

Chronic Lithium Chloride Administration Attenuates Brain NMDA Receptor-Initiated Signaling via Arachidonic Acid in Unanesthetized Rats

Mireille Basselin^{*1}, Lisa Chang¹, Jane M Bell¹ and Stanley I Rapoport¹

¹Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

It has been proposed that lithium is effective in bipolar disorder (BD) by inhibiting glutamatergic neurotransmission, particularly via N-methyl-D-aspartate receptors (NMDARs). To test this hypothesis and to see if the neurotransmission could involve the NMDAR-mediated activation of phospholipase A₂ (PLA₂), to release arachidonic acid (AA) from membrane phospholipid, we administered subconvulsant doses of NMDA to unanesthetized rats fed a chronic control or LiCl diet. We used quantitative autoradiography following the intravenous injection of radiolabeled AA to measure regional brain incorporation coefficients k^* for AA, which reflect receptor-mediated activation of PLA₂. In control diet rats, NMDA (25 and 50 mg/kg i.p.) compared with i.p. saline increased k^* significantly in 49 and 67 regions, respectively, of the 83 brain regions examined. The regions affected were those with reported NMDARs, including the neocortex, hippocampus, caudate-putamen, thalamus, substantia nigra, and nucleus accumbens. The increases could be blocked by pretreatment with the specific noncompetitive NMDA antagonist MK-801 ((5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate) (0.3 mg/kg i.p.), as well by a 6-week LiCl diet sufficient to produce plasma and brain lithium concentrations known to be effective in BD. MK-801 alone reduced baseline values for k^* in many brain regions. The results show that it is possible to image NMDA signaling via PLA₂ activation and AA release *in vivo*, and that chronic lithium blocks this signaling, consistent with its suggested mechanism of action in BD.

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INTRODUCTION

Lithium (Li⁺) has been used to treat bipolar disorder (BD) for about 50 years, but its mechanism of action is not agreed on (Barchas *et al*, 1994; Cade, 1999). One suggestion has been that it acts in part by inhibiting glutamatergic neurotransmission, particularly via N-methyl-D-aspartate (NMDA) receptors (NMDARs) (Bauer *et al*, 2003; Farber *et al*, 2002; Krystal *et al*, 2002; Stewart and Reid, 2002; Zarate *et al*, 2003). Supporting this is evidence of disturbed markers of NMDA functioning in the BD brain (Itokawa *et al*, 2003; Mundo *et al*, 2003; Scarr *et al*, 2003), and data that drugs effective against BD, such as lamotrigine, carbamazepine and valproic acid, have direct or indirect

NMDA antagonist properties (Farber *et al*, 2002; Hough *et al*, 1996; Kubota *et al*, 1994; McIntyre *et al*, 2004; Zeise *et al*, 1991).

Ionotropic NMDARs, cation channels with a high permeability to Ca²⁺ when activated, consist of heterotetrameric assemblies of NR1, NR2A-D, and NR3 subunits. The subunits are assembled cotranslationally in the endoplasmic reticulum, from where they are transported and then inserted at glutamatergic synapses. Release of NMDARs from the endoplasmic reticulum requires phosphorylation of NR1 and NR2 by protein kinases A and C (PKA and PKC), or by serine or tyrosine kinases (Scott *et al*, 2003; Wenthold *et al*, 2003). Binding of glutamate or NMDA to synaptic NMDARs allows Ca²⁺ into the cell to activate Ca²⁺-dependent enzymes such as calcium/calmodulin-dependent protein kinase II (CaMKII), PKC, phospholipase C γ , and phospholipase A₂ (PLA₂) (Colbran, 2004; Fukunaga *et al*, 1992; Gurd and Bissoon, 1997; Weichel *et al*, 1999; Wenthold *et al*, 2003).

Exposure of neurons from different brain regions to NMDA is reported to activate PLA₂ through the Ca²⁺ mechanism, to release the second messenger, arachidonic acid (AA, 20:4 n-6), from membrane phospholipid

*Correspondence: Dr M Basselin, Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bldg 9, Room 1S126-MSC 0947, Bethesda, MD 20892, USA, Tel: +1 301 594 5522, Fax: +1 301 402 0074, E-mail: mirvasln@mail.nih.gov
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(Dumuis *et al*, 1988; Kim *et al*, 1995; Kolko *et al*, 1999, 2003; Lazarewicz *et al*, 1990; Pellerin and Wolfe, 1991; Sanfeliu *et al*, 1990; Tapia-Arancibia *et al*, 1992; Tencé *et al*, 1994; Weichel *et al*, 1999). The AA release could be blocked by the nonspecific PLA₂ inhibitor, meprazine, or in the rat hippocampus by bilobalide, a constituent of Ginkgo biloba, and by a specific inhibitor of cPLA₂ (cytosolic PLA₂) (see below) (Sanfeliu *et al*, 1990; Tapia-Arancibia *et al*, 1992; Tencé *et al*, 1994; Weichel *et al*, 1999). In contrast, NMDA did not stimulate AA release in hippocampal astroglia (Sanfeliu *et al*, 1990).

Optical methods have been used to image the Ca²⁺ signal in response to exogenous NMDA in brain slices or cell culture (Hashimoto *et al*, 2002; Ismailov *et al*, 2004; Mainen *et al*, 1999; Takita *et al*, 1997). However, no method exists, as far as we know, to image the Ca²⁺ signal or its downstream effects in the brain *in vivo*. In this paper, we present such a method, which extends our published method of imaging brain PLA₂ activation in unanesthetized rats given drugs that act at receptors coupled a G-protein to PLA₂ (Basselin *et al*, 2005a,b, 2003; Bhattacharjee *et al*, 2005; DeGeorge *et al*, 1991; Qu *et al*, 2003)—cholinergic muscarinic M_{1,3,5}, dopaminergic D₂-like and serotonergic 5-HT_{2A/2C} receptors (Axelrod, 1995; Bayon *et al*, 1997; Felder *et al*, 1990; Vial and Piomelli, 1995).

There are three classes of brain PLA₂ enzymes: AA-selective Ca²⁺-dependent cPLA₂, sPLA₂ (secretory PLA₂), and iPLA₂ (Ca²⁺-independent PLA₂) (thought to be selective for docosahexaenoic acid (DHA, 22:6 n-3) rather than AA). All have been identified in neurons (Kishimoto *et al*, 1999; Strokin *et al*, 2004; Yang *et al*, 1999; Yegin *et al*, 2002). cPLA₂ has been localized at postsynaptic neuronal membranes in the brain (Basavarajappa *et al*, 1998; Ong *et al*, 1999; Pardue *et al*, 2003) and is activated by 300 nM to 1 μM Ca²⁺ (Clark *et al*, 1995), in the physiological range of intracellular Ca²⁺ during neuronal activation (Ismailov *et al*, 2004). sPLA₂ is activated at much higher Ca²⁺ concentrations, 16–18 mM (Dennis, 1994), and is localized in presynaptic vesicles that are released by exocytosis during membrane depolarization (Matsuzawa *et al*, 1996; Wei *et al*, 2003).

In elaborating our *in vivo* fatty acid imaging method when stimulating receptors coupled to PLA₂ (see above), we concluded that a fraction of the AA that is released following PLA₂ activation is rapidly reincorporated into phospholipid, whereas the remainder is lost by conversion to eicosanoids or other products, or by β-oxidation (Rapoport, 2001, 2003; Robinson *et al*, 1992). Unesterified AA in plasma rapidly replaces the quantity lost, as AA is nutritionally essential and cannot be synthesized *de novo* in vertebrate tissue or elongated significantly from its precursor, linoleic acid (18:2 n-6) (DeMar *et al*, 2004b; Holman, 1986; Washizaki *et al*, 1994). Replacement is proportional to PLA₂ activation and can be imaged by injecting radiolabeled AA intravenously, then measuring regional brain radioactivity by quantitative autoradiography. A regional AA incorporation coefficient *k** (regional brain radioactivity/integrated plasma radioactivity), calculated in this way, has been shown to be independent of changes in cerebral blood flow and to represent the plasma-derived AA reincorporated into brain phospholipid (Basselin *et al*, 2003; Chang *et al*, 1997; DeGeorge *et al*, 1991;

DeMar *et al*, 2004a; Rapoport, 2001, 2003; Robinson *et al*, 1992).

