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THE USE OF INTRACEREBRAL MICRODIALYSIS TO ELUCIDATE ENVIRONMENTALLY INDUCED NEUROTOXIC MECHANISMS

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Abstract

The technique of microdialysis permits the assessment of neurotransmitter activity and the monitoring of other cellular entities in tissue extracellular fluid. The method is widely used for quantifying biogenic amine and amino acid transmitters, peptides, administered drugs, and other molecules in response to various experimental treatments. This unit provides an overview of the manner in which the methodology of intracerebral microdialysis is utilized in the field of neurotoxicology to elucidate the actions of environmental agents. The technique is employed in a variety of creative ways to address specific experimental goals involving a myriad of toxicants. With appropriate consideration of method parameters investigators have also been able to address mechanistic issues in their studies. These investigations consist of sampling of neurotransmitters in extracellular fluid after various protocols of environmental metal exposure as well as assessments of blood-brain barrier permeability, the detection of reactive oxygen species, and description of the toxicodynamics of environmental agents. The purpose of this examination is not to review the investigational findings *per se*, but to highlight the various approaches utilized with this methodology and the experimental questions that have been addressed.

INTRODUCTION

The technique of microdialysis permits the assessment of neurotransmitter activity and the monitoring of other cellular entities in tissue extracellular fluid. The method is widely used for quantifying biogenic amine and amino acid transmitters, peptides, administered drugs, and other molecules in response to various experimental treatments. The list of potential microdialysis analytes is limited only by the availability of appropriately sensitive analytical methods for their determination. However, the methodology has also been creatively applied to many other experimental goals, resulting in a voluminous literature extending back to the 1960's.

This overview will examine the manner in which this methodology has been utilized in the field of neurotoxicology. These studies encompass much of the period of the widespread use of microdialysis going back 30 years and consist of sampling of neurotransmitters in extracellular fluid after various protocols of environmental metal exposure as well as

assessments of blood-brain barrier permeability, the detection of reactive oxygen species, and description of the toxicodynamics of environmental agents. The purpose of this examination is not to review the investigational findings *per se*, but to highlight the various approaches utilized with this methodology and the experimental questions that have been addressed in the hope of encouraging further development of applications of microdialysis in the field of neurotoxicology.

DEVELOPMENT OF MICRODIALYSIS

History

The initial efforts directed to the methodology were spurred by several laboratory groups in the 1960's and -70's attempting to develop an approach that would permit sampling of focused interstitial tissue in small animals on a minute basis. Ungerstedt and colleagues substantially improved the design and efficiency of a microdialysis probe consisting of a rigid cannula with a small loop of semi-permeable tubing at its tip (Ungerstedt and Pycock, 1974; Ungerstedt *et al.*, 1982). Thus, a small brain area could be perfused in the absence of anesthesia by connecting the probe to a liquid swivel and using a harness to restrain the animal within the confines of a small test chamber. The results of successful use of microdialysis to quantify biogenic amine levels in brain tissue were first reported by Ungerstedt and colleagues at international conferences in 1981–82, and this contributed significantly to the worldwide spread of the technique. The applicability of microdialysis as a tool for examining neurotransmitter release in the central nervous system or assessing the metabolic activity of any other tissue is now well documented, and the extension of the method to other experimental goals has been summarized in multiple excellent reviews (e.g., Chefer *et al.*, 2009; Herrera-Marschitz *et al.*, 2010).

Description

The microdialysis probe is a central component of the method, functioning as the actual site of sampling of the extracellular space. Probes consist of a segment of tubular dialysis membrane through which a solution is perfused continually. The dialysis membrane is semi-permeable with transport limited to cellular entities with molecular weights less than that specified for the tubing, typically less than 20,000 daltons. Once the probe is inserted into the tissue and perfusion begins, compounds in the extracellular milieu surrounding the dialysis membrane diffuse through the membrane into the probe down their concentration gradient (see Figure 1). The outflow of the probe is collected by various means for analyte determination. Typical perfusion rates are in the range of 0.5 – 2.0 $\mu\text{L}/\text{min}$ and depend heavily on the analytical sensitivity needed with sample collection times ranging from 1 – 30 min.

Various factors that affect the relationship between the analyte concentration in the extracellular fluid and the measured concentration in the perfusate can be expressed collectively as the extraction fraction. Analytical instrumentation has progressed to the point that several endogenous substances in the dialysates can be determined simultaneously. These compounds include biogenic amine and amino acid neurotransmitters, neuropeptides, and metabolism products, and have been typically quantified by HPLC systems utilizing a

variety of detection mechanisms. Typically then, dialysate analytes are measured under both baseline and physiologically or pharmacologically stimulated conditions.

Tenets of Microdialysis

During the microdialysis process analytes move from an area of higher concentration to a space of lower concentration. The direction of this movement is determined by the experimental conditions, which bias the process so that the analyte concentration is higher on one side of the microdialysis probe membrane. Accordingly, the methodology is employed to either sample a substance from a tissue compartment or to deliver a drug or molecule to such a compartment (i.e., in the process of retrodialysis) or both. These manipulations permit the investigator to draw conclusions about extracellular levels of analyte related to behavioral, pharmacological, or physiological interventions.

The microdialysis probe is a major component of the experimental construct, and while probes of different designs have been utilized, those of concentric design are commonly employed in studies of brain function. The probe is stereotactically inserted into the brain target area through a guide cannula surgically implanted several days earlier. The probe is then constantly perfused with physiological solution (e.g., Ringer's) at a low flow rate. This perfusing solution does not contact the surrounding tissue, but the semi-permeability of the dialysis membrane permits mass transfer to occur. The transfer also is affected by the analyte's molecular weight, structure, and polarity. These entities can move through the membrane freely in both directions. Over time a concentration equilibrium develops.

It should be noted that the tissue exchanging with the perfusate is confined to a relatively thin layer of surrounding matrix that is physically close to the probe surface. However, despite this small volume of tissue, depletion of the analyte pool by the outward flux of molecules does not typically occur, as the analyte concentration is maintained by replenishment from more distant areas of the extracellular space. Sophisticated mathematical models of these compartmented diffusion systems were developed and have demonstrated that the analyte concentration in the tissue exchange layer is a function only of the tissue exit of the analyte and its neuronal or glial recapture (Morrison *et al.*, 1991; Chefer *et al.*, 2009).

DETERMINATION OF ANALYTE EXTRACTION EFFICIENCY

Probe recovery is the important quantity underlying probe efficiency. The recovery value expresses the proportion of analyte transferred and retained in the perfusion solution relative to the tissue concentration. In general, recovery is a function of the perfusate flow rate, the diffusion resistance of the tissue, structural features of the molecule, and surface dimensions of the dialysis membrane.

