Extracellular-derived calcium does not initiate in vivo neurotransmission involving docosahexaenoic acid

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Abstract In vitro studies show that docosahexaenoic acid (DHA) can be released from membrane phospholipid by Ca^{2+}-independent phospholipase A_2 ($iPLA_2$), Ca^{2+}-independent plasmalogen PLA_2 or secretory PLA_2 ($sPLA_2$), but not by Ca^{2+}-dependent cytosolic PLA_2 ($cPLA_2$), which selectively releases arachidonic acid (AA). Since glutamatergic NMDA (N-methyl-D-aspartate) receptor activation allows extracellular Ca^{2+} into cells, we hypothesized that brain DHA signaling would not be altered in rats given NMDA, to the extent that in vivo signaling was mediated by Ca^{2+}-independent mechanisms. Isotonic saline, a subconvulsive dose of NMDA (25 mg/kg), MK-801, or MK-801 followed by NMDA was administered i.p. to unanesthetized rats. Radiolabeled DHA or AA was infused intravenously and their brain incorporation coefficients k*, measures of signaling, were imaged with quantitative autoradiography. NMDA or MK-801 compared with saline did not alter k* for DHA in any of 81 brain regions examined, whereas NMDA produced widespread and significant increments in k* for AA. In conclusion, in vivo brain DHA but not AA signaling via NMDA receptors is independent of extracellular Ca^{2+} and of $cPLA_2$. DHA signaling may be mediated by $iPLA_2$, plasmalogen PLA_2, or other enzymes insensitive to low concentrations of Ca^{2+}. Greater AA than DHA release during glutamate-induced excitotoxicity could cause brain cell damage.—Ramadan, E., A. O. Rosa, L. Chang, M. Chen, S. I. Rapoport, and M. Basselin. **Extracellular-derived calcium does not initiate in vivo neurotransmission involving docosahexaenoic acid. *J. Lipid Res.* 2010. 51: 2334–2340.**

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Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are abundant polyunsaturated fatty acids (PUFA) in brain, where they and their metabolites influence neurotransmission, membrane remodeling, gene transcription, blood flow, neuroinflammation, and other parameters (1–3). These PUFAs are concentrated in the stereospecifically numbered-2 position of synaptic membrane phospholipids, from which they can be hydrolyzed by phospholipases A_2 enzymes (PLA_2, EC 3.1.1.4) (3–5).

Four major PLA_2 subclasses are described in mammalian brain based on in vitro studies: Ca^{2+}-dependent cytosolic $cPLA_2$ type IV [$cPLA_2\alpha$ (type IVA) and β are Ca^{2+}-dependent, $cPLA_2\gamma$ is not], Ca^{2+}-dependent secretory $sPLA_2$, Ca^{2+}-independent $iPLA_2$ (β and γ), and Ca^{2+}-independent plasmalogen PLA_2 (6, 7). $cPLA_2$ (α and β) requires a low concentration of Ca^{2+} for its translocation to the membrane and activation (0.3–1 μM) and is selective for AA during acute stimulation of cells by diverse agents in vitro (8, 9). $iPLA_2$ does not require Ca^{2+} for activation (10–12) and is selective for DHA in isolated glial cells and in the test tube (13). Plasmalogen PLA_2 releases DHA from plasmalogen ethanolamine (7, 14). $sPLA_2$ requires millimolar Ca^{2+} concentrations for activation and releases AA and DHA in vitro (6, 7).

$cPLA_2$ and $iPLA_2$ have been identified at postsynaptic sites and glia in the vertebrate brain, $sPLA_2$ at presynaptic sites, and plasmalogen PLA_2 in neurons and glia (7, 15–17). Despite their different brain distributions and varying Ca^{2+} dependencies shown by in vitro studies, little information is available about the in vivo roles of the different PLA_2 enzymes to brain signaling involving AA and DHA. We therefore thought it of interest to examine and compare how in vivo signaling involving the two PUFAs depends on entry of extracellular Ca^{2+} in cells.

