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# High-resolution mapping of neuronal activity using the lipophilic thallium chelate complex TlDDC: Protocol and validation of the method

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ABSTRACT

In neurons the rate of $K^+$-uptake increases with increasing activity. $K^+$-analogues like the heavy metal ion thallium ($Tl^+$) can be used, therefore, as tracers for imaging neuronal activity. However, when water-soluble $Tl^+$-salts are injected systemically only minute amounts of the tracer enter the brain and the $Tl^+$-uptake patterns are influenced by regional differences in blood–brain barrier (BBB) $K^+$-permeability. We here show that the BBB-related limitations in using $Tl^+$ for imaging neuronal activity are no longer present when the lipophilic $Tl^+$ chelate complex thallium diethyldithiocarbamate (TlDDC) is applied. We systemically injected rodents with TlDDC and mapped the $Tl^+$-distribution in the brain using an autometallographic (AMG) technique, a histochemical method for detecting heavy metals. We find that $Tl^+$-doses for optimum AMG staining could be substantially reduced, and regional differences attributable to differences in BBB $K^+$-permeability were no longer detectable, indicating that TlDDC crosses the BBB. At the cellular level, however, the $Tl^+$-distribution was essentially the same as after injection of water-soluble $Tl^+$-salts, indicating $Tl^+$-release from TlDDC prior to neuronal or glial uptake. Upon sensory stimulation or intracortical microstimulation neuronal $Tl^+$-uptake increased after TlDDC injection, upon muscimol treatment neuronal $Tl^+$-uptake decreased. We present a protocol for mapping neuronal activity with cellular resolution, which is based on intravenous TlDDC injections during ongoing activity in unrestrained behaving animals and short stimulation times of 5 min.



## Introduction

Imaging or mapping neuronal activity at the single-cell level in the vertebrate brain still poses severe methodological challenges. Optical methods, especially calcium imaging techniques (for a review see Göbel and Helmchen, 2007), can provide temporal resolutions on a millisecond time-scale but are limited in penetration depth and field of view. In addition, these techniques are sensitive to motion artifacts which make applications in unrestrained behaving animals difficult. Immediate early gene expression techniques (for a review see Kovács, 2008), in contrast, can readily be applied to behaving animals but the expression patterns are affected by factors other than neuronal activity and quantitative data on the different degrees to which individual neurons are active cannot be obtained. None of the currently available methods is able to map cell-by-cell the spatial patterns of neuronal activity in the entire population of neurons in a vertebrate brain.

In a first attempt to establish such a method we introduced thallium autometallography, TlAMG (Goldschmidt et al., 2004). In TlAMG the monovalent thallium ion $Tl^+$, a well-established potassium analogue (Britten and Blank, 1968; Douglas et al., 1990; Gehring and Hammond, 1967; Gill et al., 2005; Landowne, 1975), is used as a tracer for mapping cerebral $K^+$-metabolism and activity-dependent changes in neuronal $K^+$-uptake. The close coupling of neuronal activity and $K^+$-uptake is well documented (Hodgkin and Keynes, 1955): upon stimulation or depolarization, respectively, neuronal potassium permeability and potassium efflux increase. During repolarization potassium influx and (re-)uptake from the extracellular space increase in return. The stimulus-dependent increase in the rate of potassium uptake from the extracellular space has been shown very clearly in the studies of

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Keynes using $^{42}K^+$ as a tracer (Keynes, 1951a,b). $K^+$-movements can be quantified using this tracer-technique as demonstrated, for example, in Keynes and Ritchie (1965), where the $K^+$-movements per impulse were determined in rabbit vagus nerve axons in culture.

As $K^+$-uptake is mediated to a large degree by the Na,K-ATPase, which consumes a dominant fraction of the ATP generated from glucose metabolism (Attwell and Iadecola, 2002), TlAMG is somewhat similar in rationale to the 2-deoxyglucose method (Sokoloff et al., 1977). In contrast to 2-DG, however, $Tl^+$ can be readily chemically fixed within the tissue and non-radioactively detected with the spatial resolution of single axons by means of a modified histochemical technique for the detection of heavy metals in the brain, the Timm-technique or autometallographic method.

Using the AMG technique we demonstrated activity-dependent neuronal $Tl^+$-uptake in the brain after intraperitoneal injections of thallium (I) acetate (Goldschmidt et al., 2004). We noted, however, that the regional distribution of $Tl^+$ was dependent on regional differences in blood–barrier permeability in essentially the same manner as described previously for $K^+$ (Smith and Rapoport, 1986). This made whole-brain analysis of neuronal activity and interregional comparisons difficult.

We here report that the blood–brain barrier related limitations in using $Tl^+$ as a tracer are no longer present when animals are injected with the lipophilic chelate complex thallium diethyldithiocarbamate (TlDDC) instead of water-soluble thallium salts. We provide evidence that, after crossing the blood–brain barrier, $Tl^+$ is released from TlDDC prior to neuronal or glial uptake. We extensively tested and validated the use of TlDDC for high-resolution mapping of neuronal activity using noxious stimulation in rats and acoustic stimulation, intracortical microstimulation and pharmacological inhibition of neuronal activity with muscimol in Mongolian gerbils.

We present a novel protocol for cellular-resolution mapping of neuronal activity based on intravenous injection of TlDDC during ongoing behavior in unrestrained animals and short stimulation times of 5 min.