Relative and absolute recovery are conceptually distinct. Relative recovery is defined as the concentration of analyte retained in the probe lumen in relation to the extracellular concentration and is typically expressed as a percentage. Absolute recovery describes the quantity of analyte transferred during a specified interval of time expressed in mass per unit time. Most commonly, experimenters seek accuracy in the extracellular analyte concentration. At flow rates optimal for recovery of endogenous compounds from tissue –

typically 0.3–2.0 $\mu\text{L}/\text{min}$ (Justice, 1993) – actual *in vivo* recovery is in the range of 5–20% depending on the analyte (Anderzhanova and Wotjak, 2013). However, because of the limitations affecting determinations of recovery, most investigators employ within and between subject comparisons of relative changes in extracellular analyte levels.

Although relative recovery estimates have been determined *in vitro* by immersing a microdialysis probe in an analyte solution of known concentration, this method of calculating extracellular levels of the compound of interest has been shown to be inaccurate (Glick *et al.*, 1994). This is due to the fact that diffusion in tissue is substantially different from diffusion in a solution (Nicholson and Rice, 1986). Alternatively, improved measurement of *in vivo* recovery of extracellular analyte concentrations can be empirically determined by any one of several quantitative methods (see Table 7 in Kho *et al.*, 2016), and these approaches will promote more accurate determination of the true extracellular values.

The three most commonly utilized quantitative methods for determination of extracellular concentrations of compounds of interest are: 1) zero-net-flux microdialysis; 2) the low flow rate method; and 3) the internal standard method. In the zero-net-flux method of *in vivo* microdialysis the tissue is perfused sequentially with several different analyte concentrations that are expected to bracket the true analyte concentration in the extracellular space. The amount of the compound of interest that is gained or lost from the probe is determined analytically, and the gains/losses are plotted as a function of the initial perfusate concentration. The line through the experimental points is determined by regression, and the intersection of this line with the abscissa axis is the extracellular analyte concentration.

Recovery is a function of the rate of perfusion, and the low flow rate method is based on the observation that relative analyte recovery approaches 100% as the flow rate is reduced to a very low value, e.g., 0.1 $\mu\text{L}/\text{min}$. This method can be impractical, as it invariably requires either the ability to analyze very small sample volumes or long collection times to produce manageable volumes. The internal standard method requires identification of a molecule whose diffusion characteristics are similar to those of the analyte, but which is not present endogenously in the extracellular fluid. The molecule is added to the perfusing solution, and its loss during dialysis is measured and this proportion of the original concentration constitutes the *in vivo* recovery value.

FACTORS SPECIFIC TO TOXICOLOGICAL INVESTIGATIONS

While the preceding sections describe the basic properties and characteristics of the microdialysis methodology, there are additional factors that must be considered for neurotoxicological investigations. These factors are based on interactions of the toxic entity with components of the perfusing solution or with endogenous extracellular ions and molecules and may also depend on the equilibrium between bound and free toxicant in tissue. Integration of these factors into the principles of microdialysis will generally enhance the complexity of the experimental investigation, but they are necessary considerations for proper interpretation of the results.

In the presence of a chronic toxic exposure with a toxicant that is a polar molecule or ion (e.g., as with heavy metals), most of the substance may be bound to storage proteins in neurons and/or glia, and thus only the free molecule/ion is assumed to be the active moiety and can directly dialyze into the perfusing solution. An equilibrium will exist between the bound and free toxicant, and if this equilibrium is slower to restore the free form in extracellular fluid than the rate of free toxicant removal by microdialysis, then a relative depletion of the active toxicant in extracellular fluid can occur (e.g., see Benveniste *et al.* 1989). If this depletion is sustained over the several hours of a typical microdialysis session, then the observed effect of the toxic exposure cannot be simply assumed to be based on the free molecule/ion. One must consider that the exposure effects may be due to structural neuronal or neurochemical changes (e.g., in signaling pathways) induced by the chronic exposure and that emanate less from the free form of the toxicant.

Another concern is that while the most commonly used perfusing solution for microdialysis is artificial cerebrospinal fluid (ACSF), other isotonic physiological solutions such as Ringer's or modified Ringer's have been effectively utilized. The concern here is that the perfusate components do not interact with the free toxic entity in extracellular fluid – primarily a concern with chronic heavy metal exposures - or otherwise disrupt the equilibrium between the bound and free toxicant. For example, PO_4^{-3} ions are known to bind Pb^{+2} in solution, so when studying chronic lead exposure in an animal model, a conservative choice for a perfusing solution might be a modified Ringer's that contains only Na^+ , K^+ , and Ca^{+2} . Such a choice would avoid any confounding or interfering effects on the experimental observations.

MICRODIALYSIS IN NEUROTOXICOLOGY – SPECIALIZED METHODS

In developing an overview of the use of intracerebral microdialysis in neurotoxicology research, published studies have been organized into two groups based on the manner, albeit subjective, in which the methodology was applied and the endpoints measured. The first group consists of a group of investigations in which microdialysis was employed in unconventional ways to achieve experimental goals. The second group includes studies utilizing various toxicants that have been studied in a more conventional manner with this methodology to assess exposure effects on neurotransmitters and cellular second messengers and metabolites. This latter group will be addressed in the next section. The primary focus of the overview of these reports is the manner and efficiency in which the methodology was employed and less so about the experimental findings *per se*.

To clarify whether manganese acts in association with synaptic neurotransmission Takeda *et al.* (2002) utilized *in vivo* microdialysis to assess the movement of Mn^{+2} during neuronal activity. These investigators focally administered 0.1 $\mu\text{L}/\text{min}$ of $^{54}\text{MnCl}_2$ (a gamma-ray emitter) via stereotactically implanted cannulae into rat amygdala or hippocampus for 50 minutes. One hour later the administration site was perfused with ACSF via microdialysis probe at 2.0 $\mu\text{L}/\text{min}$. Following 60 minutes of this perfusion the solution was changed to ACSF containing 100 mM K^+ for 30 minutes to depolarize the nerve endings. Takeda *et al.* (2002) demonstrated that an increased amount of $^{54}\text{Mn}^{+2}$ was released into the dialysate from both brain areas during the period of high K^+ perfusion, and that the increase could be

blocked by co-administration of 1 μ M tetrodotoxin. These workers also reported high correlations between the increases in $^{54}\text{Mn}^{+2}$ release and that of glutamate during high K^{+} perfusion, suggesting that Mn^{+2} is released from glutamatergic nerve terminals. Subsequently, they also showed that administration of unlabeled 200 nM MnCl_2 by microdialysis into the same brain regions resulted in decreases in extracellular glutamate, aspartate, and GABA, signifying that Mn^{+2} may influence Ca^{+2} -dependent synaptic transmission. In later work (Takeda *et al.*, 2003) these same authors used microdialysis to replicate in rat striatum the high K^{+} -induced release of previously taken up $^{54}\text{Mn}^{+2}$. However, when the striatal tissue was directly perfused with 200 nM MnCl_2 via microdialysis probe, only extracellular GABA levels were diminished. Nevertheless, this was additional evidence that Mn^{+2} could modulate synaptic transmission.