We thought it important to see if we could image brain AA signaling in response to NMDA, and if chronic LiCl would attenuate this signaling. In this study, we injected radiolabeled AA intravenously into unanesthetized rats to determine *k** for AA in each of 83 brain regions in response to i.p. saline, and to each of two i.p. doses of NMDA, 25 and 50 mg/kg, neither of which is reported to produce convulsions although capable of inducing paroxysmal EEG activity (Ormandy *et al*, 1991). We also imaged regional *k** responses to the selective noncompetitive NMDA antagonist, MK-801 ((5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate) (Wong *et al*, 1986, 1988), given at a dose of 0.3 mg/kg i.p. alone or prior to NMDA administration. Alone, this dose is reported to produce ataxia, hyperlocomotion, and stereotypies (Dai *et al*, 1995) accompanied by cortical spike-wave discharges (Feinberg *et al*, 1995). Finally, we measured *k** for AA in response to NMDA in animals fed a control or LiCl diet for 6 weeks. The LiCl diet produces plasma and brain lithium concentrations of about 0.8 mM, reported to be therapeutic in BD (Basselin *et al*, 2003; Bosetti *et al*, 2002b; Calabrese *et al*, 1995; Chang *et al*, 1996). An abstract of part of this work has been presented (Basselin *et al*, 2004).

MATERIALS AND METHODS

Animals and Diets

Experiments were conducted following the 'Guide for the Care and Use of Laboratory Animals' (National Institute of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of the National Institute of Child Health and Development. Male Fischer CDF (F-344)/CrlBR rats (2 months old) (Charles River Laboratories, Wilmington, MA) were fed a control or LiCl diet as previously described (Basselin *et al*, 2003).

Drugs

NMDA (25 or 50 mg/kg, RBI Signaling Innovation, Sigma-Aldrich, Natick, MA), (+)-MK-801 hydrogen maleate (0.3 mg/kg, RBI Signaling Innovation) or 0.9% NaCl (saline) was administered i.p. [1-¹⁴C]AA in ethanol (53 mCi/mmol, >98% pure, Moravek Biochemicals, Brea, CA) was evaporated and resuspended in HEPES buffer, pH 7.4, containing 50 mg/ml BSA, as described (DeGeorge *et al*, 1989). To confirm tracer purity, gas chromatography was performed after the [1-¹⁴C]AA was converted to a methyl ester using 1 ml of 1% H₂SO₄ in anhydrous methanol (Makrides *et al*, 1994).

Surgical Procedures and Tracer Infusion

Surgery was performed as described (Basselin *et al*, 2003; Chang *et al*, 1996). A rat was wrapped loosely in a fast-setting plaster cast taped to a wooden block, and allowed to recover from anesthesia for 3–4 h. Body temperature was maintained at 36.5°C using a feedback-heating device. Arterial blood pressure and heart rate were measured

8 and 28 min after NMDA and MK-801 injection, respectively. Behavior was monitored immediately after drug administration until the end of the experiment. Repeated cycles consisting of an activity followed by a calm period were produced by NMDA. We measured the mean duration of each of these periods, as well as of the net cycling period.

At 10 min after NMDA or 30 min after MK-801 injection [$1\text{-}^{14}\text{C}$]AA (170 $\mu\text{Ci/kg}$, 2 ml, 400 $\mu\text{l/min}$) was infused for 5 min via the femoral catheter using an infusion pump (Harvard Apparatus Model 22, Natick, MA). For studies with both drugs, MK-801 was administered 30 min prior to NMDA, which in turn was given 10 min before [$1\text{-}^{14}\text{C}$]AA. Timed arterial blood samples were collected during radiotracer infusion, to time of death at 20 min, when the rat was killed using an overdose of sodium pentobarbital (100 mg/kg i.v.) and immediately decapitated. The brain was removed and quickly frozen in 2-methylbutane at -40°C , stored at -80°C , and later sectioned for autoradiography.

Chemical Analysis

Plasma was separated from arterial blood by centrifugation, and lipids were extracted using a modification of Folch's method (DeGeorge *et al*, 1991; Folch *et al*, 1957). Total radioactivity in the organic phase was measured by liquid scintillation counting.

Quantitative Autoradiography

Quantitative autoradiography was performed as described previously (Basselin *et al*, 2003). A total of 83 brain regions were identified by comparing autoradiographs of coronal brain sections with a coronal atlas of the rat brain (Paxinos and Watson, 1987). The average of three bilateral optical density readings for each brain region from each animal was used to calculate regional radioactivity. Regional brain incorporation coefficients of AA from plasma into the stable brain lipid compartment, k^* (ml/s/g brain), were calculated as

$$k^* = \frac{c_{\text{brain}}^*(20 \text{ min})}{\int_0^{20} c_{\text{plasma}}^* dt} \quad (1)$$

where $c_{\text{brain}}^*(20 \text{ min})$ nCi/g equals brain radioactivity at the time of death (20 min), c_{plasma}^* nCi/ml equals plasma AA radioactivity determined by scintillation counting, and t equals time after onset of infusion.

Statistical Analyses

In each of 83 brain regions, effects on k^* of NMDA and MK-801 alone and in combination in control diet rats were compared to effects of saline by one-way ANOVA with Dunnett's post-test with correction for four comparisons, using GraphPad Prism version 3.0a for Macintosh (GraphPad Software, San Diego CA, www.graphpad.com). Corrections for multiple comparisons across regions were not made because this was an exploratory study (Bland, 2000) to identify the regions that were involved in individual drug effects. Additionally, a two-way ANOVA, comparing Diet (LiCl *vs* control) with Drug (NMDA *vs* saline), was performed for each region using SPSS 10.0 for Macintosh

(SPSS Inc., Chicago, IL, <http://www.spss.com>). At regions in which Diet \times Drug interactions were statistically insignificant, probabilities of Main effects of Diet and Drug were separately calculated. At regions in which interactions were statistically significant, these probabilities were not calculated because they cannot be interpreted with certainty (Tabachnick and Fidell, 2001). Instead, unpaired t -tests were used to test for individual significant differences between means. Data are reported as means \pm SD, with statistical significance taken as $p \leq 0.05$.

RESULTS

Physiological Parameters

At the time of surgery, the LiCl-fed animals weighed significantly less than the control diet group (257 ± 42 *vs* 299 ± 15 g, $n = 36$, $p < 0.0001$), as reported previously (Basselin *et al*, 2003).

In control diet rats, 25 mg/kg NMDA produced repeated cycles of head weaving and body movements lasting 4 ± 1 s (activity period), following by a 'calm period' lasting 9 ± 1 s (Table 1). The net time during which cycling continued was 90 ± 15 s. NMDA at 50 mg/kg also caused cycling, but with a significantly shorter 'calm period' (5 ± 2 s) than the lower dose, and a longer 'net cycling period' (180 ± 10 s). No significant difference in cycling periods was observed in animals fed the LiCl *vs* those fed the control diet. MK-801 provoked head-weaving and body movements from 5 min after injection to the end of the experiment.

NMDA at both doses significantly decreased heart rate by about 20% in both control and LiCl diet groups, but did not significantly change arterial blood pressure, whereas MK-801 increased arterial blood pressure by 30% in the control diet group (Table 1). Such changes, reported previously, have been ascribed to a centrally mediated increase in sympathetic nerve activity by MK-801 (Lewis *et al*, 1989). MK-801 given before NMDA abolished NMDA's significant effect on heart rate.

