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Omega-3 fatty acid deficiency impairs frontostriatal recruitment following repeated amphetamine treatment in rats: A_7 Tesla *in vivo* phMRI study

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Abstract

Although attention deficit hyperactivity disorder is associated with deficits in docosahexaenoic acid (DHA), an omega-3 fatty acid implicated in dopamine and glutamate synaptic plasticity, its role in neuroplastic brain changes that occur following repeated amphetamine (AMPH) treatment are not known. This study used pharmacological magnetic resonance imaging to investigate the impact of repeated AMPH exposure and alterations in brain DHA levels on AMPH-induced brain activation patterns. Male rats were fed a diet with no n-3 fatty acids (Deficient, DEF, $n = 20$), a diet fortified with preformed DHA (fish oil, FO, $n = 20$), or a control diet fortified with alpha-linolenic acid ($n = 20$) from P21 to P90. During adolescence (P40–60), one- half of each diet group received daily AMPH injections escalated weekly (0.5, 1.0, 2.5, 5.0 mg/kg/d) or drug vehicle. Following a 30-d abstinence period blood oxygen level dependent (BOLD) responses were determined in a 7 T Bruker Biospec system following an AMPH challenge (7.5 mg/kg, i.v). Postmortem erythrocyte and forebrain DHA composition were determined by gas chromatography. Compared with control rats, forebrain and erythrocyte DHA levels were significantly lower in DEF rats and significantly higher in FO rats. Across AMPH doses DEF rats exhibited greater locomotor activity compared to control and FO rats. In AMPH-naive rats, the AMPH challenge increased BOLD activity in the substantia nigra and basal forebrain and no diet group differences were observed. In AMPH-pretreated control and FO rats, the AMPH challenge similarly increased BOLD activation in the bilateral caudate putamen, thalamus, and motor and cingulate cortices. In contrast, BOLD activation in AMPH-pretreated DEF rats was similar to AMPH-naive DEF animals, and AMPH-pretreated DEF rats exhibited attenuated frontostriatal BOLD activation compared with AMPH-pretreated control and FO rats. These findings demonstrate that chronic

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Ethics approval All experimental procedures were approved on 2012-present by the University of Cincinnati and Children's Hospital Institutional Animal Care and Use Committees, and adhere to the guidelines set by the National Institutes of Health.

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escalating AMPH treatment induces enduring frontostriatal recruitment and that peri-adolescent deficits in brain DHA accrual impair this response.

Keywords

Omega-3 fatty acids; Docosahexaenoic acid (DHA); Sensitization; Magnetic resonance imaging; Amphetamine; Rat

Introduction

Because psychostimulant drugs including amphetamine (AMPH) are an effective treatment for youth with attention deficit hyperactivity disorder (ADHD), understanding their long-term effects on brain structure and function may provide insight into the pathophysiology of ADHD. Prior rodent studies have found that repeated treatment with AMPH leads to enduring synaptic reorganization within pre- frontal and basal forebrain circuits. Specifically, chronic treatment with, or self-administration of, an escalating dose of AMPH increases dendritic spine density in the medial prefrontal cortex, nucleus accum- bens, and hippocampus (CA1), and reduces dendritic spine density in parietal and orbitofrontal cortices.[1–3] Repeated AMPH exposure is also associated with a long-standing modification in mesolimbic dopamine release and behavioral sensitization,[4,5] and regional alterations in glutamate neurotransmission[6–8] and neuronal excitability.[9,10] Despite this body of evidence for regional neuroadaptations following repeated AMPH exposure, their effects on global and regional brain activation patterns remain poorly understood.

Meta-analyses indicate that adults and children with ADHD exhibit significant blood omega-3 polyunsaturated fatty acid (*n*-3 PUFA) deficits,[11] and that adjunctive n-3 PUFA supplementation may have therapeutic benefits in ADHD patients.[12] Docosahexaenoic acid (DHA) is the most abundant *n*-3 PUFA in the mammalian brain, and animal studies indicate that DHA has neurotrophic, anti-inflammatory, and neuroprotective properties.[13] In rodents, deficits in brain DHA accrual during development induce enduring changes in mesolimbic and mesocortical dopamine systems,[14–17] and abnormalities in dopamine-mediated behavior.[18,19] Brain DHA deficits are also associated with impaired glutamate synaptogenesis, synaptic plasticity, and homeostasis,[20–22] as well as deficits in regional functional connectivity.[23] In contrast, dietary DHA supplementation increases dendritic spine density[24] and frontal cortex dopamine levels.[25] While these findings suggest that DHA modulates dopamine and glutamate circuit plasticity, its role in the progressive neuroplastic brain changes that occur in response to repeated AMPH treatment are not known.

The present study investigated the impact of repeated AMPH exposure on AMPH-induced regional brain activation patterns *in vivo* using pharmacological magnetic resonance imaging (phMRI). MRI measures regional brain changes in blood oxygen level dependent (BOLD) signal which are attributable to a rise in oxygen-dependent synaptic activity and associated increases in local blood volume.[26] Prior rat phMRI studies have demonstrated that an acute AMPH challenge is associated with increased BOLD signal in dopamine terminal regions, and that this response is correlated with increases in extracellular dopamine levels and mediated by dopamine receptors.[27–29] To investigate the role of brain DHA levels on

AMPH-induced brain activation patterns, a peri-adolescent feeding model was used to generate three groups of rats with graded brain DHA levels.[21] Based on the evidence reviewed above it was hypothesized that repeated prior AMPH exposure would be associated with enduring changes in regional BOLD activation, and that these changes will be augmented in rats with high DHA levels and blunted in rats with low DHA levels.