Qi *et al.* (2013) developed a simple, inexpensive and rapid method to detect Pb^{+2} in biological samples by preparing graphene oxide functionalized with 3,9-dithia-6-monoazaundecane (DMA). They also knew that tryptophan can coordinate with metal ions through its carboxylate and amino groups and exhibits a natural fluorescence. In their system in the presence of the graphene quantum dot-DMA composite Pb^{+2} would serve as a selective cross-linker to the tryptophan, resulting in a significant increase in fluorescence and a limit of detection of 9 pM. No other heavy metals exhibited significant changes of fluorescence in this system. These workers subsequently added the composite and tryptophan to the perfusing solution and employed *in vivo* microdialysis of rat cerebrospinal fluid to demonstrate the applicability of this method. They reported that the concentrations of Pb^{+2} determined with this method were in good agreement with measures obtained by inductively coupled plasma-atomic emission spectroscopy. The method thus exhibited a high level of selectivity and sensitivity.

Su *et al.* (2014) coupled microdialysis sampling to highly sensitive inductively coupled plasma-mass spectrometry (ICP-MS) to evaluate the *in vivo* permeability of the blood-brain barrier to four arsenic metabolites, molybdate, and methylmercury (MeHg). They also developed a solid phase extraction step based on ion-nonfunctionalized polymer interactions to separate the metal species from the salt-heavy microdialysis perfusing solution and produce samples more amenable to ICP-MS. The microdialysis probe membrane was polyarylethersulfone with a molecular weight cutoff of 20 kDa and was implanted in the rat hippocampus. The system components and sampling rates were integrated in a manner that permitted continual monitoring of the microdialysate stream by ICP-MS. The animals were injected i.p. independently with 1 mg As/kg for each of the arsenicals or 1 mg molybdate/kg or 10 mg MeHg/kg. Microdialysate levels of the analytes were corrected by recovery values determined *in vitro*. Clearly observable blood-brain barrier permeability occurred with the arsenicals and molybdate with peak extracellular concentrations exhibited 3–5 hours after injection. However, the recovery of Hg^{+2} with the probe membrane was prohibitively low, resulting in values for MeHg below the detection limit even at higher administered doses. The authors proposed that the MeHg crossing the blood-brain barrier may have been bound to neurons or glia. Nonetheless, the system proved viable for monitoring arsenical and molybdate toxicokinetics.

Yokel and co-workers utilized microdialysis in various ways in an investigation of aluminum toxicokinetics in brain that extended over 6 publications and 10 years. Allen and Yokel (1992) implanted microdialysis probes in rat frontal cortex and lateral ventricle to assess blood-brain barrier permeation of the metal and in the jugular vein to determine unbound levels of aluminum. The metal was administered intravenously, and peak concentrations were achieved at all three implant sites within 10 minutes. The aluminum brain/blood ratios were higher than the cerebrospinal fluid/blood ratios, suggesting that the primary site of aluminum permeation is at the cerebral capillaries. Yokel *et al.* (1994) examined the neurobehavioral toxicity of aluminum in rabbits at different developmental stages using eyeblink reflex classical conditioning and microdialysis. In hippocampus aluminum pretreatment was found to reduce basal and K^+ -induced extracellular acetylcholine compared to control animals. This aluminum-diminished overflow was associated with a delay in conditioned eyeblink acquisition in the animals. This group also sought to measure aluminum levels in blood, brain, and lateral ventricle – the metal was found to primarily enter brain tissue through the cerebral microvasculature and exhibited brain/blood ratios much < 1 . That is, aluminum rapidly enters the brain, but there appears to be an active process responsible for its efflux. Allen *et al.* (1994) then explored the toxicokinetics of the maltolate compound of aluminum in rats, noted for its toxicity *in vitro* or when centrally administered *in vivo*. The steady state volume of distribution for this compound indicated limited distribution outside the vascular compartment. Microdialysis probes were implanted again in frontal cortex and the jugular vein to sample brain extracellular fluid and plasma after systemic aluminum maltolate administration. Aluminum was not immediately measurable in the frontal cortical probe, but was evident later after steady state blood and brain extracellular fluid concentrations were achieved. The brain/blood ratio for aluminum maltolate was low and lower than that calculated for other aluminum salts at equimolar doses, suggesting that the maltolate compound exhibited little permeation of the blood-brain barrier.

Allen *et al.* (1995) further investigated the mechanism of aluminum permeation of the blood-brain barrier, using microdialysis probes to sample extracellular fluid in the frontal cortex and blood. Over an extended range of aluminum citrate concentrations the brain/blood ratio was consistently much < 1 , suggesting the presence of an efficient carrier that moves Al^{+3} out of brain extracellular fluid into brain cells or blood. These ratios remained unchanged when a permeability marker indicated a partially opened blood-brain barrier. Yokel *et al.* (1999) again employed microdialysis to measure brain and blood extracellular Al^{+3} . Aluminum-transferrin, the predominant aluminum species in plasma, did not readily enter brain tissue, and may have entered by transferrin receptor-mediated endocytosis. Aluminum citrate rapidly enters brain tissue. Further microdialysis studies of brain and blood extracellular Al^{+3} indicate brain efflux exceeds influx, typical of a carrier-mediated process. Additional data supported the authors' proposal that Al^{+3} efflux occurs via the monocarboxylate transporter (MCT) with a residual fraction of the metal that remains in the tissue long term.

Finally, Yokel (2002) utilized microdialysis to further delineate aluminum toxicokinetics. Appearance of Al^{+3} in brain extracellular fluid after intravenous aluminum citrate injection was too rapid to attribute to diffusion, suggesting another carrier-mediated process. Previous

evidence pointed to the MCT as the Al^{+3} efflux carrier, but the MCT1 isoform was not a good substrate for aluminum citrate uptake into rat erythrocytes. Uptake of the citrate into mouse brain endothelial cells appeared to be carrier-mediated and could be inhibited by substrates/inhibitors of the MCT and organic anion transporter families. Experiments with ^{26}Al (a beta-emitter) indicated that aluminum-transferrin and aluminum citrate cross the blood-brain barrier by different mechanisms, that much Al^{+3} in brain extracellular fluid is rapidly effluxed, and that some aluminum is retained in the tissue for an extended period.

