropriate for the recording system, and the soldered connections are embedded in epoxy.

Animals: Eight male Long-Evan rats (~275-300 g on arrival) were obtained from Charles River and singly housed. One week after their arrival, animals were handled for several minutes daily for 1 week to habituate them to handling. All animal procedures were consistent with the U.S. National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and were approved by the Institutional Animal Care and Use Committee of Albert Einstein College of Medicine.

Surgical implantation: Rats were prepared for surgery and placed in a stereotaxic instrument as described previously (Ambroggi et al. 2008; Ishikawa et al. 2008; Nicola et al. 2004a; Nicola et al. 2004b; Yun et al. 2004). Anesthesia was induced with ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p) and maintained with isoflurane (.5-3%). Animals received 60,000 U/kg penicillin-G antibiotic the evening before surgery, just prior to surgery, and 12 hr after surgery. After surgery, the scalp was treated with Neo-Predef to prevent infection and the animals were allowed one week of recovery before proceeding with experiments. For post-surgery analgesia, animals were given 10 mg/kg of the non-steroidal anti-inflammatory drug Ketoprofen. Cannulated arrays were implanted bilaterally into the dorsal NAc core (1.4 mm anterior and ±1.5 mm lateral from bregma, and 6.5 mm ventral from the skull) using the following procedure. Bone screws are first affixed to the skull, and a craniotomy above the target structure is performed. The dura is then removed. Prior to mounting the microdrive on the moveable arm of the stereotaxic instrument, the stage is driven upwards from the hub such that when the drive hub rests on the top of the skull, the protective collar extends into the craniotomy to just below the surface of the brain and the electrodes are at their intended target. To mount the drive in the
stereotaxic instrument, an 18 ga syringe needle is inserted into a collet electrode holder (Kopf instruments) mounted on a moveable stereotaxic arm. This syringe needle is then inserted into the empty mount tube (Fig. 1A) of the microdrive, which is then aligned so that it is lengthwise in the sagittal plane. A small amount of bone wax can be placed in the bottom of the mount tube to prevent intrusion of dental cement during implantation.

The electrodes and cannula are lowered through the brain to just dorsal to the target structure. The drive is then embedded in acrylic cement, which does not impede movement of the cannula/electrodes because the protective collar surrounds these components above the brain surface. The silver wire is implanted on the posterior cortical surface for use as a ground electrode. The reservoir bulb of a plastic transfer pipette (Fisherbrand, polyethylene, 13-711-7M) is cut from the pipette shaft, placed over the microdrives as a protective cover, and embedded in cement. A small window is cut into the severed bulb to provide access to the microdrive mechanism and cannulae. An electrical connector is also embedded in the cement. Obturators (Plastics One, part #C315IDC), which are pre-trimmed to be flush with the end of the cannula, are inserted into cannulae at the end of surgery.

**Configuration of the recording/injection system:** Our system allows injections of drugs without interruption of the animal's behavior, as well as free movement of the animal unimpeded by the recording cable and fluid lines. A syringe pump (KD Scientific model 200) that accommodates two syringes is mounted outside the operant chamber. The fluid lines from the syringes are connected to a double-barreled fluid swivel, and fluid lines from the swivel are passed through the bore hole of a 24 channel slip ring commutator (Fig. 3, Table 2), along the recording cable to the animal's head. The recording cable is purchased with a protective metal coil (Plexon, Inc., HSC/165o-G1-R00-L2A1B-2), which is then wrapped in Silastic tubing and secured with electrical tape and miniature cable ties. This arrangement provides rigidity to prevent twisting of the recording cable when it is attached to the connectors embedded in acrylic cement on the
animal's head. The recording cable terminates in a male DB25 connector, which connects to its female counterpart attached to the commutator (Fig. 3). To attach the DB25 connector to the commutator, the cable hole of the DB25 casing is widened to 1.25 cm using a drill press. Then, a 2.5 cm lengthwise window is cut in a 1.25 cm diameter, 5.4 cm hollow link tube, which is inserted 1 cm into the neck of the DB25 case. A hole is drilled through both the DB25 case and the link tube, which are then secured to one another with a screw embedded in two-ton epoxy.

