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Chronic lithium feeding reduces upregulated brain arachidonic acid metabolism in HIV-1 transgenic rat

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Abstract

Background—HIV-1 transgenic (Tg) rats, a model for human HIV-1 associated neurocognitive disorder (HAND), show upregulated markers of brain arachidonic acid (AA) metabolism with neuroinflammation after 7 months of age. Since lithium decreases AA metabolism in a rat lipopolysaccharide model of neuroinflammation, and may be useful in HAND, we hypothesized that lithium would dampen upregulated brain AA metabolism in HIV-1 Tg rats.

Methods—Regional brain AA incorporation coefficients k^* and rates J_{in} , markers of AA signaling and metabolism, were measured in 81 brain regions using quantitative autoradiography, after intravenous [$1-^{14}C$] AA infusion in unanesthetized 10-month-old HIV-1 Tg and age-matched wildtype rats that had been fed a control or LiCl diet for 6 weeks.

Results— k^* and J_{in} for AA were significantly higher in HIV-1 Tg than wildtype rats fed the control diet. Lithium feeding reduced plasma unesterified AA concentration in both groups and J_{in} in wildtype rats, and blocked increments in k^* (19 of 54 regions) and J_{in} (77 of 81 regions) in HIV-1 Tg rats.

Conclusion—These *in vivo* neuroimaging data indicate that lithium treatment dampened upregulated brain AA metabolism in HIV-1 Tg rats. Lithium may improve cognitive dysfunction and be neuroprotective in HIV-1 patients with HAND through a comparable effect.

Keywords

HIV-1; lithium; arachidonic acid; brain imaging; phospholipase A₂; metabolism

Introduction

Human immune deficiency virus-1 (HIV-1)-associated neurocognitive disorders (HAND) have been recently subdivided in three classes depending on the degree of cognitive impairment and associated changes in everyday functioning: asymptomatic neurocognitive impairment, HIV-associated mild neurocognitive disorder, and HIV-associated dementia (HAD) (Antinori et al. 2007). Neurocognitive dysfunction in patients is considered to be initiated and driven by HIV-1 virus invasion and replication within the brain parenchyma, largely through productive infection of perivascular macrophages and endogenous microglia, and it is associated with neuroinflammation, reduced synaptic and dendritic density, and neuronal loss (Gray et al. 1991; Masliah et al. 1997; Yadav and Collman 2009;

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Kaul 2009; Bertin et al. 2012). Despite the great success of highly active anti-retroviral therapy (HAART), drug resistance, toxicity, large HIV-1 genetic variability and inter-subtype circulating recombinant forms, as well as limited penetration of anti-retroviral drugs into the brain, remain concerns for many patients (Schouten et al. 2011; Sigal et al. 2011; Varatharajan and Thomas 2009). Since there is no cure or therapeutic vaccine as yet for HIV-1, identifying new drugs and new drug targets could be helpful.

Lithium is approved by the FDA for the prophylaxis and treatment of bipolar disorder, a disease associated with excitotoxicity, neuroinflammation, apoptosis and synaptic loss in brain (McCarthy et al. 2010; Chiu and Chuang 2010; Kim et al. 2010; Rao et al. 2010). Lithium has been shown to have many neuroprotective activities in experimental animal models. Indeed, lithium pretreatment protected against HIV-glycoprotein (gp)120-mediated neurotoxicity in mice, while post-treatment with lithium had minimal effects (Everall et al. 2002). In a mouse model of HIV-1 encephalitis, lithium post-treatment for 7 days protected against HIV-1 neurotoxicity by diminishing neuronal apoptosis and protecting synaptic density (Dou et al. 2005). Lithium pretreatment for 3 weeks reduced serum and brain interleukin-6 levels and glial fibrillary acidic protein upregulation following lipopolysaccharide (LPS) infusion in mice (Beurel and Jope 2009). Lithium for 6 weeks reduced tau phosphorylation and reversed memory impairments induced by LPS in the triple transgenic (Tg)-Alzheimer disease mouse model (Sy et al. 2011). Additionally, post-insult treatment with lithium ameliorated neurodegeneration and behavioral performance, and suppressed neuroinflammation in a mouse model of traumatic brain injury (Yu et al. 2012).

Similar to the data obtained in rodents *in vivo*, small pilot clinical studies suggest that lithium treatment for 10–12 weeks is beneficial in HIV-1 infected patients with cognitive impairment (Letendre et al. 2006; Schifitto et al. 2009). Randomized trials showed that long-term lithium treatment also improved cognitive and biological outcomes in people with amnesic mild cognitive impairment (Forlenza et al. 2011), and increased brain-derived neurotrophic factor (BDNF) serum levels in early Alzheimer's disease patients (Leyhe et al. 2009). Furthermore, in a large epidemiological study of HIV-1 infected patients, continued lithium treatment was associated with reduction of the rate of dementia to the same level as that for the general population (Kessing et al. 2008). These results suggest that treatment with lithium may prevent or delay the onset and progression of both HAND and Alzheimer's disease. In this regard, a current clinical trial (NCT01348282) is testing whether lithium reduces neurocognitive impairment in HIV-1 patients (<http://clinicaltrials.gov/ct2/show/NCT01348282?term=lithium+hiv&rank=1>).

We recently showed that feeding LiCl to rats for 6 weeks, to produce plasma and brain lithium concentrations therapeutically relevant to bipolar disorder, attenuated the upregulated brain arachidonic acid (AA) metabolic cascade in a rat model of neuroinflammation, produced by a 6-day intracerebroventricular infusion of LPS (Basselin et al. 2010; Basselin et al. 2007). During the neuroinflammatory response, phospholipase A₂ (PLA₂) enzymes were activated, resulting in AA release from neuronal membrane glycerophospholipids and generation of lipid mediators, including prostaglandins (PG) and thromboxanes (TX). Brain AA signaling and metabolism can be measured in unanesthetized rodents by infusing radiolabeled AA intravenously, quantifying integrated plasma radioactivity, and using quantitative autoradiography to determine regional brain radioactivity, which represents tracer AA incorporated from plasma into membrane phospholipid. A mathematical model is applied to calculate AA incorporation coefficients and rates, k^* and J_{in} , respectively (Rapoport 2005; Robinson et al. 1992). Since the brain AA lost by metabolism cannot be synthesized *de novo* from 2-carbon fragments, or elongated significantly (< 1%) from its shorter-chain plasma-derived polyunsaturated precursor, linoleic acid (18:2n-6) (DeMar et al. 2006), k^* and J_{in} represent AA loss from

brain following its release from phospholipid. Using this method, we reported that LPS infusion increases k^* and J_{in} , markers of AA signaling and metabolism, activities of AA-selective Ca^{2+} -dependent cytosolic cPLA₂ and of secretory sPLA₂, and brain concentrations of unesterified AA, and of its PGE₂ and TXB₂ metabolites. Many of these changes were prevented by lithium (Basselin et al. 2010; Basselin et al. 2007). Moreover, in the same rat model, chronic lithium feeding increased brain levels of 17-hydroxydocosahexaenoic acid, which is a precursor of anti-inflammatory resolvins and neuroprotectins (Basselin et al. 2010; Hong et al. 2003).

A non-infectious HIV-1 Tg rat has been developed that demonstrates HIV-associated neuropathology and behavioral abnormalities at 7–9 months of age (Peng et al. 2010; Reid et al. 2001). We reported elevated brain markers of AA metabolism (k^* and J_{in} , PLA₂, PGE₂) and of neuroinflammation in association with lower levels of BDNF and of postsynaptic dendritic-spine drebrin in this aged HIV-1 Tg rat (Basselin et al. 2011; Rao et al. 2011). In view of the above clinical and preclinical studies, we hypothesized that feeding lithium for six weeks to 8-month-old HIV-1 Tg rats would attenuate the upregulation of their brain AA metabolism. To test this, we used our *in vivo* fatty acid technique to quantitatively image AA incorporation into the brain of unanesthetized 10-month-old HIV-1 Tg rats and age-matched wildtype controls, each fed a LiCl or control diet for 6 weeks (Robinson et al. 1992). Incorporation coefficients k^* and rates J_{in} of unesterified circulating AA were determined in 81 brain regions using quantitative autoradiography. Briefly, we found that lithium treatment dampened upregulated brain AA metabolism in HIV-1 Tg rats. An abstract of part of this work has been published (Ramadan et al. 2012).