In the search for an improved method to measure highly reactive oxygen species (ROS) Freinbichler *et al.* (2008) found sodium terephthalate to be a robust and sensitive chemical trap molecule. Reaction of the lowly fluorescent terephthalate with hydroxyl radicals or ferryl-oxo species resulted in the formation of a brilliant fluorophore, 2-hydroxyterephthalate (OH-TA). While this study was not a neurotoxicological investigation *per se*, the report describes an analytical procedure that is of wide usefulness to workers in this field. These authors then validated the procedure by determining ROS formation employing *in vivo* microdialysis with terephthalate in the perfusing solution. Experiments demonstrated that the neurotoxin kainate induced ROS production in a dose-dependent manner using this procedure. Notably, derivatization of the ROS samples with α -phthalaldehyde for amino acid detection did not affect OH-TA fluorescence, which could be readily resolved from the amino acid derivatives by liquid chromatography. Moreover, the presence of terephthalate in the perfusate did not affect the release of any of the transmitter amino acids. A disadvantage of the method is that it will not detect hydrogen peroxide or superoxide. In a later publication by this same group (Misini *et al.*, 2015) the terephthalate method was further developed by inclusion of a sensitive fluorescence detector with a capillary flow cell that permitted continuous monitoring of ROS formation in real time during microdialysis in the rat striatum. This online method exhibited comparable sensitivity to the Freinbichler *et al.* (2008) procedure described above.

The articles described in this group are summarized in Table 1.

MICRODIALYSIS IN NEUROTOXICOLOGY – ENVIRONMENTAL AGENTS

This section describes the use of microdialysis in studies utilizing various environmental agents that have been studied in a more conventional manner with this methodology to assess exposure effects on neurotransmitters and cellular second messengers and metabolites. The distinctive features of the methodology as employed and the insights that resulted will be highlighted.

Organophosphates

Current treatment of organophosphate exposure has multiple shortcomings, and poisoning victims might suffer from lasting cognitive impairments and sleep-wake disturbances. Joosen *et al.* (2009) studied the long-term effects of soman exposure on learning, memory, and neurogenesis in rats, and employed microdialysis to monitor the changes in striatal acetylcholine levels. They observed that 200 $\mu\text{g}/\text{kg}$ s.c. soman elevated extracellular acetylcholine 15-fold over baseline in the presence of more than 95% inhibition of acetylcholinesterase. The authors utilized atropine and the oxime HI-6 to reactivate the

enzyme, and results were correlated with behavioral and neurogenesis data. Acon-Chen *et al.* (2016) also studied the acute effects of soman and tested several drugs for their post-exposure neuroprotectant properties versus seizures and neuropathology in rats. Striatal microdialysis was employed to monitor the changes in extracellular acetylcholine induced by soman: the neurotransmitter peaked 30 min post-exposure at 8-fold levels over baseline. At 40 minutes after soman treatment only 10 mg/kg midazolam and 60 mg/kg cyclopentyladenosine of the drugs tested produced a significant reduction of acetylcholine in dialysate fractions. These workers concluded that delayed treatment with these drugs was effective in reducing acetylcholine elevation, seizure activity, and neuropathology.

Liu *et al.* (2015) compared the effects of parathion and chlorpyrifos on striatal acetylcholinesterase and endocannabinoids using striatal microdialysis and LC-MS/MS analyses. Endocannabinoids are endogenous neuromodulators that regulate presynaptic neurotransmitter release in central and peripheral neurons. Doses of 27 mg/kg parathion or 280 mg/kg chlorpyrifos inhibited acetylcholinesterase 85–96%, but only parathion produced signs of toxicity. In general, chlorpyrifos increased endocannabinoid levels while parathion had no or lesser effects. The authors concluded that the contrasting changes in endocannabinoids and metabolites may contribute to the agent-specific neurological signs after acute intoxication.

Herbicides

Glyphosate is the active ingredient of several herbicide formulations, and studies in humans and animals have suggested that the compound is neurotoxic. Hernandez-Plata *et al.* (2015) evaluated the effects of this compound on the rat nervous system with six i.p. injections of 50–150 mg/kg glyphosate over two weeks (1 dose/experimental group). These workers assessed dopaminergic markers and their relationship with locomotor activity. Microdialysis experiments demonstrated that acute administration of 150 mg/kg glyphosate decreased basal extracellular dopamine levels and diminished K⁺-evoked striatal dopamine release, though the two-week regimen did not decrease monoamine tissue levels. Further studies are necessary to better define glyphosate's actions on the dopaminergic system.

Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are persistent organic pollutants present in the food chain and in human blood and milk. Exposure to these compounds during pregnancy and lactation is known to lead to cognitive problems in children, but the underlying mechanisms have not been defined. Piedrafita *et al.* (2008a) investigated whether exposure of rats to PCB126 (dioxin-like) or PCB153 (non-dioxin-like) during early development affected the ability of the offspring to learn a behavioral task or disrupted the function of the cerebellar glutamate-nitric oxide-cGMP pathway as measured by microdialysis at young (3 months) or adult (7–8 months) ages. These workers showed both PCBs impaired learning ability and function of the glutamate-nitric oxide-cGMP pathway in young but not adult rats. The age-dependent decline in glutamate pathway activity in control animals was not present in PCB-exposed rats. The authors concluded that the impairment of the glutamate pathway activity may be one of the mechanisms contributing to the cognitive decrements found in children exposed to these compounds during early development. Aroclor 1254 is a mixture of PCBs and was

studied by Hilgier *et al.* (2012) in adult rats for its effects on learning and memory and how PCBs may be related to hippocampal dysfunction. Microdialysis was utilized to assess the glutamate-nitric oxide-cGMP pathway in this brain region after daily intragastric administration of 10 mg/kg Aroclor 1254 for 14 days. These investigators reported substantial neuronal damage and loss in the hippocampus at this dose in exposed animals as well as markedly decreased *N*-methyl-D-aspartate (NMDA)-evoked extracellular accumulation of newly loaded Ca^{+2} and cGMP. This regimen also diminished basal levels of the nitric oxide precursor arginine, indicating inhibition of the NMDA-nitric oxide-cGMP pathway. Baseline extracellular levels of glutamate and glutamine were also depressed, suggesting an insufficient supply of neurotransmitter glutamate, while aspartate and taurine were not affected. The authors concluded that inhibition of NMDA receptor-mediated glutamatergic signaling is potentially a significant contributor to the PCB-related cognitive deficits.