Materials and methods

Animals and diets

Post-weaning (P20) male Long-Evans hooded rats were purchased (Harlan Farms, Indianapolis, IN, USA) and randomized (n = 20/diet group) to one of three diets (Harlan-TEKLAD, Madison, WI, USA) upon arrival (P21) until the end of the experiment (P90). Control rats were maintained on an a-linolenic acid (ALA, 18:3n-3)-fortified diet (TD. 04285), and deficient (DEF) rats were maintained on an ALA- free diet (TD.04286). n-3 PUFA-enriched rats were maintained on diet containing 1.1% fish oil in place of ALA (FO, TD.110837). Diets were closely matched for all non-fat nutrients and fatty acid composition with the exception of ALA, which was absent from the DEF and FO diets, and preformed DHA and eicosapentaenoic acid (EPA, 20:5n-3) which were present in the FO diet but not the control or DEF diets (Supplemental Table 1). Rats were pair-housed under standard vivarium conditions on a 12:12 hour light:dark cycle with free access to food and water. All experimental procedures were approved on March 12, 2012-present by the University of Cincinnati and Children's Hospital Institutional Animal Care and Use Committees, and adhere to the guidelines set by the National Institutes of Health.

AMPH pretreatment

From P40 to P60 rats received daily subcutaneous injections with D-AMPH sulfate (Sigma-Aldrich Co. LLC, St. Louis, MO, USA)(n = 10/diet group) or an equivalent volume of drug vehicle (0.9% saline, ml/kg, n = 10/diet group). Each dose (0.5, 1.0, 2.5, 5.0 mg/ml/kg) was administered for 5 consecutive days, followed by 2 drug-free days, and the AMPH dose was escalated weekly over 4 consecutive weeks. An escalating AMPH dosing regimen was selected based on prior evidence that it produces robust behavioral sensitization as well as frontostriatal synaptic reorganization.[1–3] Injections were administered in automated activity chambers (46 × 24 × 20 cm) equipped with an external 8 × 4 photo beam array positioned 6 cm above the chamber floor (San Diego Instruments, San Diego, CA, USA). Horizontal locomotor activity (indexed by number of beam breaks) was collected for 1 hour post-injection on the first and fifth injection day of each dose week. Activity chambers were located in a separate room and rats were returned to their home cage following each session. Following the completion of this treatment protocol, rats resided in their home cage for a 30 d abstinence period prior to scanning on P90.

phMRI

Image acquisition—On P90, rats from each treatment group were anesthetized with 1.5–2.5% isoflurane in air, positioned supine with their teeth in a bite bar, and the head centered inside a 38 mm Litz coil (Doty Scientific, Inc., Columbia, SC, USA). Respiration was monitored and body temperature was maintained at 36–38°C using an animal monitoring

system (SAI Inc., Stony Brook, NY, USA). The coil and animal were positioned in a 7 T Bruker Biospec system (Bruker BioSpin, Ettlingen, Germany), and a set of localizers from each orthogonal plane were collected. Following localizer acquisition, RARE images (Axial images: effective TE 45 ms, TR 3500 ms, RARE factor 16, matrix 256 × 256, FOV 36 mm, 11 slices in the axial direction; and sagittal images: effective TE 45 ms, TR 2000ms, RARE factor 16, matrix 256 × 192, FOV 51.2 × 38.4 mm 11 slices in the sagittal direction) were collected. A 3D data set was also acquired for registration of the MRI data (Sagittal orientation, effective TE 60.8 ms, TR 1000 ms, RARE factor 16, matrix 256 × 160 × 180, FOV 51.2 × 32 × 36 mm^3). MRI data were then acquired using a RARE acquisition (repetition time 2000 ms, effective echo time 20 ms, RARE factor 8, 80 repetitions, 38.4 mm x 38.4 mm field-of-view, matrix 128 × 128, slice thickness 1mm, 11 slices). The AMPH challenge (7.5 mg/kg) was administered via an indwelling intravenous catheter following the 10th repetition and scanning continued for the remaining repetitions for approximately 30 minutes post-injection. Pilot studies found that this challenge dose produced consistent forebrain activation under the current experimental parameters.

Image processing—MRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). To ensure compatibility with the software, image data were cropped to contain primarily brain and the resolution was scaled by a factor of 10. Registration to a standard space based on the Paxinos and Watson atlas[30] was carried out using FLIRT.[31,32] The following pre-statistics processing were applied; motion correction using MCFLIRT,[32] spatial smoothing using a Gaussian kernel of FWHM 5 mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma =1120.0s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction.[33] Z (Gaussianised T/F) statistic images were thre- sholded using clusters determined by Z > 2.3 and a (corrected) cluster significance threshold of $P \leq 0.05$.[34]

Tissue collection

Isoflurane-anesthetized rats were sacrificed by decapitation immediately following scanning. Whole venous trunk blood was collected into EDTA-coated tubes, and centrifuged for 20 minutes (1500×g). Plasma and buffy coat were then removed and erythrocytes washed three times with 0.9% NaCl and stored at −80°C. The brain was dissected on ice to isolate the forebrain which was stored at −80°C.