Materials and methods

Animals

Eight- to nine-month-old male HIV-1 Tg rats ($n = 18$) derived from Fisher 344/NHsd Sprague-Dawley rats, and age-matched parental wildtype inbred Fisher 344/Hsd non-Tg rats ($n = 18$) (Harlan, Indianapolis, IN), were housed under a 12 h light/dark cycle with *ad libitum* access to water, and were fed a Teklad lithium-free global 18% protein diet, 2018S (sterilized) for wildtype and 2918 (irradiated) for HIV-1 Tg rats (Harlan). The 2018 diet contained soybean oil but no fishmeal or alfalfa, and had 5% crude fat by weight. Gas-liquid chromatography showed that fatty acid concentrations in each diet were (as % total fatty acid): 16.7% saturated, 21.8% monounsaturated, 54.8% linoleic, 6.2% α -linolenic, 0.03% AA, 0.02% eicosapentaenoic and 0.06% docosahexaenoic acids (Basselin et al. 2011). For lithium treatment, the 2018 and 2918 diets with LiCl addition were customized (Harlan Teklad). Wildtype and HIV-1 Tg rats ($n = 9$, each group) were fed with 1.70 g LiCl/kg for 4 weeks, followed by chow containing 2.55 g LiCl/kg for 2 weeks. This regimen produces plasma and brain lithium concentrations of about 0.7 mM, therapeutically relevant to bipolar disorder (Bosetti et al. 2002b). NaCl solution (0.45 M) was available *ad libitum* to the four groups of rats to prevent hyponatremia. 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 the Eunice Kennedy Shriver National Institute of Child Health and Human Development. One HIV-1 Tg rat fed lithium died during the surgery, and two others were sacrificed on the day of surgery because they were too sick.

Surgical procedures and tracer infusion

After a rat was anesthetized with 2–3% isoflurane/O₂, catheters were inserted into the right femoral artery and vein (Basselin et al. 2011). The rat was allowed to recover from anesthesia for 3 h in a sound-dampened, temperature-controlled chamber with its

hindquarters loosely wrapped and taped to a woodblock. During recovery, body temperature was maintained at 37°C with a rectal probe and a feedback heating element (TACT-2DF Temperature controller, Physitemp Instruments, Clifton, NJ). Arterial blood pressure and heart rate were recorded (CyQ 103/302; Cybersense, Nicholasville, KY). [1-¹⁴C]AA (170 µCi/kg; 49.2 mCi/mmol, > 99% pure, Moravек Biochemicals, Brea, CA) in 5 mM HEPES buffer (pH 7.4), containing 50 mg/ml fatty acid-free bovine serum albumin, was infused through the femoral vein at a constant rate (5 min, 400 µl/min) using an infusion pump (Harvard Apparatus Model 22, Natick, MA). Fifteen min later, the rat was euthanized with NembutalR (80 mg/kg, i.v.) and decapitated. The brain was rapidly removed, divided in two hemispheres, frozen in 2-methylbutane at -40°C, and stored at -80°C.

Chemical analysis

Thirteen arterial blood samples (150 µl) were collected before, during and after intravenous [1-¹⁴C]AA infusion and were centrifuged (30 s, 18,000 g). Total lipids were extracted from plasma (30 µl) with chloroform:methanol (3 ml, 2:1, v/v) and 0.1 M KCl (1.5 ml) (Folch et al. 1957). Greater than 97% of plasma radioactivity, as determined in the organic phase (100 µl) by liquid scintillation counting, was [1-¹⁴C]AA at the end of the 5-min infusion (DeGeorge et al. 1989). Unlabeled, unesterified fatty acid concentrations were determined in the arterial plasma (100 µl). Total lipids were extracted and separated by thin layer chromatography on 60 silica gel plates, heptane:diethylether:glacial acetic acid (60:40:3, v/v/v). Unesterified fatty acids were scraped from the plates and converted to fatty acid methyl ester derivatives (1% H₂SO₄ in methanol, 3 h, 70°C), which then were analyzed by gas chromatography with flame ionization detection and quantified relative to an internal standard, heptadecanoic acid (17:0).

Quantitative autoradiography

Twenty-µm thick brain sections were cut from the left hemisphere and then placed with [¹⁴C]methylmethacrylate standards (Amersham, Arlington Heights, IL) on Ektascan C/RA film (Eastman Kodak, Rochester, NY) for 5 weeks. Radioactivity (nCi/g wet brain) in 81 anatomically identified regions (Paxinos and Watson 1987) was determined by quantitative densitometry (NIH Image 1.62). Regional AA incorporation coefficients k^* (ml/s/g brain) were calculated as (Robinson et al. 1992),

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

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

Regional incorporation rates of unesterified unlabeled AA from plasma into brain, J_{in} (nmol/s/g), were calculated as,

$$J_{in} = k^* c_{plasma} \quad (\text{Eq. 2})$$

Statistical analyses

A two-way ANOVA, comparing diet (LiCl vs. control) with genotype (HIV-1 Tg vs. wildtype) was performed for physiology parameters, the input function, k^* and J_{in} using GraphPad Prism (GraphPad Software Inc., La Jolla, CA). If genotype x diet interactions were statistically insignificant, probabilities of main effects of genotype and diet were reported. When the interactions were statistically significant, these probabilities were not reported because they cannot be interpreted clearly (Tabachnick and Fidell 2001). A one-way ANOVA with Newman-Keuls post-hoc test with correction for 3 comparisons (wildtype rats under LiCl diet vs. wildtype rats under control diet, HIV-1 Tg rats under control diet vs. wildtype rats under control diet, and HIV-1 Tg rats under LiCl diet vs. HIV-1 Tg rats under control diet) was performed. Data are reported as means $\pm$ SD with statistical significance taken as $p < 0.05$.

Results

Physiology and arterial plasma radioactivity

Body Weight—A two-way ANOVA showed that both genotype and diet had a significant negative main effect without significant interaction on body weight (Table 1). Wildtype rats on the LiCl diet weighed 21% ($p < 0.001$) less than those on the control LiCl-free diet. This is consistent with prior data (Basselin et al. 2010; Basselin et al. 2007), and was ascribed to decreased food consumption by 20%. HIV-1 Tg rats weighed 18% ($p < 0.001$) less than wildtype rats consistent with prior data (Basselin et al. 2011) and consumed less food (−17%).

Heart Rate—A two-way ANOVA showed a significant genotype x diet interaction on heart rate. Subsequent one-way ANOVA with Newman-Keuls post-hoc tests indicated that HIV-1 Tg rats on LiCl diet had a significantly lower heart rate (−14%, $p = 0.013$) compared to rats on control diet (Table 1).

Blood Pressure—A two-way ANOVA showed no significant genotype x diet interaction. However, diet had a significant main effect by decreasing arterial systolic blood pressure by 9% in HIV-1 Tg and wildtype rats (Table 1).

Arterial plasma radioactivity—A two-way ANOVA for mean integrated radioactivity in the plasma organic fraction, the input function for determining k^* (Eq. 1), showed neither a significant genotype x diet interaction nor main effects of LiCl or genotype. Values (nCi.sec/ml plasma) for integrated radioactivity were: wildtype rats under control diet, $200,104 \pm 45,400$ ($n = 9$); wildtype rats under LiCl diet, $179,356 \pm 33,026$ ($n = 9$); HIV-1 Tg rats under control diet, $196,253 \pm 32,927$ ($n = 9$); and HIV-1 Tg rats under LiCl diet, $175,270 \pm 21,482$ ($n = 6$).

Plasma unlabeled unesterified fatty acid concentration

A two-way ANOVA showed a significant genotype effect, which increased the plasma concentration of unesterified AA by 33% (Table 2). LiCl diet had a significant main negative effect by decreasing the plasma concentrations of unesterified AA, palmitic, palmitoleic, oleic, linoleic, α -linolenic, and docosahexaenoic acids.

Regional brain AA incorporation coefficients, k^*

Baseline—Using an unpaired t-test, we compared baseline regional values of k^* for AA between the two genotypes. Baseline k^* was significantly higher in 58 of 81 (72%) brain

regions in HIV-1 Tg compared with wildtype rats, each under control diet (Table 3), consistent with our previous data (Basselin et al. 2011).

Genotype and diet—Mean values of k^* were determined in each of 81 brain regions in HIV-1 Tg and wildtype rats given control and LiCl diets, and compared using a two-way ANOVA. Statistically significant interactions between genotype (HIV-1 Tg vs. wildtype) and diet (LiCl vs. control diet) were found in 27 regions (27/81 = 33%), indicating that lithium feeding altered k^* values differently in HIV-1 Tg and wildtype rats. In each of the 27 regions, a one-way ANOVA with Newman-Keuls post-hoc test showed that HIV-1 Tg rats under control diet showed significant increases (30%–96%) in k^* compared to wildtype under control diet (Table 3, Figure 1). Affected regions were prefrontal cortex layers I (57%) and IV (53%), primary olfactory cortex (36%), frontal cortex (10) layers I (54%) and IV (52%), frontal cortex (8) layers I (57%) and IV (59%), pyriform cortex (43%), suprachiasmatic nucleus (58%), olfactory tubercle (37%), basomedial and basolateral amygdala nuclei (43%), nucleus accumbens (75%), lateral and medialisepal nuclei (40%–43%), lateral and medial habenular nuclei (32%–39%), anteromedial nucleus of the thalamus (53%), supraoptic nucleus (30%), hypothalamus [anterior (42%), periventricular (72%), ventromedial (36%)], interpeduncular nucleus (36%), flocculus (96%), molecular layer of the cerebellar grey matter (36%), corpus callosum (45%), subfornical organ (72%), and median eminence (40%). The same one-way ANOVA showed that LiCl feeding for 6 weeks compared to control diet did not change k^* significantly in wildtype rats (except for the interpeduncular nucleus), whereas it blocked 19 of the 27 (70%) increments of k^* that were observed in HIV-1 Tg rats under the control diet.

