Chapter 2
Silicon Probe Techniques for Large-Scale Multiunit Recording

Hendrik W. Steenland and Bruce L. McNaughton

Abstract Chapter 1 laid the groundwork for extracellular electrophysiology and the history of microelectrode and microdrive development. However, one of the most technically advanced areas of electrode fabrication is found in the microprobe (e.g., silicon probes) industry where nanoscale fabrication techniques are used to increase recorded neuron yield. To date, these probes have the highest number of contacts per probe and can be combined with integrated circuits, optogenetic control, and drug delivery. This chapter will review a history of development in this field, emphasizing technical advances and what it means for the investigation of neurons.

Keywords Silicon probes • High-density electrophysiology • Integrated circuits

Introduction

If one were to reverse engineer any piece of foreign electronics by blindly picking through each piece of hardware for which there was no part number, this task would be nearly impossible. The same situation exists when trying to understand the hardware of the brain. Given the incredible number of cells in the brain, in combination with the enormous number of synapses which change their connection weights, it becomes clear that the goal of reverse engineering even 1 mm^3 of brain tissue is a daunting task. Microelectrode technology can record multiple neurons simultaneously and extract general themes or patterns of brain function, but it is a far cry from reverse engineering. Moreover, the shaft of a standard microelectrode does not have any contacts for which to pick up neural activity; rather neural activity is only detected at the tip. Silicon microprobes represent one attempt to increase the detection of the number of neurons, without compromising the size of the probe. This chapter will focus on the development of varieties of silicon microprobes and their use in neuroscience.

H.W. Steenland, Ph.D. • B.L. McNaughton, Ph.D. (✉)
Department of Neuroscience, University of Lethbridge,
4401 University Drive W, Lethbridge, AB, Canada, TlK3M4
e-mail: hendrik.steenland@uleth.ca; bruce.mcnaughton@uleth.ca

M. Tatsuno (ed.), *Analysis and Modeling of Coordinated Multi-neuronal Activity*, Springer Series in Computational Neuroscience 12,
DOI 10.1007/978-1-4939-1969-7_2

The Passive Silicon Probe

In the 1970s, Wise et al. [1] united the silicon chip industry with neuroscience through the production of the first in vivo silicon probe. Figure 2.1a, b shows an illustration of their silicon probe. This microprobe was considered a passive probe since it did not contain any powered electronic circuitry for amplifying or filtering collected data. Many years have passed since this time, and microprobes have advanced considerably. A more recent passive probe designed by Kindlundh et al. [2] is depicted in Fig. 2.1c. In this case however, the electrode contacts can be seen as many small, recessed windows, populating the shaft of the probe.

Before the advantages of silicon probes are discussed, the basic process of silicon probe production will be outlined. The fabrication process typically consists of four steps: substrate preparation, metallization, insulation, and finishing. Substrate preparation often includes applying a photoresist, to protect regions of the silicon which will define the shape of the electrode. The silicon can then be etched around this protected region forming a mesa (raised region). The metallization step consists of plating a metal (e.g., gold) onto silicon substrate. The insulation step in the case of Wise [1] consisted of the deposition of silicon dioxide (glass) over the metal contact; however, many different types of insulation may be used. Electrical contacts can be exposed by selective removal of insulation from regions of the probe using a photoresist in combination with etching. The finishing procedure consists of removing the probe from the rest of the silicon wafer using an etchant process and connecting the probe to output wires. These processes are constantly under refinement, and the reader is referred to a more extensive review [3].

It can readily be appreciated that there are several fundamental advantages that the silicon microprobes have over classical microelectrodes. (1) For example, there is a high degree of reproducibility of these microprobes and their construction can be efficient if the appropriate process hurdles have been overcome. (2) The packing density of electrode contacts per given volume is well above that of traditional microelectrodes. (3) The microprobes have reproducible recording impedances compared to microelectrodes. This means that rejecting common mode signals, via referencing to one of the channels in the probe, is advantageous for the removal of biological, mechanical, and stimulus artifacts [4]. (4) The geometry of the circuit is known, and so it is easy to decipher where the electrode contact is in the brain, relative to the other contacts. (5) The use of silicon substrates makes it possible to incorporate integrated circuits for amplification, filtering and multiplexing into the microprobe (known as an active microprobe, see below). (6) The precision of the spacing of a microprobe offers the advantage of recording across different layers of the cortex, which can provide insights into the origin and propagation of seizure and brain waves [5]. (7) The silicon substrate can act as a ground plane when it is placed in the brain to reduce crosstalk between adjacent channels [1]. (8) So little metal is used in microprobe fabrication techniques, so shunt capacitance is small relative to a standard microelectrode [1]. (9) The ability to detect many neurons increases the probability that one can detect monosynaptic connections between neurons

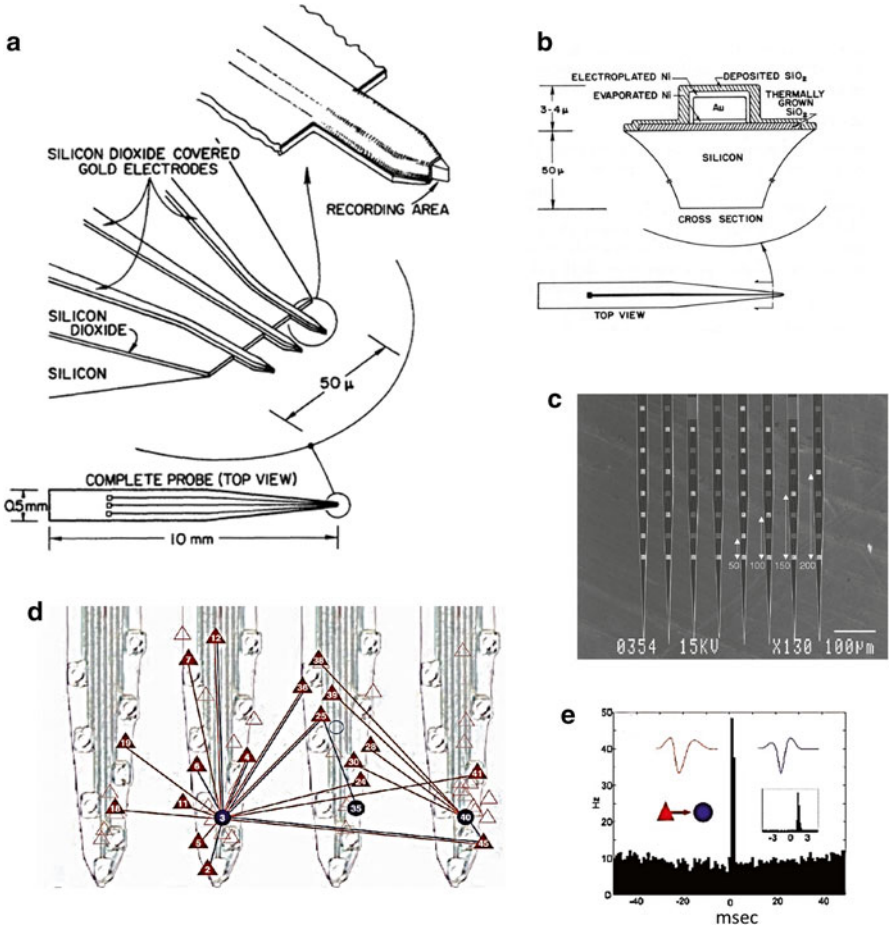

Fig. 2.1 The first passive silicon probe for in vivo spike recording. (**a**) A silicon multielectrode with three recording contacts and insulated with silicon dioxide. The exposed recording area/tip can be seen peeking out from under the silicon dioxide. © 1970 IEEE. Reprinted, with permission, from Wise KD, Angell JB, Starr A. An integrated-circuit approach to extracellular microelectrodes. IEEE Transactions on Bio-Medical Engineering. 1970;17(3):238–47. (**b**) Cross section through silicon probe, depicting the various layers including: nickel (Ni), silicon dioxide (SiO_2), gold (Au), and silicon. © 1970 IEEE. Reprinted, with permission, from Wise KD, Angell JB, Starr A. An integrated-circuit approach to extracellular microelectrodes. IEEE Transactions on Bio-Medical Engineering. 1970;17(3):238–47. (**c**) Scanning electron micrograph of a 64-site probe where selected windows have been open to make contacts (*bright squares*). Reprinted from Sensors and Actuators, 102(1), Kindlundh M, Peter N, Hofmann UG, A neural probe process enabling variable electrode configurations, 51–8, Copyright 2004, with permission from Elsevier. (**d**) Detecting functional connections with silicon probes. *Filled red triangles* are pyramidal neurons, and *filled blue circles* are inhibitory neurons. *Red lines* indicate monosynaptic excitation, and *blue lines* represent monosynaptic inhibitions. Connections were detected by cross-correlation analysis (not shown). Reproduced with permission from Bartho P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsaki G. Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. Journal of Neurophysiology. 2004;92(1):600–8. (**e**) Example of monosynaptic excitatory connection as calculated with cross-correlation analysis. *Red triangle* represents a putative pyramidal cell, while the *blue circle* represents a putative interneuron. Reproduced with permission from Bartho P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsaki G. Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. Journal of Neurophysiology. 2004;92(1):600–8

(Fig. 2.1d, e) [6]. (10) Finally, the extracellular potential conduction can be recorded along a neuron from the dendritic tree to the axon hillock, because of the precise spatial distribution of the electrode sites.

