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HISTOLOGICAL STUDIES OF THE EFFECTS OF CHRONIC IMPLANTATION OF CERAMIC-BASED MICROELECTRODE ARRAYS AND MICRODIALYSIS PROBES IN RAT PREFRONTAL CORTEX

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Abstract

Chronic implantation of neurotransmitter measuring devices is essential for awake, behavioral studies occurring over multiple days. Little is known regarding the effects of long term implantation on surrounding brain parenchyma and the resulting alterations in the functional properties of this tissue. We examined the extent of tissue damage produced by chronic implantation of either ceramic microelectrode arrays (MEAs) or microdialysis probes. Histological studies were carried out on fixed tissues using stains for neurons (cresyl violet), astrocytes (GFAP), microglia (Iba-1), glutamatergic nerve fibers (VGLUT1), and the blood-brain barrier (SMI-71). Nissl staining showed pronounced tissue body loss with microdialysis implants compared to MEAs. The MEAs produced mild gliosis extending 50–100 µm from the tracks, with a significant change in the affected areas starting at 3 days. By contrast, the microdialysis probes produced gliosis extending 200–300 µm from the track, which was significant at 3 and 7 days. Markers for microglia and glutamatergic fibers supported that the MEAs produce minimal damage with significant changes occurring only at 3 and 7 days that return to control levels by one month. SMI-71 staining supported integrity of the blood brain barrier out to 1 week for both the microdialysis probes and the MEAs. This data support that the ceramic MEAs small size and biocompatibility are necessary to accurately measure neurotransmitter levels in the intact brain. The minimal invasiveness of the MEAs reduce tissue loss, allowing for long term (>6 month) electrochemical and electrophysiological monitoring of brain activity.

Keywords

neurotransmitter; microelectrode; amperometry; microglia; Immunohistochemistry; biosensor

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Introduction

Many microelectrodes and implantable probes have been developed to study the dynamics of neurotransmitter signaling dynamics in the intact mammalian central nervous system of laboratory animals. The most widely used have been carbon fiber microelectrodes, hydrogel-coated microsensors and microdialysis probes (Bourdelaïs and Deutch, 1994; Taber and Fibiger 1994; Brake *et al.*, 1997; Belay *et al.*, 1998; Oldenziel *et al.*, 2006a; Oldenziel *et al.*, 2006b; Joyce *et al.*, 2007; Swamy and Venton 2007). These methodologies can address some of the needs for understanding neurochemical signaling in the CNS, however, none of these current technologies can address all the needs. Carbon fiber microelectrodes are minimally invasive, have rapid response times and have been used primarily to study rapid changes in dopamine, norepinephrine and serotonin. However, they have had limited utility for measuring basal levels of neurotransmitters (Daws *et al.*, 2005; Peters *et al.*, 2004). The gold standard for neurochemical measures has been microdialysis, which can be used in awake animals to measure multiple neurotransmitters at low levels of detection (Bourdelaïs and Deutch, 1994; Taber and Fibiger 1994; Takahata and Moghaddam 1998; Singer *et al.*, 2004). However, the sampling technique is limited in its spatial and temporal recording capabilities. In addition, it has been shown that they produce damage up to 1.4 mm remote from the implant site, have slow response times, may alter brain metabolism, and produce an astrocytic barrier following chronic implantation (Georgieva *et al.*, 1993; Tucci *et al.*, 1997; Belay *et al.*, 1998; Clapp-Lilly *et al.*, 1999; Kennedy *et al.*, 2002; Borland *et al.*, 2005; Schiffer *et al.*, 2006).

In vivo amperometric recordings combined with enzyme-based multisite ceramic microelectrode arrays (MEAs) have recently been used to investigate rapid changes in extracellular levels of glutamate, choline, acetylcholine and lactate in the brains of anesthetized and awake animals (Burmeister *et al.*, 2005; Parikh *et al.*, 2004; Nickell *et al.*, 2005; Bruno *et al.*, 2006; Day *et al.*, 2006 P; Parikh *et al.*, 2007; Rutherford *et al.*, 2007; Burmeister *et al.*, 2008; Hascup *et al.*, 2008). This recording technique has several advantages over other commonly used *in vivo* methods. Preliminary studies support that it is minimally invasive (Rutherford *et al.*, 2007), has the capability to chronically record a variety of neurotransmitters with low levels of detection, the recording technique has at least 2 Hz temporal resolution, and it is capable of recording resting neurotransmitter levels using self referencing methods (Day *et al.*, 2006; Rutherford *et al.*, 2007; Hascup *et al.*, 2008).