In the 54 of the 81 brain regions where genotype and diet interaction was statistically insignificant, 27 of 54 regions had a significant positive genotype effect. In addition, 6 of the 54 regions had a significant positive LiCl diet effect, indicating that increments in k^* following LiCl feeding were equally robust in HIV-1 Tg and wildtype rats. Regions were visual cortex (layers I, IV and VI), gray layer of superior colliculus, pretectal area, and inferior colliculus (Table 3).

In conclusion, HIV-1 Tg rats showed elevated k^* in 54 (27 with interactions + 27 without interactions) of the 81 brain regions analyzed, and lithium feeding for 6 weeks blocked increments in k^* in 19 of the 54 regions.

Regional incorporation rates

Rates of incorporation of unlabeled unesterified AA from plasma into brain, J_{in} , (data not shown) were calculated by Eq. 2 from regional k^* (Table 3) and c_{plasma} for AA (Table 2). Since the mean plasma unesterified AA concentration was 33% higher in HIV-1 Tg than wildtype rats under control diet, J_{in} was significantly higher than k^* to this extent in the 81 brain regions. A two-way ANOVA showed no statistically significant interaction between genotype and diet in any of the 81 brain regions. However, genotype had a significant main effect in each of the 81 brain regions and lithium diet had a significant negative main effect in 77 of 81 regions. As illustrated in three brain regions (Figure 2), J_{in} was increased in HIV-1 Tg rats compared to wildtype rats under control diet and decreased in wildtype rats under LiCl diet. J_{in} values in HIV-1 Tg rats under LiCl diet are similar to those of wildtype rats under control diet. In conclusion, HIV-1 Tg rats showed elevated J_{in} in all 81 brain regions, and lithium feeding for 6 weeks blocked increments in J_{in} in 77 of the 81 regions.

Discussion

The present study shows that a LiCl diet for 6 weeks, sufficient to produce plasma and brain lithium concentrations of about 0.7 mM, therapeutically relevant to bipolar disorder, blocked

the significant increments in k^* (19 of 54 regions) and in J_{in} (77 of 81 regions) observed in 10-month-old HIV-1 Tg rats fed a control diet. To the extent that the upregulated AA signaling in HIV-1 Tg rats has neuropathological consequences, the reported effects of lithium treatment in cognitively dysfunctional HIV-1 patients could be related to downregulation of the brain AA cascade by lithium.

The regional values in k^* for AA in wildtype and HIV-1 Tg rats under control diet are consistent with our published values (Basselin et al. 2011). The elevated values of k^* in the HIV-1 Tg rats corresponded to significantly higher incorporation rates J_{in} , the product of k^* and plasma unesterified AA concentration, which was elevated by 33% as previously reported. Of 15 unesterified fatty acids assayed in plasma, only the AA concentration was elevated significantly in HIV-1 Tg rats under control diet, consistent with our previous study (Basselin et al. 2011). The significant positive effect in wildtype rats of lithium on k^* in visual (visual cortex layers I, IV and VI, the grey layer of the superior colliculus) and auditory (inferior colliculus) systems (Brodal 1981) also agrees with prior observations (Basselin et al. 2005a, 2006; Basselin et al. 2003, 2005b; Basselin et al. 2007). The pretectal area, in which k^* also was elevated by LiCl feeding, participates in visual-oculomotor-auditory circuitry (Clarke et al. 2003). These effects may underlie lithium's ability to potentiate auditory and visual evoked responses in the human brain (Fenwick and Robertson 1983; Hegerl et al. 1990; Jung and Reme 1994; Pfeilschifter et al. 1988; Ulrich et al. 1990).

LiCl feeding significantly decreased plasma concentrations of 7 of 8 measured unlabeled unesterified fatty acids, including AA, indicating a widespread effect on whole body fatty acid metabolism. This reduction may reflect a liver effect of the lithium. In this regard, lithium decreased total lipids, unesterified fatty acids and triglycerides in the rat liver (Fleischman et al. 1974); and circulating unesterified fatty acids are derived by hydrolysis of esterified fatty acids that are secreted by the liver and adipose tissue (Gao et al. 2009; Gibbons et al. 2004; Purdon et al. 1997). These results are not consistent with other studies using F344/Ducl rats under a 5001 diet containing alfalfa and fishmeal (Basselin et al. 2007; Chang et al. 1999). However, in the current study the substrain of rats (F344/NHsd) and the diets (no fish meal, no alfalfa) were different. Unesterified palmitoleic, stearic, AA and docosahexaenoic concentrations in the 2018 diet are lower compared to those in the 5001 diet, while linoleic and α -linolenic acid concentrations are higher (Basselin et al. 2010; Basselin et al. 2011). Consequently, J_{in} was decreased in wildtype and HIV-1 Tg rats under the LiCl diet in 77 of 81 brain regions, indicating that the regional rate of metabolic AA loss from brain is lower in these animals (DeMar et al. 2006). We also cannot rule out a decrease in dietary intake caused by lithium, since we showed that lithium feeding decreased food pellet consumption in both wildtype and HIV-1 Tg rats by 20%. Lithium feeding also causes anorexia (Opitz and Schafer 1976), decreases exploratory activity, rearing, aggression, and induces conditioned taste aversion (O'Donnell and Gould 2007). In this study, lithium lowered arterial systolic blood pressure in both genotypes, which may reflect decreased activity of the angiotensin-converting enzyme that plays a role in the regulating blood pressure as well as cardiovascular function (Das and Bhargava 1985). The reduction of heart rate by lithium in HIV-1 Tg rats may be associated with polyuria resulting in dehydration (O'Kelly et al. 1965).

The decreases of k^* and J_{in} by lithium in many brain regions of HIV-1 Tg rats are consistent with the reported widespread distribution of lithium in rat brain (Bond et al. 1975) (after 6 weeks of feeding LiCl, brain and plasma levels are equivalent in the rat (Bosetti et al. 2002b), and its reported effects in amygdala (Johnson et al. 2009; Foland et al. 2008), nucleus accumbens (Dzirasa et al. 2010), suprachiasmatic nucleus (Iwahana et al. 2004), hypothalamus (Ficek 1982), and prefrontal and frontal cortex (Montezinho et al. 2007; Rao et al. 2005).

Lithium's ability to attenuate increases in k^* and J_{in} for AA could have been due to downregulation of cPLA₂ and cyclooxygenase-2 expression, and brain PGE₂ concentration, which are all increased in the HIV-1 Tg rat brain (Chang and Jones 1998; Bosetti et al. 2002a; Rao et al. 2005; Rintala et al. 1999; Rao et al. 2011), or to attenuation of brain N-methyl-D-aspartate receptor-initiated signaling *via* AA (Basselin et al. 2006; Ma and Zhang 2003) (Fig. 3). Lithium also may have inhibited glycogen synthetase kinase-3 beta (Dou et al. 2005; O'Brien et al. 2011; Maggirwar et al. 1999) or interfered with the cyclin-dependent kinase 5 (Wang et al. 2007; Jorda et al. 2005). Lithium's blocking of intracellular events is consistent with evidence that it has neuroprotective properties (see Introduction). Indeed, chronic LiCl increased brain BDNF (Fukumoto et al. 2001), hippocampal neurotrophin-3 (Walz et al. 2008) and anti-apoptotic factor B-cell lymphoma-2 (Chen et al. 1999), and reversed amphetamine-induced downregulation of BDNF and of neurotrophin-3 in rat brain (Walz et al. 2008; Frey et al. 2006). Given that the HIV-1 Tg rat brain and serum of HIV-1 patients have reduced BDNF (Rao et al. 2011; Avdoshina et al. 2011), these actions may contribute to lithium's neuroprotective effects in HAND, an HIV-1 neurocognitive-deficient state that is progressive and associated with apoptosis (Kaul et al. 2005).

In conclusion, LiCl feeding for 6 weeks dampened upregulation of brain AA metabolic changes that were observed in untreated HIV-1 Tg rats, in which neuroinflammation has been reported. Combined with measurements of brain AA metabolism and neuroinflammation, imaging with labeled AA should help to evaluate effects of anti-inflammatory and other drugs in this transgenic rat model of HIV-1 infection. Furthermore, it now is possible to measure regional brain AA incorporation in humans, using intravenously injected [^{11}C]AA and positron emission tomography, so that lithium's brain effects in HIV-1 patients might be studied directly. A brain imaging study in Alzheimer disease patients with this method demonstrated increased brain AA incorporation indicative of neuroinflammation (Esposito et al. 2008).