One of the major problems with the passive electrode is attaching a sufficient number of leads so that all the recording channels can be sampled. In addition, as the number of leads increases so will the difficulty of having such probes float in the brain. Multiplexing is an excellent means to reduce the number of data lines connected to an active microprobe (see below). A few other requirements should be mentioned for the design of an ideal microprobe. Firstly, tissue displacement from an electrode array must be small enough to avoid disrupting the physiological system under study [7]. In addition, the height of the probe shank above the cortex needs to be kept to a minimum (<1 mm, in primates) so that it can float with the brain tissue [7]. Finally, given the small size of neurons, the minimal placement of electrode contacts on a silicon probe should be between 50 and 100 μm.

The Active Silicon Probe

Passive microprobes are probes that are similar to microelectrodes in that they simply act as channels to take information from the brain and relay it to integrated circuits. By contrast, active microprobes include dedicated integrated circuits for processing information as it is detected from the brain [8]. To explain how this is possible, we will take a step back and explain what makes silicon substrates special.

Silicon is a semiconductor, meaning that under particular conditions, electrical conduction through this material can be increased. This is the fundamental property that makes the modern transistor possible. When the atomic lattice of a silicon wafer is bombarded with ions such as boron, the electrical conductivity of the chip can be biased, changing the composition of the silicon wafer into a semiconductor. By applying voltage to semiconducting silicon, one can change the flow of current, sort of like controlling the flow of water by altering the size of a bottleneck. Largely, this is what the transistor does, with the exception that electrical current is controlled. Connections between these transistors can then be laid down in combination with resistive and capacitive components to make an integrated circuit on the silicon wafer. The fundamental benefit to silicon probe manufacture is that buffering, amplification, filtering, multiplexing, and wireless transmission can all be carried out on the probe using these components. An example of this active silicon technology including many of these components can be seen in Fig. 2.2.

Two major issues tend to influence the active silicon probe. The first is providing sufficient encapsulation for the integrated circuit, so that it is protected from fluid. The second issue is the amount of heat dissipated from the microprobe to the surrounding tissue. Temperature must be kept to a minimum so that it does not lesion surrounding areas [8]. Powering active probes can cause heat dissipation, and according to Wise [7], this power dissipation must be lower than 20–30 mW.

If the final goal is to turn a passive probe into an active probe, scientists are then constrained in their techniques for the production of silicon probes. For example, deep boron diffusion is used to direct the shape of the silicon probes, but the area where integrated circuits are included need to be masked off from this doping process [9]. In addition, the substrate which supports the recording region of the probe needs to contain silicon, so that semiconductor properties can be exploited. The process must also be efficient to produce many probes in a cost-effective way (e.g., Peckerar et al. [10]). Finally, an ideal neural implant would be wireless; however, wireless devices would require a method for powering the device, possibly through inductive battery charging. Moreover, the high quantity of data collected with high-density probes would require sufficient bandwidth. Indeed, there has been effort and progress made toward integrating these features with silicon probes [11].

Active and passive probes have often been produced by circumscribed groups of researchers operating out of a particular institute. The most well known have been the Michigan and Utah groups, so their work will be discussed next according to the particular development group.

Worldwide Silicon Microprobe Developments

Michigan Probes

In its infancy, microprobe construction was relatively slow and required difficult fabrication sequences [9]. In 1985, Najafi and Wise developed a 10-channel, gold-contact microprobe which measured up to 3 mm in length and was about 50 μm wide by 15 μm thick using a single-sided wafer fabrication process [9]. Recordings were made from the cerebellar cortex, detecting action potentials as large as 200 μV.

Drake et al. [12] described the recording capabilities of a 15 μm thick by 90 μm wide microprobe built on a silicon substrate using photolithographic techniques. The probe was inserted through the pia matter into the cortex of an anesthetized rat. Interestingly, the authors report that electrode contacts on the face of the probe tended to have better amplitude recordings (up to 675 μV) than along the edge of the probe. Significantly, when adjacent recording sites were spaced at 100 μm or less, the same cell could be picked up on both recording sites. Consistent with the methods used in tetrode recording [13], this can be useful to discern the identity of individual neurons among a field of action potentials.

Najafi and Wise [14, 15] also produced the first active microprobes for in vivo investigation. These probes contained integrated chip CMOS (complementary metal–oxide–semiconductor) circuitry for signal amplification, multiplexing, and self-testing. In addition, realizing that not every site of the probe would have a good quality signal, the probe could be multiplexed between 32 analog channels from which to collect the best neural data. Later versions of probes developed by this group included electrical stimulation technology (see section “Silicon Probes and

Electrical Stimulation"). Moreover, an 8×16 3D array (inter-shank spacing 200–400 μm) of such microprobes was created with an astonishing 1,024 recording sites [16]. Since the attachment of wires to each of the recording channels would add forces which would disrupt the ability of the probe to float in the brain, multiplexing technology was required to reduce the data transmission lines. In addition, similar to the Utah probe (see section "Utah Array"), a special device was needed to insert the array through the arachnoid and pia matter and into the brain. In contrast to the Utah method, this device was spring loaded, and the array was held in place by a vacuum. The authors report that cortical units had amplitudes as high as 500 μV (peak to peak).

Kipke et al. [17] tested the utility of a floating Michigan probe in both the auditory and somatosensory cortices of chronically instrumented rats. The amplitude of recorded spike activity ranged from 50 to 800 μV peak to peak. The authors found that signal could be recorded for up to 6–28 weeks with 80 % of the recording contacts detecting neural spike activity. Michigan probes with active components, including unity gain, preamplification, and multiplexing, needed to be put to the test. One of the main limitations in developing and implementing such a device was due to low-frequency/DC drift of the preamplifier [18]. Olsson attempted to solve this problem through the addition of a high-pass filter which included a subthreshold-biased MOS transistor [18]. In this case, the MOS transistor acted as a resistor, forcing the output offset voltage to be equal to the input offset voltage. This same component also permitted the tuning of the high-pass filter right on the silicon probe. Olsson [18] also demonstrated the feasibility of multiplexing silicon probe data acquisition in vivo at 5 kHz.

The small size, reliable manufacture and high-density of recording sites make silicon microprobes potentially ideal for neuro-prosthetic applications. However, a useful implantable device would be preferred to float with the brain and would require a low profile. Yao et al. [19] developed a low-profile three-dimensional active silicon probe array (256 recording sites) (Fig. 2.2b, c). What was unique about this design is that the integrated circuits for stimulating and recording could be folded over to reduce the height of the device. Remarkably, the probe assembly only required seven leads for control and to acquire data.

An interesting offshoot for silicon probes has been to use them for detecting monoamine concentrations in the extracellular space [20, 21]. In such developments, the contacts of the silicon probe are sputter coated with carbon and coated with Nafion (for voltammetry recording). Some probes can detect both neural activity and changes in monoamine concentration from different sites within the same probe. Currently, the Michigan probe can be purchased from the company NeuroNexus.

Utah Array

Interest in the Utah array was initiated with the hope of restoring sensory function with visual cortex prosthesis [22]. This Utah array consisted of a 4.2×4.2×0.12 mm thick substrate from which 100 conductive silicon needles project (Fig. 2.3a. b).