Regional Brain AA Incorporation Coefficients, k^* , in Control Diet Rats

Plasma input function. As shown by equation (1), values of k^* for individual brain regions were calculated by dividing brain radioactivity by the brain exposure to plasma [$1\text{-}^{14}\text{C}$]AA, the plasma input function equal to mean integrated arterial plasma radioactivity between 0 and 20 min. This function did not differ significantly between any groups. Mean integrated radioactivity, nCi/s/ml, for control diet rats: saline (159003 ± 23885), NMDA 25 mg/kg (162630 ± 23409), NMDA 50 mg/kg (155796 ± 12953), MK-801 0.3 mg/kg (167647 ± 16279), and MK-801 + NMDA 50 mg/kg (179785 ± 10361), and for the LiCl diet rats: saline (177699 ± 14678), NMDA 25 mg/kg (166619 ± 19830), NMDA 50 mg/kg (160236 ± 15150). We have reported that chronic LiCl administration does not alter plasma unesterified fatty acid concentrations, including the AA concentration (Chang *et al*, 1999).

NMDA administration to control diet-fed rats. As illustrated in Figure 1, autoradiographs of coronal brain

Table 1 Physiological Parameters and Behavior Activity Following Drug Administration in Unanesthetized Rats

	Control diet			LiCl diet		
	NMDA 25	NMDA 50	MK-801	NMDA 50+MK-801	NMDA 25	NMDA 50
<i>Heart rate (beats/min)</i>						
Before	447 ± 32	467 ± 26	480 ± 0	473 ± 21	427 ± 20	427 ± 20
After	360 ± 0***	360 ± 0***	480 ± 0	473 ± 21	360 ± 0***	367 ± 20***
<i>Arterial blood pressure (mmHg)</i>						
<i>Before</i>						
Systolic	112 ± 9	106 ± 5	129 ± 10	121 ± 4	108 ± 5	113 ± 7
Diastolic	83 ± 7	83 ± 5	84 ± 10	71 ± 8	83 ± 5	82 ± 7
<i>After</i>						
Systolic	109 ± 10	101 ± 8	167 ± 17***	154 ± 15***	106 ± 8	115 ± 7
Diastolic	80 ± 5	78 ± 5	113 ± 7***	98 ± 5***	79 ± 3	83 ± 7
<i>Behavior: duration (s)</i>						
<i>Cycle</i>						
Activity	4 ± 1	4 ± 1	ND	ND	4 ± 1	4 ± 1
Calm	9 ± 1	5 ± 2***	ND	ND	10 ± 1	6 ± 2***
Net cycling	90 ± 15	180 ± 10***	ND	ND	110 ± 10	190 ± 15***

ND, not determined.

Data are presented as mean ± SD ($n=9$ except for MK-801+NMDA, $n=8$).

*** $p<0.0001$.

sections from control rats showed that NMDA 50 mg/kg compared with saline generally increased k^* for AA whereas MK-801 generally decreased k^* . There was no apparent difference between the autoradiographs from the LiCl-fed and control diet rat at baseline (i.p. saline), whereas NMDA in the LiCl-fed rat did not appear to have an effect on k^* . Net regional data derived from the autoradiographs are presented in Tables 2 and 3 below.

As shown in Table 2, NMDA (25 mg/kg) compared with saline significantly increased k^* for AA in 49/83 regions examined (25 regions at $p<0.05$ and 24 regions at $p<0.001$). k^* was elevated significantly in the prefrontal, frontal, motor, somatosensory, auditory and visual cortical areas (18–38%), olfactory tubercle (22%), hippocampus (20–29%), caudate-putamen (17–20%), lateral geniculate nucleus dorsal (22%), regions of the thalamus (15–20%), and substantia nigra (20%), with mean increments of $21.8 \pm 5.1\%$ in these areas. Also, 67 of 83 regions, including the nucleus accumbens (39%) (10 regions at $p<0.05$ and 57 regions at $p<0.001$), showed significant increments in k^* following 50 mg/kg NMDA compared with saline. Consistent with a dose-response relation, the mean of the significant increments equaled $30.6 \pm 8.0\%$, which was significantly greater ($p<0.0001$) than that following 25 mg/kg NMDA.

MK-801 in control diet rats. Compared with saline, MK-801 given to control diet rats decreased k^* for AA significantly in 21/83 regions (Table 2, Figure 1), including

the frontal cortex (16%), pyriform cortex (20%), preoptic area (21%), suprachiasmatic nucleus (24%), globus pallidus (25%), hippocampus (21%), thalamus (22–26%), and hypothalamus (32–49%). The mean reduction in the significantly affected regions was $29.8 \pm 10.9\%$.

MK-801 followed by NMDA in control diet rats. In control diet rats, MK-801 followed by NMDA 50 mg/kg, when compared with saline decreased k^* significantly in 21/83 regions, of which 17 showed a significant decrease following MK-801 alone (see above).

Insignificant Diet × Drug interactions: As illustrated in Table 3, 18/83 regions had a statistically insignificant Diet (LiCl diet vs control diet) × Drug (NMDA vs saline) interaction with regard to k^* for AA*. In seven of the 18, NMDA compared with saline had a significant positive main Drug effect on k^* , elevating k^* to the same extent in both the control diet and LiCl-fed rats. In two of the 18 regions (preoptic area and thalamus posterior), LiCl compared with control diet had a significant main effect on k^* , elevating k^* to the same extent following saline or NMDA injection. Of the 83 regions, 10 did not have a significant Drug or Diet main effect.

Significant Diet × Drug interactions: Diet × Drug interactions were statistically significant in 65/83 regions, including the prefrontal, frontal, motor, pyriform, anterior cingulate, somatosensory and visual cortex, preoptic area, superchiasmatic nucleus, globus pallidus, hippocampus, caudate-putamen, habenular nucleus, lateral geniculate

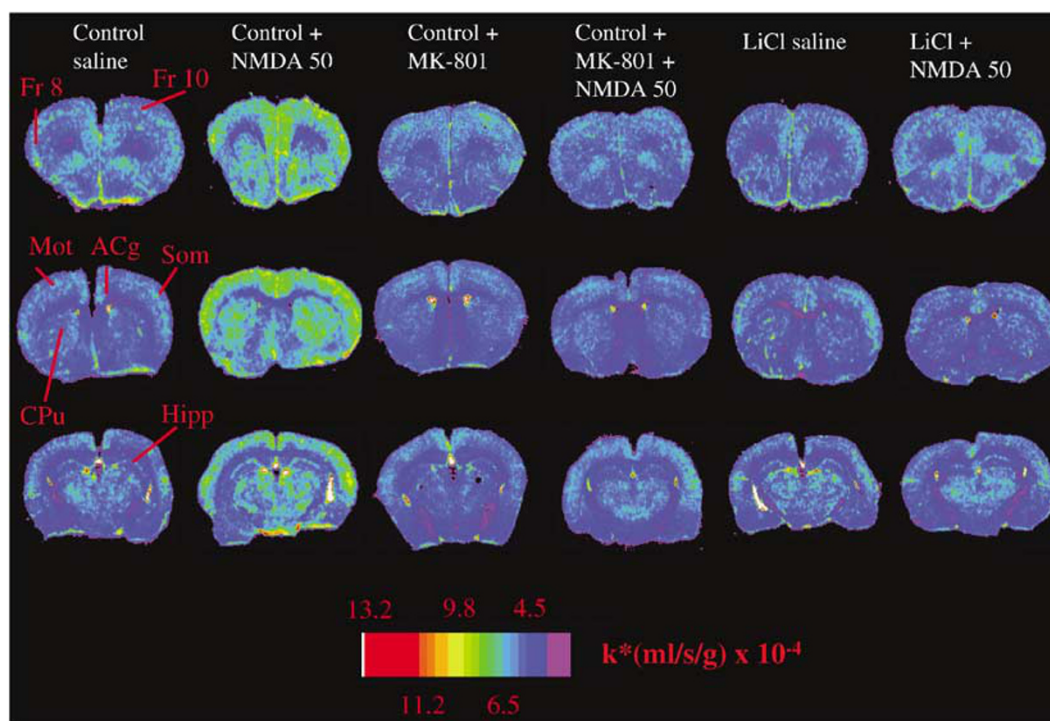


Figure 1 Effect of NMDA and MK-801 on AA incorporation into brain of control and LiCl fed rats. Regional incorporation coefficients k^* are illustrated in autoradiographs from coronal brain sections obtained 20 min after infusing [^{14}C]AA. Fr, frontal cortex; Mot, motor cortex; ACg, anterior cingulate cortex; Som, somatosensory cortex; CPu, caudate putamen; Hipp, hippocampus.