To mount this assembly on the commutator, the link tube is inserted into the commutator bore hole, so that it protrudes 2 cm above the top lip of the commutator. This link tube is then attached to the commutator lip using a shaft collar. Next, a screw is inserted through the shaft collar above the level of the commutator. Before the nut of this screw is tightened, a stiff piece of wire is wrapped several times around the screw and is connected to the spring tether mount on the fluid swivel. Then the nut is tightened. This arrangement creates strong direct coupling between the counterbalance arm, commutator and fluid swivel, so that they rotate together as the animal moves about the chamber.

The recording cable is attached to a counterbalance arm, which is constructed from a 23 cm length of 8 ga hypodermic tube suspended with a wire hinge from the DB25 connector on the commutator (Fig. 3). Small round spacers are placed at the bend of the hinge to prevent shifting of the counterbalance arm relative to the hinge. A 10 cm window is cut into the counterbalance arm, which allows the recording cable to fit securely inside. This arrangement strongly tethers the recording cable to the arm. Stainless steel nuts are attached to the other end of the arm and secured with shaft collars so that the arm rests horizontally with respect to the ground. This configuration minimizes the weight on the head of the rat and provides sufficient flexibility to allow natural movements of the animal during recording sessions. The animal's lateral and vertical movements are translated into opposing movements of the counterbalance arm, which take up or release slack in the recording cable and fluid lines to allow the animal free
movement. The rat’s rotational movement is efficiently transmitted by the fairly rigid recording cable to the counterbalance arm, freely turning the commutator and fluid swivel.

**Neural recording and lidocaine injections:** After 1 week of post-surgical recovery, animals received microinjections every other day. On test days, the fluid lines were securely taped to both the counterbalance arm and recording cable. Both the fluid lines and microinjectors were filled with mineral oil prior to drawing up the drug solution, and the level of the oil-aqueous interface was marked to enable post-hoc confirmation that the drug was injected. To prepare animals for neural recording and injection, obturators were removed and the recording cable was plugged into the electrical connector on the animal. The animal was gently restrained while the microinjectors (loaded with drug) were inserted into the animal’s guide cannulae.

We recorded pre-injection baseline firing rates lasting 2-20 min, after which the syringe pump was activated either manually or by a programmed electronic signal. Flow rates were optimized in pilot studies prior to these experiments. We found that flows > 0.06 μl/min resulted in a low yield of neurons in subsequent more ventral recordings, and that this problem was eliminated by slowing the rate of injection. Therefore, we used a flow rate of 0.046 μl/min in these experiments. Over 12 min, we injected 0.55 μl of freshly prepared 5% lidocaine hydrobromide monohydrate (Sigma-Aldrich) dissolved in 0.9% normal saline, or saline alone as a control. Recordings continued for 30-45 min after the injection so we could assess drug effects and recovery. After the experiment, the microdrive carrying the electrode arrays was advanced ~160 μm in order to obtain a new set of neurons for the next experiment two days later.

We recorded from 181 neurons in 36 recording/injection sessions. The voltage signals were amplified and filtered, and neural spikes detected and sorted, as previously described (Ambroggi et al. 2008; Nicola et al. 2004a; Nicola et al. 2004b; Yun et al. 2004). Discrimination of single units was performed with Offline Sorter (Plexon, Inc., Dallas, Texas) using principal
component analysis of spike waveforms. Units with spike amplitudes less than 75 μV were discarded because they cannot be reliably separated from the noise levels (~25 μV). Interspike interval distributions, autocorrelograms and cross-correlograms were used to verify isolation of single units.

Analysis of neural data: Timestamps of verified spikes were analyzed with custom routines in the R software environment (R.Development.Core.Team 2005). To determine if a neuron was affected by lidocaine or saline, each neuron’s spike timestamps were divided into 10 sec bins, and the Poisson probability distribution function (PDF) was calculated for the baseline period prior to injection of lidocaine or saline. A neuron was considered affected by a treatment if it exhibited 6 consecutive bins (60 sec) in which the firing rate was below the lower limit of the 99% confidence interval of the mean baseline firing rate.