Gas chromatography

Erythrocyte and forebrain total fatty acid composition were determined by gas chromatography with a Shimadzu GC-2014 Shimadzu Scientific Instruments Inc., Columbia, MD, USA), as described in detail previously.[35] The column was a DB-23 (123–2332): 30 m (length), I.D. 0.32 mm wide bore, film thickness of 0.25 μM (J&W Scientific, Folsom, CA, USA). Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap, PA, USA). Analysis of fatty acid methyl esters is based on areas calculated with EZstart 7.4 software. Fatty acid composition is expressed as weight percent of total fatty acids (mg fatty acid/100

mg fatty acids). All samples were processed by a technician blinded to treatment. The primary measures of interest were DHA, arachi- donic acid (AA, 20:4*n*-6), and the AA/DHA ratio.

Statistical analyses

Group differences in fatty acid measures were determined with a two-way ANOVA, with Diet (control, FO, DEF) and Treatment (SAL, AMPH) as the independent variables. *Post hoc* comparisons were made with Fisher's LSD tests. Locomotor activity data (total number of beam breaks 30 minutes post-injection) were analyzed with a four-way ANOVA with repeated measures, with Diet (control, FO, DEF) and Treatment (SAL, AMPH) as the between subjects variables, and with Dose (0.5, 1.0, 2.5, 5.0 mg/kg) and injection Day (1st, 5th) as the repeated measures. Additionally, three-way ANOVAs (Diet × Drug × Day) with Day as a repeated measure were performed for each dose. Post hoc comparisons were made using Fisher's LSD tests. Statistical analyses were completed using the Statistical Package for the Social Sciences (SPSS, IBM Corp., IL, USA).

Results

Fatty acid composition

Significant main effects of Diet were observed for erythrocyte DHA, $F(2,57) = 284.7$, $P \leq 0.0001$, AA, $F(2,57) = 394.9$, $P \leq 0.0001$, and the AA/DHA ratio, $F(2,57) = 455.2$, $P \leq 0.0001$, and the main effects of Treatment and the Diet × Treatment interaction were not significant. (Fig. 1A-C). Significant main effects of Diet were observed for forebrain DHA, $F(2,57) = 556.3$, $P \leq 0.0001$, AA, $F(2,57) = 9.7$, $P \leq 0.0001$, and the AA/DHA ratio, $F(2,57) = 3.1$, $P \leq 0.0001$. The main effect of Treatment and the Diet × Treatment interaction were not significant (Fig. 1D- F). After collapsing across treatment groups, forebrain DHA composition was significantly lower in DEF rats (−35%, $P \leq 0.0001$) and significantly higher in FO rats (+6%, $P \leq 0.0001$) compared with control rats (Fig. 1D). Compared with control rats, AA composition was significantly higher in DEF rats (+4%, $P = 0.002$) and significantly lower in FO rats (−5%, $P \leq 0.0001$) (Fig. 1E). Compared with control rats, the AA/DHA ratio composition was significantly higher in DEF rats (+37%, $P \leq 0.0001$) and significantly lower in FO rats (−10%, $P \leq 0.0001$) (Fig. 1F). Across all treatment groups ($n = 60$), forebrain and erythrocyte DHA ($r = +0.95$, $P \leq 0.0001$), AA ($r = +0.66$, $P \leq 0.0001$), and AA/DHA ratio ($r = +0.89$, $P \leq 0.0001$) were positively correlated.

Locomotor activity

There was a significant overall main effect of drug, $F(1,41) = 432.3$, $P < 0.0001$, with AMPH-treated rats exhibiting greater locomotor activity across all doses compared with saline-treated rats (Fig. 2A). The overall main effect of diet was also significant, $F(2,41) = 5.6$, $P = 0.007$, with DEF rats being significantly more active than control ($P = 0.029$) and FO ($P = 0.002$) rats across doses. Locomotor responses on the first day of each dose are illustrated in Fig. 2B. At the 0.5 mg/kg dose, the main effect of diet was not significant, $F(2,42) = 1.0$, $P = 0.36$, and the main effect of day, $F(1,42) = 5.9$, $P = 0.019$, and the Day × Drug Interaction, $F(1,42) = 11.1$, $P = 0.002$, were significant. Locomotor activity decreased from day 1 to day 5 in rats receiving saline, whereas locomotor activity increased from day 1

to day 5 in rats receiving AMPH. At 0.5 mg/kg AMPH-treated DEF rats were more active relative to AMPH-treated control rats ($P = 0.046$). The main effect of diet was significant for 1.0 mg/kg, $F(2,41) = 5.481$, $P = 0.008$, with DEF rats exhibiting greater locomotor activity compared with control ($P = 0.007$) or FO ($P = P = 0.001$) rats. The main effect of diet was not significant at 2.5 mg/kg, $F(2,42) = 1.5$, $P = 0.2$, or 5.0 mg/kg, $F = 2.9$, $P = 0.07$, nor were the main effects of day or interaction terms.