In this regard, binding of glutamate or N-methyl-D-aspartic acid (NMDA) to ionotropic NMDA receptors that are highly expressed in brain (18) is known to cause extracellular Ca^{2+} entry into the cell and stimulate Ca^{2+}-sensitive

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Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; NMDA, N-methyl-D-aspartic acid; PLA2, phospholipase A2; cPLA2, Ca^{2+}-dependent cytosolic PLA2; iPLA2, Ca^{2+}-independent PLA2; sPLA2, secretory PLA2; PUFA, polyunsaturated fatty acid.

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cPLA$_2$ to release AA from membrane phospholipid (19–21). Increments in intracellular Ca^{2+} following NMDA are ≤1 μM (22, 23). Using our imaging method involving the intravenous infusion of [1-^{14}C]AA (24, 25), we reported that administration of a subconvulsive dose of NMDA (25 mg/kg i.p.) to unanesthetized rats increased AA signaling, measured as increased AA incorporation coefficients k*, in wide areas of brain (26–28). The increases could be blocked by pretreatment with the NMDA receptor antagonist MK-801, which by itself reduced baseline values of k* by 16–49% (26).

In the present study, we employed our in vivo imaging method to determine the dependence of in vivo brain DHA signaling on extracellular Ca^{2+}, compared to AA signaling. We hypothesized that the DHA signal would not be altered following NMDA, to the extent that it is mediated by iPLA$_2$, plasmalogen PLA$_2$, or another Ca^{2+}-independent enzyme (see above). We administered (i.p.) isotonic saline, a subconvulsive dose of NMDA (25 mg/kg), MK-801, or MK-801 followed by NMDA, to unanesthetized male adult rats. Radiolabeled DHA (or AA as a control) was infused intravenously for 5 min, and brain incorporation coefficients k* were imaged using quantitative autoradiography at 20 min. We confirmed that NMDA widely increased AA incorporation coefficients k* in brain, whereas it had no significant effect on DHA incorporation coefficients in any of 81 measured brain regions. Thus, extracellular-derived Ca^{2+} and cPLA$_2$ are not involved in brain DHA signaling in vivo.

MATERIALS AND METHODS

Animals

Experiments were conducted following the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of Eunice Kennedy Shriver National Institute of Child Health and Human Development. Three-month-old male Fischer F344 rats (Taconic Farms, Rockville, MD) were acclimated for 1 week in an animal facility having regulated temperature, humidity, and a light-dark cycle. The rats had ad libitum access to water and food (Rodent NIH-31 auto 18-4 diet, Zeigler Bros, Gardners, PA). The diet contained (as percent of total fatty acids) 20.0% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% α-linolenic, 0.2% AA, 2.0% eicosapentaenoic, and 2.3% DHA acids (29).

Drugs

NMDA and MK-801 ((5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate) were purchased from Sigma-Aldrich (Saint Louis, MO) and 0.9% NaCl (saline USP) was purchased from Hospira (Lake Forest, IL). [1-^{14}C]AA in ethanol (53 mCi/mmol, >98% pure (Moravek Biochemicals, Brea, CA) or [1-^{14}C]DHA in ethanol (56 mCi/mmol, >98% pure, Moravek) was evaporated and resuspended in HEPES buffer, pH 7.4, containing 50 mg/ml BSA, as described (30). To confirm tracer purity, thin-layer chromatography was performed and radioactivity of each band was measured by scintillation counter. 98% of radioactivity was detected in the fatty acid band. Gas chromatography was performed to confirm the identity of the fatty acid (31).

Surgical procedures and tracer infusion

In the morning of an experiment, a rat was anesthetized with 2–3% halothane in O_2. Polyethylene catheters were inserted into the right femoral artery and vein, as described (26). The rat was allowed to recover from anesthesia for 3 h in a sound-dampened, temperature-controlled box with its hindquarters loosely wrapped and taped to a wooden block. Arterial blood pressure and heart rate were measured with a blood pressure recorder (CyQ 103/302; Cybersense, Inc., Nicholasville, KY). Arterial blood pH, partial pressure of oxygen (pO_2), and partial pressure of carbon dioxide (pCO_2) were measured with a blood gas analyzer (Model 248, Bayer Health Care, Norwood, MA).