To determine the latency of lidocaine effects across the electrode array, 1 sec bins were used to calculate the PDF of the pre-injection baseline period. Because smaller bins were used than for the previous analysis, the lower tail of the 99% confidence interval of the baseline firing rate spanned zero in some neurons (i.e., those with very low firing rates). These 39 neurons (out of 100 affected by lidocaine) were discarded for the latency analysis. The time of onset of the drug effect was determined as the first bin of at least 60 consecutive bins with spike counts below the lower 99% confidence interval of the baseline period. To analyze the variability in latency to onset of lidocaine’s effects across neurons recorded in individual sessions, we determined which neuron in each session first demonstrated a lidocaine-induced reduction in firing rate (as defined above) and then calculated the latency between this neuron’s drug onset time and those of the other neurons recorded from the same hemisphere.

To determine the fraction of neurons that recovered from the effects of lidocaine, 1 min bins were constructed and the spike count for the last complete bin in each session was calculated
as a percent of the mean spike count during the pre-injection baseline period. Time to recovery
from lidocaine treatment was determined by isolating the time at which the firing rate of affected
neurons returned to a value greater than the lower limit of the 99% confidence interval.

RESULTS

To characterize the effectiveness of the cannulated array system, we performed a series of
microinjections of the Na⁺ channel blocker lidocaine into the NAc core to determine flow rates
and volumes that produce efficient diffusion of drugs to recorded neurons. We chose lidocaine
because recovery from its effects tends to be rapid (Hille 1966; Sandkuhler et al. 1987;
Tehovnik and Sommer 1997), enabling us to differentiate drug effects from displacement of the
neuron from the recording electrode. We determined that 0.55 μl of 5% lidocaine dissolved in
saline, injected over 12 min, resulted in reductions in firing rate that were temporally and
quantitatively similar across neurons recorded from electrodes surrounding the injection site.

Fig. 4A shows an example of this similarity. Lidocaine injection reduced the firing of 4 neurons
simultaneously recorded on different electrodes to ~0 Hz, whereas saline injection into the same
hemisphere (on a different day) had no effect (Fig. 4B). The long interval between injection and
drug effects is explained by the extremely slow injection rate; similar volumes are injected over
2 min in typical behavioral pharmacology experiments (Ambroggi et al. 2008; Delfs et al. 2000;
Mueller et al. 2008; Nicola 2010; St Onge and Floresco 2010).

Consistent with lidocaine’s blockade of Na⁺ channels, the waveforms of recorded neurons
changed just prior to the lidocaine-induced elimination of spiking (Fig. 4C). Both the peak
amplitude and initial slope of the waveforms decreased, and recovered when firing rate returned
to baseline. In contrast, saline injections caused no observable changes in waveform shape
(Fig. 4D). Effects of lidocaine similar to those described in Fig. 4A (firing rate reduced below the
99% confidence interval of the baseline for ≥ 60 sec) were observed in 100 out of 105 neurons (95.2%) during 19 simultaneous recording and lidocaine injection sessions (Fig. 4E). Of these 100 neurons, 68 recovered to within the 95% confidence interval of their pre-injection baseline firing rates computed from 1 min bins (Fig. 4F). Only 15 of the 100 neurons failed to demonstrate any appreciable recovery (remaining at 0 – 10% of baseline by the end of the recording session). Excluding these 15 neurons, there was no significant difference between the mean firing rate of affected neurons during the pre-injection baseline (mean±SEM: 4.4 ± 0.44 Hz) and the recovery period (4.3 ± 0.4 Hz) (t_{84}=0.16, p=0.6, n=85). On average, lidocaine effects lasted 11.5±1.4 min (n=70 neurons). In contrast, saline injections reduced the firing of only 6 of 76 neurons (7.9%) recorded during 17 injections (Fig. 4E). These results demonstrate that the recording/microinjection system can be used to obtain robust and reliable drug effects on the vast majority of neurons recorded during the injection.