One of the central issues of the MEA technology is that they are capable of measuring neurotransmitter signals for over 2 weeks *in vivo* and the MEAs without enzyme coatings can record single unit neuron activity through electrophysiology for up to 6 months *in vivo* (Rutherford *et al.*, 2007; Burmeister *et al.*, 2008; Hascup *et al.*, 2008; unpublished data). For this reason, and as we extend the possible recording lifespan of the MEA technology, it is important to establish the effects of chronic implantation of the bare ceramic MEA on surrounding tissue. While the MEA tip is small (65 μm width, 125 μm thickness) and minimally invasive, the issue of tissue damage associated with chronic implantation of our ceramic MEAs has not been fully investigated. In the present study, we examined changes in astrocyte (glial fibrillary acidic protein; GFAP) and microglia (ionized calcium-binding adapter molecule 1; Iba1) levels, as well as glutamatergic nerve fibers (VGLUT1) and the blood brain barrier (SMI-71) surrounding the MEA in the prefrontal cortex (PFC) of rats at 1, 3 or 7 days and 1 or 6 months following implantation. In addition, to allow for direct comparisons, microdialysis probes were implanted into the PFC of rats for 1, 3 or 7 days. No recordings were done with the MEAs or microdialysis probes to allow for a more accurate comparison of damage between the two devices.

Results

The MEAs and non-dialyzed microdialysis probes were chronically implanted into the right PFC of Long Evans rats. Following 1, 3 or 7 days or 1 or 6 months (only 1, 3 or 7 days for microdialysis probe implants) rats were sacrificed and their brains were extracted and sectioned. MEA and microdialysis probe placements in the PFC were verified with cresyl violet staining, which at 7 days following implantation showed the minor tracks produced by the MEA (Figure 1A) and a pronounced track produced by the microdialysis probe (Figure 1B). Of particular interest is the amount of tissue drop out routinely seen from the effects of the microdialysis probes, which typically created a highly visible track not observed with the MEAs. In general changes in cell bodies and tissue drop out were minimally observed next to the MEA tracks over the 1 day to 6 months of implantation. An increase in cresyl violet (nissl) staining was observed directly surrounding the implantation site of the microdialysis probe, which correlates with the increase in Iba1 (microglia) observed directly surrounding the microdialysis implant site at the same time period (Figure 2f).

The GFAP staining was used to visualize astrocyte composition in the PFC of rats chronically implanted with either Nafion[®] coated MEAs or non-dialyzed microdialysis probes at 1, 3 or 7 days following implantation (Figure 2). The mean GFAP-staining density surrounding the microdialysis tracks was significantly elevated at 3 days (11.93 ± 2.46 , $p < 0.01$) and 1 week (26.95 ± 4.59 , $p < 0.001$) compared to control brain tissue (3.77 ± 0.77) (Figure 3a) and was elevated up to 300 μm from the edge of the tracks (Figure 2). Significant changes were also observed with GFAP staining in MEA implants at 3 days (12.47 ± 2.35 , $p < 0.05$) and 1 week (16.15 ± 6.61 , $p < 0.01$) following implantation. Of particular note is that the elevated GFAP staining was confined to within 50–100 μm surrounding the MEA implantation site, regardless of the duration of the implant, while elevated GFAP staining extended 200–300 μm from the edge of the microdialysis probe tracks. Additionally, we observed a non-significant small decrease in GFAP levels adjacent to the tracks at one day following implantation with both the MEAs and microdialysis probes. This may suggest a change in morphology surrounding the implant site at the one day time point.

The Iba1 staining was used to visualize microglia composition in the PFC of rats chronically implanted with either Nafion[®] coated MEAs or non-dialyzed microdialysis probes at 1, 3 or 7 days following implantation. As observed with the GFAP staining, increased Iba1 staining was within 50–100 μm of the MEA implantation site and up to 300 μm surrounding the microdialysis implant site (Figure 2). Again, in rats implanted with MEAs there were significant changes in the mean levels of Iba1 adjacent to the tracks at three days (12.99 ± 2.76 , $p < 0.05$) and 1 week (20.14 ± 7.33 , $p < 0.001$) following implantation. There was also a significant elevation in Iba1 staining adjacent to the microdialysis tracks at three days (19.45 ± 7.49 , $p < 0.05$) and 1 week (39.73 ± 9.05 ; $p < 0.001$) following implantation compared to control (4.80 ± 0.56 ; Figure 3b).