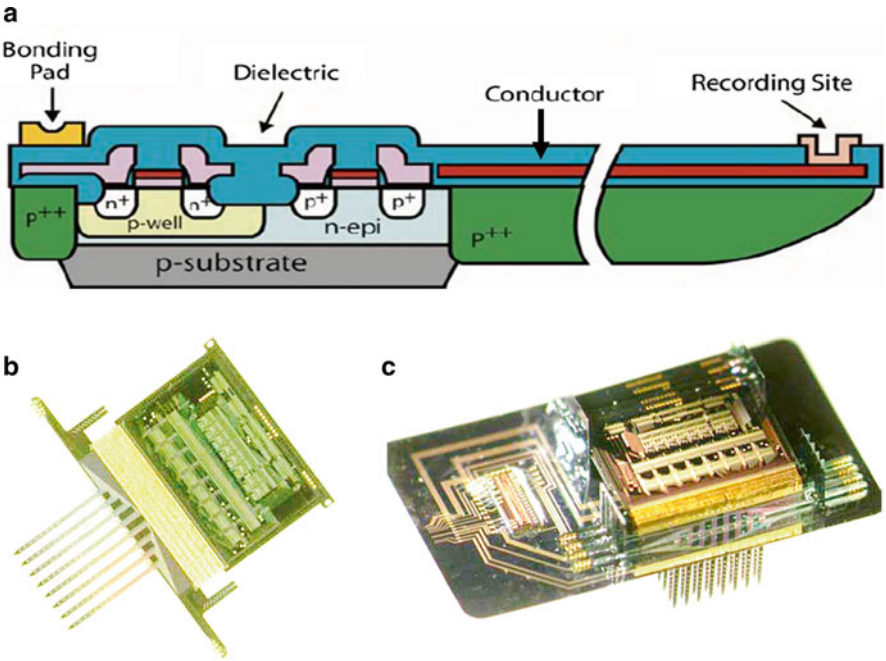

Fig. 2.2 Active silicon probe. (**a**) Cross section of a silicon probe with different layers of insulation (*blue* dielectric), conducting signals (*red* conductor), recording site (peach), etchant stop layer (p^{++}silicon), transistor gate (*small red* regions), and oxide layer (*small pink* regions) interposed between doped p+(sources) and p+(drains) and n+(sources) and n+(drains). p-well and n-epi are doped regions controlled by their respective base regions. The combination of these complimentary npn and pnp MOSFETs forms a CMOS transistor and can be exploited for active electronic functions of the silicon probe [45]. © 2004 IEEE. Reprinted, with permission, from Wise K, Anderson DJ, Hetke J, Kipke DR, Najafi A. Wireless Implantable Microsystems: High-Density Electronic Interfaces to the Nervous System. Proceedings of the IEEE. 2004;92(1):76–97. (**b**) Active stimulating and recording probe. Probe has active CMOS circuitry including on-chip digital-to-analog conversion for stimulation and preamplifiers to record spikes [19]. © 2005 IEEE. Reprinted, with permission, from Yao Y, Gulari MN, Ghimire S, Hetke JF, Wise KD. A low-profile three-dimensional silicon/parylene stimulating electrode array for neural prosthesis applications. Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference. 2005;2:1293–6. (**c**) The recording probe in (**b**) can be assembled in to an array. The probes had a flexible region which permitted the active circuitry to fold over, limiting the profile of the device [19]. © 2005 IEEE. Reprinted, with permission, from Yao Y, Gulari MN, Ghimire S, Hetke JF, Wise KD. A low-profile three-dimensional silicon/parylene stimulating electrode array for neural prosthesis applications. Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference. 2005;2:1293–6

The needles are 1.5 mm long and 90 μm at their base with polyimide as an insulator. The sharpened ends of the needles were coated with platinum to facilitate charge transfer with the interfacing tissue. However, several modifications had to be made before this final version. What is most innovative about the original manufacture of these probes is that the scientists doped specific regions of the silicon with

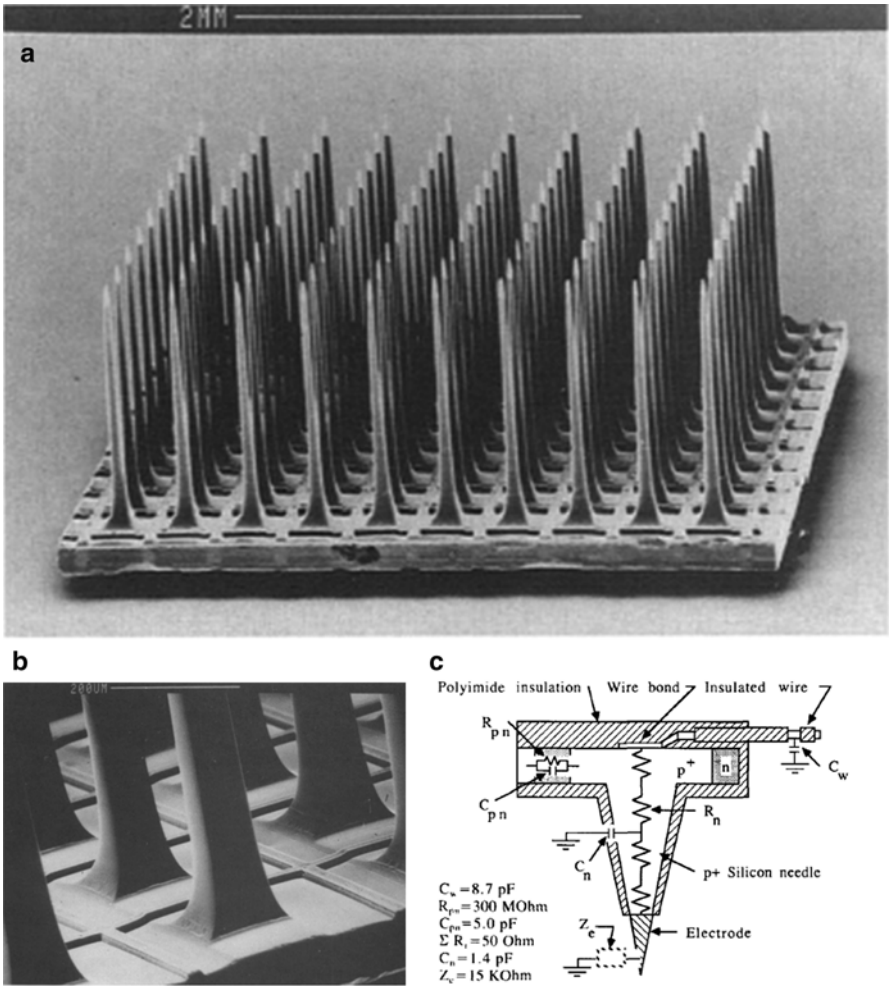

Fig. 2.3 The Utah array. (**a**) Scanning electron micrograph of a revised Utah electrode array. Reprinted from Brain Research, 726(1–2), Nordhausen CT, Maynard EM, Normann RA, Single unit recording capabilities of a 100 microelectrode array, 129–140, Copyright 1996, with permission from Elsevier. (**b**) Scanning electron micrograph showing the glass insulating regions as raised bumps between the columns. With kind permission from Springer Science+Business Media: Annals of Biomedical Engineering, A glass/silicon composite intracortical electrode array, 20(4), 1992, 423–37, Jones KE, Campbell PK, Normann RA. (**c**) The original equivalent circuit schematic of the thermo-migrated Utah array probe. The circuit shows wire capacitances (C_w), pn junction capacitance (C_{pn}) and resistance (R_{pn}), silicon needle resistance (R_n) and insulation capacitance (C_n), and the electrode contact impedances (Z_e). Polyimide was used as insulation, and the electrode tip was plated with platinum. The p and n refer to doped regions of the probe which as a consequence of their spatial arrangement reduce current leakage to adjacent probes (not shown). © 1991 IEEE. Reprinted, with permission, from Campbell PK, Jones KE, Huber RJ, Horch KW, Normann RA. A silicon-based, three-dimensional neural interface: manufacturing processes for an intracortical electrode array. IEEE Transactions on Bio-Medical Engineering. 1991;38(8):758–68

aluminum through a process of thermo-migration. The result is 100 columns of p+-doped semiconducting material. (An equivalent circuit can be seen in Fig. 2.3c.) Since the columns were realized in a solid block of material, the investigators used a computer-controlled, dicing saw to produce a 10×10 array of rectangular columns and thus separate the p+-doped silicon. The final shape of the needle was created by chemically etching the 100 columns of silicon to a point. Notably, this was the first array to be constructed vertically, while all other probes had been constructed on their flank. Subsequent alteration to this array included a glass dielectric to provide insulation between individual electrode needles in the array [23] (Fig. 2.3b). In this design, the thermo-migration procedure was not used to dope the silicon; rather a pre-doped p-type silicon substrate was used, and was sawn into an array. This method was found to improve interelectrode impedance and capacitance.