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References

- Anthony IC, Bell JE. The Neuropathology of HIV/AIDS. *Int Rev Psychiatry*. 2008; 20 (1):15–24. [PubMed: 18240059]
- Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, Gisslen M, Grant I, Heaton RK, Joseph J, Marder K, Marra CM, McArthur JC, Nunn M, Price RW, Pulliam L, Robertson KR, Sacktor N, Valcour V, Wojna VE. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology*. 2007; 69 (18):1789–1799. [PubMed: 17914061]
- Avdoshina V, Garzino-Demo A, Bachis A, Monaco MC, Maki PM, Tractenberg RE, Liu C, Young MA, Mocchetti I. HIV-1 decreases the levels of neurotrophins in human lymphocytes. *AIDS*. 2011; 25 (8):1126–1128. [PubMed: 21422985]
- Basselin M, Chang L, Bell JM, Rapoport SI. Chronic lithium chloride administration to unanesthetized rats attenuates brain dopamine D₂-like receptor-initiated signaling via arachidonic acid. *Neuropsychopharmacology*. 2005a; 30:1064–1075. [PubMed: 15812572]
- Basselin M, Chang L, Bell JM, Rapoport SI. Chronic lithium chloride administration attenuates brain NMDA receptor-initiated signaling via arachidonic acid in unanesthetized rats. *Neuropsychopharmacology*. 2006; 31 (8):1659–1674. [PubMed: 16292331]
- Basselin M, Chang L, Seemann R, Bell JM, Rapoport SI. Chronic lithium administration potentiates brain arachidonic acid signaling at rest and during cholinergic activation in awake rats. *J Neurochem*. 2003; 85 (6):1553–1562. [PubMed: 12787074]

- Basselin M, Chang L, Seemann R, Bell JM, Rapoport SI. Chronic lithium administration to rats selectively modifies 5-HT_{2A/2C} receptor-mediated brain signaling via arachidonic acid. *Neuropsychopharmacology*. 2005b; 30 (3):461–472. [PubMed: 15562295]
- Basselin M, Kim HW, Chen M, Ma K, Rapoport SI, Murphy RC, Farias SE. Lithium modifies brain arachidonic and docosaehaenoic metabolism in rat lipopolysaccharide model of neuroinflammation. *J Lipid Res*. 2010; 51 (5):1049–1056. [PubMed: 20040630]
- Basselin M, Ramadan E, Igarashi M, Chang L, Chen M, Kraft AD, Harry GH, Rapoport SI. Imaging upregulated brain arachidonic acid metabolism in HIV-1 transgenic rats. *J Cereb Blood Flow Metab*. 2011; 31 (2):486–493. [PubMed: 20664612]
- Basselin M, Villacreses NE, Lee HJ, Bell JM, Rapoport SI. Chronic lithium administration attenuates up-regulated brain arachidonic acid metabolism in a rat model of neuroinflammation. *J Neurochem*. 2007; 102:761–772. [PubMed: 17488274]
- Bertin J, Barat C, Methot S, Tremblay MJ. Interactions between prostaglandins, leukotrienes and HIV-1: Possible implications for the central nervous system. *Retrovirology*. 2012; 9 (1):4. [PubMed: 22236409]
- Beurel E, Jope RS. Lipopolysaccharide-induced interleukin-6 production is controlled by glycogen synthase kinase-3 and STAT3 in the brain. *J Neuroinflammation*. 2009; 6:9. [PubMed: 19284588]
- Bond PA, Brooks BA, Judd A. The distribution of lithium, sodium and magnesium in rat brain and plasma after various periods of administration of lithium in the diet. *Br J Pharmacol*. 1975; 53 (2): 235–239. [PubMed: 1148484]
- Bosetti F, Rintala J, Seemann R, Rosenberger TA, Contreras MA, Rapoport SI, Chang MC. Chronic lithium downregulates cyclooxygenase-2 activity and prostaglandin E₂ concentration in rat brain. *Mol Psychiatry*. 2002a; 7:845–850. [PubMed: 12232777]
- Bosetti F, Seemann R, Bell JM, Zahorchak R, Friedman E, Rapoport SI, Manickam P. Analysis of gene expression with cDNA microarrays in rat brain after 7 and 42 days of oral lithium administration. *Brain Res Bull*. 2002b; 57:205–209. [PubMed: 11849827]
- Brodal A. *Neurological Anatomy in Relation to Clinical Medicine*. 3. Oxford University Press; Oxford: 1981.
- Chang MCJ, Bell JM, Purdon AD, Chikhale EG, Grange E. Dynamics of docosaehaenoic acid metabolism in the central nervous system: lack of effect of chronic lithium treatment. *Neurochem Res*. 1999; 24:399–406. [PubMed: 10215514]
- Chang MCJ, Jones CR. Chronic lithium treatment decreases brain phospholipase A₂ activity. *Neurochem Res*. 1998; 23:887–892. [PubMed: 9572678]
- Chen G, Zeng WZ, Yuan PX, Huang D, Jiang YM, Zhao ZH, Manji HK. The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. *J Neurochem*. 1999; 72:879–882. [PubMed: 9930766]
- Chiu CT, Chuang DM. Molecular actions and therapeutic potential of lithium in preclinical and clinical studies of CNS disorders. *Pharmacol Ther*. 2010; 128 (2):281–304. [PubMed: 20705090]
- Clarke RJ, Zhang H, Gamlin PD. Primate pupillary light reflex: receptive field characteristics of pretectal luminance neurons. *J Neurophysiol*. 2003; 89 (6):3168–3178. [PubMed: 12611972]
- D'Aversa TG, Eugenin EA, Berman JW. NeuroAIDS: contributions of the human immunodeficiency virus-1 proteins Tat and gp120 as well as CD40 to microglial activation. *J Neurosci Res*. 2005; 81 (3):436–446. [PubMed: 15954144]
- Das S, Bhargava HN. Effect of lithium treatment on blood pressure and angiotensin-converting enzyme activity in normotensive Wistar-Kyoto and spontaneously hypertensive rats. *Arch Int Pharmacodyn Ther*. 1985; 276 (1):82–91. [PubMed: 2996454]
- DeGeorge JJ, Noronha JG, Bell JM, Robinson P, Rapoport SI. Intravenous injection of [1-¹⁴C]arachidonate to examine regional brain lipid metabolism in unanesthetized rats. *J Neurosci Res*. 1989; 24:413–423. [PubMed: 2512392]
- DeMar JCJ, Lee HJ, Ma K, Chang L, Bell JM, Rapoport SI, Bazinet RP. Brain elongation of linoleic acid is a negligible source of the arachidonate in brain phospholipids of adult rats. *Biochim Biophys Acta*. 2006; 1761 (9):1050–1059. [PubMed: 16920015]
- Dinarello CA. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol*. 2002; 20 (5 Suppl 27):S1–13. [PubMed: 14989423]

- Dou H, Ellison B, Bradley J, Kasiyanov A, Poluektova LY, Xiong H, Maggirwar S, Dewhurst S, Gelbard HA, Gendelman HE. Neuroprotective mechanisms of lithium in murine human immunodeficiency virus-1 encephalitis. *J Neurosci*. 2005; 25 (37):8375–8385. [PubMed: 16162919]
- Dzirasa K, Coque L, Sidor MM, Kumar S, Dancy EA, Takahashi JS, McClung CA, Nicoletis MA. Lithium ameliorates nucleus accumbens phase-signaling dysfunction in a genetic mouse model of mania. *J Neurosci*. 2010; 30 (48):16314–16323. [PubMed: 21123577]
- Esposito G, Giovacchini G, Liow JS, Bhattacharjee AK, Greenstein D, Schapiro M, Hallett M, Herscovitch P, Eckelman WC, Carson RE, Rapoport SI. Imaging neuroinflammation in Alzheimer's Disease with radiolabeled arachidonic acid and PET. *J Nucl Med*. 2008; 49:1414–1421. [PubMed: 18703605]
- Everall IP, Bell C, Mallory M, Langford D, Adame A, Rockenstein E, Masliah E. Lithium ameliorates HIV-gp120-mediated neurotoxicity. *Mol Cell Neurosci*. 2002; 21 (3):493–501. [PubMed: 12498789]
- Fenwick PB, Robertson R. Changes in the visual evoked potential to pattern reversal with lithium medication. *Electroencephalogr Clin Neurophysiol*. 1983; 55:538–545. [PubMed: 6187547]
- Ficek W. Neurosecretory and microstructural changes in the hypothalamus of rats following administration of lithium chloride and ³H-thymidin. *Z Mikrosk Anat Forsch*. 1982; 96 (4):720–730. [PubMed: 6295017]
- Fleischman AI, Lenz PH, Bierenbaum ML. Effect of lithium upon lipid metabolism in rats. *J Nutr*. 1974; 104 (10):1242–1245. [PubMed: 4413066]
- Foland LC, Altshuler LL, Sugar CA, Lee AD, Leow AD, Townsend J, Narr KL, Asuncion DM, Toga AW, Thompson PM. Increased volume of the amygdala and hippocampus in bipolar patients treated with lithium. *Neuroreport*. 2008; 19 (2):221–224. [PubMed: 18185112]
- Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*. 1957; 226:497–509. [PubMed: 13428781]
- Forlenza OV, Diniz BS, Radanovic M, Santos FS, Talib LL, Gattaz WF. Disease-modifying properties of long-term lithium treatment for amnesic mild cognitive impairment: randomised controlled trial. *Br J Psychiatry*. 2011; 198 (5):351–356. [PubMed: 21525519]
- Frey BN, Andreazza AC, Cereser KM, Martins MR, Valvassori SS, Reus GZ, Quevedo J, Kapczinski F. Effects of mood stabilizers on hippocampus BDNF levels in an animal model of mania. *Life Sci*. 2006; 79 (3):281–286. [PubMed: 16460767]
- Fukumoto T, Morinobu S, Okamoto Y, Kagaya A, Yamawaki S. Chronic lithium treatment increases the expression of brain-derived neurotrophic factor in the rat brain. *Psychopharmacology (Berl)*. 2001; 158 (1):100–106. [PubMed: 11685390]
- Gao F, Kiesewetter D, Chang L, Ma K, Bell JM, Rapoport SI, Igarashi M. Whole-body synthesis-secretion rates of long-chain n-3 PUFAs from circulating unesterified {alpha}-linolenic acid in unanesthetized rats. *J Lipid Res*. 2009; 50 (4):749–758. [PubMed: 19074373]
- Gibbons GF, Wiggins D, Brown AM, Hebbachi AM. Synthesis and function of hepatic very-low-density lipoprotein. *Biochem Soc Trans*. 2004; 32 (Pt 1):59–64. [PubMed: 14748713]
- Gray F, Haug H, Chimelli L, Geny C, Gaston A, Scaravilli F, Budka H. Prominent cortical atrophy with neuronal loss as correlate of human immunodeficiency virus encephalopathy. *Acta Neuropathol*. 1991; 82 (3):229–233. [PubMed: 1927280]
- Hegerl U, Herrmann WM, Ulrich G, Muller-Oerlinghausen B. Effects of lithium on auditory evoked potentials in healthy subjects. *Biol Psychiatry*. 1990; 27:555–560. [PubMed: 2310809]
- Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J Biol Chem*. 2003; 278 (17):14677–14687. [PubMed: 12590139]
- Iwahana E, Akiyama M, Miyakawa K, Uchida A, Kasahara J, Fukunaga K, Hamada T, Shibata S. Effect of lithium on the circadian rhythms of locomotor activity and glycogen synthase kinase-3 protein expression in the mouse suprachiasmatic nuclei. *Eur J Neurosci*. 2004; 19 (8):2281–2287. [PubMed: 15090054]