The blood-brain barrier was examined in rats implanted in the PFC with either MEAs or microdialysis probes at the 1, 3 or 7 day time points using the SMI-71 stain. Rats implanted with MEAs and those implanted with the microdialysis probes showed an intact blood-brain barrier surrounding the tracks (for example, day 3 is shown in Figure 4) supporting that differences observed between the two probes were not due to a disruption of the blood brain barrier. Previous MEA studies in our laboratory have reported reliable glutamate measures in awake rats and mice for at least one week following implantation (Rutherford *et al.*, 2007; Hascup *et al.*, 2008). Resting glutamate values obtained using the MEA in the PFC (44.9 ± 4.7 μM glutamate; Rutherford *et al.*, 2007) were elevated compared to values obtained by others using microdialysis (~ 2 – 9 μM glutamate; Baotell *et al.*, 1995; Rocha *et al.*, 1996). One possible explanation for the discrepancies in resting glutamate values could be damage near

the implantation site. Therefore, we wanted to examine glutamatergic nerve fibers near the MEA or microdialysis tracks. We observed consistent glutamatergic fiber innervations surrounding the MEA tracks at 1 week following implantation. However, at the same time point we observed a large area produced by the implantation of the microdialysis probe that did not contain any glutamatergic innervation as well as possible diminished glutamatergic fiber innervation adjacent to the microdialysis probe implant tracks (Figure 5) at the sampling area. GFAP and Iba1 were also used to examine the extent of damage adjacent to the MEA tracks at the one month and six month time points, in addition to the 1, 3 and 7 day time points. We observed slight glial scarring at six months, which is commonly seen in all types of chronic implants in this time range (Figure 6). At both the one month and six month time points, increases in GFAP and Iba1 were limited to within 50–100 μm from the MEA tracks, similar to our findings at 1, 3 or 7 days following implantation. We observed significantly elevated GFAP levels at the one (14.07 ± 4.34 , $p < 0.05$) and six (15.36 ± 2.25 , $p < 0.01$) month following implantation, however, no significant differences were observed in Iba1 staining at the same time points (Figure 7), indicating acceptance of the MEA by the surrounding brain tissue.

Discussion

The current studies investigated the histopathological effects of chronic MEA implantation at various time points from one day to six months *in vivo*. In addition, we made a direct comparison with microdialysis probes at relevant time points of 1, 3 and 7 days following implantation. Taken together, the data support that chronic implantation of MEAs produces minimal damage to the CNS tissue and less extensive than the effects (100–600 μm) often produced by silicon-based microelectrodes (Turner *et al.*, 1999; Szarowski *et al.*, 2003; Biran *et al.*, 2005; Biran *et al.*, 2007). In addition, the MEAs produce far less damage to CNS tissue as compared to microdialysis probes (Clapp-Lilly *et al.*, 1999; Borland *et al.*, 2005). Finally, we were able to show histological data supporting minimal tissue damage caused by the ceramic MEAs for up to 6 months following implantation, supporting the potential for long term electrophysiological applications of the MEAs, which has been carried out in the hippocampus of awake behaving rats (Gerhardt *et al.*, 2004).

At close evaluation, both GFAP (astrocytes) and Iba1 (microglia) staining were seen to surround the MEA tracks extending into tissue on average by 50 – 100 μm at all time points examined from 1 day through 6 months. GFAP levels were significantly elevated at all time points between three days and six months while Iba1 was significantly elevated only at three and seven days following implantation. The containment (50 – 100 μm) of the changes that did occur and the return to control levels of Iba1 by one month support that the ceramic MEAs are well tolerated by the brain for up to 6 months *in vivo* and did not lead to apparent rejection of the microelectrode. This is an improvement compared to recent studies conducted using shank silicon microelectrodes where activated microglia were elevated more than 500 μm and decreased neuronal density up to 230 μm from the microelectrode surface at time points ranging from one to twelve weeks (Biran *et al.*, 2005; Biran *et al.*, 2007; Leung *et al.*, 2008).