In theory, a two-dimensional array of electrodes should produce a bed-of-nails effect when inserted into the brain. To circumvent this problem, and implant the Utah array, Rousche and Normann [24] designed a pneumatically actuated impact insertion system. To implant the Utah array through the meninges, a minimum speed of 8.3 m/s was required, with the resultant electrode depth at 1.5 mm. With the electrical properties refined and the insertion method standardized, the recording quality of the Utah probe was tested in cat striate cortex [25]. 58.6 % of the electrodes in the array detected evoked neural responses as large as 200 μV. In these recordings, signals were not yet multiplexed, so all 100 wires were connected between the array and a 100-channel printed circuit board. Currently, the Utah probe is commercially available from Blackrock Microsystems in Salt Lake City, Utah.

MIT Probe

Kuperstein and Whittington [26] designed a 24-contact microprobe with 85 μm contract spacing (Fig. 2.4a, b) at the Massachusetts Institute of Technology. Contacts were arranged along the edge of the probe, rather than the face, to increase the contact with the brain. With this design, neural signals were recorded from the cat visual cortex [26] and the olfactory and hippocampal cortices of the rat, with signal to noise similar to conventional microelectrodes [27]. Molybdenum was used as a substrate to increase the strength of the probe; however, the use of this substance as a substitute for silicon practically excludes the possibility for silicon-based integrated circuitry.

Hopkins Probe

Like the MIT probe, the Hopkins probe contains a strong and flexible molybdenum substrate with polyimide insulation (Fig. 2.4c) [4]. This probe was developed at Johns Hopkins University by Blum et al. [4]. The probe was 19 μm thick with up to

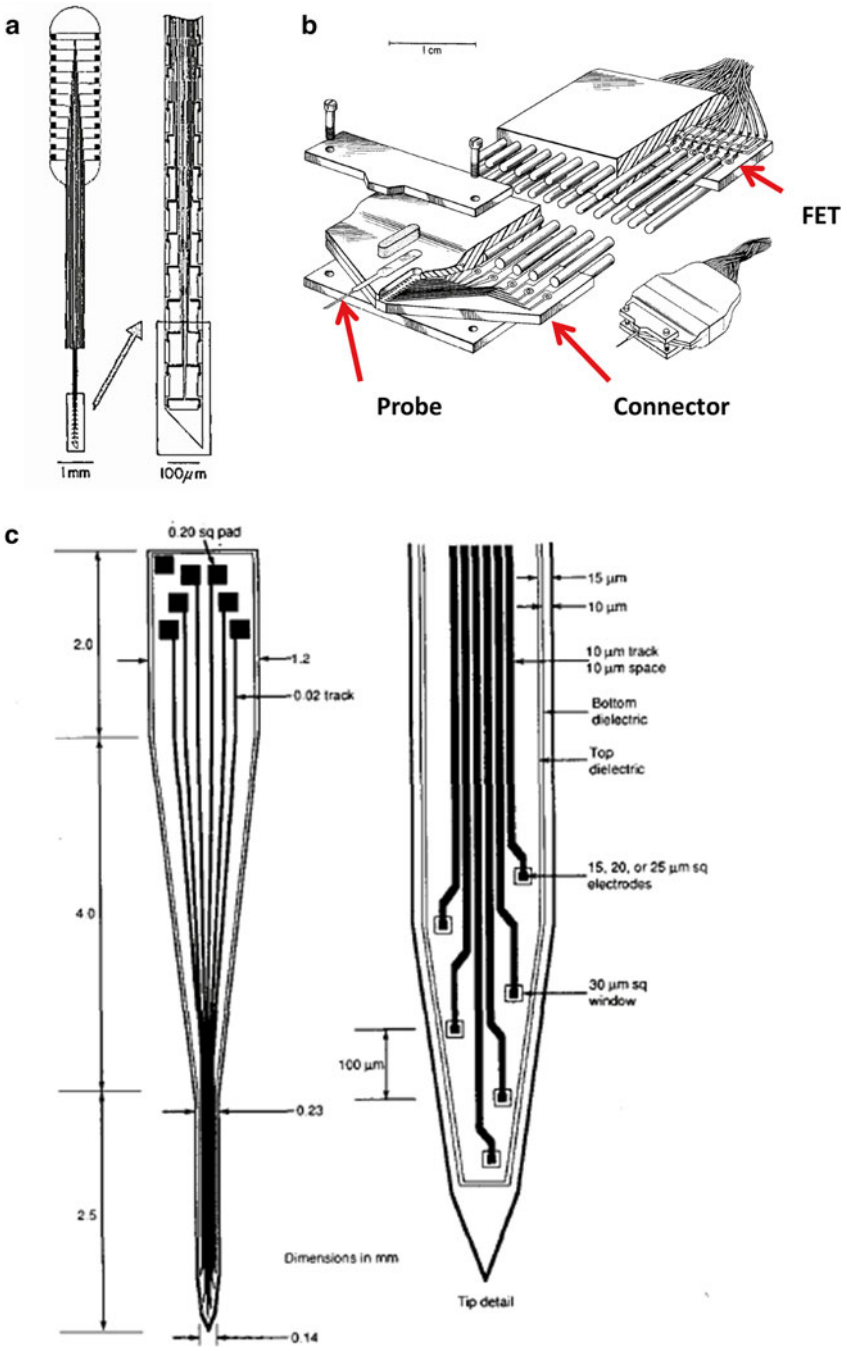

Fig. 2.4 The MIT and Hopkins probes. (**a**) MIT probe with contacts located around the perimeter. Reprinted from Neuroscience, 15(3), Kuperstein M, Eichenbaum H, Unit activity, evoked potentials and slow waves in the rat hippocampus and olfactory bulb recorded with a 24-channel

eight gold recording channels [4]. The advantage of this probe is that it did not require advanced semiconductor processing technology. Since molybdenum is conductive, it needed to be separated from the electrical recording contacts; therefore, the electrodes were constructed on top of a thin layer of polyimide which adhered to the substrate. A final layer of polyimide is placed over channels and small holes in the polyimide permit the electrical contact with the extracellular recording environment. This probe was reported to record action potentials greater than 100 μV from the rat spinal cord.

Caltech Probes

Silicon microprobes typically have one side with recording contacts, because of constraints on the manufacturing process. However, if electrical contacts could be etched on both sides of the probe, neuron recording yield could be increased. At Caltech University, Du et al. [28] developed a dual-sided microprobe on a silicon substrate with 16 individually addressable recording sites on each shank. Moreover, two silicon substrates were stacked parallel to one another to make a 3D array. As a consequence, the neuronal source could be triangulated much like a tetrode. Recordings from the locust nervous system with this microprobe yielded spikes as high as 800 μV.

Du et al. [29] also developed a silicon microprobe with nanofabricated high-density recording leads using an electron-beam lithography technique. This technique does not require the use of a mask and can produce submicron features. This microprobe was manufactured with signal amplification, band-pass filtering, and 32:1 multiplexing, impedance testing, and required a mere 6 lines to control power and data acquisition. The small electrical routing sizes (<300 nm) permitted an increase in the number of recording channels on this probe (up to 64) and the spread of recording contacts across the face of the probe (Fig.2.5a–c). The maximum spike amplitude recorded in the thalamus was on average 150 μV. With a mere 40 μm spacing of electrodes, adjacent channels could be used to look at local circuit connections using cross-correlation.