nucleus dorsal, geniculate medial, areas of the thalamus and hypothalamus, substantia nigra, and inferior colliculus (Table 3). In 10 of the 65 regions, unpaired *t*-tests showed that the LiCl diet, compared to the control diet, elevated k^* significantly at baseline (in response to saline). These regions included visual cortex layers I, IV, and VI, lateral habenular nucleus, medial geniculate nucleus, and inferior colliculus, which belong to central auditory and visual pathways (Brodal, 1981). We reported this observation (Basselin *et al*, 2003, 2005b), and suggested that it may underlie lithium's ability in human subjects to increase the amplitudes of P1/N1 components of auditory-evoked responses and of 65-P95 and P95-N125 components of visual-evoked responses (Fenwick and Robertson, 1983; Hegerl *et al*, 1990; Ulrich *et al*, 1990).

In 65/83 regions, NMDA increased k^* significantly in the control diet but not in the LiCl-fed rats. Thus, LiCl blocked 97% of the positive k^* responses to 50 mg/kg NMDA (65/67).

DISCUSSION

This study shows that (1) the *in vivo* fatty acid method (see Introduction) (Rapoport, 2001, 2003) can be used to image PLA₂ activation coupled to NMDARs in unanesthetized rats; (2) regional increments in k^* for AA in response to subconvulsant NMDA doses in control diet rats are blocked by pretreatment with MK-801; (3) MK-801 alone (or when given prior to 50 mg/kg NMDA) produces widespread baseline reductions in k^* for AA in control diet rats; and (4) a 6-week LiCl diet, sufficient to produce plasma and

brain lithium concentrations therapeutically relevant to BD (Bosetti *et al*, 2002b; Chang *et al*, 1996), largely (97%) prevents the significant NMDA-induced increments in k^* seen in control diet rats.

NMDA at 25 and 50 mg/kg significantly increased k^* for AA by an average of 22% and 31%, respectively, in significantly affected brain regions. These regions, reported to have NMDARs (Bernard and Bolam, 1998; Ikeda *et al*, 2003; Jarvis *et al*, 1987; Monaghan and Cotman, 1986; Pal *et al*, 1999; Petralia *et al*, 1994), included cerebral cortex, caudate-putamen, globus pallidus, hippocampus, substantia nigra, and nucleus accumbens. NMDA has also been reported to increase the regional cerebral metabolic rate for glucose, rCMR_{glc}, a marker of axonal activity (Sokoloff, 1999), in the rat suprachiasmatic nucleus (Shibata *et al*, 1992), frontal cortex layers V-VI, globus pallidus, caudate-putamen, substantia nigra, thalamus, nucleus accumbens, and lateral habenular nucleus (Browne *et al*, 1998).

The blocking of increments in k^* for AA following NMDA by MK-801 is consistent with the increments being mediated by NMDARs. Furthermore, the ability of MK-801, given alone or prior to NMDA, to significantly reduce k^* for AA by 16–49%, suggests that NMDA-initiated PLA₂ activation contributes substantially to the baseline release of AA in control brain. This interpretation is consistent with evidence that glutamatergic receptors constitute about 75% of the synapses in the mammalian cortex, and that many of these synapses include NMDARs (Braitenberg and Schüz, 1998; Fonnum, 1984; Raichle and Gusnard, 2002).

The increments in k^* for AA in response to the subconvulsant doses of NMDA were most likely due to enhanced Ca²⁺ entry into the cell via NMDARs, activating

Table 2 Mean Values of Arachidonic Acid Incorporation Coefficients k^{*a} in Control Diet-Fed Rats, at Baseline (in Response to Saline) and in Response to NMDA^b, MK-801^c, and MK-801+NMDA^d

Brain region	Control diet				
	Saline (n = 9)	NMDA 25 (n = 9)	NMDA 50 (n = 9)	MK-801 (n = 9)	MK-801+NMDA 50 (n = 8)
<i>Telencephalon</i>					
Prefrontal cortex layer I	6.15 ± 0.54	7.64 ± 0.79***	7.81 ± 0.83***	5.58 ± 0.36*	5.77 ± 0.58
Prefrontal cortex layer IV	6.41 ± 0.62	8.11 ± 0.56***	8.22 ± 0.94***	6.35 ± 0.44	6.57 ± 0.58
Primary olfactory cortex	5.90 ± 0.82	7.42 ± 0.93***	8.04 ± 0.94***	4.98 ± 0.47	4.72 ± 0.69*
<i>Frontal cortex (10)</i>					
Layer I	6.37 ± 0.61	7.76 ± 0.64***	8.62 ± 1.06***	5.69 ± 0.40	5.75 ± 0.59
Layer IV	6.77 ± 0.68	8.18 ± 0.64***	8.87 ± 0.90***	6.85 ± 0.63	6.99 ± 0.43
<i>Frontal cortex (8)</i>					
Layer I	6.46 ± 0.59	8.04 ± 0.69***	8.43 ± 1.00***	5.42 ± 0.48***	5.39 ± 0.41***
Layer IV	6.89 ± 0.68	8.58 ± 0.79***	8.95 ± 1.13***	6.06 ± 0.51	5.98 ± 0.31
Pyramidal cortex	5.64 ± 0.52	6.29 ± 0.73	6.93 ± 0.82***	4.53 ± 0.61***	4.13 ± 0.77***
Anterior cingulate cortex	7.34 ± 1.10	8.59 ± 0.64*	9.87 ± 1.29***	6.60 ± 0.17	7.01 ± 1.22
<i>Motor cortex</i>					
Layer I	6.43 ± 0.67	7.57 ± 0.54*	8.35 ± 1.35***	5.95 ± 0.08	5.64 ± 0.90
Layer II–III	6.54 ± 0.68	7.78 ± 0.49*	8.34 ± 1.33***	6.03 ± 0.33	6.05 ± 0.86
Layer IV	6.88 ± 0.77	8.40 ± 0.81***	9.22 ± 1.36***	6.60 ± 0.38	6.46 ± 0.92
Layer V	5.31 ± 0.44	6.54 ± 0.37***	7.14 ± 1.00***	4.99 ± 0.33	4.86 ± 0.61
Layer VI	5.25 ± 0.55	6.24 ± 0.36***	6.90 ± 0.93***	4.73 ± 0.32	4.86 ± 0.68
<i>Somatosensory cortex</i>					
Layer I	6.32 ± 0.66	7.83 ± 0.54*	9.15 ± 1.6***	5.61 ± 0.26	5.38 ± 0.92
Layer II–III	6.36 ± 0.75	7.86 ± 0.56***	9.00 ± 1.30***	5.74 ± 0.28	5.81 ± 0.77
Layer IV	6.93 ± 0.59	8.65 ± 0.80*	9.79 ± 1.84***	6.25 ± 0.31	6.30 ± 0.94
Layer V	6.22 ± 0.73	7.72 ± 0.53*	8.99 ± 1.62***	5.70 ± 0.42	5.74 ± 0.80
Layer VI	6.16 ± 0.85	7.32 ± 0.44*	8.29 ± 1.53***	5.60 ± 0.51	5.70 ± 0.90
<i>Auditory cortex</i>					
Layer I	6.37 ± 0.39	7.53 ± 1.08	7.75 ± 2.37	6.29 ± 0.36	5.63 ± 0.23
Layer IV	7.06 ± 0.41	8.45 ± 1.05	8.74 ± 2.31*	6.74 ± 1.14	6.17 ± 0.64
Layer VI	6.02 ± 0.59	7.95 ± 0.89***	8.01 ± 1.55***	6.99 ± 0.71	6.69 ± 0.41
<i>Visual cortex</i>					
Layer I	5.28 ± 0.92	7.29 ± 1.00***	7.50 ± 1.35***	6.12 ± 0.95	5.83 ± 0.94
Layer IV	6.07 ± 0.54	7.74 ± 0.94***	7.97 ± 1.54***	6.51 ± 0.81	6.31 ± 0.96
Layer VI	5.64 ± 0.55	7.09 ± 0.86***	7.36 ± 1.24***	5.93 ± 0.98	5.86 ± 1.16 ^A
<i>Preoptic area (LPO/MPO)</i>					
Suprachiasmatic nu	5.70 ± 0.88	6.56 ± 0.71	6.82 ± 0.69*	4.49 ± 0.66***	4.46 ± 0.88***
Globus pallidus	5.53 ± 0.77	6.50 ± 0.77*	5.55 ± 0.99	4.23 ± 0.61***	3.92 ± 0.65***
Bed nu stria terminalis	5.31 ± 0.80	6.38 ± 0.75*	6.42 ± 0.69***	3.98 ± 0.73***	3.92 ± 0.67***
Olfactory tubercle	5.68 ± 0.82	6.44 ± 0.94	6.98 ± 0.50***	4.16 ± 0.56***	4.12 ± 0.49***
<i>Diagonal band</i>					
Dorsal	5.83 ± 0.57	7.12 ± 0.55***	7.42 ± 0.93***	5.13 ± 0.39	5.04 ± 0.69
Ventral	5.75 ± 0.33	7.04 ± 0.82***	8.04 ± 0.57***	4.94 ± 0.70	5.10 ± 0.76
Amygdala basolat/med	5.61 ± 0.60	6.96 ± 0.84***	8.04 ± 0.87***	4.99 ± 0.67	4.70 ± 0.64
Amygdala basolat/med	5.28 ± 0.95	5.88 ± 0.51	6.42 ± 0.64***	4.23 ± 0.77*	4.18 ± 0.72*

