Long-term cognitively challenging experiences modulate metabolite concentrations in the healthy brain: the case of bilingualism

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Abstract

Cognitively demanding experiences, including complex skills acquisition and processing, have been shown to induce brain adaptations, at least at the macroscopic level, e.g. on brain volume and/or functional connectivity. However, the neurobiological bases of these adaptations, including at the microstructural cellular level, remain poorly understood. Here we use bilingualism as a case study to investigate the microscopic correlates of experience-based brain adaptations. We employ Magnetic Resonance Spectroscopy to measure concentrations of metabolites in the ventral striatum, a region critical to language control which is reshaped by bilingualism. Our results revealed increased concentration of myo-Inositol in bilinguals compared to monolinguals. This metabolite is linked to synaptic pruning, a process underlying experience-based brain restructuring. Crucially, concentration was predicted by relative amount of bilingual engagement. Our results suggest that (degree of) long-term cognitive experiences have measurable effects at the microcellular level, which might accompany, if not drive, the observed macroscopic brain adaptations.

Research over the past two decades has unequivocally shown that human brain structure is not static. Rather, it is affected by learning new skills via environmental experiences of individuals. Notably, this malleability applies to the healthy brain as well as to the diseased one, and appears to be relatively independent of critical periods and other developmental milestones[1,2]. For example, grey and white matter adaptations have been reported for cognitively demanding experiences such as learning a new language, mastering complex visuospatial tasks and memory training[3–5], and, crucially, these adaptations tend to be observed in brain regions significantly associated with the task at hand, as well as white matter tracts that provide connectivity between these regions. Moreover, adaptations themselves are not static but dynamic. They are dependent on the quality and quantity of the experiences that induce them, e.g. the intensity and degree of novel skill training[4] and bilingualism[6].

Although the exact processes underlying the observed structural neuroplasticity are still under investigation, several suggestions link them to (cognitive) experiences. For example, volumetric changes in grey matter structure have been interpreted as a mechanism that temporarily increases the availability of neural pathways for a new skill to be acquired, most likely via the generation of new dendritic spines. This is subsequently followed by pruning of idle connections (and spines) while retaining the most efficient networks. In turn, this leads to volumetric renormalisation of previously grown regions[4]. Conversely, changes to white matter structure have been interpreted as increases in the availability of myelin as a result of increased axonal activity, which, in turn, can be brought about by new skill learning. These effects are very likely to be linked to increased activity of glial cells, especially oligodendrocytes[7].

Most of the relevant evidence in humans comes from MRI studies that utilise macroscopic indices, such as volume and thickness for grey matter, fractional anisotropy and

mean diffusivity for white matter. However, it is worth noting that all proposed processes are energy demanding. Therefore, there is scope for studying neuroplasticity at the microscopic level, and specifically with methods that tap local metabolic activity and the neurochemical processes that underlie it. A method that has been increasingly used for this purpose is proton Magnetic Resonance Spectroscopy (^{1}H-MRS)[8]. MRS has been typically used in clinical settings in order to estimate average metabolite concentrations in a specific region of interest (RoI) in the brain *in vivo*[9]. To date, MRS research has mainly focused on a few major metabolites: these include N-acetylaspartate (NAA), a marker of neural density and viability, Choline (CHO), related to the density and integrity of the cell membrane, Creatine (CRE), considered essential in cellular energy metabolism, and myo-Inositol (INS), a marker of glial proliferation and glial size (for a review, see[10]).

Disruptions of metabolic activity can relate to a breakdown of cognitive functions; as such, metabolic activity has been studied in neurodegenerative diseases such as Parkinson's[11], Huntington's[12] and Alzheimer's[13] Diseases, Multiple Sclerosis[14], and Primary Progressive Aphasia[15], where disruptions have been typically treated as precursors of the disease in pre-symptomatic patients, and/or as predictors for related cognitive deficits. Crucially, similar approaches have been used with healthy participants, where metabolite concentrations have been used as predictors of cognitive functionality in domains such as reading[16], episodic memory[17] , executive control[18] among others. In the domain of ageing, while metabolite concentrations are also typically used as predictors of cognitive abilities, it is not uncommon that they are also treated as *outcomes* of the ageing processes (for a review, see[19]). For example, Chiu and colleagues[20] suggest that changing levels of metabolites such as CHO, CRE and NAA in the elderly can be viewed as a proxy for the study of several age-related neural processes. These

include glial proliferation which acts as a compensatory mechanism in challenging situations where there is an increased energy demand that cannot be supported by regional blood flow. It is worth noting that these changes can vary between different metabolites and different brain regions; specifically, NAA has been shown to *decrease* in the basal ganglia but *increase* in the ACC, INS has generally been shown to increase with age, and CHO and CRE to stay stable, whereas GLX is less well studied and understood, especially as far as subcortical regions are concerned (for a recent systematic review of these findings, see[21]).