Ten min after i.p. saline or i.p. NMDA (25 mg/kg), or 30 min after MK-801 (0.3 mg/kg i.p.), [1-^{14}C]DHA (170 μCi/kg) was infused into the femoral vein for 5 min at a rate of 400 μl/min using an infusion pump (Harvard Apparatus Model 22, Natick, MA) (26). For studies with both drugs, MK-801 was administered 30 min prior to NMDA, which was given 10 min before the radioisotope. For the AA study, saline or NMDA (25 mg/kg) was injected 10 min before [1-^{14}C]AA (170 μCi/kg) infusion. Twenty min after beginning tracer infusion in either case, the rat was euthanized with Nembutal® (80 mg/kg, i.v.). The brain was removed within 30 s, frozen in 2-methylbutane maintained at −40°C with dry ice, and stored at −80°C.

Chemical analysis

Thirteen arterial blood samples, collected before, during, and after [1-^{14}C]AA or [1-^{14}C]DHA infusion, were centrifuged immediately (30 s at 18,000 *g*) to determine unesterified plasma DHA or AA radioactivity. Total lipids were extracted from 30 μl plasma with 3 ml chloroform:methanol (2:1, v/v) and 1.5 ml KCl (0.1 M) (32). Radioactivity was determined in 100 μl of the organic phase by liquid scintillation counting. As reported, after a 5 min [1-^{14}C]AA or [1-^{14}C]DHA infusion, >97% of plasma radioactivity was the infused radiolabeled PUFA (33, 34).

Quantitative autoradiography

Frozen brains were cut in serial 20-μm thick coronal sections on a cryostat at −20°C, then placed for 4–5 weeks with calibrated [^{14}C]methylmethacrylate standards (Amersham Life Science, Arlington Heights, IL) on Ektascan C/RA film (Eastman Kodak, Rochester, NY). Radioactivity (nCi/g wet weight brain) in 81 identified regions (35) was measured bilaterally six times by quantitative densitometry, using the public domain NIH Image program 1.62. Regional incorporation coefficients k* (ml/s/g brain) of AA or DHA were calculated as follows (equation 1):

$$k^* = \frac{c^*_{brain}(20\,\text{min})}{\int_0^{20} c^*_{plasma}\,dt} \qquad \textit{(Eq. 1)}$$

c^*_{brain} (nCi/g wet brain wt) is brain radioactivity 20 min after beginning infusion, c^*_{plasma} (nCi/ml plasma) is arterial labeled unesterified fatty acid as determined by scintillation counting, and t (min) is time after beginning [1-^{14}C]fatty acid infusion. Integrated plasma radioactivity (input function in denominator) was determined in each experiment by trapezoidal integration and used to calculate k* for each experiment.

Statistical analyses

Physiological parameters were analyzed by paired *t*-tests in the same animal before and after drug injection (GraphPad Prism Software, San Diego, CA). Statistical significance of drug on arterial plasma radioactivity and on k* for each brain region was determined by one-way ANOVA (ANOVA) with Dunnett's post-test

for DHA infusion or an unpaired *t*-test for AA infusion. Corrections for multiple comparisons across regions were not made because this was an exploratory study to identify regions that are involved in individual drug effects. Data are reported as mean ± SD, with statistical significance set at $P \leq 0.05$.

RESULTS

Physiology and arterial plasma radioactivity

For the DHA infusion study, NMDA (25 mg/kg, i.p.) compared with saline significantly decreased heart rate by 11% (387 ± 46 versus 442 ± 25 beats/min, $P < 0.05$) but did not have a significant effect on arterial blood pressure, whereas MK-801 alone or before NMDA increased systolic arterial blood pressure by 9–13% in the unanesthetized rats ($P < 0.05$; n = 7–8). Arterial blood pressure (mmHg) was as follows: before MK-801 (169 ± 10); after MK-801 (184 ± 9); before MK-801 and NMDA (176 ± 10); after MK-801 and NMDA (199 ± 22). Such changes, reported previously, have been ascribed to a centrally mediated increase in sympathetic nerve activity by MK-801 (26). MK-801 given before NMDA abolished NMDA's significant effect on heart rate and decreased plasma pH (data not shown).

A one-way ANOVA with Dunnett's test showed that MK-801 significantly ($P < 0.01$) increased mean integrated radioactivity by 42% in the plasma organic fraction, the input function for determining k* in equation 1 during [1-^{14}C]DHA infusion. Mean integrated radioactivity (nCi/s)/ml) was as follows: saline (144,723 ± 34,385); NMDA (141,659 ± 34,451); MK-801 (205,895 ± 33,515); MK-801 + NMDA (181,283 ± 40,200).