Table 2 Continued

Brain region	Control diet				
	Saline (n = 9)	NMDA 25 (n = 9)	NMDA 50 (n = 9)	MK-801 (n = 9)	MK-801+NMDA 50 (n = 8)
<i>Hippocampus</i>					
CA1	4.70 ± 0.53	5.95 ± 0.70***	6.16 ± 0.44***	4.13 ± 0.43	4.30 ± 0.49
CA2	4.99 ± 0.78	6.03 ± 0.61***	6.58 ± 0.44***	3.93 ± 0.51***	4.13 ± 0.53*
CA3	5.34 ± 0.93	6.23 ± 0.68*	6.66 ± 0.44***	4.20 ± 0.56***	4.48 ± 0.56*
Dentate gyrus	5.74 ± 0.83	6.52 ± 0.64	7.54 ± 1.16***	5.28 ± 0.98	5.69 ± 0.81
SLM	5.48 ± 0.75	7.08 ± 0.84***	8.08 ± 1.19***	5.43 ± 0.46	5.94 ± 0.47
Accumbens nucleus	5.92 ± 0.78	6.19 ± 0.56	8.24 ± 1.57***	5.62 ± 0.66	5.92 ± 0.50
<i>Caudate putamen</i>					
Dorsal	6.17 ± 0.57	7.21 ± 0.65***	8.22 ± 0.86***	5.71 ± 0.47	5.79 ± 0.49
Ventral	6.11 ± 0.64	7.12 ± 0.66*	8.25 ± 1.04***	5.57 ± 0.65	5.87 ± 0.55
Lateral	6.06 ± 0.60	7.28 ± 0.68*	8.32 ± 1.15***	5.72 ± 0.63	6.22 ± 0.76
Medial	6.12 ± 0.69	7.26 ± 0.70*	8.21 ± 0.96***	5.51 ± 0.71	5.78 ± 0.67
Septal nucleus lateral	5.61 ± 0.74	6.16 ± 0.63	7.28 ± 1.00***	4.34 ± 0.99***	4.20 ± 0.82***
Medial	5.93 ± 0.49	6.29 ± 0.71	7.88 ± 0.92***	4.90 ± 0.90*	5.01 ± 0.70*
<i>Diencephalon</i>					
Habenular nu lateral	7.00 ± 0.95	8.33 ± 1.43	9.50 ± 2.63*	6.52 ± 1.00	7.12 ± 0.43
Medial	7.22 ± 0.78	8.01 ± 1.23	9.45 ± 2.65*	6.53 ± 0.99	6.19 ± 0.82
Lat geniculate nu dorsal	6.42 ± 0.46	7.85 ± 0.99***	8.92 ± 1.03***	6.36 ± 0.70	6.17 ± 0.61
Geniculate medial	6.28 ± 0.81	7.74 ± 0.94	7.96 ± 2.08*	6.04 ± 0.52	6.21 ± 0.71
<i>Thalamus</i>					
Ventroposterior lat nu	6.28 ± 0.47	6.79 ± 0.75	7.52 ± 0.79***	5.52 ± 0.93	5.90 ± 0.86
Ventroposterior med nu	6.09 ± 0.44	6.72 ± 0.69	7.54 ± 0.64***	5.60 ± 0.94	5.44 ± 0.55
Paraterial nu	6.52 ± 0.67	7.48 ± 0.71	8.64 ± 1.31***	4.88 ± 0.52***	5.37 ± 0.73*
Anterovertral nu	7.68 ± 1.10	9.25 ± 1.04*	10.74 ± 1.75***	6.18 ± 0.69	7.19 ± 1.00
Anteromedial nu	6.51 ± 0.66	7.80 ± 0.71***	8.66 ± 0.93***	5.06 ± 0.58***	5.67 ± 0.55
Reticular nu	6.55 ± 0.56	7.77 ± 0.81*	8.95 ± 0.91***	5.14 ± 0.61***	6.13 ± 1.17
Paraventricular nu	6.58 ± 0.72	7.65 ± 0.67*	8.64 ± 1.28***	4.84 ± 0.50***	5.35 ± 0.54*
Parafascicular nu	6.06 ± 0.56	6.94 ± 0.63*	7.03 ± 0.64***	6.10 ± 1.03	5.93 ± 0.55
Subthalamic nucleus	6.51 ± 0.61	7.52 ± 1.07*	8.33 ± 0.82***	6.90 ± 0.93	6.70 ± 0.28
<i>Hypothalamus</i>					
Supraoptic nu	5.31 ± 0.60	6.28 ± 0.75*	6.76 ± 0.99***	4.16 ± 1.11	5.28 ± 0.60
Lateral	5.93 ± 0.61	6.59 ± 0.72	6.72 ± 0.84*	4.05 ± 0.58***	4.24 ± 0.21***
Anterior	5.79 ± 0.63	6.56 ± 0.83	6.72 ± 0.82*	3.94 ± 0.64***	4.37 ± 0.17***
Periventricular	6.34 ± 0.39	6.87 ± 0.81	7.84 ± 0.97***	3.21 ± 0.55***	3.58 ± 0.20***
Arcuate	5.79 ± 1.12	5.88 ± 0.75	6.59 ± 0.70	3.36 ± 1.01***	3.10 ± 1.32***
Ventromedial	5.61 ± 0.96	6.01 ± 0.63	6.50 ± 0.55*	3.49 ± 0.93***	3.16 ± 1.16***
Posterior	6.34 ± 0.39	6.87 ± 0.81	7.84 ± 0.97***	5.03 ± 1.28	3.37 ± 1.12***
Mammillary nucleus	6.03 ± 0.78	6.82 ± 0.90	7.10 ± 1.30	5.64 ± 0.92	6.83 ± 0.94
<i>Mesencephalon</i>					
Interpeduncular nucleus	8.18 ± 0.69	9.62 ± 1.32	9.70 ± 2.33	7.89 ± 0.24	7.51 ± 0.61
<i>Substantia nigra</i>					
Pars reticulata	5.57 ± 0.43	6.57 ± 0.46*	6.39 ± 0.60	5.16 ± 1.47	4.66 ± 0.47
Pars compacta	5.57 ± 0.47	6.74 ± 0.56*	6.42 ± 0.57	5.11 ± 1.47	4.53 ± 0.42*
Pretectal area	6.39 ± 0.61	7.56 ± 0.93	6.88 ± 0.57	6.38 ± 0.99	6.36 ± 0.76