phMRI

No significant negative BOLD responses were observed in any analysis. In SAL-pretreated (AMPH- naive) control rats, the AMPH challenge induced bilateral increases in BOLD activity in the substantia nigra and basal forebrain dopamine structures including the islands of Calleja, ventral pallidum, anterior amygdala, lateral hypothalamus, as well as the subiculum, and a similar response pattern was observed in SAL- pretreated FO and DEF rats (Fig. 3A). There were no significant diet group differences among SAL-pretreated rats ($P \leq 0.05$ corrected). In AMPH-pretreated control rats, the AMPH challenge induced widespread bilateral BOLD activation in multiple regions, including substantia nigra, ventral tegmentum, caudate putamen, amygdala, hippocampus, thalamus, and cin- gulate and motor cortices (Fig. 3B). A similar, albeitless robust, pattern was observed in AMPH-pretreated FO rats, whereas AMPH-pretreated DEF rats did not exhibit widespread bilateral BOLD activation (Fig. 3B). Compared with SAL-pretreated control rats, AMPH-pretreated control rats revealed significantly greater BOLD activation in multiple cortical and subcortical regions, including the bilateral caudate putamen, thalamus, frontoparietal and motor cortices, and a similar pattern was observed between SAL- and AMPH-pretreated FO rats (Fig. 3C). Contrasts of SAL- and AMPH-pretreated DEF rats did not reveal any significant differences in BOLD activation. Compared with AMPH-pretreated control and FO rats, AMPH-pretreated DEF rats exhibited significantly lower BOLD activity in bilateral caudate putamen, thalamus, frontoparietal and motor cortices, and there were no differences between AMPH-pre- treated control and FO rats (Fig. 4).

Discussion

This phMRI study investigated the hypothesis that repeated prior AMPH exposure would be associated with enduring changes in regional BOLD activation, and that these changes would be augmented in rats with high DHA levels and blunted in rats with low DHA levels. Compared with control rats, forebrain and erythrocyte DHA levels were significantly lower in DEF rats and significantly higher in FO rats. Across AMPH pretreatment doses DEF rats exhibited greater locomotor activity compared with control and FO rats. In AMPH-naive rats, the AMPH challenge increased activation (BOLD signal) primarily in basal forebrain dopamine terminal regions, including the islands of Calleja, ventral pallidum, and lateral hypothalamus, and no diet group differences were observed. In support of our hypothesis, prior chronic escalating AMPH treatment was associated with an enduring (30 day) increase in AMPH-induced activation in multiple regions beyond the basal forebrain, including the bilateral caudate putamen, thalamus, and cingulate and motor cortices (hereafter referred to as ‘frontostriatal’ recruitment), and this response was blunted in DEF rats. In contrast to our hypothesis, frontostriatal recruitment was not augmented in FO rats, though the increase in

forebrain DHA levels observed in FO rats relative to control rats (+6%) may not have been sufficiently robust to further increase activation. Together, these results demonstrate that chronic escalating AMPH treatment is associated with enduring frontostriatal recruitment and that this response is markedly blunted in DHA- deficient rats.

In response to the AMPH challenge AMPH-naive control rats exhibited increased activation in the substantia nigra and basal forebrain dopamine terminal regions including the islands of Calleja, ventral pallidum, and lateral hypothalamus. It is notable that this relatively discrete activation pattern differs from the widespread activation observed in response to acute AMPH challenge in prior phMRI studies.[27,28] The reason for this discrepancy may be due in part to methodological differences including anesthetic (isoflurane vs. halothane), magnet strength (7 T vs. 2.3–4.7 T), AMPH challenge dose (7.5 mg/kg vs. 3.0 mg/kg), and rat strain (LEH vs. Sprague-Dawley). However, the BOLD pattern observed in AMPH-naive control rats is very similar to the pattern observed following a methylphenidate challenge in a recent phMRI study that also used isoflurane anesthetic.[36] In the present study, we additionally demonstrate that this activation pattern was not significantly altered by either increases or decreases in brain DHA levels. Prior *in vivo* microdialysis studies indicate that larger brain DHA deficits (−70%) resulting from *perinatal n*-3 fatty acid deficiency produce long-standing deficits in AMPH- or tyramine-stimulated increases in extracellular dopamine levels in the basal forebrain and pre- frontal cortex.[16,17] Although the effects of moderate reductions in brain DHA levels observed in the present study on dopamine release dynamics are not known, the present phMRI evidence would suggest that they are not robustly impacted by peri-adolescent *n*-3 fatty acid deficiency.

A central finding of the present study is that prior exposure to chronic escalating AMPH dosing is associated with an enduring expansion of activation in multiple regions outside of the basal forebrain, including the bilateral caudate putamen, thalamus, and cingulate and motor cortices. While the neuroplastic mechanisms mediating this ‘recruitment’ of frontostriatal structures following prior AMPH exposure are poorly understood, chronic escalating AMPH dosing was previously found to induce enduring synaptic reorganization in frontostriatal regions which may mediate increased regional functional connectivity.[2,3] However, other mechanisms including the sensitization of mesocortical and mesostriatal dopamine neurotransmission also likely play a role and additional studies are warranted to delineate mechanisms. It is notable that a similar pattern of frontostriatal recruitment was observed in a recently published study that used an AMPH dosing regimen found to have dopamine terminal neurotoxic effects.[36] Moreover, frontostriatal recruitment was not observed in rats challenged with AMPH following 3-week treatment with methylphenidate, which does not have dopamine terminal neurotoxic effects.[37] Although we did not evaluate indices of dopamine neurotoxicity, the AMPH dosing regimen used in the present study has previously been shown to not be associated with dopamine terminal degeneration.[38] Nevertheless, additional studies will be required to better understand the mechanisms mediating neuroplastic changes within frontostriatal circuitry following repeated AMPH exposure.