Fig. 2.4 (continued) microelectrode, 703–12, Copyright 1985, with permission from Elsevier. (**b**) Specialized connector and MIT probe (*left*) with a preamplifier (*right*). The FETs of the preamplifier are represented by the small pads located between the pins and the jumper wires (which connect to the output leads). *Bottom right* is the assembled probe, connector, and amplifier. Reprinted from Neuroscience, 15(3), Kuperstein M, Eichenbaum H, Unit activity, evoked potentials and slow waves in the rat hippocampus and olfactory bulb recorded with a 24-channel microelectrode, 703–12, Copyright 1985, with permission from Elsevier. (**c**) Depiction of the spatial arrangement on the Hopkins probe. © 1991 IEEE. Reprinted, with permission, from Blum NA, Carkhuff BG, Charles HK, Jr., Edwards RL, Meyer RA. Multisite microprobes for neural recordings. IEEE transactions on bio-medical engineering. 1991;38(1):68–74

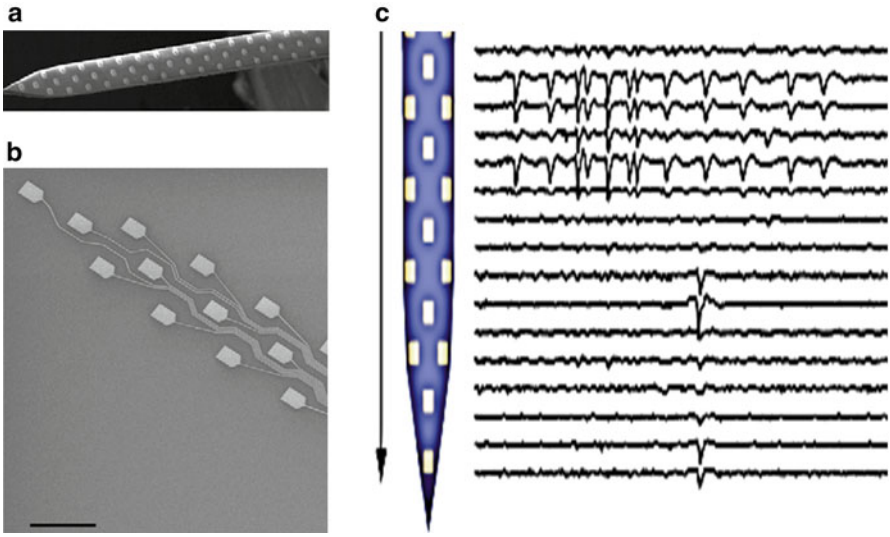

Fig. 2.5 Caltech probe. (**a**) High-density microprobe configuration with scale bar is 200 μm. (**b**) Gold recording sites composed (*rectangles*) and leads (290–1,000 nm). Scale bar is 50 μm. (**c**) Parallel recording from all leads of the microprobe after data was multiplexed. Recordings were made from the mouse thalamus. Figures reproduced with permission from Du J, Blanche TJ, Harrison RR, Lester HA, Masmanidis SC. Multiplexed, high density electrophysiology with nanofabricated neural probes. PloS one. 2011;6(10):e26204

Silicon Probes and Actuation

Microprobes typically have enough contacts to span many cortical layers; accordingly there is little need to adjust the depth of the electrode while it is in the cortex at its final destination. However, there have been some attempts to permit adjustment of the silicon probe even after implantation in the brain. One option is to design micromechanical structures to directly interface with the silicon probe. This concept was made apparent with a review by Wise in 1991 [3], which showcased development of micromachined gears and other micromechanical components.

The closest approximation to what was envisioned by Wise appears to be the recent actuating devices developed by Muthuswamy et al. [30]. This device microactuated a 3-channel polysilicon microelectrode array (Fig. 2.6). The probes are moved with thermal actuators with a step resolution of 8.8 μm and a total range of 5 mm. The actuators can heat up to several hundred degrees, but because the actuators are so small, the heat is not appreciably transferred to the recording probe. The actuators are coupled to a ratcheting system that drives a shuttle up and down. The device was able to sample neural activity of the somatosensory cortex of chronically implanted adult rats in the range of 400–900 μV. Subsequently, Muthuswamy et al. [30] developed an electrostatic driving mechanism for actuating polysilicon

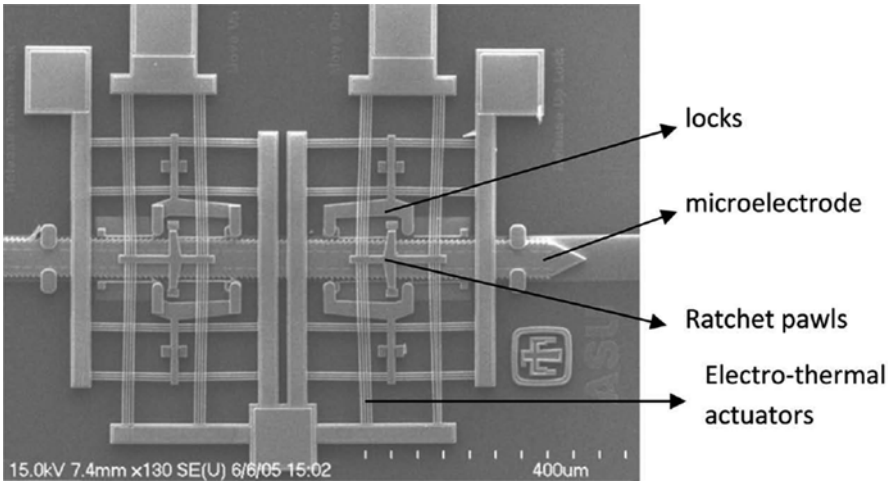

Fig. 2.6 Actuating probes. An electron micrograph of an electrothermal microactuator. The microelectrode is a mere 50 μm wide. Reproduced with permission from Muthuswamy J, Anand S, Sridharan A. Adaptive movable neural interfaces for monitoring single neurons in the brain. Frontiers in Neuroscience. 2011;5:94 [46]

microelectrodes with gears down to 1 μm precision. Electrodes can be moved a total distance of 5 mm. Recordings were obtained chronically from the somatosensory cortex of rats yielding units of amplitudes ranging from 100 to 500 μV.

In Chap. 1, the use of hyperdrives was discussed for actuating microelectrodes in the brain. Recently, a similar use has been appropriated for silicon probes. Vandecasteele et al. [31] describe a method to implant a 4-shank, 32-site silicon probes using a movable microdrive. In this example, connecting the silicon probe to the headstage required a mini-flexible polyimide cable.

Silicon Probes and Electrical Stimulation

A microprobe that has the potential to both record and respond to neural activity has practical application in neural prosthesis. Based on this practical possibility, neuroscientists have tried to build microprobes with combinations or recording and stimulation. This first stimulating microprobe consisted of 8–16 silicon contacts that could not only detect neural activity but also have the capacity to respond and influence neural activity through the application of electrical stimulation [32]. Subsequently, Kim et al. [33] developed a 64-recording-site probe which was also capable of stimulation (Fig. 2.7a, b). The probe would use a serial bit stream to supply the probe with an address and the magnitude of the current (+127 to −127 μA). On-chip integrated circuits were used for analog data amplification and filtering. Among the many interesting circuit features, this probe also had on-chip impedance