Carbon Monoxide

Acute carbon monoxide (CO) intoxication in humans results in motor deficits, which suggest possible disturbance of central dopaminergic neuronal systems. A series of 9 papers have been published by the same group of workers attempting to define the CO mechanism(s) of toxicity and the basis for the increased production of free hydroxyl radicals employing microdialysis in rats. These investigators administered 3000 ppm CO for 40 minutes and initially reported a stimulation of depolarization-induced striatal dopamine release (Hara *et al.* 2002) and an apparent suppression of striatal nitric oxide production (Hara *et al.*, 2003). Hydroxyl radical generation in this latter work was assessed by the inclusion of salicylic acid in the perfusing solution to produce 2,3-dihydroxybenzoic acid, which can be measured by electrochemical detection. Additional studies suggested that the CO exposure regimen induced Na^{+} influx through voltage-dependent Na^{+} channels in rat striatum resulting in enhanced free hydroxyl radical generation (Hara *et al.*, 2004), and transiently enhanced striatal extracellular cGMP in a manner that did not resemble that produced by hypoxia (Hara *et al.*, 2007a). Subsequent work examined the potential role of nitric oxide in the generation of free hydroxyl radicals by CO by the microdialysis administration of nitric oxide synthase inhibitors or arginine, but Hara *et al.* (2007b) could not definitively demonstrate nitric oxide involvement in the generation of these hydrogen species. Hara *et al.* (2009) further assessed whether ascorbate or iron played a role in the CO-stimulated production of hydroxyl radicals and related neuronal injury. Intrastriatal administration of an oxidase to degrade ascorbate strongly reduced hydroxyl radical generation, and CO exposure elevated striatal extracellular ascorbate, suggesting a role for ascorbate in the production of the hydrogen species. In addition, administration of an iron chelator eliminated the CO-induced hydroxyl radical generation. These workers then turned their attention to CO exposure and the production of cAMP in rat striatum (Hara *et al.*, 2011a). A high level of CO exposure caused an increase in extracellular cAMP that could be negated by tetrodotoxin, results analogous to those of free hydroxyl radicals after CO administration. After a further series of experiments with P2Y_{11} receptor antagonists the authors concluded that a threshold for cAMP production through these receptors exists, but is reached only in cases of severe CO exposure. Hara *et al.* (2011b) further examined the role of hypoxia in the CO-induced generation of hydroxyl radicals in rat striatum: While 3000

ppm CO and 5% O₂ induced comparable hypoxic conditions, the results suggested that CO poisoning may be independent of hypoxia *per se*. After further experiments with P2Y₁₁-like receptor antagonists and ATP and other high energy phosphates Hara *et al.* (2014) concluded that the mechanism of free hydroxyl radical production may differ between CO exposure and exogenously applied ATP. Inhibition of NADPH oxidase was found to markedly reduce CO-stimulated hydroxyl radical production as well as the production of the hydrogen species induced by forskolin, which stimulates cAMP formation. The authors concluded that CO-induced free hydroxyl radical production is likely a result of NADPH oxidase activation through cAMP signaling pathways.

Microdialysis obviously played a central role in the conduct of these studies by Hara and co-workers, measuring not only the hydroxyl radicals, signaling molecules, and transmitters, but also delivering by microdialysis the various drugs and agents to test the mechanistic hypotheses under investigation. This work is one of the best extended examples of the integral use of microdialysis in the pursuit of experimental goals.

Methylmercury

Prenatal exposure to methylmercury (MeHg) contaminated food, especially fish, may affect brain development in children, resulting in long term impairments in cognitive function. Juarez *et al.* (2002) tested the hypothesis that MeHg neuronal damage is caused by excitotoxicity following disruption of astrocytic glutamate uptake. Concentrations of MeHg (10 or 100 μ M) were administered through a microdialysis probe into rat frontal cortex, and extracellular glutamate was monitored. Perfusion of MeHg for 90 min resulted in immediate and significant elevations in glutamate, suggesting that glutamatergic dysfunction plays a significant role in MeHg neurotoxicity. Faro *et al.* (2002) asked whether NMDA receptor antagonists and nitric oxide synthase inhibitors would exhibit protective effects on MeHg-induced striatal dopamine release using *in vivo* microdialysis to administer the drugs and monitor dopamine. Prior infusion of NMDA receptor antagonists diminished the MeHg stimulation of dopamine release by 50–70%, while perfusate nitric oxide synthase inhibitors reduced the dopamine response to MeHg perfusion by 40–50%. The authors concluded that a component of MeHg action involves an overstimulation of NMDA receptors with possible nitric oxide production to release dopamine, and that the tested receptor antagonists and enzyme inhibitors protect from this MeHg mechanism.

Piedrafita *et al.* (2008b) raised the question of whether developmental exposure to MeHg diminished cognitive function through actions on the glutamate-nitric oxide-cGMP pathway similarly to the effects of PCBs. The pathway was assessed via the use of microdialysis. MeHg was administered in the drinking water to pregnant dams from gestational day 7 through postnatal day 21 with the ingested dose adjusted daily to maintain 0.5 mg/kg/day. This perinatal MeHg exposure was found to impair function of the cerebellar glutamate pathway in the offspring and was associated with diminished learning ability in the Y maze task at three months of age. The authors concluded that these results support the notion that the function of this pathway modulates acquisition of the Y maze task.

Lead

Probably no toxicant has received more attention from the toxicological research community than inorganic lead, and this is reflected in the number of published papers utilizing microdialysis in animal models to study its neurotoxic properties. Furthermore, because of lead's neurobehavioral effects in children resulting from exposure during early development, many studies have utilized widely available analytical methods to investigate biogenic amine and amino acid neurotransmitter function in animals.

Nation and Burkey (1994) exposed adult rats to lead in the drinking water for two months, and then employed microdialysis in the nucleus accumbens to compare the response of extracellular dopamine in exposed and control animals after 10 mg/kg, i.p. cocaine. The time course of dopamine efflux was found to be diminished in the lead-exposed group compared to control animals. Similarly, Kala and Jadhav (1995) placed microdialysis probes in nucleus accumbens of adult rats exposed to lead for three months and assessed basal and K⁺-induced extracellular dopamine concentrations. These workers also reported reductions in baseline and depolarization-induced release of dopamine in lead-exposed rats compared to controls, suggesting a decreased dopamine availability in the exposed group. Devoto *et al.* (2001) exposed rats to lead beginning at gestational days 2–6 or from 28 days of age and investigated the accumbens dopaminergic system at three months of age by transverse microdialysis. In this version of the methodology the probes are implanted on a mediolateral axis rather than the more conventional dorsal-ventral axis. They observed that the group whose exposure began during gestation exhibited a significantly smaller response to 60 mM K⁺ than either control animals or the lead group exposed postweaning. The gestational lead group also responded with lower dopamine release to 1 mg/kg, i.p. amphetamine administration, but the three experimental groups did not differ in response to the D₂-D₃ dopaminergic receptor agonist quinpirole locally administered to nucleus accumbens in the perfusing solution. The authors concluded that lead exposure preferentially affected dopamine synthesis and/or release.