Prior to experiments, the microinjectors (pre-loaded with drug) were inserted into the cannulae before the acquisition of a neural baseline. A major advantage of this approach is that it is unnecessary to unplug the animal to perform an injection. Consequently, neural activity can be continuously monitored before, during and after injection of drugs. However, one potential concern is that lidocaine might leak out of the microinjector prior to activation of the infusion pump, thus preventing the acquisition of a drug-free baseline. Such leakage is unlikely because the fluid system is closed to gravity. Nevertheless, to determine whether lidocaine leaked from the microinjectors prior to activation of the pump, we examined the pre-injection firing rates as a percentage of the average firing rate of the entire baseline period for all 100 neurons that were affected by lidocaine (Fig. 5A). The baselines were very stable over the entire pre-injection period, which ranged from 2-20 min (baselines of at least 15 min were obtained prior to 13 of 19 injections). The lack of a trend towards a reduction in firing rate over time in any neurons during
the baseline strongly suggests that, if lidocaine leaked from the microinjector during this period, it did so in quantities insufficient to affect neuronal firing.

In many of our initial experiments, the injection pump was started and stopped manually and therefore we do not have precise time stamps for these events. Consequently, we chose a subset of later experiments, conducted with precise electronically-determined injection onset and offset times, to obtain an estimate of the time course of the onset of the lidocaine effect. In these experiments, a 10 min neural firing baseline was obtained, after which the animal was injected with lidocaine or saline. All 12 neurons exposed to lidocaine in 2 injections exhibited a sharp reduction in firing rate that dropped to 0 Hz between 13 and 14 min after the end of the injection, and 8 of these neurons either fully or partially recovered from the injection (Fig. 5B, black traces). There was no effect on any of the 14 neurons exposed to saline in 2 injections (Fig. 5B, gray traces).

One advantage of our method is that the electrodes are all equidistant from the cannula, so that the time course of the effects of an injected drug should be similar across all recorded neurons. To determine whether lidocaine did in fact reach sampled neurons at the same time, we examined the variability in the latency of lidocaine’s effects on firing rate. Within each recording session, the neuron that first showed a lidocaine effect was identified, and the difference in latency to a lidocaine effect relative to this reference neuron’s latency was calculated for all other neurons recorded in the same hemisphere. (During 4 sessions, we recorded from only one neuron, and so we were unable to calculate latency differences for these experiments. In addition, 39 out of 100 neurons affected by lidocaine had 99% confidence intervals spanning zero when spike counts were binned at 1 second, and thus were not included in this analysis.) The calculated latency differences were at most 6 sec (Fig. 5C). We categorized these latencies according to whether they were taken from neurons recorded on the same or different electrode than the reference neuron. There was no statistically significant difference in the latencies
between these two groups (t test, p = 0.43), and all 42 calculated latency differences (across 15 injections) were < 7 sec. These results indicate that the circular design of the array facilitates temporally uniform diffusion of injected drugs to neurons recorded on all electrodes, with very little variability in the latency to drug onset.

**DISCUSSION**

This study presents evidence that our novel drivable cannulated electrode array can be used to obtain robust and repeatable effects of drugs injected directly into the vicinity of neurons recorded in freely moving rats. After lidocaine injection into the NAc core, the vast majority of the recorded NAc neurons typically ceased firing entirely, and the majority of the affected neurons (68%) recovered to within the 95% confidence interval of their baseline firing rates. These drug effects were evident ~13.5 min after the end of the injection, they lasted 11.5 min on average, and the onset of the effect was nearly simultaneous in all neurons recorded in the same hemisphere during a given injection. Furthermore, saline injections using identical parameters had no effect on the vast majority of NAc neurons. These results demonstrate that the approach we describe offers a reliable method for the study of the action of pharmacological agents on neural activity in freely moving rats.