- Johnson SA, Wang JF, Sun X, McEwen BS, Chattarji S, Young LT. Lithium treatment prevents stress-induced dendritic remodeling in the rodent amygdala. *Neuroscience*. 2009; 163 (1):34–39. [PubMed: 19501634]
- Jorda EG, Verdager E, Canudas AM, Jimenez A, Garcia de Arriba S, Allgaier C, Pallas M, Camins A. Implication of cyclin-dependent kinase 5 in the neuroprotective properties of lithium. *Neuroscience*. 2005; 134 (3):1001–1011. [PubMed: 15979805]
- Jung H, Reme C. Light-evoked arachidonic acid release in the retina: illuminance/duration dependence and the effects of quinacrine, mellitin and lithium. Light-evoked arachidonic acid release. *Graefes Arch Clin Exp Ophthalmol*. 1994; 232 (3):167–175. [PubMed: 8188067]
- Kaul M. HIV-1 associated dementia: update on pathological mechanisms and therapeutic approaches. *Curr Opin Neurol*. 2009; 22 (3):315–320. [PubMed: 19300249]
- Kaul M, Zheng J, Okamoto S, Gendelman HE, Lipton SA. HIV-1 infection and AIDS: consequences for the central nervous system. *Cell Death Differ*. 2005; 12(Suppl 1):878–892. [PubMed: 15832177]
- Kessing LV, Sondergard L, Forman JL, Andersen PK. Lithium treatment and risk of dementia. *Arch Gen Psychiatry*. 2008; 65 (11):1331–1335. [PubMed: 18981345]
- Kim HW, Rapoport SI, Rao JS. Altered expressions of apoptotic factors and synaptic markers in postmortem brain from bipolar disorder patients. *Neurobiol Dis*. 2010; 37 (3):596–603. [PubMed: 19945534]
- Lane TE, Buchmeier MJ, Watry DD, Fox HS. Expression of inflammatory cytokines and inducible nitric oxide synthase in brains of SIV-infected rhesus monkeys: applications to HIV-induced central nervous system disease. *Mol Med*. 1996; 2 (1):27–37. [PubMed: 8900532]
- Letendre SL, Woods SP, Ellis RJ, Atkinson JH, Masliah E, van den Brande G, Durelle J, Grant I, Everall I. Lithium improves HIV-associated neurocognitive impairment. *AIDS*. 2006; 20 (14):1885–1888. [PubMed: 16954730]
- Leyhe T, Eschweiler GW, Stransky E, Gasser T, Annas P, Basun H, Laske C. Increase of BDNF serum concentration in lithium treated patients with early Alzheimer's disease. *J Alzheimers Dis*. 2009; 16 (3):649–656. [PubMed: 19276559]
- Luschen S, Adam D, Ussat S, Kreder D, Schneider-Brachert W, Kronke M, Adam-Klages S. Activation of ERK1/2 and cPLA(2) by the p55 TNF receptor occurs independently of FAN. *Biochem Biophys Res Commun*. 2000; 274 (2):506–512. [PubMed: 10913368]
- Ma J, Zhang GY. Lithium reduced N-methyl-D-aspartate receptor subunit 2A tyrosine phosphorylation and its interactions with Src and Fyn mediated by PSD-95 in rat hippocampus following cerebral ischemia. *Neurosci Lett*. 2003; 348 (3):185–189. [PubMed: 12932824]
- Maggirwar SB, Tong N, Ramirez S, Gelbard HA, Dewhurst S. HIV-1 Tat-mediated activation of glycogen synthase kinase-3 β contributes to Tat-mediated neurotoxicity. *J Neurochem*. 1999; 73 (2):578–586. [PubMed: 10428053]
- Marcoli M, Cervetto C, Paluzzi P, Guarnieri S, Raiteri M, Maura G. Nitric oxide-evoked glutamate release and cGMP production in cerebellar slices: control by presynaptic 5-HT_{1D} receptors. *Neurochem Int*. 2006; 49 (1):12–19. [PubMed: 16469416]
- Masliah E, Heaton RK, Marcotte TD, Ellis RJ, Wiley CA, Mallory M, Achim CL, McCutchan JA, Nelson JA, Atkinson JH, Grant I. Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC Group. The HIV Neurobehavioral Research Center. *Ann Neurol*. 1997; 42 (6):963–972. [PubMed: 9403489]
- McCarthy MJ, Leckband SG, Kelsoe JR. Pharmacogenetics of lithium response in bipolar disorder. *Pharmacogenomics*. 2010; 11 (10):1439–1465. [PubMed: 21047205]
- Montezinho LP, Mork A, Duarte CB, Penschuck S, Geraldles CF, Castro MM. Effects of mood stabilizers on the inhibition of adenylate cyclase via dopamine D(2)-like receptors. *Bipolar Disord*. 2007; 9 (3):290–297. [PubMed: 17430304]
- O'Brien WT, Huang J, Buccafusca R, Garskof J, Valvezan AJ, Berry GT, Klein PS. Glycogen synthase kinase-3 is essential for beta-arrestin-2 complex formation and lithium-sensitive behaviors in mice. *J Clin Invest*. 2011; 121 (9):3756–3762. [PubMed: 21821916]
- O'Donnell KC, Gould TD. The behavioral actions of lithium in rodent models: leads to develop novel therapeutics. *Neurosci Biobehav Rev*. 2007; 31 (6):932–962. [PubMed: 17532044]