A second important finding is that the frontostriatal recruitment observed in control and FO rats following chronic AMPH exposure was not observed in DEF rats. Indeed, the regional

activation pattern following the AMPH challenge in AMPH-pretreated and AMPH-naive DEF rats did not differ significantly. While the reason for this blunted response is not known, prior evidence indicates that DHA deficiency is associated with impaired glutamatergic synaptic plasticity (i.e. long-term potentiation)[20] whereas increasing DHA intake increases dendritic spine density.[24] Therefore, DHA deficiency may have impaired regional alterations in dendritic spine density and synaptic remodeling observed following chronic AMPH exposure.[1–3] Additionally or alternatively, DHA has been found to be protective against neurotoxic dopamine lesions which may be mediated in part by elevated pro-inflammatory signaling,[39–41] and larger brain DHA deficits are associated with constitutive increases in pro-inflammatory cytokine levels[42] and dopamine cell loss.[14] However, a neurotoxic mechanism is not supported by the observation that DEF rats exhibited greater locomotor activity during AMPH pretreatment, and frontostriatal recruitment is also observed following a neurotoxic AMPH dosing regimen.[36] Nevertheless, additional studies are warranted to determine whether AMPH- induced dopamine neurotoxicity and/or synaptic remodeling are altered in DEF rats.

The present findings may take on additional significance in view of evidence that medication-naive ADHD patients exhibit blunted frontostriatal activation and functional connectivity during performance of cognitive tasks, and that blunted frontostriatal activation is normalized following psychostimulant treatment.[43–47] The present results additionally suggest that DHA deficits commonly observed in ADHD patients[11] may hinder AMPH-induced neuroplastic adaptations in frontostriatal circuits, and that the therapeutic benefits associated with *n-3* PUFA supplementation in ADHD patients may be mediated in part by promoting frontostriatal activation.[12] It is also notable that DEF rats exhibited elevated locomotor activity during AMPH pretreatment across doses, suggesting that impaired frontostriatal recruitment is associated with behavioral hyperactivity rather than hypoactivity. The latter, apparently paradoxical, effect may be due in part to a failure to recruit medial prefrontal-mediated inhibitory feedback on ventral striatum dopamine systems (i.e. nucleus accumbens)[48] as is observed following medial prefrontal cortex lesions.[49] These and other findings support the translational hypothesis that optimal n-3 PUFA levels are required for AMPH- induced frontostriatal recruitment, and prospective fMRI studies are warranted to evaluate this mechanism in medication-naive ADHD patients.

This study has a number of limitations. First, rats were anesthetized with isoflurane during MRI acquisition which may have artificially altered BOLD signal. However, all groups were anesthetized with similar levels of isoflurane and reliable AMPH- induced changes in BOLD activity were observed. Second, we did not obtain locomotor activity data at P90 to confirm that the AMPH pretreatment regimen resulted in behavioral sensitization. Third, we did not determine indices of dopamine neurotoxicity or synaptic remodeling (i.e. dendritic spine density) to evaluate their potential contribution to the present findings. Strengths of this study include high magnetic field strength (7 Tesla), voxel-based determination of regional brain activation patterns *in vivo,* measurement of behavioral responses to AMPH treatment, and selective manipulation of diets and postmortem evaluation of fatty acid levels.

In conclusion, the present findings provide preclinical evidence that chronic escalating AMPH treatment leads to an enduring recruitment of frontostriatal regions, and that this