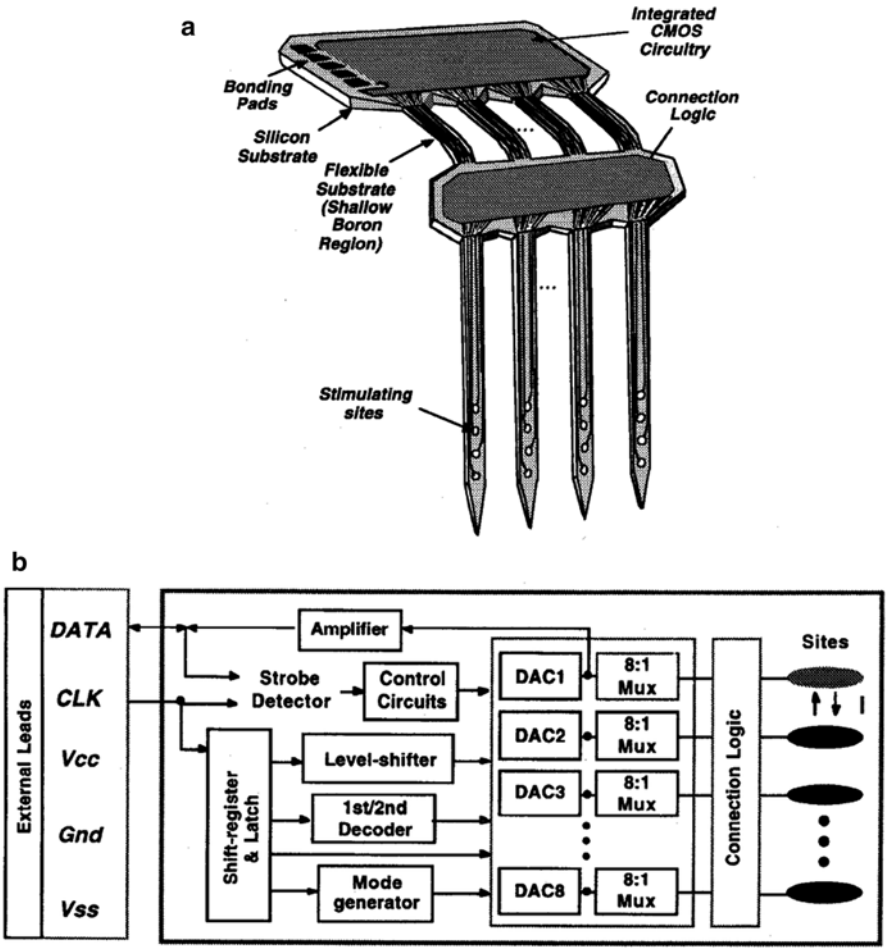

Fig. 2.7 Electrical stimulation microprobe. (**a**) Basic structure of a multiplexed neural probe. The contacts on the shanks can act as both stimulating and recording electrodes controlled with onboard circuits. A flexible substrate permits the probe to fold [34]. (**b**) Block diagram of on-chip circuit. The data line carries 8:1 multiplexed input. The on-chip DAC or digital-to-analog converters are used to send electrical stimulation to the recording sites. Bidirectional data flow permits control signals to be sent for stimulation and acquire signals to be sent for data acquisition [34]. © 1997 IEEE. Figures reprinted, with permission, from Kim C, Wise K. Low-Voltage Electronics for the Stimulation of Biological Neural Networks Using Fully Complementary BiCMOS Circuits. IEEE Journal of Solid-State Circuits. 1997;32(10):1483–90

testing for all channels of the probe. Subsequently, Kim et al. [34] developed a chronic stimulation and recording Michigan probe with BiCMOS technology. This permitted the advantages of CMOS for digital circuitry but reduced the power consumption with integrated bipolar transistor technology. In 2005 [18] Michigan probe technology, combined with multisite stimulation, was tested in an in vivo preparation. One hundred microsecond pulses were found to evoke spikes in nearby recorded neurons with currents as low as 4–8 μA.

Silicon Probes and Drug Delivery

Given the silicon probe's utility in acquiring high-density neural data, it became desirable to modulate neural activity with more selectivity than what electrical stimulation offers. Chen et al. [35] designed a silicon probe capable of delivering pharmacological agents as well as simultaneously recording from neurons. The silicon substrate possessed multiple flow channels (10 μm) and orifices near the electrode recording sites (Fig. 2.8a–c). At the back end of the probe, the channels are fitted with polyimide tube, so that pharmacological agents could be perfused. The authors found that they could inhibit local neuron activity with the perfusion of gamma-aminobutyric acid. Cheung et al. [36] introduced a silicon probe fabrication method based on SOI (silicon on insulator) technology to produce a silicon probe with a sprinkler-style fluidic channel. The holes for this sprinkler (3×3 μm) were spaced 50 μm apart along a 6 mm length of the probe.

One of the most complex drug delivery probes to date was developed by Spieth et al. [37]. This probe consisted of a 3D array of 4×4 probes, which has a form that is similar to that of the Utah array. Each probe was significantly longer (8 mm) than the Utah array (1.3 mm) and had multiple recording contacts (Fig. 2.9a–d). Microchannels were incorporated into the probes for drug delivery and a special elastic microfluidic cable was developed so that fluid could be delivered to the probe while

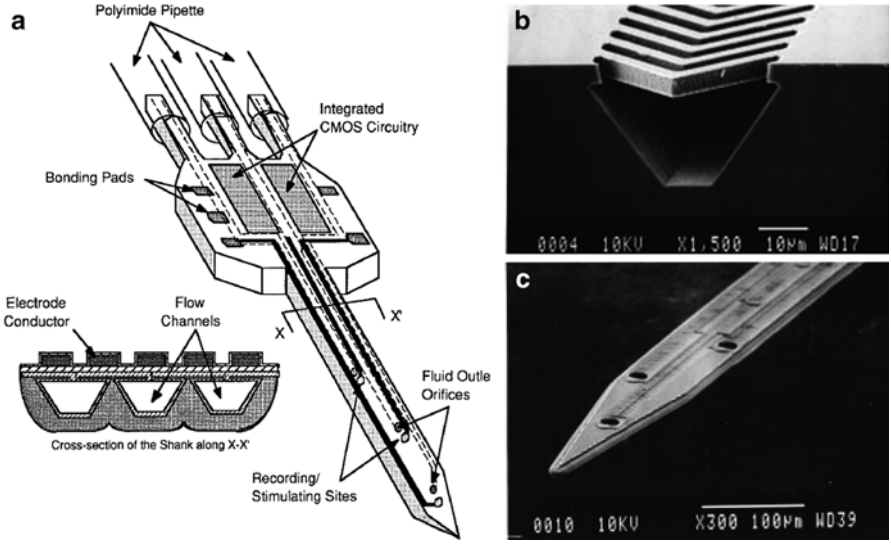

Fig. 2.8 Drug delivery microprobe. (**a**) Micromachined probe which has three channels for drug delivery in addition to recording and stimulation sites. Note the integrated CMOS and the polyimide contact sites. (**b**) Electron micrograph cross section of the etched micro-channel. The chevron pattern will later be sealed for the finished product. (**c**) Electron micrograph of a probe containing three drug delivery orifices. © 1997 IEEE. Figures reprinted, with permission, from Chen J, Wise KD, Hetke JF, Bledsoe SC, Jr. A multichannel neural probe for selective chemical delivery at the cellular level. IEEE transactions on bio-medical engineering. 1997;44(8):760–9

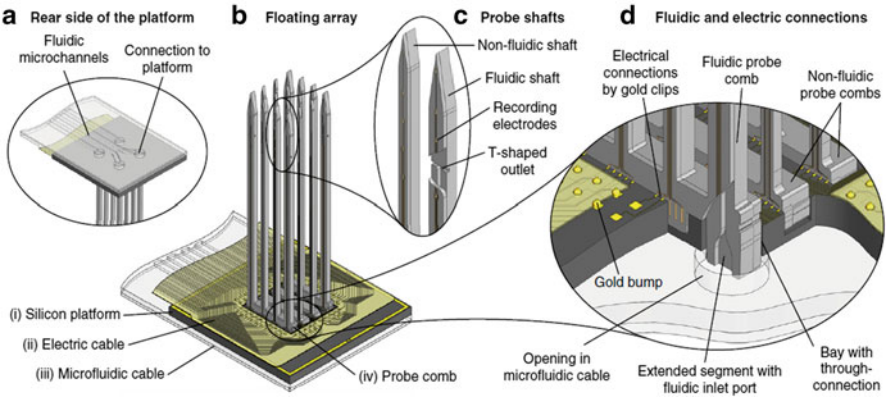

Fig. 2.9 Drug delivery microprobe array. (**a**) Floating 3D array for recording and drug delivery. (**b**) Underside of the probe assembly with flexible cable for liquid delivery. (**c**) Combined fluidic and non-fluidic probes in the same shaft. (**d**) Fluidic and electronic connections. Figures reproduced with permission from Spieth S, Brett O, Seidl K, Aarts AA, Erismis M, Herwik S, et al. A floating 3D silicon microprobe array for neural drug delivery compatible with electrical recording. Journal of Micromechanics and Microengineering. 2011;21:1–16. © IOP Publishing. Reproduced by permission of IOP Publishing. All rights reserved

the probe floats in the brain. The infusion rate of the probe could range from 1 to 5 μL/min. Future developments will likely see refinements in design, integrated circuits, and possibly glutamate biosensors.

Silicon Probes and Optogenetics

As discussed in Chap. 1, optogenetics is a very powerful tool for neuroscientists, permitting the possibility of turning on and off neurons using light. Integrating optics and silicon probes will permit the ability to specifically stimulate local neurons while monitoring neural responses.

Rather than design a new silicon probe, the most expeditious way to combine fiber optics with high-density electrophysiology is simply to glue a fiber optic assembly to a microprobe. Stark et al. [38] constructed a fiber optic stimulator probe by attaching a diode to an optical fiber. The end of the fiber optic probe was etched to a fine tip and was attached to the silicon probe, above the level of the recording contacts. Since the light source was a diode, it was feasible to drive each fiberoptic probe independently and with varying levels of stimulation, controlled by regulating current to the diode. Probes that were implanted into the hippocampus were found to reliably evoke neural activity in both rats (expressing exogenous opsins) and transgenic mice (expressing ChR2 (channelrhodopsin 2) under the control of a CaMKII or PV promoter).