For the reference AA infusion study, NMDA significantly decreased heart rate by 16% (data not shown). Unpaired *t*-tests showed that NMDA significantly increased integrated plasma radioactivity during [1-^{14}C]AA infusion by 21% ($P = 0.04$) [NMDA (114,173 ± 20,236) versus saline (94,118 ± 15,356) nCi/s/ml].

Regional brain DHA and AA incorporation coefficients, k*

As shown in **Table 1** and **Fig. 1**, NMDA compared with saline did not increase k* for DHA significantly in any of the 81 regions examined. Also, MK-801 given alone or 30 min prior NMDA had no effect on k* for DHA in any region (data not shown). In contrast, NMDA compared with saline significantly increased k* for AA by 20–86% in 54 of 81 regions. Affected regions included prefrontal cortex (38–46%), frontal cortex (23–30%), piriform cortex (32%), motor cortex (40–58%), somatosensory cortex (32–41%), auditory cortex (25–39%), hippocampus (40–86%), nucleus accumbens (26%), caudate putamen (39–48%), septal nuclei (44–70%), some regions of the thalamus (20–38%), hypothalamus (24–72%), two white matter regions (58–77%), and two nonblood brain barrier regions (51–69%).

DISCUSSION

An acutely administered subconvulsive dose of NMDA (25 mg/kg i.p.) in unanesthetized rats, compared with i.p. saline control, failed to induce a significant change in the brain DHA incorporation coefficient k* in any of 81 regions imaged using quantitative autoradiography. MK-801 also had no effect on baseline k* for DHA. In contrast, we confirmed the report that this dose of NMDA significantly increased k* for AA in wide areas of the brain (26–28).

The baseline values of k* for DHA and AA in this study agree with published data (26–28, 30). k* for each PUFA largely reflects its metabolic loss following its release from membrane phospholipid, as DHA and AA cannot be synthesized de novo in vertebrate tissue and only negligible amounts (< 1%) can be elongated in brain from their circulating precursors α-linolenic (18:3n-3) and linoleic acid (18:2n-6), respectively. Loss is replaced entirely by unesterified PUFA from plasma (2, 29, 36).

The statistically significant increments in k* for AA following NMDA are thought to arise from entry of extracellular Ca^{2+} into the cell through ionic NMDA receptors to activate $cPLA_2$ and selectively hydrolyze AA from synaptic membrane phospholipid (4, 6, 8, 9, 20, 21). NMDA receptor activation results in intracellular Ca^{2+} increments of ≤ 1 μM (22, 23), sufficient for displacement of $cPLA_2$ to the membrane, which requires 0.3–1 μM Ca^{2+}. In contrast, the absence of DHA responses to NMDA indicates that the DHA signal is independent of extracellular-derived Ca^{2+} and $cPLA_2$, and thus likely mediated by a PLA_2 whose activation is independent of entry of increments in intracellular Ca^{2+} of about 1 μM. As discussed above, potential Ca^{2+}-independent candidates are $iPLA_2$ (β and γ) and plasmalogen PLA_2, and $cPLA_2\gamma$ (6, 7, 10–13, 37, 38). $sPLA_2$ is an unlikely candidate because of its presynaptic location and its mM requirement for Ca^{2+} (6, 39). Supporting a role for $iPLA_2\beta$ in DHA signaling in vivo, k* for DHA at rest or in response to stimulation of G-protein-coupled cholinergic muscarinic $M_{1,3,5}$ receptors by arecoline is decreased in unanesthetized heterozygous and homozygous $iPLA_2\beta$ (VIA)-deficient mice (40). Additionally, in rats whose diets are deficient in n-3 PUFAs, mRNA, protein, and activity levels of $iPLA_2\beta$ are reduced in relation to reduced DHA turnover in brain phospholipid (41, 42).