Nowak *et al.* (2008a) wished to explore the role of developmental lead exposure on a putative dopaminergic-nitric interaction in the nigrostriatal pathway. Pregnant rats were exposed to lead only during gestation, and at 12 weeks of age control and lead offspring were tested by striatal microdialysis. Gestational lead had no effect on basal extracellular dopamine or the dopaminergic response to 1 mg/kg, i.p. amphetamine. Neither did prenatal lead alter free hydroxyl radical formation (monitored by the microdialysis perfusion of salicylate to react with the radicals and form 2,5-dihydroxybenzoic acid) in the striatum after combined administration of amphetamine and a nitric oxide synthase inhibitor. However, the facilitatory effect of the inhibitor on extracellular dopamine was attenuated as a result of gestational lead exposure compared to the control group. The authors concluded that lead exposure distorted the nigrostriatal dopaminergic-nitric interaction, but did not affect ROS. Nowak *et al.* (2008b) performed analogous experiments in medial prefrontal cortex, but found that gestational lead had no effect on dopaminergic neurotransmission in response to amphetamine and/or a nitric oxide synthase inhibitor, and did not alter the formation of hydroxyl radical species measured by microdialysis salicylate perfusion. It was concluded

that dopamine-rich brain areas such as striatum and nucleus accumbens are more intricately associated with lead neurotoxicity than frontal cortex.

Mansouri *et al.* (2013) examined chronic lead exposure in adult rats that produced blood lead concentrations below those producing overt neurological deficits in occupationally exposed workers. Microdialysis was employed to assess biogenic amine neurotransmitter activity. Six months exposure produced motor and cognitive alterations with the lead-induced hyperactivity in the open field associated with an increase in extracellular acetylcholine in prefrontal cortex. Cortical extracellular dopamine was unaffected by lead, while extracellular serotonin was decreased by lead exposure. Mansouri and co-workers asserted that these data unveiled new cellular mechanisms underlying the neurobehavioral effects of chronic lead exposure in adulthood.

Other investigators explored the actions of chronic developmental lead exposure on hippocampal glutamatergic and GABAergic neurotransmission, and how the resulting neurochemical alterations corresponded to associated changes in neurophysiological measures. In a series of six papers Lasley and Gilbert and co-workers utilized the same exposure protocols to examine the manner in which different exposure periods and exposure levels altered the lead-related impairments in long term potentiation (LTP), paired pulse functions, and the K^+ -stimulated responses in hippocampal extracellular glutamate and GABA. The amino acid systems were assessed by microdialysis and analyzed by binary gradient HPLC.

Lasley and Gilbert (1996) posed the question as to whether glutamate and GABA efflux were impaired in intact animals continuously exposed to lead from birth. Pregnant dams received 0.2% lead in the drinking water at parturition, and male offspring were weaned to the same solution as that given their dams and tested in young adulthood. Microdialysis probes implanted in the hippocampal CA1-dentate area were perfused with a modified Ringer's solution. Transmitter release was stimulated by perfusion with 150 mM K^+ combined with a glutamate reuptake inhibitor. Half the animals in each group were tested with Ca^{+2} present in the perfusing solution (i.e., total release was operationally defined as the combination of Ca^{+2} -dependent and -independent components) and the other half tested with Ca^{+2} absent in the presence of the Ca^{+2} chelator EGTA and $MgCl_2$ (Ca^{+2} -independent release). K^+ -stimulated total glutamate release was reduced in lead-exposed animals compared to controls; no group differences were observed under Ca^{+2} -free microdialysis, indicating that Ca^{+2} -dependent glutamate release was diminished in exposed animals. In contrast, no group differences in K^+ -induced total GABA release were evident, but there existed an augmentation in extracellular GABA that was uncovered under Ca^{+2} -free conditions in exposed rats. As a result of paired pulse testing in identically exposed animals, the lead-induced suppression of paired pulse facilitation was found to be consistent with the exposure-related decreases in total glutamate release. These authors concluded that the effects of exposure on hippocampal glutamate activity may contribute to the effects of lead on synaptic plasticity models such as LTP.

Lasley *et al.* (1999) then studied how the lead effects on hippocampal glutamatergic neurotransmission changed as a function of exposure during different developmental

periods. Experimental groups were exposed continually beginning at conception (Gestational-Life) or two weeks after weaning (Wean-Life). Exposure in a third group began at conception but ended at weaning (Gestational-Wean). Transmitter release was stimulated in adult animals by high K^+ in the presence and absence of Ca^{+2} in the microdialysis perfusing solution as in the earlier work with the exception that EGTA was replaced by a Ca^{+2} channel blocker and total release and Ca^{+2} -independent release were determined in the same animal in consecutive testing sessions. Reductions in total glutamate and GABA efflux were found in the Gestational-Life and Wean-Life groups compared to controls, decrements that could be traced to exposure-induced reductions in Ca^{+2} -dependent release. The pattern of changes in the Gestational-Life group resembled that described above in a group continuously exposed from birth, indicating that gestational exposure did not further enhance the burden of exposure beginning at birth when exposure in both groups extended into adulthood. Similar exposure-related changes were also found in the Wean-Life group, indicating that lead during early development is not a requirement to produce changes in glutamate/GABA release. Lead-related diminished responses were also observed in the Gestational-Wean group: Ca^{+2} -dependent glutamate release was decreased, and reductions in total and Ca^{+2} -dependent GABA release were similar to those in the other two lead exposure groups. Thus, exposure limited to early development was also sufficient to produce deficits in evoked transmitter efflux. Moreover, the lead-related decreases in glutamate responses correspond to exposure-induced impairments in LTP (Gilbert *et al.*, 1999a), providing further support for the contention that the lead effects on glutamate release are a critical factor in the associated changes found in LTP.

Lasley and Gilbert (2002) then conducted further studies to determine the effects of lead on hippocampal glutamate and GABA release as a function of exposure level. Groups of rats were exposed continuously to single levels of lead in the range of 0.1–1.0% in the drinking water beginning at gestational day 15–16. Transmitter release was induced in adult males via microdialysis as described above; Ca^{+2} was present and absent with a Ca^{+2} channel blocker and Mg^{+2} in the perfusing solution in consecutive experimental sessions in the same animal. Chronic lead produced decrements in total (Ca^{+2} present in perfusate) K^+ -evoked glutamate and GABA release at exposure levels of 0.1–0.5% Pb. Maximal effects were observed in the 0.2% group, and changes in total efflux could be directly traced to alterations in the Ca^{+2} -dependent component. However, these effects were less evident in the 0.5% group and were no longer present in the 1.0% Pb group, thus defining U-shaped dose-effect relationships. Moreover, in the absence of Ca^{+2} in the dialysis perfusate K^+ -induced release was elevated in the two highest exposure groups, suggesting a Pb^{+2} -induced enhancement in evoked release. This pattern of results indicates the presence of two actions of exposure on *in vivo* transmitter release: a more potent suppression of stimulated release seen at lower exposure levels combined with Ca^{+2} -mimetic actions to induce exocytosis that is exhibited at higher exposure levels. That is, the ability of Pb^{+2} to substitute for Ca^{+2} in the process of exocytosis became the dominant stimulus at high exposure levels in the relative absence of extracellular Ca^{+2} . Furthermore, significant similarities in the dose-effect relationships uncovered in measures of evoked glutamate release and hippocampal LTP (Gilbert *et al.*, 1999b) reinforced the conclusion that exposure-related effects on glutamate release played a significant role in the lead-induced effects on synaptic plasticity.