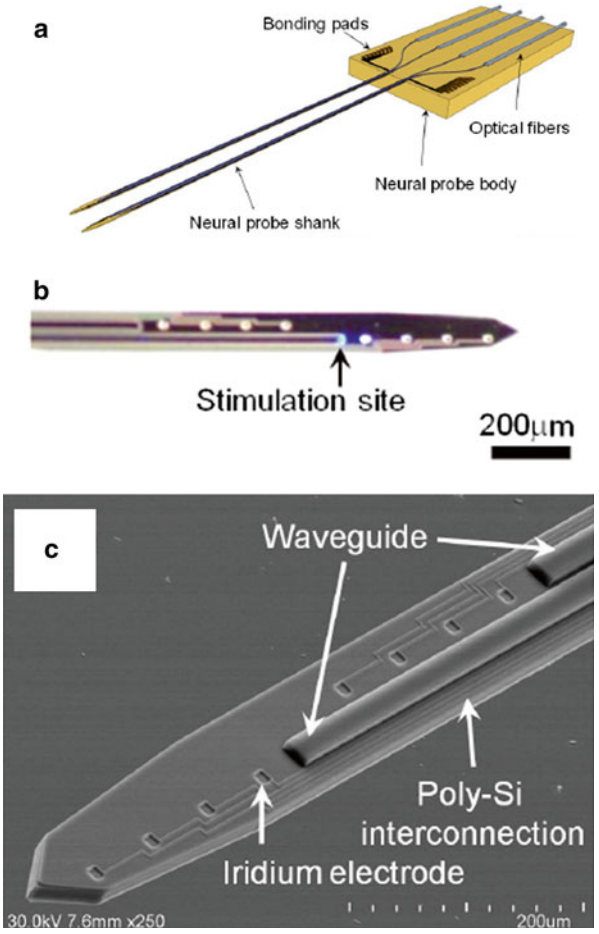

Fig. 2.10 Combined optogenetic silicon probe. (**a**) Drawing of a dual-shank neural probe with waveguides for conducting light to the brain for optogenetic control. (**b**) Light transmission through a waveguide. (**c**) Single shank showing the waveguide and the iridium electrode contact. © 2011 IEEE. Figures reprinted, with permission, from Im M, Cho IJ, Wu F, Wise KD, Yoon E. A dual-shank neural probe integrated with double waveguides on each shank for optogenetic applications. Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Conference. 2011;2011:5480–3

Im et al. [39] developed a combination silicon and optogenetic probe. The process of making this probe involved the use of a photo-definable polymer to pattern dual 15 μm waveguides on an 8-channel recording shank of the probe (Fig. 2.10a–c). In addition, optical fiber grooves were etched into the back of the probe so that optical lines could be attached to the probe. A variation of this design, with a single waveguide composed of oxynitride was used to test the feasibility of optogenetic activation of recorded neurons [40]. The probe functioned well, being able to entrain neural activity in the rat hippocampus to 25 Hz sinusoidal light stimulation in ChR2 expressing rats.

Schwaerzle et al. [41] designed an optical stimulation microprobe by using onboard bare laser diode chips. The probe shanks measured 8 mm long, carrying four electrodes and a waveguide that interfaced with the laser diodes. The addition

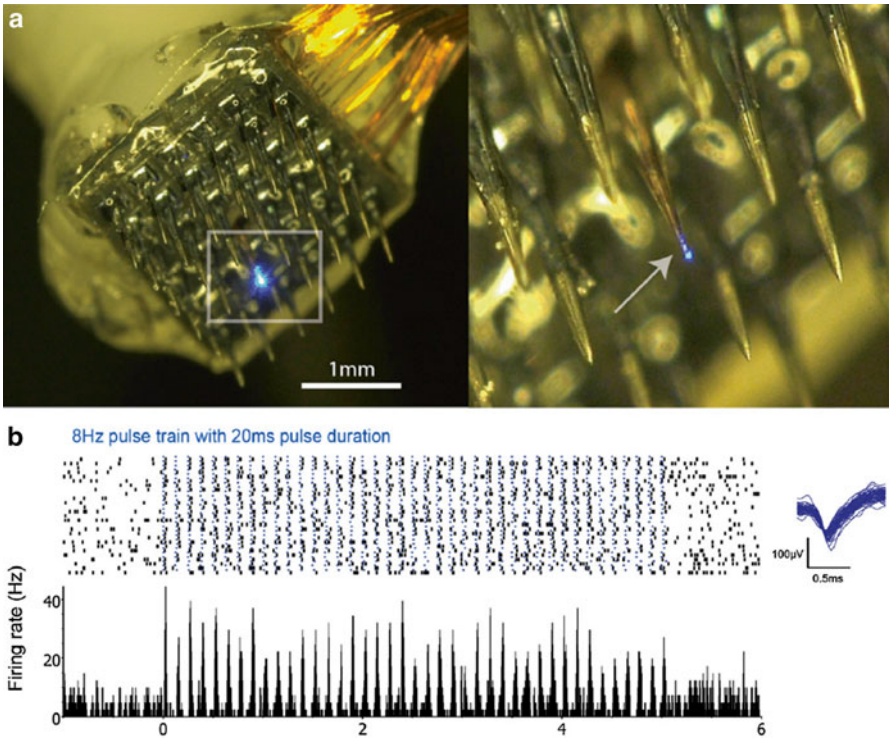

Fig. 2.11 Utah-optogenetic probe. (**a**) 6×6 multielectrode array with one of the elements replaced by a sharpened optrode. Electrode shank length is 1 mm, and spacing between electrodes is 400 **μ**m. A *blue* laser light can be seen emitting from the optrode tip. (**b**) Peristimulus time histogram of a neuron that is entrained to optogenetic stimulation at 8 Hz. Figures reproduced with permission from Wang J, Wagner F, Borton DA, Zhang J, Ozden I, Burwell RD, et al. Integrated device for combined optical neuromodulation and electrical recording for chronic in vivo applications. Journal of neural engineering. 2012;9(1):016001 © IOP Publishing. Reproduced by permission of IOP Publishing. All rights reserved

of these laser diodes was an important advance as it reduced the necessity for contact with fiberoptic cabling and obviates the need for a hybrid optical–electrical commutator.

Not only have the 2D style microprobes been outfitted for optogenetics, but a 6×6 Utah array has also been fit with optogenetic capabilities [42, 43]. In this realization, a sharpened optical probe was swapped with one of the original recording shanks in the Utah array (Fig. 2.11a). Thus, light could be delivered to neurons surrounding that shank, and the response of neurons in other shanks could be recorded. When the array was implanted into ChR2 expressing rats, pulses of light were found to entrain both local field potentials and neurons (Fig. 2.11b).

Summary

Silicon microprobes have evolved dramatically since their inception including the number of recordable channels, the repeatability of their manufacture, and superior integration with a variety of stimulation methods, including optogenetic, electrical, and pharmacological methods. Moreover, microprobes offer the possibility of a fully integrated silicon recording device. One of the big hurdles which remain is a standardized technology to broadcast neuronal signals from these microprobes with a transmitter. Moreover, there may still be a need to increase signal quality of microprobes, as sharp microelectrodes tend to record signals of higher magnitude than silicon probes (compare Chap. 1) and therefore may lend to better spike sorting. Alternatively, tetrode-configured silicon probes are improving upon our ability to isolate neurons. Altogether, the future of high-density microprobes appears to be requisite for the extraction of both the fine details and functional engineering principles of the brain.

Acknowledgments Special thanks to Mary Steenland, Erik Hopkins, and Dr. Masami Tatsuno for a careful editing of this chapter.