- O'Kelly LI, Hatton GI, Tucker L, Westall D. Water regulation in the rat: heart Rate as a function of hydration, anesthesia, and association with reinforcement. *J Comp Physiol Psychol.* 1965; 59:159–165. [PubMed: 14288337]
- Opitz K, Schafer G. The effect of lithium on food intake in rats. *Int Pharmacopsychiatry.* 1976; 11 (4): 197–205. [PubMed: 1010709]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 3. Academic Press; New York: 1987.
- Peng J, Vigorito M, Liu X, Zhou D, Wu X, Chang SL. The HIV-1 transgenic rat as a model for HIV-1 infected individuals on HAART. *J Neuroimmunol.* 2010; 218:94–101. [PubMed: 19913921]
- Pfeilschifter J, Reme C, Dietrich C. Light-induced phosphoinositide degradation and light-induced structural alterations in the rat retina are enhanced after chronic lithium treatment. *Biochem Biophys Res Commun.* 1988; 156:1111–1119. [PubMed: 2847726]
- Purdon D, Arai T, Rapoport S. No evidence for direct incorporation of esterified palmitic acid from plasma into brain lipids of awake adult rat. *J Lipid Res.* 1997; 38 (3):526–530. [PubMed: 9101433]
- Ramadan, E.; Basselin, M.; Rapoport, SI. Chronic lithium administration dampens the upregulated brain arachidonic acid metabolism in HIV-1 Tg rats. Paper presented at the 43rd Ann Meeting Amer Soc Neurochemistry; Baltimore, MD. March 3–7, 2012; 2012. p. Abstract PTW03–05
- Ramadan E, Rosa AO, Chang L, Chen M, Rapoport SI, Basselin M. Extracellular-derived calcium does not initiate in vivo neurotransmission involving docosahexaenoic acid. *J Lipid Res.* 2010; 51 (8):2334–2340. [PubMed: 20388940]
- Rao JS, Harry GJ, Rapoport SI, Kim HW. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. *Mol Psychiatry.* 2010; 15 (4):384–392. [PubMed: 19488045]
- Rao JS, Kim HW, Kellom M, Greenstein D, Chen M, Kraft AD, Harry GJ, Rapoport SI, Basselin M. Increased neuroinflammatory and arachidonic acid cascade markers, and reduced synaptic proteins in brain of HIV-1 transgenic rat. *J Neuroinflammation.* 2011; 8(1):101. Erratum in: *J Neuroinflammation* (2012), 2019, 2019. [PubMed: 21846384]
- Rao JS, Lee HJ, Rapoport SI, Bazinet RP. Mode of action of mood stabilizers: is the arachidonic acid cascade a common target? *Mol Psychiatry.* 2008; 13 (6):585–596. [PubMed: 18347600]
- Rao JS, Rapoport SI, Bosetti F. Decrease in the AP-2 DNA-binding activity and in the protein expression of AP-2 alpha and AP-2 beta in frontal cortex of rats treated with lithium for 6 weeks. *Neuropsychopharmacology.* 2005; 30:2006–2013. [PubMed: 15827566]
- Rapoport SI. In vivo approaches and rationale for quantifying kinetics and imaging brain lipid metabolic pathways. *Prostaglandins Other Lipid Mediat.* 2005; 77 (1–4):185–196. [PubMed: 16099403]
- Reid W, Sadowska M, Denaro F, Rao S, Foulke J Jr, Hayes N, Jones O, Doodnauth D, Davis H, Sill A, O'Driscoll P, Huso D, Fouts T, Lewis G, Hill M, Kamin-Lewis R, Wei C, Ray P, Gallo RC, Reitz M, Bryant J. An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. *Proc Natl Acad Sci U S A.* 2001; 98 (16):9271–9276. [PubMed: 11481487]
- Rintala J, Seemann R, Chandrasekaran K, Rosenberger TA, Chang L, Contreras MA, Rapoport SI, Chang MCJ. 85 kDa cytosolic phospholipase A₂ is a target for chronic lithium in rat brain. *Neuroreport.* 1999; 10:3887–3890. [PubMed: 10716228]
- Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: Review and critical analysis. *Brain Res Brain Res Rev.* 1992; 17:187–214. [PubMed: 1467810]
- Schifitto G, Zhong J, Gill D, Peterson DR, Gaugh MD, Zhu T, Tivarus M, Cruttenden K, Maggirwar SB, Gendelman HE, Dewhurst S, Gelbard HA. Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. *J Neurovirol.* 2009; 15 (2):176–186. [PubMed: 19306230]
- Schouten J, Cinque P, Gisslen M, Reiss P, Portegies P. HIV-1 infection and cognitive impairment in the cART era: a review. *AIDS.* 2011; 25 (5):561–575. [PubMed: 21160410]
- Sigal A, Kim JT, Balazs AB, Dekel E, Mayo A, Milo R, Baltimore D. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. *Nature.* 2011; 477 (7362):95–98. [PubMed: 21849975]

- Six DA, Dennis EA. The expanding superfamily of phospholipase A₂ enzymes: classification and characterization. *Biochim Biophys Acta*. 2000; 1488 (1–2):1–19. [PubMed: 11080672]
- Sy M, Kitazawa M, Medeiros R, Whitman L, Cheng D, Lane TE, Laferla FM. Inflammation induced by infection potentiates tau pathological features in transgenic mice. *Am J Pathol*. 2011; 178 (6): 2811–2822. [PubMed: 21531375]
- Tabachnick, BG.; Fidell, LS. *Computer-Assisted Research Design and Analysis*. Boston: Allyn and Bacon edn; 2001. p. 184–188.
- Ulrich G, Herrmann WM, Hegerl U, Muller-Oerlinghausen B. Effect of lithium on the dynamics of electroencephalographic vigilance in healthy subjects. *J Affect Disord*. 1990; 20 (1):19–25. [PubMed: 2147187]
- Varatharajan L, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary of current knowledge and recommendations for further research. *Antiviral Res*. 2009; 82 (2):A99–109. [PubMed: 19176219]
- Walz JC, Frey BN, Andreazza AC, Cereser KM, Cacilhas AA, Valvassori SS, Quevedo J, Kapczinski F. Effects of lithium and valproate on serum and hippocampal neurotrophin-3 levels in an animal model of mania. *J Psychiatr Res*. 2008; 42 (5):416–421. [PubMed: 17512948]
- Wang Y, White MG, Akay C, Chodroff RA, Robinson J, Lindl KA, Dichter MA, Qian Y, Mao Z, Kolson DL, Jordan-Sciutto KL. Activation of cyclin-dependent kinase 5 by calpains contributes to human immunodeficiency virus-induced neurotoxicity. *J Neurochem*. 2007; 103 (2):439–455. [PubMed: 17897354]
- Yadav A, Collman RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. *J Neuroimmune Pharmacol*. 2009; 4 (4):430–447. [PubMed: 19768553]
- Yu F, Wang Z, Tchantchou F, Chiu CT, Zhang Y, Chuang DM. Lithium ameliorates neurodegeneration, suppresses neuroinflammation, and improves behavioral performance in a mouse model of traumatic brain injury. *J Neurotrauma*. 2012; 29 (2):362–374. [PubMed: 21895523]

Figure 1.

Coronal brain autoradiographs showing effects of LiCl feeding for 6 weeks on regional AA incorporation coefficients k^* in wildtype and HIV-1 Tg rats. Values of k^* (ml/s/g brain) $\times 10^{-4}$ are given on a color scale. ACg, anterior cingulate cortex; CPu, caudate putamen; Ctx, cortex; Hipp, hippocampus; Mot, motor cortex; Som, somatosensory cortex.

Figure 2.

J_{in} for AA (Eq. (2)) in each of three brain regions, in wildtype and HIV-1 Tg rats fed a control or LiCl diet for six weeks. Means $\pm$ SD. Statistics were calculated by a 2-way ANOVA, ($n = 9$, except for HIV-1 Tg rats under LiCl diet $n = 6$).

Figure 3A

Figure 3B

Figure 3.

Proposed sites of action of lithium on brain arachidonic acid (AA) cascade (3a) and the cascade as it is upregulated in brain HIV-1 infection (3b). Figure 3a. As illustrated, AA is released from phospholipid following activation of cPLA₂ by a receptor-mediated mechanism. Unesterified AA is a substrate for COX-2 and other oxidative enzymes, and forms PGE₂ and other bioactive products that have cellular actions. Remaining AA is recycled into phospholipid *via* acyl-CoA synthetase 4 (Acsl4) and an acyltransferase. Based on animal studies and as illustrated, lithium can downregulate the AA cascade by interfering with: NMDA receptor mediated release of Ca²⁺ to activate cPLA₂, transcription of cPLA₂

by the transcription factor activator protein (AP)-2, expression levels of cytosolic cPLA₂ (mRNA, protein and activity), turnover of AA within the deacylation-reacylation cycle, the unesterified brain AA concentration, protein and activity levels of COX-2, and activity of secretory sPLA₂ (not shown). Adapted from Rao et al. (Rao et al. 2008). Figure 3b.

Suggested brain targets related to AA cascade in HIV-1 brain infection. Following entry of circulating macrophages containing the HIV-1 virus into brain *via* blood brain barrier, the macrophages or released virus infect resident microglia (productive infection) or astrocytes (nonproductive infection), *via* CD4, CCR5 and other receptors. Released glycoprotein-120 (gp-120) also can activate microglia. Activated microglia release cytokines that bind to astrocytic IL-1 β and TNF- α receptors that are coupled to activation of cPLA₂ and sPLA₂, to initiate the AA cascade by releasing AA from membrane phospholipid (see 3A). Inducible nitric oxide synthetase (iNOS) in activated microglia releases nitric oxide (NO). NO stimulates release of glutamate by presynaptic glutamatergic nerve terminals, and excess glutamate binds to postsynaptic NMDA receptors (NMDAR) to release extracellular Ca²⁺ into the neuron, which in turn activates Ca²⁺-dependent cPLA₂ to release AA and increase its conversion to PGE₂ within the AA cascade. Excess glutamate also accumulates in the synaptic cleft because of reduced expression of the astrocytic glutamate reuptake receptor (EEAT2). Lithium's actions, identified by interrupted arrows, are suggested to dampen (–) upregulated AA cascade parameters designated by $\uparrow$ in astrocytes and post-synaptic neuronal elements, and to block the NMDAR on post-synaptic membrane (Anthony and Bell 2008; Basselin et al. 2006; Basselin et al. 2010; Basselin et al. 2007; Ramadan et al. 2012; Ramadan et al. 2010; Rao et al. 2011; Luschen et al. 2000; Dinarello 2002; Lane et al. 1996; Marcoli et al. 2006; D'Aversa et al. 2005; Six and Dennis 2000).