response in impaired in rats subjected to peri-adolescent deficits in brain DHA accrual. These findings compliment prior evidence for enduring synaptic reorganization in frontostriatal regions following similar chronic escalating AMPH dosing, and support additional research to determine whether enduring synaptic reorganization within frontostriatal structures contributes to enduring increases in activation in response to AMPH. Together these findings may have implications for understanding the neurobiological substrates mediating the therapeutic actions of psychostimulant medications in ADHD, and further suggest that these neuroplastic processes are impaired by low brain DHA levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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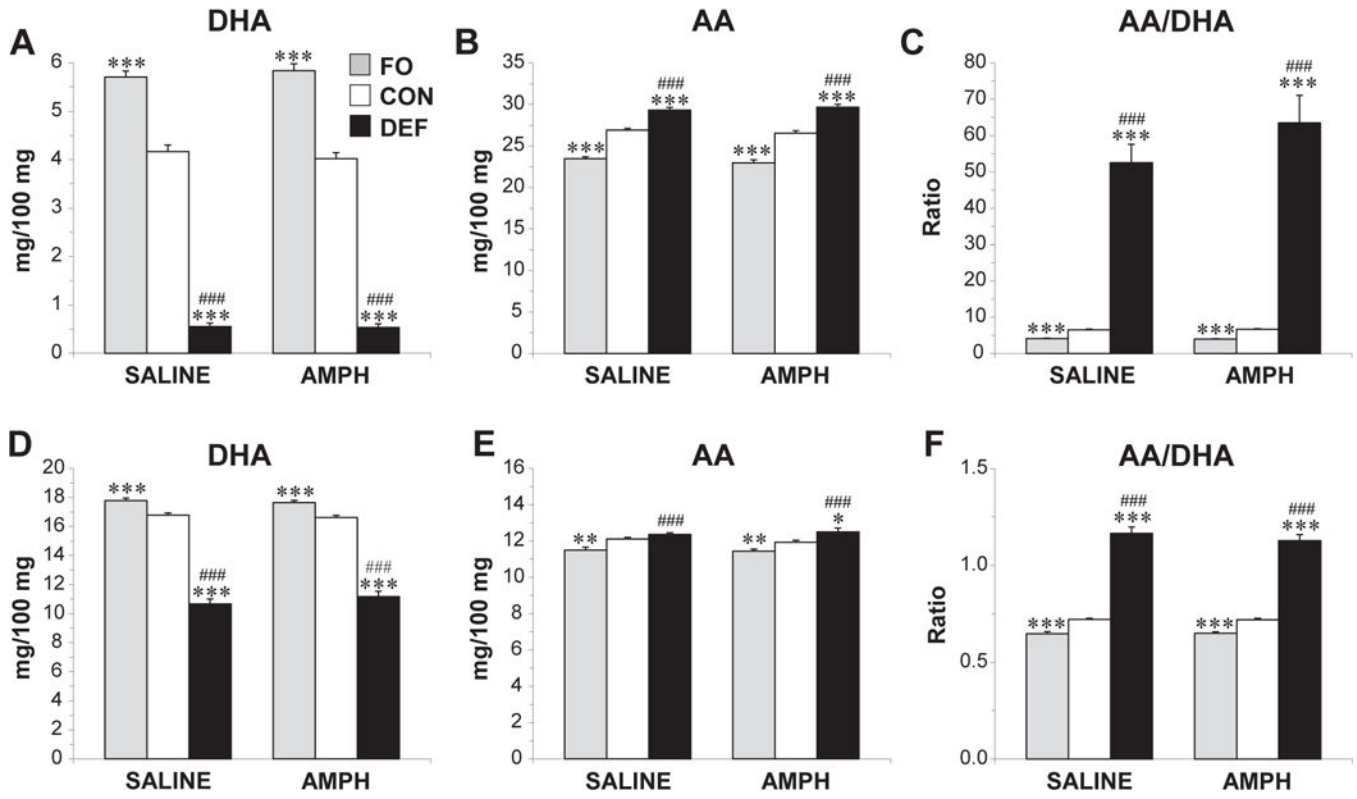

Figure 1.

DHA (A,D) and arachidonic acid (AA) (B,E) composition, and the AA/DHA ratio (C,F) in erythrocytes (A–C) and forebrain, (D–F) of adult rats maintained on the control diet (CON), *n*-3-free diet (DEF), and fish oil-fortified diet (FO) during peri-adolescent, development. Diet groups are separated into those that received prior saline or AMPH dosing. Values are group mean ± S.E.M., $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.0001$ vs. CON, $^{###}P \leq 0.0001$ vs. FO