Sheng *et al.* (2005) then utilized microdialysis in the hippocampal dentate gyrus to investigate the changes produced in extracellular glutamate and GABA in rat offspring exposed to 0.5% lead from birth to weaning at postnatal day 21. These investigators were interested in the effects of exposure before and after 50 days of training on a fixed-interval operant task. Compared to pre-training values, extracellular glutamate increased to a greater extent in the exposed group than in controls, while extracellular GABA decreased significantly in the lead group and control values did not change. The authors concluded that lead exposure altered the responses of glutamate and GABA transmission to the fixed-interval testing.

Manganese

Despite clear distinctions in their respective toxicities studies of the neurotoxic actions of manganese utilizing *in vivo* microdialysis have in many respects resembled the analogous investigations in lead-exposed animals described in the preceding section. One basis for these similar approaches is concerns about exposure to children during early development leading to neurobehavioral impairments. Thus, studies concerning manganese and employing microdialysis have involved developmental exposure regimens.

McDougall *et al.* (2008) posed the question of whether a high dose manganese regimen during the postnatal period would depress presynaptic dopaminergic activity. Rat offspring received oral doses of 0.75 mg Mn/day from postnatal days 1–21, and on day 90 dopamine transporter protein expression, dopamine uptake, and *in vivo* microdialysis were performed in striatum and nucleus accumbens. Postnatal manganese was shown to produce long term decreases in transporter expression and dopamine uptake as well as persistent reductions in striatal dopamine efflux in response to 20 mg/kg, i.p., cocaine. The authors concluded that the postnatal manganese exposure resulted in long term declines in various markers of presynaptic dopaminergic activity. These findings were associated with a significant increase in amphetamine-induced stereotypy and delayed acquisition of a fixed-ratio behavioral task.

Khalid *et al.* (2011) examined the effects of subacute exposure to manganese on striatal dopaminergic activity in adult mice. Animals were given 50 mg/kg, s.c. MnCl₂ injections every three days over a 7-day period. Increased striatal manganese concentrations persisted throughout the 21-day post-exposure monitoring period. Fast scan cyclic voltammetry performed *ex vivo* on striatal slices found diminished dopamine release 7 and 21 days after exposure. Basal levels of extracellular dopamine determined by zero net flux microdialysis were lower in manganese mice at 7 days post-exposure, and K⁺-induced increases in extracellular dopamine in microdialysate were reduced at 1, 7, and 21 days post-exposure. These results clearly demonstrated an effect of subacute manganese administration on striatal dopaminergic activity.

Developmental manganese exposure is associated with motor dysfunction in children and animal models, but little is known about the underlying neurochemical mechanisms or the potential for amelioration by pharmacotherapy. Beaudin *et al.* (2015) investigated the issue of whether methylphenidate would alleviate the fine motor dysfunction resulting from chronic postnatal manganese exposure (Beaudin *et al.*, 2013), and whether microdialysis of biogenic amines in the striatum and prefrontal cortex would elucidate a possible basis for the

motor toxicity. Rats were orally exposed to 0, 25 or 50 mg Mn/kg/day from postnatal day 1 until the end of the study, and the staircase test was used to assess skilled forelimb function in adult animals. Manganese exposure was found to impair reaching and grasping skills as well as the stimulated release of norepinephrine in frontal cortex and dopamine in striatum and prefrontal cortex. Oral methylphenidate fully alleviated the fine motor deficits in the exposed rats. These findings support the belief that diminished striatal and cortical catecholaminergic function may underlie the fine motor dysfunction induced by developmental manganese exposure.

Previous reports have indicated that brain manganese accumulation is enhanced by dietary iron deficiency, and disturbances in noradrenergic function have also been noted. Anderson *et al.* (2009) undertook efforts to investigate the effects of manganese exposure (0.1% in drinking water) and iron deficiency (6 mg iron/kg diet)-associated manganese accumulation in rats and assess noradrenergic neuronal activity in multiple brain regions. Decreased norepinephrine uptake in locus coeruleus was associated with increased extracellular manganese concentrations in this region, and decreased iron levels were associated with reduced norepinephrine uptake in caudate-putamen and locus coeruleus. Microdialysis experiments determined that basal extracellular norepinephrine was reduced in caudate-putamen in response to manganese exposure and iron deficiency. Elevated brain extracellular manganese and decreased iron also resulted in marked declines in norepinephrine transporter and α_2 -adrenergic receptor protein expression, which were proposed to be the basis for the reduced transmitter uptake and extracellular levels. The same workers employed an identical experimental design to assess GABA transporter and receptor protein expression and extracellular GABA concentrations in weanling rats (Anderson *et al.*, 2008), based on previous data from this group indicating decreased GABA tissue content and uptake due to manganese exposure and iron deficiency. An increase in striatal basal extracellular GABA was uncovered via microdialysis as a result of manganese exposure and iron deficiency. No other systematic changes were observed across brain regions in gene and protein expression of the GAT-1 GABA transporter and GABA receptors. The authors suggested that elevated extracellular manganese concentrations result in increased extracellular GABA levels due to altered gene and protein expression of transport and receptor proteins.

This same lab group further investigated the effects of brain extracellular manganese accumulation on rat striatal GABAergic function by determining whether the previously observed manganese-induced increase in extracellular GABA was due to changes in GABA transporter function, and if manganese exposure disrupts other amino acid neurotransmitters such as taurine and glycine (Fordahl *et al.*, 2010). Manganese exposure was administered in drinking water as described above. *In vivo* microdialysis was employed to determine extracellular manganese and amino acid levels plus to administer a GABA transporter inhibitor, nipecotic acid. The increase in extracellular GABA in response to the transporter inhibitor was attenuated by manganese, and a large increase in extracellular taurine was observed in controls after the nipecotic acid was withdrawn. Manganese suppressed the increase in extracellular taurine and nearly eliminated taurine levels throughout the collection period. The authors suggested that manganese increases extracellular GABA by inhibiting function of the transporter GAT-1 and by deranging taurine homeostasis.

The articles described in this group are summarized in Table 2.