The absence of significant reductions in k* for DHA following MK-801, in contrast to 16–49% reductions reported for k* for AA following MK-801(26), further indicates that endogenous glutamate is not involved in baseline DHA release. In agreement, exposure of eosinophilic leukemia cells or platelets to a Ca^{2+}-ionophore did not release DHA while releasing AA through $cPLA_2$ activation (9), and chronic NMDA administration to rats increased $cPLA_2$-IVA but not $iPLA_2$-VI expression in brain (43). Under some in vitro conditions, acute NMDA receptor activation may release Ca^{2+} from intracellular stores by Ca^{2+}-induced Ca^{2+} release (CICR) (44, 45), which may indirectly stimulate $iPLA_2$ (46–48). However, our in vivo data do not support this signaling pathway with the dose of NMDA administered.

Our observations may be relevant to excitotoxicity, which involves high brain glutamate concentrations. In rodent models, excitotoxicity is associated with increased brain concentrations of AA and of its proinflammatory metabolites and with increased $cPLA_2$-IVA expression,

TABLE 1. Docosahexaenoic and arachidonic acid incorporation coefficients k* in rats in response to saline and NMDA

	DHA		AA	
Brain Region	Saline (n = 8)	NMDA (n = 7)	Saline (n = 8)	NMDA (n = 8)
Prefrontal cortex layer I	8.68 ± 2.28	8.09 ± 2.59	8.45 ± 0.87	12.33 ± 2.83[a]
Prefrontal cortex layer IV	9.56 ± 2.77	9.42 ± 4.03	10.09 ± 1.39	13.90 ± 3.30[a]
Primary olfactory cortex	8.45 ± 1.25	8.80 ± 1.53	10.06 ± 2.15	12.36 ± 2.60
Frontal cortex (10)				
Layer I	8.43 ± 2.25	7.73 ± 2.39	9.02 ± 1.49	11.10 ± 3.42[a]
Layer IV	9.51 ± 2.71	9.43 ± 4.60	10.85 ± 1.54	13.87 ± 3.11[b]
Frontal cortex (8)				
Layer I	9.04 ± 2.06	7.39 ± 1.40	10.84 ± 2.06	14.05 ± 2.88[b]
Layer IV	9.18 ± 1.98	9.60 ± 1.10	12.70 ± 1.99	15.78 ± 2.80[b]
Piriform cortex	6.52 ± 1.27	5.74 ± 2.37	6.92 ± 3.90	9.13 ± 2.10[a]
Anterior cingulate cortex	8.65 ± 2.27	9.16 ± 1.56	12.50 ± 2.29	14.64 ± 2.75
Motor cortex				
Layer I	8.43 ± 2.36	8.53 ± 3.66	7.30 ± 2.06	11.50 ± 2.32[a]
Layer II – III	8.53 ± 2.65	8.81 ± 3.75	8.39 ± 1.66	12.35 ± 2.28[a]
Layer IV	9.76 ± 2.33	10.45 ± 4.10	9.74 ± 1.43	13.59 ± 2.23[a]
Layer V	7.41 ± 2.32	8.19 ± 3.19	9.18 ± 2.12	12.92 ± 2.70[a]
Layer VI	7.82 ± 2.47	8.80 ± 3.30	8.62 ± 1.84	12.36 ± 2.17[a]
Somatosensory cortex				
Layer I	9.57 ± 2.53	9.64 ± 3.57	8.12 ± 2.25	11.21 ± 5.14
Layer II–III	9.53 ± 2.65	10.42 ± 4.86	9.02 ± 2.07	12.72 ± 2.89[b]
Layer IV	9.21 ± 1.69	10.69 ± 1.09	11.01 ± 1.62	14.49 ± 2.60[a]
Layer V	8.69 ± 2.18	9.01 ± 1.68	9.95 ± 1.27	13.31 ± 2.71[a]
Layer VI	8.28 ± 1.92	8.35 ± 1.45	9.31 ± 1.86	13.00 ± 2.65[a]
Auditory cortex				
Layer I	8.66 ± 2.41	8.24 ± 2.59	10.32 ± 2.92	13.45 ± 1.97[b]
Layer IV	8.17 ± 2.52	6.91 ± 1.81	13.02 ± 2.76	16.34 ± 1.94[b]