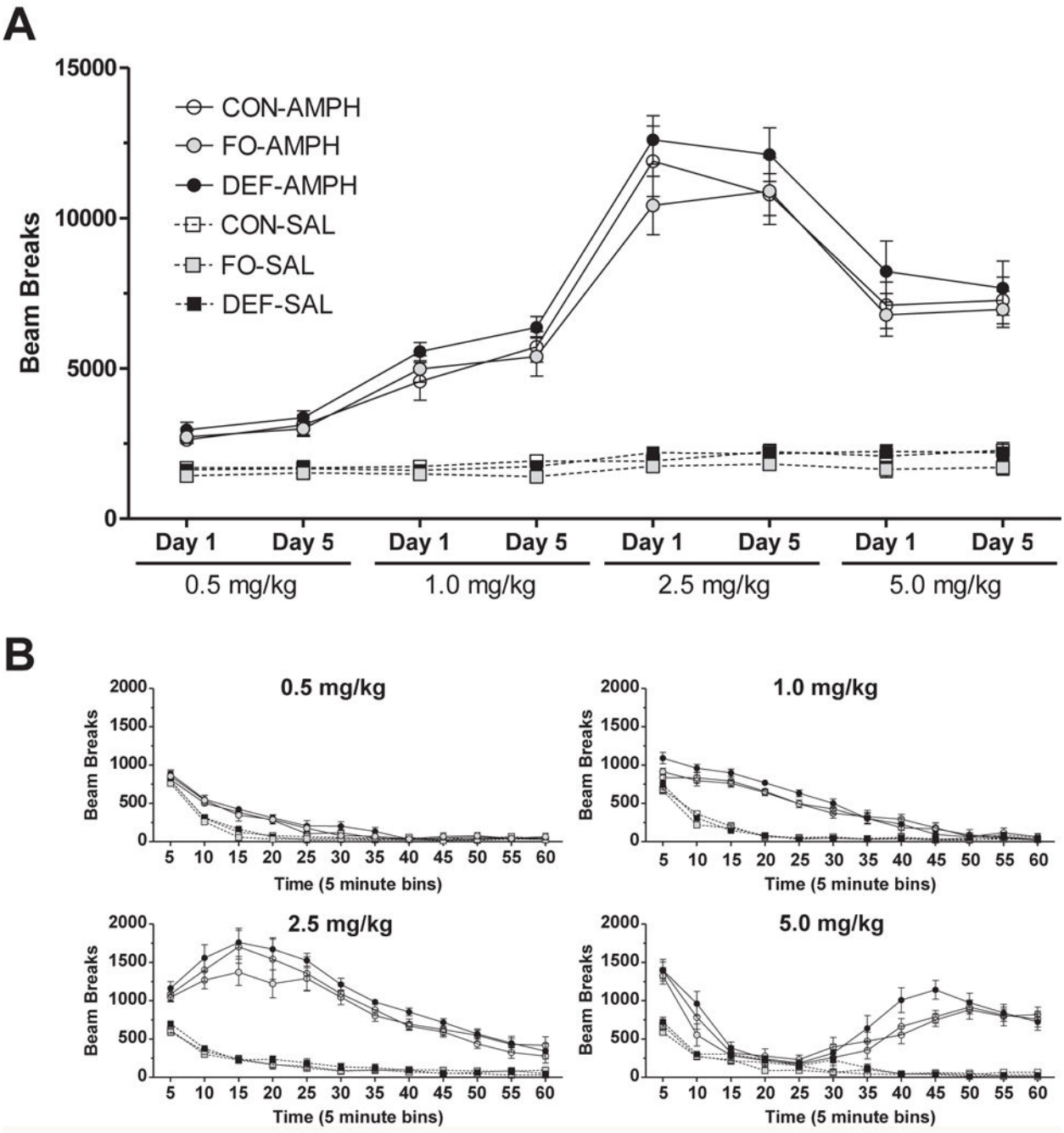

Figure 2.

(A) Horizontal locomotor activity (beam breaks) for the first 30 minutes following administration of saline (SAL, 1.0 ml/kg) or escalating AMPH doses (0.5, 1.0, 2.5, 5.0 mg/kg) on the first (Day 1) and fifth (Day 5) injection day of each dose week in adolescent rats maintained on the control diet (CON), *n*-3-free diet (DEF), and fish oil-fortified diet (FO). (B) Horizontal locomotor activity following administration of SAL or escalating AMPH doses on the first day of each dose in rats maintained on CON, FO, or DEF diets. Values are group mean beam breaks ± S.E.M.