Table 1

Physiological parameters in wildtype and HIV-1 Tg rats under control and lithium diets

	Wildtype		HIV-1 Tg		Genotype x Diet Interaction P-value	Genotype Effect P-value	Diet Effect P-value
	Control Diet (n = 9)	Lithium Diet (n = 9)	Control Diet (n = 9)	Lithium Diet (n = 6)			
Body Weight (g)	433 ± 18	342 ± 27	354 ± 34	300 ± 18	0.0518	<0.0001	<0.0001
Food Consumption (g/rat/day)	18 ± 1	14 ± 2	15 ± 1	12 ± 2	0.7054	0.0012	<0.001
Rectal Temperature (°C)	37.0 ± 0.4	36.9 ± 0.5	37.2 ± 0.5	36.8 ± 0.3	0.3477	0.7527	0.1225
Heart Rate (beats/min)	367 ± 22	367 ± 37	402 ± 42	344 ± 31*	0.0228		
Arterial blood pressure (mmHg)							
Systolic	165 ± 9	150 ± 20	175 ± 9	159 ± 12	0.9159	0.0524	0.0026
Diastolic	109 ± 2	110 ± 6	109 ± 5	110 ± 7	0.8859	0.6672	0.3192

Values are presented as mean ± SD.

* P < 0.05 mean different from HIV-1 Tg under control diet.

Table 2
Effects of Lithium on Unesterified Plasma Fatty Acid Concentrations in wildtype and HIV-1 Tg rats

	Wildtype		HIV-1 Tg		Genotype x Diet Interaction P-value	Genotype Effect P-value	Diet Effect P-value
	Control Diet (n = 9)	Lithium Diet (n = 9)	Control Diet (n = 9)	Lithium Diet (n = 6)			
Palmitic (16:0)	211 ± 73	103 ± 58	268 ± 88	128 ± 23	0.511	0.099	<0.0001
Palmitoleic (16:1n-9)	21 ± 11	5 ± 3	25 ± 18	5 ± 1	0.617	0.617	<0.0001
Stearic (18:0)	26 ± 14	30 ± 12	30 ± 17	29 ± 12	0.619	0.765	0.765
Oleic (18:1 n-9)	170 ± 63	102 ± 65	232 ± 87	112 ± 20	0.278	0.136	0.0004
Linoleic (18:2 n-6)	300 ± 103	183 ± 124	371 ± 101	195 ± 40	0.417	0.256	0.0003
α-Linolenic (18:3 n-3)	12 ± 11	11 ± 8	20 ± 9	7 ± 3	0.060	0.519	0.0297
Arachidonic (20:4 n-6)	40 ± 12	27 ± 13	53 ± 16	37 ± 5	0.741	0.016	0.0031
Docosahexaenoic (22:6 n-3)	9 ± 3	5 ± 2	11 ± 4	7 ± 1	1.000	0.057	0.0004

Concentrations are nmol/ml plasma. Values are means ± SD measured from arterial plasma collected after [1-¹⁴C] AA infusion.

Table 3
Arachidonic Acid Incorporation Coefficients, k^* , in HIV-1 Tg and Wildtype rats under Control and LiCl Diets.

Brain Regions	Wildtype		HIV-1 Tg		Genotype x Diets Interaction P-value	Diet Effect P-value
	Control Diet (n = 9)	Lithium Diet (n = 9)	Control Diet (n = 9)	Lithium Diet (n = 6)		
Prefrontal cortex layer I	3.65 ± 0.95	3.92 ± 0.39	5.73 ± 1.53**	4.40 ± 1.00*	0.0402	
Prefrontal cortex layer IV	4.18 ± 0.96	4.46 ± 0.37	6.38 ± 1.57***	5.01 ± 0.81*	0.0328	
Primary olfactory cortex	3.72 ± 0.66	3.93 ± 0.38	5.06 ± 0.92**	4.06 ± 0.97*	0.0301	
Frontal cortex (10)						
Layer I	3.66 ± 0.90	4.12 ± 0.51	5.63 ± 1.43**	4.58 ± 0.74	0.0365	
Layer IV	4.25 ± 0.86	4.75 ± 0.56	6.45 ± 1.55**	5.33 ± 1.20	0.0457	
Frontal cortex (8)						
Layer I	3.80 ± 0.66	4.12 ± 0.54	5.96 ± 1.50***	4.60 ± 0.97*	0.0226	
Layer IV	4.36 ± 0.92	4.74 ± 0.59	6.95 ± 1.65***	5.36 ± 0.91*	0.0169	
Pyriform cortex	3.08 ± 0.64	3.49 ± 0.33	4.41 ± 0.98**	3.56 ± 1.06*	0.0291	
Anterior cingulate cortex	4.81 ± 0.80	5.47 ± 0.70	6.72 ± 1.44	5.94 ± 1.14	0.0622	0.8685
Motor cortex						
Layer I	3.36 ± 0.58	3.78 ± 0.59	5.05 ± 0.95	4.29 ± 1.41	0.0684	0.5851
Layer II–III	3.97 ± 0.64	4.26 ± 0.56	5.44 ± 1.11	4.81 ± 1.26	0.1573	0.5932
Layer IV	4.71 ± 0.75	5.04 ± 0.46	6.57 ± 1.38	5.72 ± 1.37	0.1164	0.4705
Layer V	4.46 ± 0.69	4.75 ± 0.39	5.74 ± 1.38	5.16 ± 1.11	0.2067	0.6728
Layer VI	4.10 ± 0.71	4.28 ± 0.48	5.60 ± 1.30	4.69 ± 1.03	0.1046	0.2689
Somatosensory cortex						
Layer I	3.58 ± 0.67	3.78 ± 0.59	5.07 ± 1.14	4.20 ± 1.41	0.1256	0.3250
Layer II–III	4.17 ± 0.74	4.30 ± 0.53	5.76 ± 1.31	5.00 ± 1.21	0.2094	0.3671
Layer IV	5.11 ± 0.83	5.38 ± 0.57	6.88 ± 1.53	5.71 ± 1.29	0.0740	0.2600
Layer V	4.60 ± 0.81	4.94 ± 0.57	6.13 ± 1.35	5.20 ± 0.90	0.0695	0.3962
Layer VI	4.42 ± 0.68	4.68 ± 0.39	5.89 ± 1.30	5.13 ± 1.02	0.1204	0.4373
Auditory cortex						
Layer I	3.99 ± 0.80	4.50 ± 0.48	4.72 ± 0.87	4.73 ± 0.79	0.3498	0.3404