Layer VI	6.77 ± 1.28	6.62 ± 1.68	10.45 ± 2.96	14.54 ± 1.70[a]
Visual cortex				
Layer I	8.93 ± 2.37	7.04 ± 1.45	8.46 ± 3.29	11.99 ± 3.97
Layer IV	9.33 ± 2.40	7.91 ± 1.99	11.23 ± 2.96	14.27 ± 3.88
Layer VI	8.91 ± 1.68	7.04 ± 1.45	9.52 ± 3.25	12.90 ± 3.42
Preoptic area (LPO/MPO)	6.04 ± 1.05	6.08 ± 1.56	6.66 ± 1.70	10.46 ± 1.84[a]
Suprachiasmatic nu	7.06 ± 1.33	6.90 ± 1.05	7.87 ± 1.43	10.78 ± 2.34[a]
Globus pallidus	6.89 ± 1.41	5.91 ± 1.54	6.97 ± 1.69	10.20 ± 1.93[a]
Bed nu stria terminalis	6.72 ± 1.54	6.37 ± 2.01	6.19 ± 2.06	8.59 ± 1.51[b]
Olfactory tubercle	7.58 ± 1.06	7.38 ± 1.37	8.73 ± 2.51	11.86 ± 1.85[b]
Diagonal band dorsal	7.76 ± 1.44	6.94 ± 1.79	8.62 ± 1.50	12.15 ± 3.73[b]
Diagonal band ventral	7.52 ± 1.95	6.46 ± 1.37	8.16 ± 1.79	12.29 ± 2.87[a]
Amygdala basolat/med	6.28 ± 1.17	6.00 ± 1.43	5.03 ± 2.58	8.99 ± 1.95[a]
Hippocampus CA1	5.90 ± 0.99	5.85 ± 1.65	4.49 ± 2.59	7.49 ± 1.79[b]
Hippocampus CA2	5.87 ± 0.72	5.23 ± 1.16	4.66 ± 2.32	8.04 ± 1.98[a]
Hippocampus CA3	5.77 ± 1.15	5.58 ± 1.73	4.58 ± 2.32	8.51 ± 1.77[a]
Hippocampus dentate gyrus	7.47 ± 1.61	7.18 ± 1.32	5.74 ± 2.76	9.49 ± 1.54[a]
Hippocampus SLM	7.69 ± 2.05	7.88 ± 2.00	9.28 ± 1.25	13.01 ± 2.43[a]
Nucleus accumbens	8.94 ± 2.18	7.74 ± 1.88	10.16 ± 1.28	12.83 ± 3.16[b]
Caudate putamen				
Dorsal	7.17 ± 1.52	7.34 ± 2.84	8.32 ± 1.67	11.75 ± 3.26[b]
Ventral	8.12 ± 1.94	8.13 ± 3.23	8.18 ± 2.14	12.08 ± 3.12[b]
Lateral	8.80 ± 2.58	7.46 ± 1.96	8.42 ± 1.96	11.99 ± 2.85[b]
Medial	8.22 ± 2.08	7.81 ± 3.34	8.26 ± 1.93	11.49 ± 2.86[b]
Septal nu lateral	7.33 ± 1.76	6.08 ± 1.94	5.63 ± 2.29	9.57 ± 2.65[a]
Septal nu medial	7.64 ± 2.24	8.68 ± 3.92	8.59 ± 1.34	12.35 ± 3.68[b]
Habenular nu lateral	10.11 ± 2.72	11.12 ± 3.78	14.00 ± 2.65	15.58 ± 2.96
Habenular nu medial	9.46 ± 2.45	7.63 ± 1.85	10.81 ± 2.08	13.43 ± 3.29
Lat geniculate nu dorsal	9.91 ± 2.70	9.50 ± 4.41	12.77 ± 2.22	15.05 ± 2.43
Geniculate medial	9.19 ± 1.94	8.90 ± 2.41	14.45 ± 3.24	16.56 ± 2.49
Thalamus				
Ventroposterior lateral nu	8.42 ± 1.59	7.75 ± 1.82	12.18 ± 1.82	14.33 ± 3.07
Ventroposterior medial nu	7.82 ± 1.52	8.66 ± 1.88	12.13 ± 1.57	14.38 ± 2.76
Paratenial nu	7.41 ± 1.48	6.60 ± 2.08	9.10 ± 1.73	11.76 ± 3.21
Anteroventral nu	10.34 ± 2.00	11.06 ± 3.03	14.34 ± 2.15	15.62 ± 2.87
Anteromedial nu	7.90 ± 1.81	9.74 ± 4.89	12.39 ± 1.52	15.15 ± 2.69[b]
Reticular nu	7.92 ± 2.62	7.97 ± 2.41	11.40 ± 1.36	13.68 ± 2.54[b]
Paraventricular nu	7.67 ± 2.09	7.72 ± 2.64	8.29 ± 2.14	11.45 ± 2.64[b]
Parafascicular nu	8.82 ± 2.18	7.77 ± 2.43	11.87 ± 2.19	14.37 ± 2.92
Subthalamic nu	9.08 ± 1.41	8.58 ± 1.54	10.45 ± 1.03	12.91 ± 3.55
Hypothalamus				
Supraoptic nu	7.87 ± 2.00	6.46 ± 3.32	9.05 ± 1.10	11.21 ± 1.62[a]
Lateral	7.09 ± 1.43	6.06 ± 1.54	6.65 ± 2.04	10.38 ± 2.62[a]
Anterior	7.44 ± 1.84	6.26 ± 1.97	6.55 ± 2.52	10.40 ± 2.27[a]