Previous microdialysis implantation studies observed a high degree of damage to the brain at the site of implant with extended damage observed up to 1.4 mm from the site of implantation within 30 hours when examined with electron microscopy and carbon fiber microelectrode measures (Clapp-Lilly *et al.*, 1999; Borland *et al.*, 2005). Our findings also showed a high degree of cell loss, nerve fiber damage, and elevated astrocyte and microglia staining up to 300 μm from the microdialysis probe tracks. The smaller area of damage observed in our study may be due to dissimilarities in procedures, including differences in perfusion of the microdialysis probes (we did not perfuse, both the previous studies perfused), the brain region (we implanted in the prefrontal cortex, the previous studies were in the striatum), and the manner of discerning damage (we used immunohistological measures, the previous studies

used light microscopy, electron microscopy, or voltammetry). By contrast, our MEAs did not disrupt brain tissue more than 50–100 μm from the microelectrode tracks and qualitatively, cell morphology remained healthy with normal processes and cell shape up to 6 months following implantation. In these studies we made a direct comparison between chronic MEA and non-dialyzed microdialysis probe tracks using cresyl violet, GFAP, Iba1, VGLUT1, and SMI-71. Both GFAP and Iba1 mean density values were significantly elevated around tracks from the microdialysis probe implants at three days ($p<0.01$ and $p<0.05$, respectively) and one week ($p<0.001$) compared to control. Additionally, GFAP levels appeared to be more concentrated immediately surrounding (within 100 μm) the microdialysis probes than observed with MEA implantation. Our data therefore suggest that differences in measured resting glutamate levels between microdialysis and ceramic MEAs may reflect differences in astrocytic mechanisms of glutamate uptake.

Furthermore, based on a two-way ANOVA analysis no significant differences were observed in Iba1 levels between microdialysis probes and MEA implants ($p=0.061$). As with GFAP, we observed increased Iba1 staining immediately surrounding (within 50 μm) the microdialysis implantation compared to MEA implantation, however, taken over the analyzed area this was not significant. Additionally, microdialysis probe implantation resulted in increased tissue loss (up to 180 μm), compared to less than 50 μm with MEAs, and decreased glutamatergic nerve fiber innervations at the sampling area directly surrounding the implantation site, which could account for discrepancies between resting glutamate levels reported with MEA recordings and microdialysis. Reduced nerve fiber density has also been shown with shank silicon microelectrodes at a distance of up to 230 μm (Biran *et al.*, 2005; Leung *et al.*, 2008). However, both the MEA and microdialysis probe implant sites showed evidence for an intact blood-brain barrier at 1, 3 and 7 days following implantation, suggesting that the observed damage was due to the implantation and not from the breakdown of the blood-brain barrier.

Upon further examination of the GFAP staining, although not significant, we observed a small decrease in GFAP stained cells near the MEA and microdialysis implant tracks after 1 day compared to control. GFAP staining reappeared at day 3 following implantation; however, staining was still remote from the surface (Figure 2). As previously mentioned, at three and seven days following implantation, GFAP staining was significantly elevated around the microdialysis probe implant tracks compared to control and staining was seen around the tracks. This supports either a loss of astrocytes surrounding the implant site or a change in astrocyte composition such that they are no longer stained with GFAP, which could potentially lead to inaccurate clearance measures at 1 day following implantation for neurotransmitters such as glutamate.

In summary, the present studies examining the implications of chronically implanting devices into the brain that are capable of measuring neurotransmitters have helped us to better understand not only the short and long term damage imposed by these implantations, but also possible implications in the effectiveness of the probes for reliably detecting neurotransmitters. Our laboratory has previously provided evidence for the need to record neurotransmitters, such as glutamate, from unanesthetized animals (Rutherford *et al.*, 2007, Hascup *et al.*, 2008) and that our ceramic MEAs are capable of recording for at least 6 months *in vivo* (Gerhardt *et al.*, 2004). Here we show that chronic implantation of the ceramic MEAs leads to less damage than other conventional methods for chronically recording neurotransmitters, such as microdialysis, and provide one possible explanation to the discrepancies observed in basal glutamate levels between the two recording methods. In addition, the MEAs appear to produce comparable or less effects on brain tissue when compared to other silicon-based microelectrodes supporting that the MEAs may be useful for chronic electrophysiological studies of single neuron activity for at least 6 months *in vivo*. The decreased disruption of tissue immediately surrounding the implant observed with the MEAs support that recordings using the MEAs allow for longer,

more reliable, and more accurate measures of neurotransmitters with a closer proximity to the synapse in addition to the added advantage of an increased time resolution over microdialysis.