In light of the above, it is reasonable to investigate whether, and to what extent, different types of cognitively demanding lifestyle enrichment activities might induce comparable changes to metabolite concentrations, even at younger ages. Recall that skill acquisition and maintenance entail increased cognitive demands and have been shown to bring about structural brain plasticity and accompanying increased energy demands. In turn, it could follow that skill acquisition and maintenance would be accompanied by metabolic changes, similar to what has been found for aging. Indeed, and related to the present investigation, recent MRS research has suggested that changes in metabolite concentrations are potentially good markers of experience-related structural brain plasticity, potentially signifying neuronal, glia and vascular changes as responses to cognitively challenging tasks[22]. For example, several studies have reported increased levels of INS, CHO and CRE in the occipital cortex of blind subjects, compared to sighted ones[23,24], and these findings have been interpreted as markers of plastic changes in the glial cells (astrocytes and oligodendrocytes) that underlie and support repurposing of the visual cortical regions for other sensory modalities in the blind. This suggestion corroborates previous findings of functional and structural adaptations of the visual cortex in the blind (for reviews, see[25,26]), suggesting that changes at the macroscopic level are very likely accompanied by effects at the

microscopic level, in this case metabolic ones. Importantly, changes in the concentrations of INS, NAA and GLX have also been reported in the occipital cortex of sighted participants after visual training[27]. This suggests that the glial cells might have a central role in brain restructuring as a result of cognitive and sensory experiences, beyond ageing or pathology.

In line with the above, it is judicious to hypothesise and empirically test that similar effects in metabolite concentrations can result from long-term, cognitively challenging experiences known to affect brain structure and function. One of these experiences is bilingualism. It is widely accepted that the mental juggling of more than one language in a single mind/brain is cognitively demanding. Indeed, the need for use between the two languages is unpredictable. As a result, both languages are continuously active at all times irrespective of apparent need or intent. This reality requires an efficient system of control for appropriate selection of one language for comprehension and production alongside simultaneous suppression of the irrelevant language to a low level of idle activation for whenever the other may become needed[28,29]. This constant competition taxes domain general executive control abilities and their underlying brain structures, leading to long-term adaptations in domain general cognition[30], and in brain function[31], structure[6] and metabolism[32]. Notably, such effects can be both observed after extended long-term exposure in a bilingual environment[33,34] and after short-term intensive language training[35,36]. Moreover, recent literature has shown that the nature and location of these effects is modulated by quantitative measures of the depth and intensity of experiences bilinguals have with using their languages and opportunities to switch between them[37–40].

Over all, it seems reasonable to predict that the continuously challenging task of handling two (or even more) languages would result in brain changes at the neurochemical level;

bilingualism requires greater and more sustained efficiency in brain regions subserving language and cognitive control, such as the anterior cingulate gyrus (ACC) and parts of the basal ganglia such as the caudate nucleus and putamen. In fact, all these regions have been shown to change in shape and/or volume as a response to bilingualism[28,41–44]. Therefore, it is possible that these structural changes might have their correlates in changes in metabolite concentrations. For example, and drawing parallels from the findings from healthy ageing and neuroplasticity in the blind[23,24], the observed restructuring of these regions might be characterised, at least partly, by expansion of glial cells, which is in itself marked by increases in regional CHO, CRE and INS. Crucially, this hypothesis could provide the biological basis of bilingualism-induced regional neuroplasticity, which is currently not well understood[6].