TABLE 1. *Continued.*

Brain Region	DHA		AA	
	Saline (n = 8)	NMDA (n = 7)	Saline (n = 8)	NMDA (n = 8)
Periventricular	5.84 ± 1.38	4.70 ± 1.45	6.34 ± 2.61	9.70 ± 2.46[b]
Arcuate	7.01 ± 1.58	6.23 ± 2.27	6.01 ± 2.40	10.11 ± 2.96[a]
Ventromedial	6.84 ± 1.38	6.19 ± 2.31	6.22 ± 2.05	10.69 ± 2.81[a]
Posterior	7.44 ± 1.81	6.73 ± 1.69	6.56 ± 1.93	10.12 ± 2.69[a]
Mammillary nu	8.47 ± 2.79	8.81 ± 4.85	6.09 ± 1.74	9.55 ± 1.80[a]
Interpeduncular nu	9.29 ± 3.13	10.53 ± 5.22	17.88 ± 2.30	18.81 ± 3.45
Substantia nigra	7.89 ± 1.72	6.09 ± 1.87	9.62 ± 2.94	11.81 ± 3.76
Pretectal area	7.82 ± 2.61	7.38 ± 1.62	13.10 ± 2.85	14.61 ± 3.38
Superior colliculus	8.87 ± 1.70	8.91 ± 2.58	14.12 ± 2.32	14.51 ± 2.73
Deep layers	7.71 ± 1.15	7.92 ± 1.91	11.93 ± 3.02	14.89 ± 2.52
Inferior colliculus	15.22 ± 4.26	15.27 ± 3.11	19.90 ± 3.11	22.75 ± 4.39
Flocculus	9.60 ± 2.61	9.58 ± 3.16	18.34 ± 2.88	19.01 ± 2.65
Cerebellar gray matter	11.51 ± 2.32	11.48 ± 3.46	14.64 ± 2.49	15.48 ± 2.71
Molecular layer cerebellar gray matter	12.92 ± 2.28	14.48 ± 2.22	17.88 ± 2.49	17.80 ± 2.98
White matter				
Corpus callosum	6.18 ± 0.96	5.10 ± 1.11	4.94 ± 1.78	8.73 ± 3.42[b]
Zone incerta	7.37 ± 1.26	8.00 ± 1.56	11.04 ± 2.00	13.00 ± 3.32
Internal capsule	4.76 ± 0.85	4.29 ± 1.78	4.01 ± 2.30	6.34 ± 1.84[b]
Cerebellar white matter	10.83 ± 3.60	7.77 ± 2.58	5.72 ± 3.00	7.76 ± 1.39
Non-blood brain barrier regions				
Subfornical organ	7.37 ± 2.14	6.91 ± 1.73	6.56 ± 2.31	9.89 ± 1.90[a]
Median eminence	7.14 ± 2.20	6.85 ± 3.61	5.93 ± 1.64	10.01 ± 3.26[b]

k* = (ml/s/g) × 1024. Each value is mean ± SD. NMDA administration: 25 mg/kg i.p. 10 min. Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; lat, lateral; LPO, lateral preoptic area; med, medial; MPO, medial preoptic area; nu, nucleus; NMDA, N-methyl-D-aspartic acid; SLM, stratum lacunosum-moleculae of hippocampus.