Experimental Procedure

Animals

Male Long Evans (Harlan) rats 3–6 months of age (350–550 g) at the time of surgery were used for all experiments. The animals were individually housed in a 12 hour light/dark cycle with food and water available *ad libitum*. All procedures were in accordance to those specified by the Association for Assessment and Accreditation of Laboratory Animal Care International. Animals were allowed at least one week to acclimate to the environment prior to any experiments. All appropriate animal care (food, water, bedding, cage cleaning, etc.) was performed by the Animal Resource Center staff. There were no procedures involving undue discomfort to the animals. Following surgery, rats were individually housed under the same conditions. Animal care was approved by the University of Kentucky Institutional Animal Care and Use Committee and was in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Surgical Procedure for Microdialysis and MEA Implantation

Surgical and implantation procedures followed those previously described (Rutherford *et al.*, 2007). Briefly, male Long Evans rats were implanted with either ceramic MEAs or microdialysis probes (CMA 11; membrane length: 2 mm) into the right PFC (AP: +3.2; ML: –0.8; DV: 4.5 mm vs. bregma; Paxinos and Watson, 1998) under isoflurane anesthesia (2%) using a hydraulic microdrive connected to a stereotaxic arm and were allowed to recover. The total implantation time, including sedation, incision, craniotomy, skull screw placement, implantation of either the microdialysis probe or the MEA, dental cement application, and regaining of consciousness was accomplished in approximately 20–25 minutes for all rats. Following implantation surgery, rats were returned to the animal facilities and remained there for either 1, 3 or 7 days, or 1 or 6 months (n=4 per group, except 1 month group n=3). Four research naïve rats were used as controls at these time points. The MEAs were coated with Nafion® as previously described but were not coated with enzyme (Rutherford *et al.*, 2007). Microdialysis probes were not perfused following implantation. There were no recording sessions with these animals. After the designated time points following surgery (1, 3, or 7 days, or 1 or 6 months), rats were anesthetized with isoflurane and transcardially perfused with 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde for three days. After three days, the storage solution was changed to 0.1 M phosphate buffer with 10% sucrose.

Histology

The brains, which were stored in 0.1 M phosphate buffer with 10% sucrose, were rinsed in sucrose and sections (14 µm) were collected from the implantation sites using a cryostat. The sections were postfixed in acetone for 3 minutes prior to being processed for indirect immunohistochemistry. Some sections were processed for studies of cell bodies using cresyl violet staining. Antibodies used were raised against GFAP (anti-mouse, diluted 1:400; Chemicon International Inc., Temecula, CA, USA) as a marker for astrocytes, Iba1 (anti-rabbit, diluted 1:1000; Wako Chemicals GmbH, Karlsruhe, Germany) as a pan-microglia marker (Imai *et al.*, 1996; Ito *et al.*, 1998), mouse anti-SMI-71 (diluted 1:500; Covance, Berkeley, CA, USA) as a marker for intact blood-brain barrier, rabbit anti-laminin (diluted 1:100; Abcam, Cambridge, UK) as a marker for all blood vessels, and guinea pig anti-vesicular glutamate transporter 1 (VGLUT1, Chemicon International Inc., Temecula, CA, USA) as a marker for glutamatergic nerve fibers and terminals. Incubations for primary antibodies were performed for 48 hours at 4°C. After washing, the sections were incubated in Alexa 488 and Alexa 594 secondary antibodies (diluted 1:500, Molecular Probes, Leiden, The Netherlands) for 1 hour

at room temperature. Antibodies were diluted in phosphate buffered saline (PBS) containing 0.3% Triton-X. After additional rinsing, the sections were mounted in 90% glycerin in PBS. Double labeling of GFAP/Iba1 and SMI-71/laminin were performed in sequence such that each type of antibody was applied one at a time. Sections were evaluated using image analysis and Improvision software (Improvision®, Sweden). The densities of GFAP and Iba1 immunoreactivities were calculated using NIH image software. Images were captured using a 20 × lens and the areas immunoreactive for GFAP or Iba1 were made as binary images. Gray values were measured over the standardized area adjacent to the track on the binary images and expressed as mean gray density. Background values from the same section were subtracted from the measured gray values. Mean values from four sections from each brain were analyzed using a one way ANOVA followed by Tukey's post hoc analyses. Furthermore, a two way ANOVA was conducted to determine significance between microdialysis probe and ceramic MEA implants in GFAP or Iba1. All measurements were performed on blind coded slices. Significance was determined at $p < 0.05$.