To the best of our knowledge, only one study has looked at correlates in metabolic concentrations of the effects of bilingualism on cognition and the brain. Weekes and colleagues[45] compared young bilingual adults and age- and education-matched monolingual controls on metabolite concentrations (NAA, CHO, CRE, INS and GLX) in the anterior cingulate cortex (ACC), a region critical for domain general cognitive control[28]. Contrary to their predictions, Weekes and colleagues reported *lower* levels of NAA, CHO, CRE and GLX in bilinguals than monolinguals[1], and interpreted this finding as an indication of more efficient control monitoring of the bilingual brain as a result of prolonged bilingual experience. However, they reported no significant correlations between these concentrations and executive control abilities as were measured by a Flanker Task, nor a significant difference in task performance between the two groups. This study suggests that there might be effects of bilingualism on brain metabolism that are not detectable behaviourally, echoing some evidence in the neuroimaging literature

[1] However, it is worth noting that Weekes et al. looked at absolute metabolite concentrations, and not relative concentrations as many of the related studies did.

suggesting that behavioural measures might not always capture latent effects of bilingualism on brain function[38]. More to the present point, this set of results provided the first evidence that bilingualism-induced neuroplasticity might have its roots in changes in metabolite concentrations.

The present study expands on Weekes et al.[45] by looking at the effects of bilingualism on metabolite concentrations in the ventral striatum, a subcortical grey matter nucleus comprising the caudate nucleus and the putamen, key structures for language selection and cognitive control[28], that have both been shown to be affected structurally by bilingualism[41,43]. In order to study such effects across the adult lifespan, the present study comprises a relatively large sample that spans an age range representative of the adult lifespan, additionally accounting for the known effects of age on metabolite concentrations [19]. We looked at relative concentrations of four key metabolites (NAA, INS, CHO, GLX) as proportions of a fifth one (CRE). Based on previous literature, we predicted that increased age will lead to increased INS and decreased NAA concentrations in the ventral striatum, potentially accompanied by increases in GLX but not CHO[19–21]. In terms of the effects of bilingualism, we consider the data in two ways: following the most traditional practice in the field[45], we split our participant groups into Bilinguals and Monolinguals according to experiences in using more than one language (for details, see Methods). For this comparison, we predicted overall higher concentrations of INS and CHO in bilinguals, compared to monolinguals, an effect that would signify glial expansion, which could contribute to the observed restructuring of the ventral striatum in bilinguals[23,24]. Moreover, if the effects of age and bilingualism are based on similar mechanisms, then the combined effect of these two factors should lead to steeper increases with age of the INS and CHO concentrations in bilinguals. Following from more recent suggestions on how bilingualism

can differentially affect brain structure and function[38,39], we also looked at our entire sample to investigate whether the concentrations the metabolites of interest might be modulated by intensity of engagement with (bilingual) language experiences. We predicted that the more intense and sustained the bilingual experience, the greater the concentration increases would manifest.

Results

i) Effects of age and bilingualism

The results from the first set of analyses are illustrated in Table 1. The analysis revealed that Age was a significant predictor of the concentrations of NAA, INS and GLX only. Specifically, while INS concentration significantly increased with age, NAA and GLX concentrations significantly decreased. Moreover, Bilinguals were found to have a significantly higher concentration of INS than Monolinguals. There were no significant Age x Bilingualism interactions for any of the metabolites of interest. Figure 1 illustrates the relative concentrations per group across age for each metabolite.

Table 1: The effects of Age and Bilingualism on the relative metabolite concentrations, expressed in p values.

	INS/CRE	**NAA/CRE**	**CHO/CRE**	**GLX/CRE**
Age	0.008*^	0.027*	0.67	<0.001*^
Bilingualism	0.024*	0.38	0.122	0.503
Age x Bilingualism	0.67	0.927	0.488	0.147

For significant effects all Fs>3.3

Note: ^: edf>1, denoting a non-linear effect

ii) Effects of individual bilingual experiences

Our second set of analyses examined how metabolite concentrations can be predicted by the degree of bilingual engagement across our entire group. This revealed that only INS concentration was significantly predicted by all metrics of interest, L2 home , L2 social and the LSBQ composite score, in that the higher the experiences the higher the INS levels. The results from the second set of analyses are presented in Table 2. Figure 2 illustrates the effects on the INS concentrations.

Table 2: The effects of L2 home, L2 social and LSBQ composite scores on the relative metabolite concentrations, expressed in p values.