[a] $P < 0.01$; unpaired t-test.

[b] $P < 0.05$; unpaired t-test.

without a significant change in DHA concentration or iPLA$_2$-VI expression (43, 49, 50). High concentrations of AA and its metabolites can be neurotoxic (51), whereas DHA and its metabolites are considered neuroprotective (1). Thus, increased release of AA but not of DHA due to excessive NMDA receptor activation by glutamate may interfere with brain function and structure (2, 52).

In summary, stimulating brain NMDA receptors in unanesthetized rats by a subconvulsive dose of NMDA does not produce a significant DHA signal, but it produces a

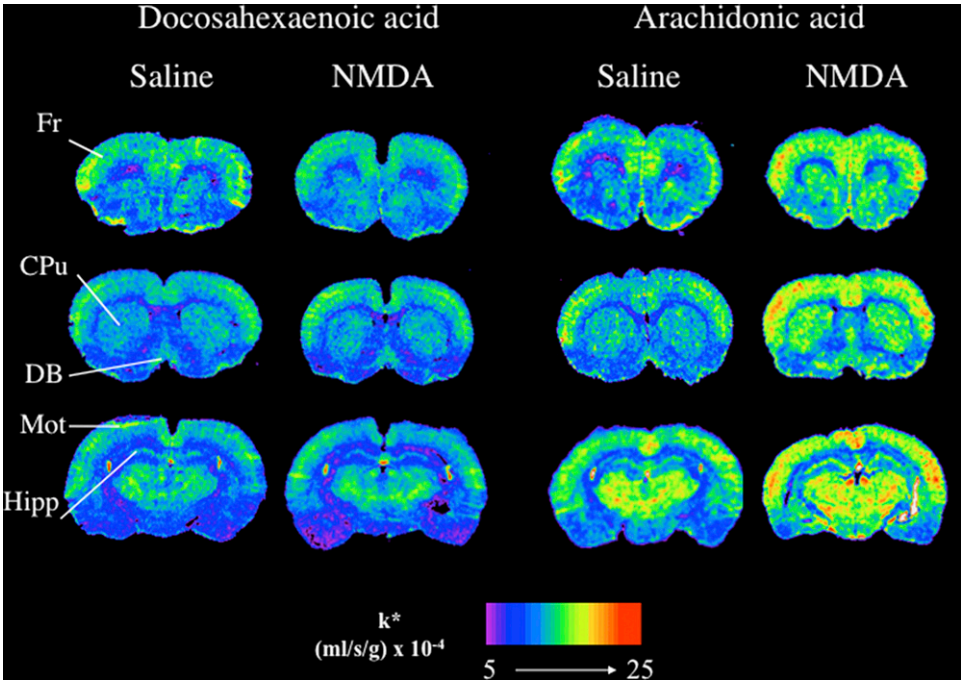

Fig. 1. Coronal autoradiographs showing effects of NMDA on regional brain docosahexaenoic and arachidonic acid incorporation coefficients k* in rats. Values of k* (ml/s/g brain × 10^{-4}) are given on a color scale. CPu, caudate-putamen; DB, diagonal band; Fr, frontal cortex; Hipp, hippocampus; Mot, motor cortex; NMDA, N-methyl-D-aspartic acid.

robust AA signal. As such stimulation allows extracellular Ca^{2+} into the cell, these in vivo results are consistent with in vitro evidence that AA is preferentially hydrolyzed from phospholipid by Ca^{2+}-dependent $cPLA_2$ and that the in vivo AA signal following NMDA is a surrogate marker of Ca^{2+} entry into cells. The absence of a DHA signal in rat brain following NMDA is consistent with in vitro evidence that DHA hydrolysis can be mediated by $iPLA_2$, plasmalogen PLA_2, and $cPLA_2\gamma$, none of which requires Ca^{2+}.

Several human diseases, including Alzheimer disease and bipolar disorder, show upregulated $cPLA_2$-IVA but not $iPLA_2\beta$ expression, with high glutamatergic function and other evidence of excitotoxicity (53–56). Excess release of AA compared with DHA from membrane phospholipid would be expected to disturb the normally balanced interactions between the two PUFAs (52) and disrupt brain function in these diseases.

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