Abbreviations used

MEA	microelectrode array
FAST -16	fast analytical sensing technology
GFAP	glial fibrillary acidic protein
PBS	phosphate buffered saline
Iba1	ionized calcium-binding adapter molecule 1
PFC	prefrontal cortex

References

- Belay A, Collins A, Ruzgas T, Kissinger PT, Gorton L, Csöregi E. Redox hydrogel based bienzyme electrode for L-glutamate monitoring. *J. Pharmaceutical and Biomedical Analysis* 1998;19(1–2):93–105.
- Biran R, Martin DC, Tresco PA. Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays. *Exp. Neurol* 2005;195(1):115–126. [PubMed: 16045910]
- Biran R, Martin DC, Tresco PA. The brain tissue response to implanted silicon microelectrode arrays is increased when the device is tethered to the skull. *J. Biomed. Mater Res A* 2007;82(1):169–178. [PubMed: 17266019]
- Boatell ML, Bendahan G, Mahy N. Time-related cortical amino acid changes after basal forebrain lesion: a microdialysis study. *J. Neurochem* 1995;64:285. [PubMed: 7798924]
- Borland LM, Guoyue S, Hua Y, Michael AC. Voltammetric study of extracellular dopamine near microdialysis probes acutely implanted in the striatum of anesthetized rat. *J. Neurosci. Methods* 2005;146:149–158. [PubMed: 15975664]
- Bourdelaïs AJ, Deutch AY. The effects of haloperidol and clozapine on extracellular GABA levels in the prefrontal cortex of the rat: an in vivo microdialysis study. *Cereb Cortex* 1994;4(1):69–77. [PubMed: 8180492]
- Brake WG, Noel MB, Boksa P, Gratton A. Influence of perinatal factors on the nucleus accumbens dopamine response to repeated stress during adulthood: an electrochemical study in the rat. *Neurosci* 1997;77(4):1067–1076.
- Bruno JP, Gash C, Martin B, Zmarowski A, Pomerleau F, Burmeister J, Huettl P, Gerhardt GA. Second-by-second measurement of acetylcholine release in prefrontal cortex. *Eur. J. Neurosci* 2006;24:2749–2757. [PubMed: 17156201]
- Burmeister JJ, Palmer M, Gerhardt GA. L-lactate measures in brain tissue with ceramic-based multisite microelectrodes. *Biosens Bioelectron* 2005;20(9):1772–1779. [PubMed: 15681193]