The diffusion time course and functional spread of intracranially injected lidocaine have been investigated in great detail in restrained or anesthetized monkeys, cats, raccoons, and rats (Boehnke and Rasmusson 2001; Martin 1991; Martin and Ghez 1999; Sandkuhler and Gebhart 1984; Tehovnik and Sommer 1997). In rats, injection of 0.5 μl of 4% lidocaine reduces the glucose metabolism of neurons at distances of up to 1.8 mm from the injection site (Martin 1991); the distance between our electrodes tips and the injection site (670 μm) is well within this range. Electrophysiological studies show that the strongest effects of 0.5 μl lidocaine injected in
the rat brain extend spherically 500 μm from the injector, and that lesser but significant effects are observed up to 1 mm from the injection site (Sandkuhler and Gebhart 1984; Tehovnik and Sommer 1997). Although our electrodes were farther from the injector than 500 μm, they were also dorsal to the injection site, and our injections were slow. Injection at lower rates is more likely to cause diffusion dorsally (towards the lower pressure area around the injector shaft) prior to outward diffusion (James and Starr 1978; Myers and Hoch 1978). Thus, the nearly complete shutdown of neural activity we observed after lidocaine injection in behaving animals is consistent with the spatial characteristics of lidocaine diffusion previously observed by others in anesthetized or restrained preparations.

The temporal characteristics of the lidocaine effects in the present work are also consistent with previous studies, which observed a 5-10 min interval to onset of firing effects after rapid injections of large volumes of lidocaine (Levy et al. 2001; Martin and Ghez 1999; Sandkuhler and Gebhart 1984; Sandkuhler et al. 1987; Tehovnik and Sommer 1997). The latency to effect was longer in our experiments (~13.5 min), but this is likely due to the slower injection rates we used. The long latency to lidocaine effects in this and previous studies is likely attributable to variables such as the low affinity of lidocaine for Na⁺ channels and the requirement that lidocaine molecules diffuse significant distances through the extracellular matrix to reach the point where their action can be observed (the soma of the recorded neurons).

Although we used lidocaine for functional tests of our system, our results suggest that other drugs, such as specific receptor blockers, could also be injected. A potential source of concern is the finding that 32% of neurons affected by lidocaine did not recover to within the 95% confidence interval of baseline firing rate, with 15% showing no recovery at all (Fig. 4F). One possibility is that the pressure injections caused displacement of neural tissue away from the electrodes, such that their firing could no longer be observed even after wash-out of the drug. Arguing strongly against this idea is that saline injections reduced the firing rate of very few
neurons (<7%). A more compelling explanation for the failure of recovery from lidocaine injections is that lidocaine is somewhat toxic (Gold et al. 1998; Kanai et al. 1998; Ready et al. 1985). Therefore, injection of less toxic drugs using this system is likely to produce a higher proportion of neurons exhibiting complete recovery of drug effects.

The use of drugs other than lidocaine with our system is likely to require some adjustment in injection parameters. Total injection volume, size of the drug molecule, and injection rate are the major factors that determine the spatial extent of drug diffusion in brain tissue (Nicholson 1985; Sykova and Nicholson 2008). In pilot experiments, we optimized the injection rate to minimize neuron loss and maximize lidocaine effects. A relatively narrow range of flow rates yielded reliable results: with fast injections (0.55 μl in < 8 minutes), a significant number of recorded neurons was lost; with very slow injections (0.55 μl in > 16 minutes), there was no discernible lidocaine effect on recorded neurons (not shown). Injection rates slower than 0.55 μl in 8 min increased the yield of neurons in subsequent experiments (after driving the electrodes and cannula ventrally). This increased yield is likely reflective of a decrease in damage due to distension of brain tissue after rapid introduction of a relatively large volume of perfusate (Demer and Robinson 1982). Thus, injection rates of ~0.046 μl/min appear to be optimal to minimize tissue damage and maximize drug effects. Using this injection rate, the drug concentration and length of injection should be empirically determined to obtain injection parameters for other drugs of interest. In addition, it should be possible to obtain drug effects more prolonged than the 11.5 min observed in our experiments simply by increasing the volume of injection while maintaining a slow injection rate, as observed by others using lidocaine (Tehovnik and Sommer 1997).