Table 2 Continued

Brain region	Control diet				
	Saline (n = 9)	NMDA 25 (n = 9)	NMDA 50 (n = 9)	MK-801 (n = 9)	MK-801+NMDA 50 (n = 8)
Superior colliculus	5.68 ± 0.91	6.95 ± 0.75*	7.24 ± 1.57*	5.42 ± 1.16	5.60 ± 0.50
Deep layers	6.60 ± 0.60	7.97 ± 0.90	7.71 ± 2.16	6.45 ± 1.21	6.02 ± 0.99
Inferior colliculus	7.18 ± 0.64	9.82 ± 0.66*	10.57 ± 1.38***	7.83 ± 0.96	8.28 ± 0.47
<i>Rhombencephalon</i>					
Flocculus	6.97 ± 0.46	7.87 ± 0.96	7.58 ± 1.33	5.81 ± 1.00	6.16 ± 0.61
Cerebellar gray matter	5.96 ± 0.88	6.88 ± 0.66*	6.63 ± 1.11*	5.80 ± 0.86	5.85 ± 0.37
Molecular layer cerebellar gray matter	7.18 ± 0.90	8.77 ± 1.08	8.82 ± 2.22	7.86 ± 1.03	8.42 ± 0.44
<i>White matter</i>					
Corpus callosum	4.78 ± 0.71	5.48 ± 0.60*	5.91 ± 0.40***	4.34 ± 0.77	4.04 ± 0.67
Zone incerta	5.48 ± 0.55	6.04 ± 0.62	6.38 ± 0.38***	5.14 ± 0.90	4.53 ± 0.51
Internal capsule	4.41 ± 1.02	4.99 ± 1.31	5.21 ± 0.47	3.40 ± 0.71	3.47 ± 0.51
Cerebellar white matter	3.29 ± 1.03	3.42 ± 0.58	4.26 ± 0.76	2.87 ± 0.98	3.35 ± 0.71
<i>Nonblood-brain barrier regions</i>					
Subfornical organ	5.31 ± 0.60	5.28 ± 0.75	5.76 ± 0.99	5.13 ± 1.27	4.62 ± 0.87
Median eminence	5.72 ± 0.84	6.58 ± 1.06	6.56 ± 0.55	4.06 ± 1.09	5.22 ± 0.44
Choroid plexus	15.2 ± 1.82	17.2 ± 2.81	16.4 ± 2.73	15.4 ± 2.82	15.7 ± 0.81

nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-moleculare of the hippocampus.

Each value is a mean ± SD.

* $p < 0.05$; *** $p < 0.001$; compared with the saline group (one-way ANOVA Dunnett tests).

$k^* = (\text{ml/s/g}) \times 10^{-4}$.

^bNMDA administration: 25 and 50 mg/kg i.p., 10 min.

^cMK-801 administration: 0.3 mg/kg i.p., 30 min.

^dMK-801+NMDA administration: 0.3 mg/kg i.p., 30 min followed by NMDA 50 mg/kg i.p., 10 min.

cPLA₂ to release AA from membrane phospholipid. NMDA-provoked EPLA₂ activation has been demonstrated in rat hippocampal slices, where it could be blocked by a specific cPLA₂ inhibitor and was accompanied by the formation of lyso-phosphatidylcholine and glycerophosphocholine, consistent with activation of the AA-selective cPLA₂ (Dennis, 1994; Weichel *et al*, 1999). Other studies also reported that Ca²⁺-mediated activation of PLA₂ in neurons released AA following NMDA (Dumuis *et al*, 1988; Lazarewicz *et al*, 1990; Pellerin and Wolfe, 1991; Tapia-Arancibia *et al*, 1992). Although both cPLA₂ and sPLA₂ were activated in some of those studies (Kim *et al*, 1995), it is likely that mainly cPLA₂ was activated in ours, as cPLA₂ is sensitive to the low Ca²⁺ concentrations, 300 nM to 1 μM (Clark *et al*, 1995; Ismailov *et al*, 2004) that occur during normal neuronal activation. sPLA₂ activation requires a much higher Ca²⁺ concentration, 16–18 mM, and is involved mainly in the presynaptic release of synaptic vesicles (Dennis, 1994; Matsuzawa *et al*, 1996; Wei *et al*, 2003). iPLA₂ is Ca²⁺-independent and selective for DHA acid rather than AA (Dennis, 1994; Strokin *et al*, 2004).

The chronic LiCl diet of this study is reported to reduce global brain mRNA, protein and activity levels of cPLA₂ in rats, but not to affect expression of sPLA₂ or iPLA₂ (Bosetti *et al*, 2002a, b; Chang and Jones, 1998; Rapoport and Bosetti,

2002; Rintala *et al*, 1999; Weerasinghe *et al*, 2004). Its cPLA₂ effect may be due a lithium-induced downregulation of Ca²⁺-dependent PKC, to reduce expression of PKC-dependent AP-2, which regulates cPLA₂ transcription (Rao *et al*, 2005; Wang *et al*, 2001). Consistent with selective cPLA₂ targeting, the LiCl diet reduces incorporation and turnover rates of AA but not of DHA in brain phospholipids of unanesthetized rats (Chang *et al*, 1996). Thus, one explanation for its suppression of the NMDA-induced increments in k^* for AA in the present study is that it directly reduces the cPLA₂ activation response to increased intracellular Ca²⁺.

Another possible explanation is that it changed the phosphorylation state of NMDAR subunits, preventing NMDAR-mediated Ca²⁺ entry into cells. For example, exposing cultured neurons to lithium, or injecting lithium into rats for 7 days, has been reported to reduce NMDAR-mediated Ca²⁺ influx by inhibiting NR2B tyrosine phosphorylation and Src tyrosine kinase (Hashimoto *et al*, 2002, 2003; Nonaka and Chuang, 1998), or by reducing NR2A tyrosine phosphorylation and interactions of the NR2A subunit with Src and Fyn mediated by PSD-95 in the rat hippocampus (Ma and Zhang, 2003; Ma *et al*, 2004). If blocking NMDAR-mediated Ca²⁺ entry into neurons were the primary reason for the reduced k^* responses, then lithium's reduction of global cPLA₂ expression in the rat

Table 3 Mean Values of Arachidonic Acid Incorporation Coefficients k^{*a} in Lithium Diet-Fed Rats, at Baseline (in Response to Saline) and in Response to NMDA^b