Brain Regions	Wildtype		HIV-1 Tg		Genotype x Diets Interaction P-value	Genotype Effect P-value	Diet Effect P-value
	Control Diet (n = 9)	Lithium Diet (n = 9)	Control Diet (n = 9)	Lithium Diet (n = 6)			
Layer IV	4.81 ± 1.01	5.77 ± 0.56	5.96 ± 0.96	6.24 ± 1.89	0.3911	0.0488	0.1259
Layer VI	4.46 ± 1.06	5.37 ± 0.64	5.25 ± 0.98	5.44 ± 1.43	0.3355	0.2427	0.1388
Visual cortex							
Layer I	3.84 ± 1.00	4.65 ± 0.71	4.12 ± 0.59	4.91 ± 1.47	0.9714	0.4282	0.0230
Layer IV	4.82 ± 1.17	5.90 ± 0.68	5.21 ± 0.82	6.24 ± 1.53	0.9541	0.3310	0.0081
Layer VI	4.29 ± 1.34	5.22 ± 0.71	4.66 ± 0.82	5.41 ± 1.16	0.8072	0.4480	0.0262
Preoptic area (LPO/MPO)	3.32 ± 0.70	3.78 ± 0.54	4.51 ± 0.99	3.87 ± 0.97	0.0627	0.0318	0.7530
Suprachiasmatic nu	3.51 ± 0.96	3.79 ± 0.47	5.55 ± 0.96***	3.74 ± 0.79***	0.0012		
Globus pallidus	3.82 ± 0.88	3.95 ± 0.38	4.86 ± 1.36	4.16 ± 0.83	0.2177	0.0697	0.3971
Bed nu stria terminalis	3.07 ± 0.74	3.44 ± 0.34	4.21 ± 1.19	4.01 ± 1.29	0.3905	0.0142	0.7951
Olfactory tubercle	3.18 ± 0.53	3.40 ± 0.48	4.36 ± 0.83**	3.36 ± 0.67*	0.0119		
Diagonal band Dorsal	3.61 ± 0.99	3.85 ± 0.48	4.96 ± 1.32	4.28 ± 1.25	0.2246	0.0225	0.5585
Ventral	4.11 ± 0.81	3.98 ± 0.42	5.09 ± 0.99	4.06 ± 1.02	0.1340	0.0830	0.0556
Amygdala basolateral/medial	3.11 ± 0.73	3.80 ± 0.62	4.44 ± 0.95**	3.13 ± 0.76**	0.0010		
CA1	3.12 ± 0.85	3.21 ± 0.54	3.86 ± 1.15	2.85 ± 0.74	0.0813	0.5368	0.1452
CA2	3.24 ± 0.74	3.55 ± 0.67	4.00 ± 1.04	3.46 ± 0.54	0.1432	0.2424	0.6773
CA3	3.37 ± 0.91	3.66 ± 0.59	4.16 ± 1.09	3.34 ± 0.71	0.0796	0.4524	0.3894
Dentate gyrus	3.78 ± 1.06	4.18 ± 0.76	4.59 ± 1.14	4.31 ± 0.91	0.3311	0.1902	0.8701
SLM	4.59± 1.16	5.18 ± 0.70	5.73 ± 1.27	5.51 ± 1.09	0.2947	0.0615	0.6304
Accumbens nucleus	3.48 ± 0.95	3.97 ± 0.51	6.08 ± 1.35***	4.15 ± 0.75***	0.0013		
Caudate putamen							
Dorsal	4.28 ± 0.70	4.73 ± 0.47	5.44 ± 1.09	4.84 ± 0.96	0.0830	0.0390	0.8084
Ventral	4.26 ± 0.71	4.60 ± 0.49	5.61 ± 1.38	4.79 ± 0.81	0.0845	0.0245	0.4736
Lateral	4.35 ± 0.72	4.69 ± 0.54	5.56 ± 1.38	4.93 ± 1.09	0.1709	0.0449	0.6861
Medial	4.09 ± 0.87	4.63 ± 0.60	5.41 ± 1.19	4.88 ± 0.83	0.1029	0.0202	0.9802
Septal nu lateral	3.16 ± 0.59	3.42 ± 0.39	4.43 ± 1.10*	3.51 ± 0.93*	0.0419		
Septal nu medial	3.24 ± 0.46	3.93 ± 0.35	4.76 ± 1.26**	3.52 ± 0.72*	0.0017		
Habenular nu lateral	6.12 ± 1.08	7.44 ± 1.05	8.38 ± 1.72**	7.10 ± 1.19	0.0085		

Brain Regions	Wildtype		HIV-1 Tg		Genotype x Diets Interaction P-value	Genotype Effect P-value	Diet Effect P-value
	Control Diet (n = 9)	Lithium Diet (n = 9)	Control Diet (n = 9)	Lithium Diet (n = 6)			
Habenular nu medial	5.28 ± 1.03	6.51 ± 0.96	7.33 ± 1.30**	6.13 ± 1.13	0.0044		
Lateral geniculate nu dorsal	5.35 ± 1.17	6.27 ± 0.94	6.33 ± 1.16	6.24 ± 1.72	0.2570	0.2860	0.3460
Medial geniculate nu	5.89 ± 1.35	6.95 ± 0.65	6.77 ± 1.39	7.17 ± 2.46	0.5344	0.3015	0.1742
Thalamus							
Ventroposterior lateral nu	5.45 ± 1.58	5.97 ± 0.67	5.86 ± 1.36	6.06 ± 1.18	0.7217	0.5775	0.4233
Ventroposterior medial nu	5.41 ± 1.68	5.92 ± 0.49	6.23 ± 1.38	5.91 ± 1.11	0.3572	0.3781	0.8282
Paratenial nu	4.49 ± 0.76	4.70 ± 0.45	6.07 ± 1.25	5.36 ± 1.06	0.1704	0.0017	0.4512
Anteroventral nu	6.79 ± 1.18	7.23 ± 0.85	8.46 ± 1.57	7.81 ± 1.77	0.2608	0.0244	0.8241
Anteromedial nu	5.04 ± 1.21	5.58 ± 0.62	7.71 ± 1.83***	5.96 ± 0.85*	0.0142		
Reticular nu	4.95 ± 0.89	5.40 ± 0.50	7.23 ± 1.75	6.13 ± 1.22	0.0742	0.0012	0.4466
Paraventricular nu	4.36 ± 0.91	4.55 ± 0.41	6.19 ± 1.01	5.47 ± 1.17	0.1573	0.0002	0.4166
Parafascicular nu	4.50 ± 0.92	5.44 ± 0.69	6.01 ± 1.37	6.00 ± 1.83	0.2727	0.0223	0.2827
Subthalamic nu	5.23 ± 1.18	5.76 ± 0.69	6.36 ± 0.97	5.83 ± 0.89	0.1266	0.0861	0.9906
Hypothalamus							
Supraoptic nu	4.22 ± 0.62	4.26 ± 0.59	5.50 ± 1.03**	4.10 ± 0.61**	0.0107		
Lateral	3.48 ± 0.78	3.93 ± 0.29	4.72 ± 1.26	3.99 ± 0.88	0.0669	0.0444	0.6736
Anterior	3.32 ± 0.75	3.96 ± 0.36	4.72 ± 0.96**	3.74 ± 0.60*	0.0034		
Periventricular	3.40 ± 0.86	3.97 ± 0.53	5.86 ± 1.53***	4.41 ± 0.76*	0.0086		
Arcuate	3.13 ± 0.69	3.80 ± 0.68	4.36 ± 0.97	4.07 ± 0.68	0.0916	0.0104	0.5010
Ventromedial	3.23 ± 0.59	4.13 ± 0.75	4.40 ± 1.10*	3.81 ± 1.01	0.0220		
Posterior	3.73 ± 0.91	3.95 ± 0.71	4.24 ± 1.09	3.51 ± 0.61	0.1330	0.9195	0.4185
Mammillary nu	3.90 ± 1.61	3.34 ± 1.38	4.09 ± 0.64	3.06 ± 0.56	0.5833	0.9117	0.0661
Interpeduncular nu	6.02 ± 1.53	7.82 ± 1.52*	8.16 ± 1.45*	7.68 ± 1.44	0.0389		
Substantia nigra	4.42 ± 1.16	5.03 ± 0.67	4.79 ± 1.36	5.46 ± 1.60	0.9453	0.3588	0.1462
Pretectal area	4.81 ± 0.92	6.30 ± 1.09	6.25 ± 1.16	6.51 ± 1.64	0.1517	0.0574	0.0458
Grey layer Superior colliculus	4.85 ± 0.82	6.49 ± 0.86	6.31 ± 1.07	6.49 ± 1.79	0.0757	0.0765	0.0302
Superior colliculus	5.21 ± 1.27	5.82 ± 1.08	6.05 ± 1.27	5.84 ± 1.46	0.3601	0.3421	0.6570
Inferior colliculus	7.58 ± 2.03	9.74 ± 1.74	8.42 ± 1.48	9.11 ± 2.31	0.2714	0.8668	0.0392

Brain Regions	Wildtype		HIV-1 Tg		Genotype x Diets Interaction P-value	Genotype Effect P-value	Diet Effect P-value
	Control Diet (n = 9)	Lithium Diet (n = 9)	Control Diet (n = 9)	Lithium Diet (n = 6)			
Flocculus	6.42 ± 0.90	7.97 ± 1.64	12.56 ± 3.20***	7.12 ± 2.24***	< 0.0001		
Cerebellar gray matter	5.12 ± 1.36	6.53 ± 0.72	6.83 ± 1.67	7.04 ± 1.86	0.2404	0.0359	0.1191
Molecular layer cerebellar grey	5.89 ± 1.70	7.49 ± 0.78	8.00 ± 1.40*	7.52 ± 1.38	0.0390		
White matter							
Corpus callosum	2.37 ± 0.78	2.97 ± 0.30	3.43 ± 0.97*	2.94 ± 0.86	0.0500		
Internal capsule	2.34 ± 0.83	2.75 ± 0.24	3.35 ± 1.05	2.77 ± 1.03	0.1007	0.0907	0.7733
Cerebellar white matter	2.79 ± 0.84	2.36 ± 0.32	3.02 ± 0.62	2.61 ± 0.62	0.9608	0.2808	0.0673
Non-blood-brain barrier regions							
Subfornical organ	3.33 ± 0.79	3.30 ± 0.42	5.72 ± 0.92***	3.58 ± 0.93***	0.0006		
Median eminence	3.45 ± 0.68	3.67 ± 0.64	4.84 ± 0.89**	3.52 ± 0.67**	0.0060		
Choroid plexus	18.11 ± 3.77	18.04 ± 3.57	20.80 ± 3.20	17.54 ± 4.89	0.2440	0.4210	0.2244

Abbreviations: nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-molecular of the hippocampus. k* = (ml/s/g) x 10⁻⁴. Each value is a mean ± S.D.

* P < 0.05,

** P < 0.01,

P < 0.001; one-way ANOVA Newman-Keuls tests, wildtype rats under LiCl diet vs wildtype rats under control diet, HIV-1 Tg rats under control diet vs. wildtype rats under control diet, and HIV-1 Tg rats under LiCl diet vs HIV-1 Tg rats under control diet.