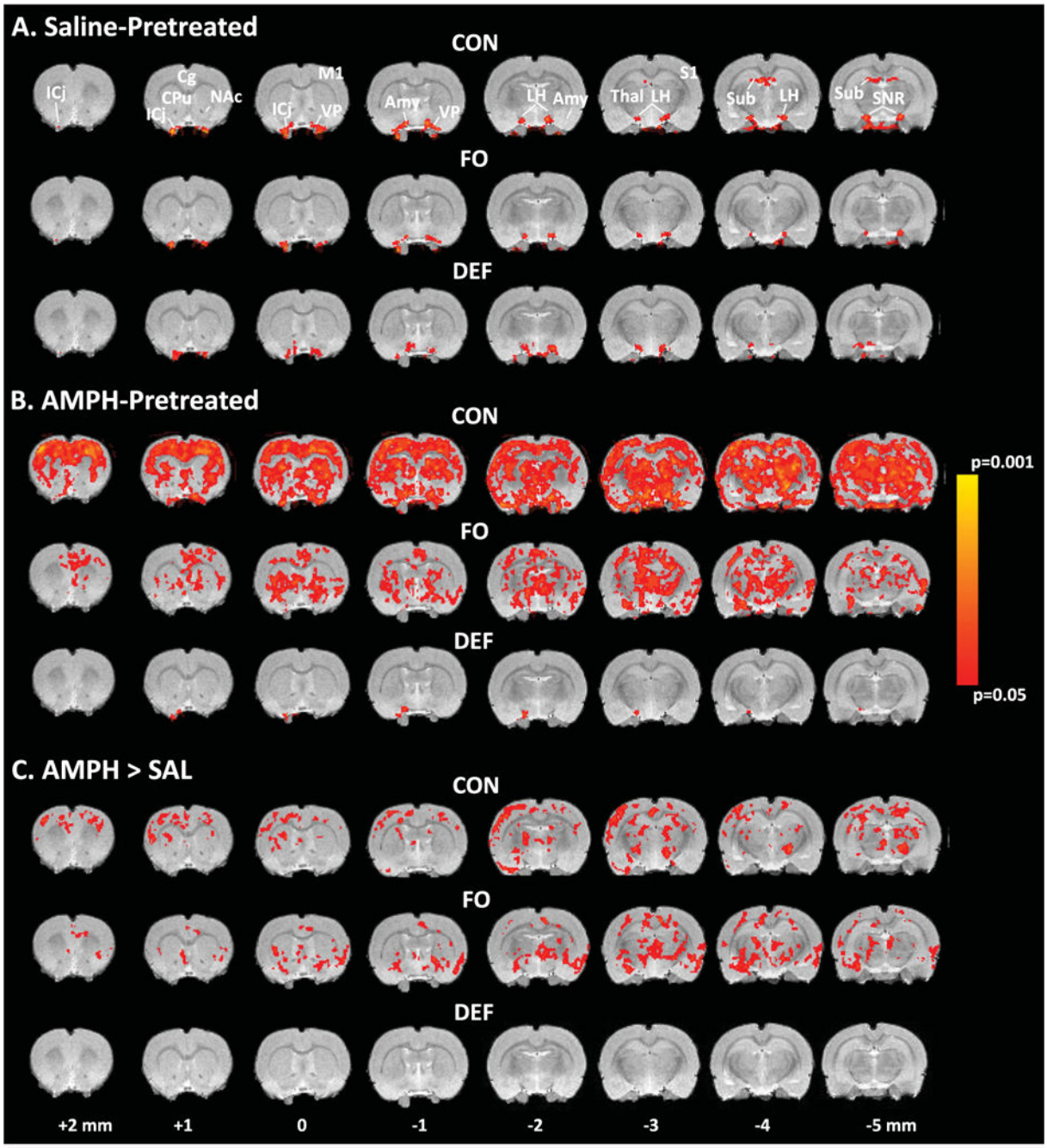

Figure 3.

Voxel-based analysis illustrating AMPH-induced BOLD activity in saline-pretreated (AMPH-naïve) (A) and AMPHpretreated, (B) adult (P90) rats maintained on the control diet (CON), fish oil-fortified diet (FO), and n-3-free diet (DEF) during periadolescent, development (P21–P90). (C) Contrasts illustrating differential AMPH-induced BOLD activity in AMPH-pretreated versus saline-pretreated rats maintained on CON, FO, or DEF diets. All contrasts used cluster significance threshold of $P \leq 0.05$ (corrected). Bregma coordinates of each 1 mmslice are indicated. Abbreviations: ICj, islands of Calleja; Cg,

cingulate cortex; M1, motor cortex; CPu, caudate putamen; NAc, nucleus accumbens; VP, ventral pallidum; Amy, amydgala; LH, lateral hypothalamus;Thal, thalamus; S1, somatosensory cortex; Sub, subiculum; SNR, substantia nigra.

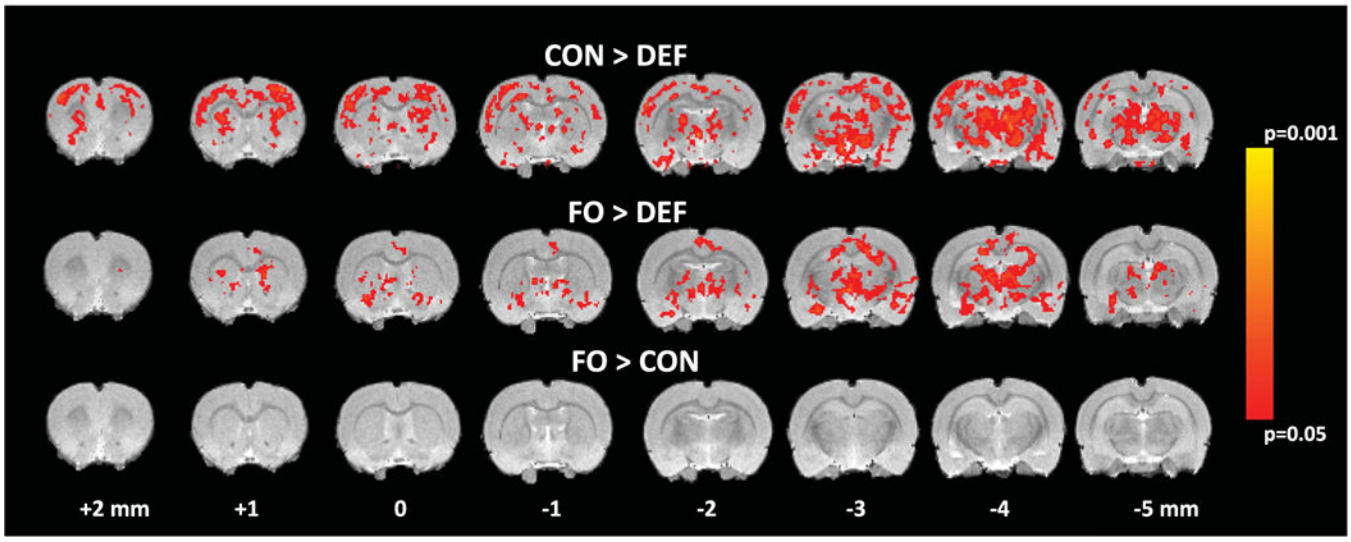

Figure 4.

Voxel-based analysis illustrating differential AMPH-induced BOLD activity in AMPH-pretreated adult rats maintained on the CON diet versus DEF diet (CON > DEF), FO diet versus DEF diet (FO > DEF), and FO diet versus CON diet (FO > CON). All contrasts used cluster significance threshold of $P \leq 0.05$ (corrected). Bregma coordinates of each 1 mm slice are indicated