## Materials and methods

### *Animals*

Six young adult male Wistar rats weighting 280 to 320 g and 24 Mongolian gerbils (*Meriones unguiculatus*) of both sexes weighing 65–85 g were used in this study. Gerbils were obtained from Tumblebrook Farms, West Brookfield, MA, USA. The experiments were approved by the animal ethics committee of Sachsen-Anhalt in accordance with the NIH guidelines for the care and use of laboratory animals.

### *Thallium application and anesthesia*

#### *Intraperitoneal injections of thallium (I) acetate*

Experiments with intraperitoneal injections of thallium (I) acetate (TlAc) were performed as described previously (Goldschmidt et al., 2004). Thallium (I) acetate was obtained from Fluka, Germany. Gerbils were injected i.p. with 400 µl of a 1% aqueous solution of TlAc. After 15 min gerbils were anesthetized with i.p. injections of a mixture of ketamine (100 mg/kg body weight) and xylazine (40 mg/kg BW).

#### *Intraperitoneal injections of thallium diethyldithiocarbamate*

Animals were injected with freshly prepared solutions of thallium diethyldithiocarbamate (TlDDC). The TlDDC-solution was prepared by mixing equal volumes of TlAc-solutions and sodium diethyldithiocarbamate trihydrate (NaDDC)-solutions. NaDDC × 3 $H_2O$ was obtained from Sigma, Deisenhofen, Germany. The solutions were mixed within the syringe used for injecting TlDDC. Syringes were first filled with a certain volume of TlAc. Then the same amount of an aqueous NaDDC solution was added. The lipophilic chelate complex TlDDC immediately forms within the syringe after adding NaDDC to TlAc. Gerbils were injected i.p. with 300 µl 0.1% TlDDC solution, prepared by mixing 150 µl 0.2% aqueous NaDDC-solution and 150 µl 0.2% aqueous TlAc-solution. I.p. injections were made in gerbils that were intracortically microstimulated (see below) and in five gerbils that were used for testing the sulfide-only perfusion.

#### *Intravenous injections of thallium diethyldithiocarbamate*

*Jugular vein catheterization.* Catheters were placed into the right external jugular vein of rats and gerbils according to the protocol provided by Thrivikraman et al. (2002). The animals were anesthetized i.p. with pentobarbital (1 ml 5% Nembutal/100 g body weight) and the right neck was shaved. An incision was made above the right clavicle and the external jugular vein was exposed. After isolating an approximately 5 mm section of the vein, two ligatures were placed at either end of the isolated vessel. The cranial ligature was tied and the catheter was inserted into the isolated portion of the jugular vein. A 10 cm long PE50-polyethylene tube (Charles River Laboratories, Sulzfeld, Germany) served as a catheter. A soft silicon tube (Rena Sil 037), 1 cm in length, was placed on the tip of the polyethylene tube to minimize vascular damage during the insertion. The catheter was connected via a 23-G needle to a 1 ml syringe. The syringe and the catheter were filled with saline (0.9% NaCl) before insertion. After inserting 2–3 cm of the catheter into the vein the caudal ligature was tied. The catheter was tunneled under the skin, exteriorized between the scapulae and filled with heparinized (50 IU/ml) saline solution. The ventral skin incision was closed with wound clips and the catheter was sealed by shortly heating and melting its end with a lighter. Animals were allowed to recover from jugular vein catheterization for 1–3 days before they were used for further experiments.

*Intravenous TlDDC application during ongoing behavior.* On the day of the experiment the jugular vein catheter was connected to a 40 cm long PE50-polyethylene tube filled with saline. Animals were shortly anaesthetized with 4% halothane, the sealed end of the animal's catheter was cut off and the PE50-tube was connected to the catheter via a short silicon tube. Animals were placed in a cage and were usually given at least 3 h to recover from the anesthesia. In the experiments with noxious stimulation in the formalin-test the recovery time was only 60 min, which is in the range of the time for maximum effects after formalin injection (Dubuisson and Dennis, 1977). Animals were placed in cages of about 30 cm height with an open top.

TlDDC-solutions were freshly prepared in a similar manner as for intraperitoneal injections, but the doses used were lower and the solutions were prepared in isotonic NaCl instead of $H_2O$. The syringe used for i.v.-injection was first filled with an aqueous solution of 0.1% TlAc. Then an equal volume of 0.1% NaDDC, dissolved in an aqueous 1.8% NaCl solution, was added. The final concentration of this solution was 0.05% TlDDC in 0.9% NaCl. Rats were injected with 1 ml of this solution prepared from 500 µl 0.1% TlAc and 500 µl 0.1% NaDDC. Gerbils were injected with 350 to 425 µl of the same solution, the exact amount depending on the experimental conditions. TlDDC was injected through the catheter from outside the cage while the animals were unrestrained and freely moving. The TlDDC-solution was injected during the initial 4 min of the experiment. Then a small volume of saline (150 µl) was injected to clear the catheter. Five minutes after starting the experiment the animals were deeply anesthetized by i.v. injection of 270–280 µl ketamine (Ketaminratiopharm, 50 mg/ml) in rats and 180 µl in gerbils. Since about 120 µl of the anesthetic remains in the catheter this corresponds to a dose of ca. 25 mg ketamine/kg body weight in rats and 40 mg/kg BW in gerbils. This dose results in an almost immediate (within seconds) onset of anesthesia.