- Burmeister JJ, Pomerleau F, Huettl P, Gash CR, Werner CE, Bruno JP, Gerhardt GA. Ceramic-based multisite microelectrode arrays for simultaneous measures of choline and acetylcholine in CNS. *Biosens Bioelectron* 2008;23(9):1382–1389. [PubMed: 18243683]
- Clapp-Lilly KL, Roberts RC, Duffy LK, Irons KP, Hu Y, Drew KL. An ultrastructural analysis of tissue surrounding a microdialysis probe. *J. Neurosci. Methods* 1999;90:129–142. [PubMed: 10513596]
- Day BK, Pomerleau F, Burmeister JJ, Huettl PF, Gerhardt GA. Microelectrode array studies of basal and potassium-evoked release of L-glutamate in the anesthetized rat brain. *J. Neurochem* 2006;96(6):1626–1635. [PubMed: 16441510]
- Georgieva J, Luthman J, Mohring B, Magnusson O. Tissue and Microdialysate Changes After Repeated and Permanent Probe Implantation in the Striatum of Freely Moving Rats. *Brain Research Bulletin* 1993;31:463–470. [PubMed: 8098651]
- Hascup KN, Hascup ER, Pomerleau F, Huettl P, Gerhardt GA. Second-by-second measures of L-glutamate in the prefrontal cortex and striatum of freely moving mice. *J. Pharmacol. Exp. Ther* 2008;324(2):725–731. [PubMed: 18024788]
- Imai Y, Iba T, Ito D, Ohsawa K, Kohsaka S. A novel gene *iba1* in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. *Biochem Biophys Res Commun* 1996;224(3):855–862. [PubMed: 8713135]
- Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuchi Y, Kosaka S. Microglia-specific localization of a novel calcium binding protein, *Iba1*. *Brain Res mol Brain Res* 1998;57(1):1–9. [PubMed: 9630473]
- Joyce BM, Glaser PE, Gerhardt GA. Adderall produces increased striatal dopamine release and a prolonged time course compared to amphetamine isomers. *Psychopharm. (Berl.)* 2007;191(3):669–677.
- Kennedy RT, Watson CJ, Haskins WE, Powell DH, Strecker RE. In vivo neurochemical monitoring by microdialysis and capillary separations. *Curr. Op. Chem. Biol* 2002;6:659–665.
- Leung BK, Biran R, Underwood CT, Tresco PA. Characterization of the microglial attachment and cytokine release on biomaterials of differing surface chemistry. *Biomaterials* 2008;29(23):3289–3297. [PubMed: 18485471]
- Nickell J, Pomerleau F, Allen J, Gerhardt GA. Age-related changes in the dynamics of potassium-evoked L-glutamate release in the striatum of Fischer 344 rats. *J. Neural Transm* 2005;112:87–96. [PubMed: 15599607]
- Oldenziel WH, Dijkstra G, Cremers TIFH, Westerink BHC. In vivo monitoring of extracellular glutamate in the brain with a microsensor. *Brain Res* 2006a;1118:34–42. [PubMed: 16956598]
- Oldenziel WH, Dijkstra G, Cremers TIFH, Westerink BHC. Evaluation of hydrogel-coated glutamate microsensors. *Anal. Chem* 2006b;78:3366–3378. [PubMed: 16689539]
- Parikh V, Pomerleau F, Huettl P, Gerhardt GA, Sarter M, Bruno JP. Rapid assessment of in vivo cholinergic transmission by amperometric detection of changes in extracellular choline levels. *European Journal of Neuroscience* 2004;20:1545–1554. [PubMed: 15355321]
- Parikh V, Kozak R, Martinez V, Sarter M. Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron* 2007;56:141–154. [PubMed: 17920021]
- Paxinos, G.; Watson, C. The rat brain: stereotaxic coordinates. New York: Academic Press; 1998.
- Peters JL, Miner LH, Michael AC, Sesack SR. Ultrastructure at carbon fiber microelectrode implantation sites after acute voltammetric measurements in the striatum of anesthetized rats. *Journal of Neuroscience Methods* 2004;137:9–23. [PubMed: 15196823]
- Pomerleau F, Day BK, Huettl P, Burmeister JJ, Gerhardt GA. Real time in vivo measures of L-glutamate in the rat central nervous system using ceramic-based multisite microelectrode arrays. *Ann. N.Y. Acad. Sci* 2003;1003:454–457. [PubMed: 14684487]
- Rocha L, Briones M, Ackermann RF, Anton B, Maidment NT, Evans CJ, Engel J Jr. Pentylentetrazol-induced kindling: early involvement of excitatory and inhibitory systems. *Epilepsy Res* 1996;26:105–113. [PubMed: 8985692]
- Rutherford EC, Pomerleau F, Huettl P, Strömberg I, Gerhardt GA. Chronic second-by-second measures of L-glutamate in the central nervous system of freely moving rats. *J. Neurochem* 2007;102:712–722. [PubMed: 17630982]