The cannulated array described here offers several advantages over existing means for local drug treatment of neurons recorded in behaving animals. Microinjection is a relatively simple
procedure compared with microdialysis and iontophoresis, and it requires little specialized
equipment that is not standard in a laboratory that performs multielectrode recordings in
behaving rats. Indeed, a major advantage over iontophoresis is that the present technique
utilizes multiple electrodes to maximize the experimental yield. Although a previous study
employed pressure injections lateral to a linear array of electrodes in the brains of awake rats
(Krupa et al. 1999), our configuration situates the electrodes equidistant from the injection
cannula, resulting in nearly simultaneous drug effects at all recorded neurons. Furthermore, our
design incorporates a microdrive so that new neuronal populations can be repeatedly sampled
in the same animal. Importantly, our design results in physical parameters (e.g., distances
between electrodes and cannula; drive dimensions) that are highly reproducible across
constructed systems, in part because of the use of a jig to position the electrodes and cannula,
and in part because of the use of precision stereolithography to produce custom-designed
plastic parts for the microdrive. Because the jig and drive components are inexpensive, our
system minimizes cost while maximizing consistency and ease of construction and use. Its
success with lidocaine injections in the NAc suggests that it could be used in a variety of brain
structures to inject many different drugs. Moreover, because of its light weight, the system is
suitable for use in mice and other small animals. Finally, with slight modification, the central
cannula could also accommodate a fiber optic cable for light activation of neurons expressing
light-sensitive ion channels.

In summary, we developed a novel drivable cannulated electrode array that facilitates robust
and quantitatively reproducible drug effects on ensembles of single neurons recorded with
multiple electrodes in the brain of awake, unrestrained rats. Lidocaine injection experiments
suggest that it could also be used to deliver agonists and antagonists of neurotransmitter
receptors, providing a reliable means to study the contribution of neurotransmitters to
behaviorally relevant neural activity.
ACKNOWLEDGEMENTS

We are grateful to Vince McGinty, Sylvie Lardeux and Sara Morrison for helpful discussions and comments on the manuscript. This work was supported by NIH grant DA019473 and by grants from the Peter F. McManus Charitable Trust, the Klarman Family Foundation and NARSAD.

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Kiyatkin EA and Rebec GV. Striatal neuronal activity and responsiveness to dopamine and glutamate after selective blockade of D1 and D2 dopamine receptors in freely moving rats. *J Neurosci* 19: 3594-3609, 1999b.


Nicola SM, Yun IA, Wakabayashi KT and Fields HL. Firing of nucleus accumbens neurons during the consummatory phase of a discriminative stimulus task depends on previous reward predictive cues. *J Neurophysiol* 91: 1866-1882, 2004b.


FIGURE LEGENDS

Figure 1. The microdrive and jig. A. A schematic of the microdrive. The microdrive consists of two plastic components: the drive stage and drive hub. A 0-80 screw threaded through the brass insert (in the drive hub) constitutes the drive mechanism. The electrodes and microinjection guide cannula will be embedded in the drive tube of the finished microdrive. The drive and mount tubes extend through holes in the stage and hub. These tubes are attached to the stage, and to lower the electrodes and microinjection cannula through the brain, the stage is lowered by turning the 0-80 screw (80 μm per ¼ turn). During microdrive construction, epoxy is applied where indicated by blue stars. B. A schematic of the jig that enables precise construction of the cannulated array. The electrodes and cannula are placed into their respective guide tubes so that they can be mounted in the microdrive. The distance from the center of the cannula guide to the center of each electrode guide is 0.45 mm. To clearly depict the configuration of the cannula and electrode guides, the top portion of the jig sleeve is not shown; all tubes that comprise the jig and sleeve are the same length and flush with each other.

Figure 2. Mounting the cannula and electrodes into the microdrive. A. Photo of the array fabrication device. The XYZ linear translator moves an arm that holds the microdrive. The Z linear translator holds a platform, on which the cannula and microwires rest while they are mounted in the microdrive. The jig is held by a rotation mount so that it can be turned 180 degrees. The rotation step protects the recording tips of the microwires from damage during placement in the electrode guide tubes of the jig. B. The cannula and 8 microelectrodes (recording tips up) are carefully placed into the guide tubes of the jig. C. The electrodes are threaded through the drive tube in the microdrive. D. The microdrive and platform are then moved until they are flush with the top and bottom of the jig, respectively, and epoxy is applied where indicated by the blue star. E. The microdrive is retracted several millimeters from the jig.
(with the microwires and cannula still in their guide tubes) and epoxy is applied where indicated by the blue star.