SUMMARY

This unit has provided an overview of the manner in which the methodology of intracerebral microdialysis has been utilized in the field of neurotoxicology to elucidate the toxicodynamics and toxicokinetics of environmental agents. Clearly, the technique has been employed in a variety of creative ways to address specific experimental goals involving a myriad of toxicants. With appropriate consideration of method parameters investigators have also been able to address mechanistic issues in their studies. With more efficient microdialysis equipment and improved methods of analyte detection the technique has retained broad relevance and potential value for toxicological research.

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Figure 1. Diagram of microdialysis diffusion occurring in tissue. Downward arrows indicate direction of perfusion flow, horizontal arrows indicate direction of diffusion of analytes or molecules of interest. (A) Conventional form of microdialysis in which molecules smaller than the molecular weight cutoff of the tubing diffuse down their concentration gradient from extracellular fluid into the perfusing solution. It is incumbent on analytical methods to separate the molecules of interest (e.g., the red spheroids) and quantify their concentration. (B) Reverse microdialysis or retrodialysis in which a toxicant, drug, or molecule present in the perfusing solution (light blue triangles) diffuses into the extracellular fluid of the surrounding tissue. Only compounds smaller than the molecular weight cutoff of the dialysis tubing will undergo this diffusion down their concentration gradient. Notice the concentration gradients that develop from the top to the bottom of the tissue extracellular fluid and perfusing solution.

Table 1.

Specialized Methods Employing Microdialysis

Toxicant	Microdialysis Site	Endpoint Measures
Manganese		
Takeda <i>et al.</i> , 2002	Rat amygdala, hippocampus	Evoked release of preloaded $^{54}\text{Mn}^{+2}$
Takeda <i>et al.</i> , 2003	Rat striatum	ECF transmitters after MD Mn^{+2}
Lead		
Qi <i>et al.</i> , 2013	Rat striatum	Striatal ECF Pb^{+2} by MD graphene conjugates
Arsenic		
Su <i>et al.</i> , 2014	Rat hippocampus	BBB permeability of As metabolites, molybdate
Aluminum		
Allen and Yokel, 1992	Rat frontal cortex, lateral ventricle, jugular vein	BBB permeability of Al^{+3}
Yokel <i>et al.</i> , 1994	Rabbit hippocampus, frontal cortex, lateral ventricle, jugular vein	Basal, evoked ECF ACh, Al^{+3} toxicokinetics
Allen <i>et al.</i> , 1994, 1995 Yokel <i>et al.</i> , 1999	Rat frontal cortex, jugular vein	BBB transit of Al-maltolate, Al-transferrin; Al^{+3} toxicokinetics
Yokel, 2002	Rat frontal cortex, jugular vein	ECF Al^{+3} , BBB permeability of IV $^{26}\text{Al}^{+3}$
Reactive Oxygen Species		
Freinbichler <i>et al.</i> , 2008 Misini <i>et al.</i> , 2015	Rat striatum	Fluorophore of Na terephthalate and ROS

Abbreviations: ECF, extracellular fluid; MD, microdialysis; BBB, blood-brain barrier; ACh, acetylcholine; ROS, reactive oxygen species

Table 2.

Use of Microdialysis to Investigate Specific Environmental Agents

Toxicant	Microdialysis Site	Endpoint Measures
Organophosphates		
Joosen <i>et al.</i> , 2009 Acon-Chen <i>et al.</i> , 2016	Rat striatum	ECF ACh
Liu <i>et al.</i> , 2015	Rat striatum	ECF endocannabinoids and metabolites
Herbicides		
Hernandez-Plata <i>et al.</i> , 2015	Rat striatum	Basal, evoked ECF dopamine
PCBs		
Piedrafita <i>et al.</i> , 2008a	Rat cerebellum	ECF glutamate-NO-cGMP pathway activity
Hilgier <i>et al.</i> , 2012	Rat hippocampus	Evoked ECF $^{45}\text{Ca}^{+2}$, cGMP, glutamate, arginine, glutamine
Carbon monoxide		
Hara <i>et al.</i> , 2002, 2003	Rat striatum	ECF NO_2^- , NO_3^- , arginine, free OH radicals, evoked dopamine
Hara <i>et al.</i> , 2004, 2007b	Rat striatum	ECF free OH radicals, glutamate
Hara <i>et al.</i> , 2007a, 2011a	Rat striatum	ECF cGMP, cAMP
Hara <i>et al.</i> , 2009, 2011b	Rat striatum	ECF free OH radicals, ascorbate
Hara <i>et al.</i> , 2014	Rat striatum	ECF free OH radicals
Methylmercury		
Juarez <i>et al.</i> , 2002	Rat frontal cortex	ECF glutamate
Faro <i>et al.</i> , 2002	Rat striatum	ECF dopamine
Piedrafita <i>et al.</i> , 2008b	Rat cerebellum	Glutamate-NO-cGMP pathway activity
Lead		
Nation and Burkey, 1994 Kala and Jadhav, 1995	Rat nucleus accumbens	Basal, stimulated ECF dopamine
Devoto <i>et al.</i> , 2001	Bilateral nucleus accumbens (transverse MD)	Basal, stimulated ECF dopamine
Nowak <i>et al.</i> , 2008a,b	Rat striatum Rat frontal cortex	Basal, stimulated ECF dopamine, free OH radicals
Mansouri <i>et al.</i> , 2013	Rat prefrontal cortex	ECF ACh, dopamine, 5-HT
Lasley and Gilbert, 1996 Lasley <i>et al.</i> , 1999 Lasley and Gilbert, 2002	Rat hippocampus	Basal, evoked ECF glutamate, GABA – Ca^{+2} -dependent and – independent components
Sheng <i>et al.</i> , 2005	Rat hippocampus	ECF glutamate, GABA
Manganese		
McDougall <i>et al.</i> , 2008	Rat striatum, nucleus accumbens	Basal, stimulated ECF dopamine

Toxicant	Microdialysis Site	Endpoint Measures
Khalid <i>et al.</i> , 2011	Mouse striatum	Basal, evoked ECF dopamine, ECF Mn ⁺²
Beaudin <i>et al.</i> , 2015	Rat striatum, prefrontal cortex	Basal, evoked ECF dopamine, norepinephrine, 5-HT
Anderson <i>et al.</i> , 2008, 2009	Rat striatum, locus coeruleus	ECF Mn ⁺² , Fe ⁺² Basal ECF norepinephrine, GABA
Fordahl <i>et al.</i> , 2010	Rat striatum	Basal, stimulated ECF GABA, taurine, ECF Mn ⁺²

Abbreviations: ECF, extracellular fluid; ACh, acetylcholine; NO, nitric oxide; 5-HT, 5-hydroxytryptamine; OH, hydroxyl