Brain region	LiCl diet			Diet × drug interaction	LiCl effect	Drug effect
	Saline (n = 9)	NMDA 25 (n = 9)	NMDA 50 (n = 9)			
<i>Telencephalon</i>						
Prefrontal cortex (10)-I	5.70 ± 0.73	5.87 ± 0.88	5.32 ± 0.83	<0.001		
Prefrontal cortex (10)-IV	6.30 ± 0.83	6.20 ± 0.91	6.03 ± 1.03	<0.001		
Primary olfactory cortex	5.57 ± 0.96	5.47 ± 0.79	5.20 ± 0.99	<0.001		
<i>Frontal cortex (10)</i>						
Layer I	5.94 ± 0.80	6.09 ± 0.87	5.58 ± 0.91	<0.001		
Layer IV	6.47 ± 0.81	6.54 ± 0.96	6.06 ± 0.95	<0.001		
<i>Frontal cortex (8)</i>						
Layer I	6.09 ± 0.97	6.11 ± 0.96	5.83 ± 1.20	0.002		
Layer IV	6.88 ± 1.00	6.99 ± 0.88	6.24 ± 0.22	<0.001		
Pyramidal cortex	4.62 ± 0.68	4.67 ± 0.66	4.11 ± 0.93	<0.001		
Anterior cingulate cortex	7.05 ± 1.12	7.50 ± 0.55	6.76 ± 1.64	0.001		
<i>Motor cortex</i>						
Layer I	6.12 ± 0.87	6.41 ± 0.61	5.23 ± 1.09	<0.001		
Layer II–III	6.15 ± 0.88	6.21 ± 0.98	5.56 ± 1.05	0.002		
Layer IV	6.54 ± 0.86	6.75 ± 1.07	5.98 ± 1.13	<0.001		
Layer V	4.92 ± 0.81	5.12 ± 0.71	4.69 ± 0.98	0.001		
Layer VI	4.82 ± 0.67	4.95 ± 0.59	4.33 ± 0.88	<0.001		
<i>Somatosensory cortex</i>						
Layer I	6.09 ± 0.81	6.39 ± 0.77	5.12 ± 0.64	NS	NS	0.005
Layer II–III	6.06 ± 0.97	6.56 ± 0.87	5.24 ± 0.47	NS	NS	0.002
Layer IV	6.83 ± 1.06	6.99 ± 0.82	5.96 ± 1.08	NS	NS	0.015
Layer V	5.86 ± 1.03	6.11 ± 0.99	5.06 ± 0.80	NS	NS	0.010
Layer VI	5.84 ± 1.06	5.95 ± 0.95	5.02 ± 1.31	0.001		
<i>Auditory cortex</i>						
Layer I	6.36 ± 0.84	7.53 ± 0.94	6.95 ± 1.60	NS	NS	0.029
Layer IV	7.20 ± 0.55	8.78 ± 1.10	8.10 ± 1.52	NS	NS	0.003
Layer VI	6.29 ± 0.63	7.27 ± 0.91	7.00 ± 1.17	NS	NS	NS
<i>Visual cortex</i>						
Layer I	6.28 ± 0.43**	6.66 ± 0.83	6.82 ± 0.49	0.009		
Layer IV	7.51 ± 0.81***	6.79 ± 0.85	6.78 ± 0.77	<0.001		
Layer VI	6.95 ± 0.93	6.42 ± 0.87	6.45 ± 0.73	NS	NS	0.008
<i>Preoptic area (LPO/MPO)</i>						
Suprachiasmatic nu	5.16 ± 0.76	5.23 ± 1.04	4.48 ± 1.21	0.016		
Globus pallidus	5.05 ± 0.67	5.41 ± 1.19	4.54 ± 1.01	NS	0.001	0.014
Bed nu stria terminalis	4.68 ± 0.67	4.66 ± 0.75	4.00 ± 0.77	0.003		
Olfactory tubercle	4.86 ± 0.83	4.88 ± 0.89	4.37 ± 1.04	0.010		
<i>Diagonal band</i>						
Dorsal	5.47 ± 0.68	5.66 ± 0.48	5.51 ± 0.72	0.003		
Ventral	5.52 ± 0.89	5.62 ± 0.91	5.10 ± 0.95	NS	NS	0.003
Amygdala basolat/med	5.42 ± 0.79	5.40 ± 0.90	5.35 ± 1.30	0.001		
	4.29 ± 1.00	4.54 ± 0.43	5.02 ± 0.72	NS	NS	0.002

Table 3 Continued

Brain region	LiCl diet			Diet × drug interaction	LiCl effect	Drug effect
	Saline (n = 9)	NMDA 25 (n = 9)	NMDA 50 (n = 9)			
<i>Hippocampus</i>						
CA1	4.22 ± 0.66	4.20 ± 0.35	4.25 ± 0.55	<0.0001		
CA2	4.48 ± 0.61	4.51 ± 0.29	4.59 ± 0.65	0.001		
CA3	4.67 ± 0.63	4.71 ± 0.39	4.80 ± 0.66	0.025		
Dentate gyrus	5.22 ± 0.89	5.14 ± 0.66	5.42 ± 1.02	0.033		
SLM	6.47 ± 0.52	5.69 ± 0.48	5.65 ± 0.37	NS	NS	0.003
Accumbens nucleus	5.33 ± 1.06	5.87 ± 1.03	5.70 ± 1.21	0.006		
<i>Caudate putamen</i>						
Dorsal	5.49 ± 0.95	5.89 ± 0.72	5.11 ± 0.94	NS	NS	0.006
Ventral	5.57 ± 1.00	5.87 ± 0.74	5.28 ± 1.00	<0.0001		
Lateral	5.48 ± 0.88	5.84 ± 0.79	5.15 ± 1.13	<0.0001		
Medial	5.41 ± 0.85	5.80 ± 0.74	5.13 ± 1.15	0.001		
Septal nucleus lateral	4.90 ± 0.57	4.90 ± 0.88	4.05 ± .84	NS	NS	NS
Medial	5.06 ± 1.09	4.81 ± 1.05	4.95 ± 0.87	0.003		
<i>Diencephalon</i>						
Habenular nu lateral	8.12 ± 0.51**	8.22 ± 0.74	8.09 ± 0.54	0.027		
Medial	7.92 ± 0.44	8.30 ± 0.72	7.93 ± 0.60	0.032		
Lat geniculate nu dorsal	6.89 ± 0.47	6.82 ± 0.55	7.01 ± 0.47	NS	NS	NS
Geniculate medial	7.25 ± 0.66*	6.90 ± 0.54	6.97 ± 0.36	0.013		
<i>Thalamus</i>						
Ventroposterior lat nu	5.31 ± 0.86	5.95 ± 0.46	5.98 ± 0.91	NS	NS	NS
Ventroposterior med nu	5.22 ± 0.925	.95 ± 0.46	6.20 ± 0.71	NS	NS	NS
Paratenial nu	6.07 ± 0.80	6.30 ± 0.73	6.36 ± 0.92	0.012		
Anterovertral nu	7.89 ± 1.08	8.66 ± 0.90	7.60 ± 1.15	NS	0.001	0.002
Anteromedial nu	6.04 ± 1.00	6.48 ± 0.80	5.80 ± 1.38	0.002		
Reticular nu	6.28 ± 0.91	6.54 ± 1.10	5.87 ± 1.17	<0.0001		
Paraventricular nu	6.18 ± 0.89	6.76 ± 0.34	6.58 ± 1.03	NS	0.0001	0.002
Parafascicular nu	5.91 ± 0.64	5.38 ± 0.42	5.98 ± 0.51	0.002		
Subthalamic nucleus	5.86 ± 0.80	5.94 ± 0.63	6.36 ± 1.03	NS	NS	NS
<i>Hypothalamus</i>						
Supraoptic nu	5.27 ± 0.83	4.16 ± 0.63	4.29 ± 1.10	<0.0001		
Lateral	4.98 ± 0.94	4.90 ± 0.66	4.41 ± 0.85	0.038		
Anterior	4.95 ± 0.78	5.03 ± 0.47	4.33 ± 0.82	0.011		
Periventricular	4.15 ± 0.65	4.19 ± 0.46	3.85 ± 0.67	NS	NS	NS
Arcuate	5.92 ± 0.76	5.85 ± 1.13	4.79 ± 0.82	0.003		
Ventromedial	5.73 ± 0.64	5.48 ± 1.09	4.35 ± 0.86	<0.0001		
Posterior	4.85 ± 0.34	5.03 ± 0.56	5.61 ± 0.54	NS	NS	0.002
Mammillary nucleus	5.13 ± 0.51	5.74 ± 0.70	6.64 ± 0.88	NS	0.001	<0.0001
<i>Mesencephalon</i>						
Interpeduncular nucleus	9.08 ± 1.12	10.09 ± 1.19	9.98 ± 0.51	NS	NS	0.02
Substantia nigra						
Pars reticulata	5.72 ± 0.51	5.19 ± 0.58	5.61 ± 0.54	<0.0001		
Pars compacta	5.65 ± 0.75	5.39 ± 0.44	5.48 ± 0.60	0.004		
Pretectal area	6.88 ± 0.71	6.06 ± 0.67	6.45 ± 0.70	0.001		