References

1. Wise KD, Angell JB, Starr A. An integrated-circuit approach to extracellular microelectrodes. IEEE Trans Bio Med Eng. 1970;17(3):238–47.
2. Kindlundh M, Peter N, Hofmann UG. A neural probe process enabling variable electrode configurations. Sensors Actuators. 2004;102(1):51–8.
3. Wise KD, Najafi K. Microfabrication techniques for integrated sensors and microsystems. Science. 1991;254(5036):1335–42.
4. Blum NA, Carkhuff BG, Charles Jr HK, Edwards RL, Meyer RA. Multisite microprobes for neural recordings. IEEE Trans Bio Med Eng. 1991;38(1):68–74.
5. Prohaska O, Olcaytug F, Womastek K, Petsche H. A multielectrode for intracortical recordings produced by thin-film technology. Electroencephalogr Clin Neurophysiol. 1977;42(3):421–2.
6. Bartho P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsaki G. Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. J Neurophysiol. 2004;92(1):600–8.
7. Wise KD. Silicon microsystems for neuroscience and neural prostheses. IEEE Eng Med Biol Mag. 2005;24(5):22–9.
8. BeMent SL, Wise KD, Anderson DJ, Najafi K, Drake KL. Solid-state electrodes for multichannel multiplexed intracortical neuronal recording. IEEE Trans Bio Med Eng. 1986;33(2):230–41.
9. Najafi K, Wise K, Mochizuki T. A high-yield IC-compatible multichannel recording array. IEEE Trans Bio Med Eng. 1985;ED-32(7):1206–11.
10. Peckerar M, Shihab H, Rebbert M, Kosakowski J, Isaacson P. Passive microelectrode arrays for recording of neural signals: a simplified fabrication process. Rev Sci Instrum. 1991;62:2276–80.
11. Yu H, Najafi K. Low-power interface circuits for bio-implantable microsystems presented at the Int IEEE Solid-State Circuit Conference (ISSCC), 2003.

12. Drake KL, Wise KD, Farraye J, Anderson DJ, BeMent SL. Performance of planar multisite microprobes in recording extracellular single-unit intracortical activity. IEEE Trans Bio Med Eng. 1988;35(9):719–32.
13. McNaughton BL, O'Keefe J, Barnes CA. The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. J Neurosci Methods. 1983;8(4):391–7.
14. Najafi K, Ji J, Wise KD. Scaling limitations of silicon multichannel recording probes. IEEE Trans Bio Med Eng. 1990;37(1):1–11.
15. Najafi K, Wise K. Implantable multielectrode array with on-chip signal processing. IEEE International Solid-State Circuit Conference, 1986, p. 98.
16. Bai Q, Wise KD, Anderson DJ. A high-yield microassembly structure for three-dimensional microelectrode arrays. IEEE Trans Bio Med Eng. 2000;47(3):281–9.
17. Kipke DR, Vetter RJ, Williams JC, Hetke JF. Silicon-substrate intracortical microelectrode arrays for long-term recording of neuronal spike activity in cerebral cortex. IEEE Trans Neural Syst Rehabil Eng. 2003;11(2):151–5.
18. Olsson 3rd RH, Buhl DL, Sirota AM, Buzsaki G, Wise KD. Band-tunable and multiplexed integrated circuits for simultaneous recording and stimulation with microelectrode arrays. IEEE Trans Bio Med Eng. 2005;52(7):1303–11.
19. Yao Y, Gulari MN, Ghimire S, Hetke JF, Wise KD. A low-profile three-dimensional silicon/parylene stimulating electrode array for neural prosthesis applications. Conf Proc IEEE Eng Med Biol Soc. 2005;2:1293–6.
20. van Horne CG, Bement S, Hoffer BJ, Gerhardt GA. Multichannel semiconductor-based electrodes for in vivo electrochemical and electrophysiological studies in rat CNS. Neurosci Lett. 1990;120(2):249–52.
21. Sreenivas G, Ang SS, Fritsch I, Brown WD, Gerhardt GA, Woodward DJ. Fabrication and characterization of sputtered-carbon microelectrode arrays. Anal Chem. 1996;68(11):1858–64.
22. Campbell PK, Jones KE, Huber RJ, Horch KW, Normann RA. A silicon-based, three-dimensional neural interface: manufacturing processes for an intracortical electrode array. IEEE Trans Bio Med Eng. 1991;38(8):758–68.
23. Jones KE, Campbell PK, Normann RA. A glass/silicon composite intracortical electrode array. Ann Biomed Eng. 1992;20(4):423–37.
24. Rousche PJ, Normann RA. A method for pneumatically inserting an array of penetrating electrodes into cortical tissue. Ann Biomed Eng. 1992;20(4):413–22.
25. Nordhausen CT, Maynard EM, Normann RA. Single unit recording capabilities of a 100 microelectrode array. Brain Res. 1996;726(1–2):129–40.
26. Kuperstein M, Whittington DA. A practical 24 channel microelectrode for neural recording in vivo. IEEE Trans Bio Med Eng. 1981;28(3):288–93.
27. Kuperstein M, Eichenbaum H. Unit activity, evoked potentials and slow waves in the rat hippocampus and olfactory bulb recorded with a 24-channel microelectrode. Neuroscience. 1985;15(3):703–12.
28. Du J, Riedel-Kruse IH, Nawroth JC, Roukes ML, Laurent G, Masmanidis SC. High-resolution three-dimensional extracellular recording of neuronal activity with microfabricated electrode arrays. J Neurophysiol. 2009;101(3):1671–8.
29. Du J, Blanche TJ, Harrison RR, Lester HA, Masmanidis SC. Multiplexed, high density electrophysiology with nanofabricated neural probes. PLoS One. 2011;6(10):e26204.
30. Muthuswamy J, Okandan M, Gilletti A, Baker MS, Jain T. An array of microactuated microelectrodes for monitoring single-neuronal activity in rodents. IEEE Trans Bio Med Eng. 2005;52(8):1470–7.
31. Vandecasteele M, M S, Royer S, Belluscio M, Berenyi A, Diba K, et al. Large-scale recording of neurons by movable silicon probes in behaving rodents. J Vis Exp. 2012; (61):e3568
32. Tanghe S, Wise K. A 16-channel CMOS neural stimulating array. IEEE J Solid State Circ. 1992;27:1819–25.

33. Kim C, Wise K. A 64-site multishank CMOS low-profile neural stimulating probe. IEEE J Solid State Circ. 1996;31(9):1230–8.
34. Kim C, Wise K. Low-voltage electronics for the stimulation of biological neural networks using fully complementary BiCMOS circuits. IEEE J Solid State Circ. 1997;32(10):1483–90.
35. Chen J, Wise KD, Hetke JF, Bledsoe Jr SC. A multichannel neural probe for selective chemical delivery at the cellular level. IEEE Trans Bio Med Eng. 1997;44(8):760–9.
36. Cheung K, Djupsund K, Dan Y, Lee L. Implantable multichannel electrode array based on SOI technology. J Microelectromech Syst. 2003;12(2):179–84.
37. Spieth S, Brett O, Seidl K, Aarts AA, Erismis M, Herwik S, et al. A floating 3D silicon microprobe array for neural drug delivery compatible with electrical recording. J Micromech Microeng. 2011;21:1–16.
38. Stark E, Koos T, Buzsaki G. Diode probes for spatiotemporal optical control of multiple neurons in freely moving animals. J Neurophysiol. 2012;108(1):349–63.
39. Im M, Cho IJ, Wu F, Wise KD, Yoon E. A dual-shank neural probe integrated with double waveguides on each shank for optogenetic applications. Conf Proc IEEE Eng Med Biol Soc. 2011;2011:5480–3.
40. Wu F, Stark E, Im M, Cho IJ, Yoon ES, Buzsaki G, et al. An implantable neural probe with monolithically integrated dielectric waveguide and recording electrodes for optogenetics applications. J Neural Eng. 2013;10(5):056012.
41. Schwaerzle M, Seidl K, Schwarz U, Paul O, Ruther P. Ultracompact optotrode with integrated laser diode chips and SU-8 waveguides for optogenetic applications. IEEE MEMS, 2013, pp. 1029–32.
42. Wang J, Wagner F, Borton DA, Zhang J, Ozden I, Burwell RD, et al. Integrated device for combined optical neuromodulation and electrical recording for chronic in vivo applications. J Neural Eng. 2012;9(1):016001.
43. Zhang J, Laiwalla F, Kim JA, Urabe H, Van Wagenen R, Song YK, et al. Integrated device for optical stimulation and spatiotemporal electrical recording of neural activity in light-sensitized brain tissue. J Neural Eng. 2009;6(5):055007.
44. Wise K, Anderson DJ, Hetke J, Kipke DR, Najafi A. Wireless implantable microsystems: high-density electronic interfaces to the nervous system. Proc IEEE. 2004;92(1):76–97.
45. Muthuswamy J, Anand S, Sridharan A. Adaptive movable neural interfaces for monitoring single neurons in the brain. Front Neurosci. 2011;5:94.