**Figure 3.** System for uninterrupted injection/recording experiments. **A.** Schematic of the custom assembly that permits online injections of drugs without interrupting neural recordings. All major components are labeled. The entire assembly is mounted on top of an operant chamber so that the fluid swivel (which is attached to an infusion pump) is above the top of the chamber while the commutator, counterbalance arm and recording cable (attached to the connectors on the animal’s head) are inside the operant chamber. Fluid lines are attached to the fluid swivel, passed through the bore hole in the commutator and taped to the recording cable. These fluid lines terminate at 33 ga microinjectors at the animal’s head.

**Figure 4.** Simultaneous recording with lidocaine or saline injections. **A.** Injection of lidocaine into the NAc inhibits firing in 4 simultaneously recorded NAc neurons. **B.** Injection of saline has no effect on 4 simultaneously recorded NAc neurons. **C.** Extracellularly recorded action potential waveforms from 2 example neurons recorded during the pre-injection baseline (left), just prior to abolition of firing by lidocaine (middle), and during recovery from lidocaine’s effects (right). **D.** Example action potential waveforms from 2 neurons before, during and after saline injections. **E.** Proportions of all neurons significantly affected and unaffected by lidocaine and saline injections. The criterion for injection effect was that 6 consecutive 10 sec bins exhibited spike counts that were below the 99% confidence interval of the baseline firing rate. **F.** Recovery was complete in most neurons. Graph shows the distribution of firing rates in the last recorded 1 min bin normalized to the pre-injection baseline for all 100 neurons affected by lidocaine. Dashed grey lines encompass those neurons that recovered to within the 95% confidence interval of their pre-injection baseline firing rates (computed from 1 min bins).
**Figure 5.** Pre-injection baseline and time course of the onset of lidocaine effects. 

**A.** Pre-injection baseline (1 min bins) presented as percent of average firing rate of total baseline period for all 100 neurons affected by lidocaine in 19 injections. Absence of a downward trend in firing rate across time in any neuron is evidence that lidocaine did not leak from the injector during the baseline period. **B.** Precise determination of the time course of the onset of lidocaine effects on firing of neurons. The black arrow indicates the end of the lidocaine infusion. The traces represent the firing rates of single neurons as a percent of the average pre-injection baseline firing rate of that neuron prior to injection of either lidocaine (black traces, n=12 in 2 injections) or saline (gray traces, n=14 in 2 injections). **C.** Variability in onset latency of lidocaine effects across simultaneously recorded neurons is low. Graph shows the distribution of the onset latency of lidocaine effects relative to the first affected neuron in each hemisphere. Neurons that were recorded on the same (gray) or different (black) electrode from the reference neuron are compared.
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Hub Stage 17 ga jig sleeve
23 ga cannula guide 0.5 mm 0.127 mm
Protective collar
0-80 screw, 25.4 mm
Brass insert, 3.175 mm
17 g tube/mount tube 11.2 mm 3.175 mm
Drive Tube

Fig. 1

A
Stage Retaining ring Drive Tube
Hub 3 mm
Protective collar
Drive Tube

B
23 ga cannula guide
0.127 mm
0.5 mm
31 ga electrode guide
17 ga jig sleeve
Fig. 4

A. Firing Rate (Hz) vs. Time (Minutes) for Lidocaine vs. Saline.

B. Percent of neurons affected by Lidocaine vs. Saline.

C. Recovery firing rate (Percent of baseline) for Baseline, Lidocaine, and Recovery.

D. Baseline, Saline, and Recovery waveforms.

E. Percentage of neurons affected and unaffected by Lidocaine and Saline.

F. Distribution of recovery firing rates with 95% confidence interval.
Fig. 5

A

B

C

Latency difference (sec)

Percent of neurons

Across wire

Same wire

n=21

n=9

n=6

n=3

n=2

n=1

0-1 1-2 2-3 5-6

Latency difference (sec)