- Schiffer WK, Mirrione MM, Biegon A, Alexoff DL, Patel V, Dewey SL. Serial microPET measures of the metabolic reaction to a microdialysis probe implant. *Journal of Neuroscience Methods* 2006;155:272–284. [PubMed: 16519945]
- Singer S, Rossi S, Verzosa S, Hashim A, Lonow R, Cooper T, Sershen H, Lajtha A. Nicotine-induced changes in neurotransmitter levels in brain areas associated with cognitive function. *Neurochem. Res* 2004;29(9):1779–1792. [PubMed: 15453274]
- Swamy BE, Venton BJ. Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo. *Analyst* 2007;132(9):876–884. [PubMed: 17710262]
- Szarowski DH, Andersen MD, Retterer S, Spence AJ, Isaacson M, Craighead HG, Turner JN, Shain W. Brain responses to micro-machined silicon devices. *Brain Res* 2003;983:23–35. [PubMed: 12914963]
- Taber MT, Fibiger HC. Cortical regulation of acetylcholine release in rat striatum. *Brain Res* 1994;639(2):354–356. [PubMed: 8205490]
- Takahata R, Moghaddam B. Glutamatergic regulation of basal and stimulus-activated dopamine release in the prefrontal cortex. *J. Neurochem* 1998;71(4):1443–1449. [PubMed: 9751176]
- Tucci S, Rada P, Sepulveda MJ, Hernandez L. Glutamate measured by 6-s resolution brain microdialysis: capillary electrophoretic and laser-induced fluorescence detection application. *J. Chromatography* 1997;694:343–349.
- Turner AJ, Shain W, Szarowski DH, Andersen M, Martins S, Isaacson M, Craighead H. Cerebral astrocyte response to micromachined silicon implants. *Experimental Neurology* 1999;156:33–49. [PubMed: 10192775]

Figure 1.

Photomicrograph showing cresyl violet staining of an MEA track (a) and a track from a microdialysis probe (b) in the right prefrontal cortex. The MEA track is clearly visible in the right prefrontal cortex in both the MEA and microdialysis probe tracks; however, the microdialysis track shows greater cell damage.

Figure 2.

Photomicrographs of GFAP and Iba1 staining in the rat prefrontal cortex following chronic implantation of MEAs or microdialysis probes.

Rats were implanted with either MEAs (a, c or e) or microdialysis probes (b, d or f) and sacrificed after 1 (a, b), 3 (c, d) or 7 (e, f) days *in vivo*. Images are shown for GFAP (red) and Iba1 (green) co-staining. Scale bar is 50 μ m.

Figure 3.

Average effects of chronic implantation of MEAs or microdialysis probes on GFAP and Iba1 staining in rat prefrontal cortex. The quantification of GFAP (a) and Iba1 (b) levels was carried out at 1, 3 or 7 days post implantation. MEA and microdialysis probe implantations lasting three and seven days had significantly elevated GFAP and Iba1 levels compared to control values. No significant differences were observed between the GFAP and Iba1 levels adjacent to the MEA and microdialysis probes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4.

Photomicrographs showing SMI-71 staining in the prefrontal cortex following chronic implantations of MEAs or microdialysis probes. Rats were implanted with either MEAs (a) or microdialysis probes (b) and sacrificed after 1, 3 or 7 days (day 3 shown). The blood-brain barrier was intact in brains implanted with MEAs and microdialysis probes at all time points. Scale bar is 25 μ m.

Figure 5.

Photomicrographs showing VGLUT1 staining for glutamatergic fibers in tracks following chronic implantation of MEAs or microdialysis probes. Rats were implanted with either MEAs (a) 1 week implant (arrow indicated MEA location), c) 6 month implant or microdialysis probes (b) 1 week. Glutamatergic nerve fibers remained intact in the MEA implants, with a glial scar clearly visible at 6 months post implantation. Glutamatergic nerve fibers were decreased in the tracks at 1 week following microdialysis probe implantation. Scale bar is 25 μ m.

Figure 6.

Photomicrographs showing GFAP (red) and Iba1 (green) staining following chronic implantation of MEAs in rat prefrontal cortex. Rats were implanted with MEAs and sacrificed after one (b) or six (c) months and compared to controls (a). GFAP levels adjacent to the MEA tracks were significantly elevated at one and six months, however, there was no significant change in Iba1 levels at one or six months. A small glial scar was observed at six months following implantation. Scale bar is 50 μ m.

Figure 7.

Average effects of chronic implantation of MEAs and microdialysis probes on GFAP and Iba1 immunoreactivity adjacent to the tracks. The bar graphs show quantification of GFAP (a) and Iba1 (b) levels observed following chronic implantation of MEAs for days 1, 3 and 7, or 1 or 6 months. GFAP levels were significantly elevated at all time points between 3 days and 6 months. Iba1 levels were only significantly elevated at 3 and 7 days following implantation and returned to control levels by 1 month. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.