Table 3 Continued

Brain region	LiCl diet					
	Saline (n = 9)	NMDA 25 (n = 9)	NMDA 50 (n = 9)	Diet × drug interaction	LiCl effect	Drug effect
Superior colliculus	7.23 ± 0.52	6.41 ± 0.68	6.40 ± 0.88	0.001		
Deep layers	7.14 ± 1.01	6.83 ± 0.61	6.87 ± 0.34	NS	NS	NS
Inferior colliculus	9.86 ± 1.07	8.65 ± 0.62	8.48 ± 1.25	NS	NS	NS
<i>Rhombencephalon</i>						
Flocculus	8.89 ± 2.08	8.83 ± 1.10	9.59 ± 0.92	NS	NS	NS
Cerebellar gray matter	6.00 ± 0.63	5.91 ± 0.90	6.22 ± 0.42	0.041		
Molecular layer cerebellar gray matter	8.13 ± 1.02	7.94 ± 0.64	8.02 ± 0.95	NS	NS	NS
<i>White matter</i>						
Corpus callosum	3.97 ± 0.74	4.44 ± 0.74	3.94 ± 0.71	0.027		
Zone incerta	4.24 ± 0.25	3.95 ± 0.37	4.70 ± 1.41	NS	NS	0.015
Internal capsule	3.17 ± 0.85	3.42 ± 0.65	3.37 ± 0.82	NS	NS	NS
Cerebellar white matter	3.10 ± 0.42	3.11 ± 0.43	3.98 ± 0.62	0.027		
<i>Nonblood–brain barrier regions</i>						
Subfornical organ	4.80 ± 0.45	4.71 ± 1.06	4.19 ± 0.60	NS	NS	NS
Median eminence	4.95 ± 0.72	4.73 ± 0.34	5.00 ± 0.74	NS	NS	NS
Choroid plexus	22.2 ± 3.25	21.6 ± 2.20	24.0 ± 2.14	NS	NS	NS

nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-molecular of the hippocampus.

Each value is a mean ± SD.

NS: not significant.

In cases of statistically significant Diet × Drug interaction, unpaired t tests were realized. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, LiCl diet-saline vs control diet-saline. No mean k^* in response to 25 or 50 mg/kg NMDA in the LiCl-fed rats differed significantly from k^* in response to saline.

^a $k^* = (\text{ml/s/g}) \times 10^{-4}$.

^bNMDA administration: 25 and 50 mg/kg i.p., 10 min.

brain (see above) could be secondary to a chronic down-regulation of Ca^{2+} entry via defective NMDARs (Du *et al*, 2004; Gray *et al*, 2003; Karkanas and Papke, 1999). In any case, blocking NMDAR signaling via cPLA₂ as contributing to lithium's therapeutic efficacy is consistent with evidence that other anti-BD drugs (Calabrese *et al*, 1995)—lamo-trigine (Farber *et al*, 2002; McIntyre *et al*, 2004), carbamazepine (Hough *et al*, 1996; Kubota *et al*, 1994), and valproic acid (Zeise *et al*, 1991) have direct or indirect NMDAR-blocking properties.

Reduced NMDAR signaling as contributing to lithium's efficacy is consistent with evidence of disturbed NMDAR subunit expression in the BD brain. NR1 and NR2 genes are reported to confer susceptibility to BD (Itokawa *et al*, 2003; Mundo *et al*, 2003), and NMDAR density is decreased in the post-mortem BD hippocampus (Scarr *et al*, 2003). In other studies, NR2D mRNA was higher in the post-mortem BD striatum (Meador-Woodruff *et al*, 2001), NR1 mRNA was decreased in the BD dentate gyrus and CA3 of the hippocampus (Law and Deakin, 2001), and NR3A mRNA was decreased in the BD prefrontal cortex (Mueller and Meador-Woodruff, 2004).

It remains possible that the NMDA response involved neuroreceptors other than NMDA. For example, dopaminergic and glutamatergic/NMDA transmission are closely

linked in the mammalian brain, particularly in the prefrontal cortex, striatum, and nucleus accumbens (Ferretti *et al*, 2005; Ikeda *et al*, 2003; Piomelli and Di Marzo, 1993; Rogue *et al*, 1990; Takeuchi *et al*, 2002; Tseng and O'Donnell, 2004; Wang *et al*, 2003). Medium-sized spiny neurons, the majority of neurons in the neostriatum, receive convergent glutamate-containing afferents from the neocortex and dopamine-containing afferents from the substantia nigra and ventral tegmentum (Murata *et al*, 2002). Dopamine receptor-linked signaling can affect the phosphorylation state and synaptic efficacy of NMDARs coexpressed on striatal neurons (Cepeda and Levine, 1998; Chase, 2004; Nair *et al*, 1998), whereas inhibition of NMDAR expression by antisense oligonucleotides can modulate pre- and postsynaptic expression of D₂-like receptors in these neurons (Murata *et al*, 2002). Furthermore, both dopaminergic and glutamatergic pathways can modulate neuroplasticity involving cAMP, DARPP-32 (dopamine, cAMP-regulated phosphoprotein of 32 kDa), and ERK (extracellular signal-regulated kinase) (Gould and Manji, 2005; Valjent *et al*, 2005). In this regard, chronic lithium increased the level of DARPP-32 in the rat frontal cortex (Guitart and Nestler, 1992), which may explain its ability to activate the ERK-signaling cascade (Einat *et al*, 2003).

In view of the interplay between D₂-like receptors and NMDARs, it is not surprising that chronic LiCl suppresses increments in *k** for AA in unanesthetized rats given quinpirole (Basselin *et al*, 2005a), an agonist of D₂-like receptors coupled to PLA₂ via a G-protein (Bhattacharjee *et al*, 2005; Hayakawa *et al*, 2001; Huo and Healy, 1991; Vial and Piomelli, 1995). This suppression agrees with evidence that drugs that interfere with dopaminergic neurotransmission can be effective in BD (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002; Fisher *et al*, 1991; Peet and Peters, 1995; Post *et al*, 1980; Sultzer and Cummings, 1989). On the other hand, the ability of chronic LiCl to potentiate *k** responses in rats to the muscarinic M_{1,3,5} receptor agonist, arecoline (Basselin *et al*, 2003), is consistent with reports that cholinomimetics can be therapeutic as well (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002). These published data, and the data of this study, suggest that lithium acts in BD by rectifying several neurotransmitter-signaling imbalances through downregulating NMDAR and D₂-like receptor signaling and upregulating M_{1,3,5} receptor signaling involving PLA₂ and the AA cascade (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002; Dumuis *et al*, 1988; Rapoport and Bosetti, 2002; Shimizu and Wolfe, 1990).

Glutamate at excessive concentrations can kill neurons by the process of excitotoxicity (Coyle and Puttfarcken, 1993; Rothman and Olney, 1995), and it has been suggested that glutamatergic excitotoxicity contributes to BD (Zarate *et al*, 2003). Lithium's ability to block NMDA-initiated increments in *k** for AA (this paper) is consistent with evidence that chronic lithium can be neuroprotective in animal models of neurodegeneration and even in BD patients (Moore *et al*, 2000; Rowe and Chuang, 2004; Zarate *et al*, 2003). Excess PLA₂-mediated release of AA, with concurrent formation of excess *lyso*-phospholipids, can engender neurotoxic sequelae (Bazan *et al*, 1995; Farooqui *et al*, 1997; Sapirstein and Bonventre, 2000).

Finally, interference in the normal NMDAR-initiated PLA₂-mediated release of AA by lithium may disturb long-term potentiation in the hippocampus, considered the basis of learning and memory (Dumuis *et al*, 1988; Nishizaki *et al*, 1999; Otmakhov *et al*, 2004; Schmitt *et al*, 2005; Williams *et al*, 1989). This may be relevant to reports that lithium treatment inhibited learning, memory, and speed of information processing in BD patients and to some extent in control subjects (Honig *et al*, 1999; Pachet and Wisniewski, 2003; Stip *et al*, 2000).

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