	INS/CRE	NAA/CRE	CHO/CRE	GLX/CRE
L2 home	0.020*^	0.592	0.302	0.729
L2 social	0.017*^	0.419	0.500	0.733
LSBQ composite	0.017*^	0.469	0.251	0.681

For significant effects all Fs>5.69

Note: ^: edf>1, denoting a non-linear effect

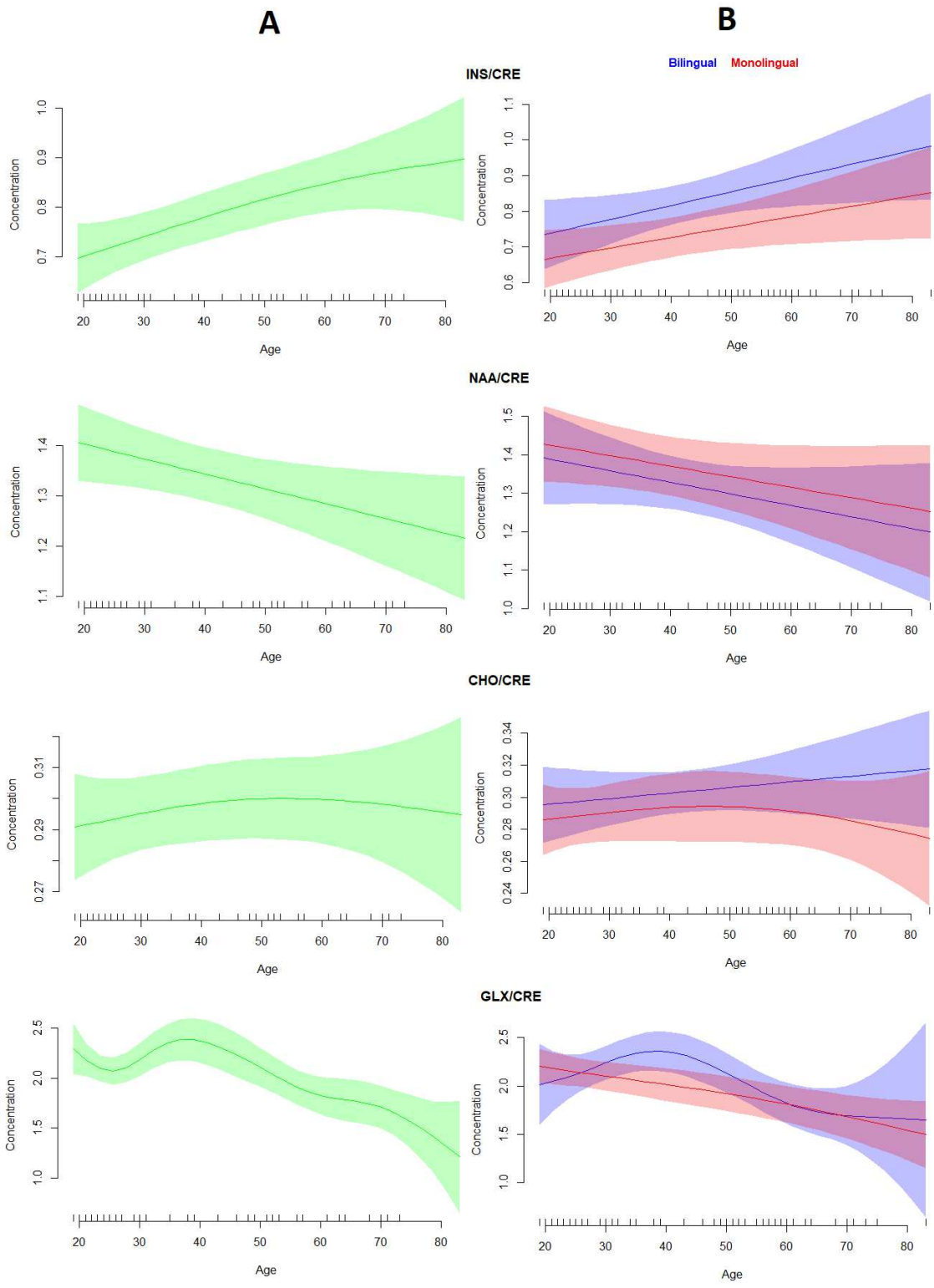
A
B
Bilingual
Monolingual
INS/CRE
NAA/CRE
CHO/CRE
GLX/CRE
Concentration
Age

Figure 1: (A) The effects of Age on the concentrations of the metabolites of interest; (B) the effects of Age split by group. Shaded regions represent confidence bands for the smoothed effects.

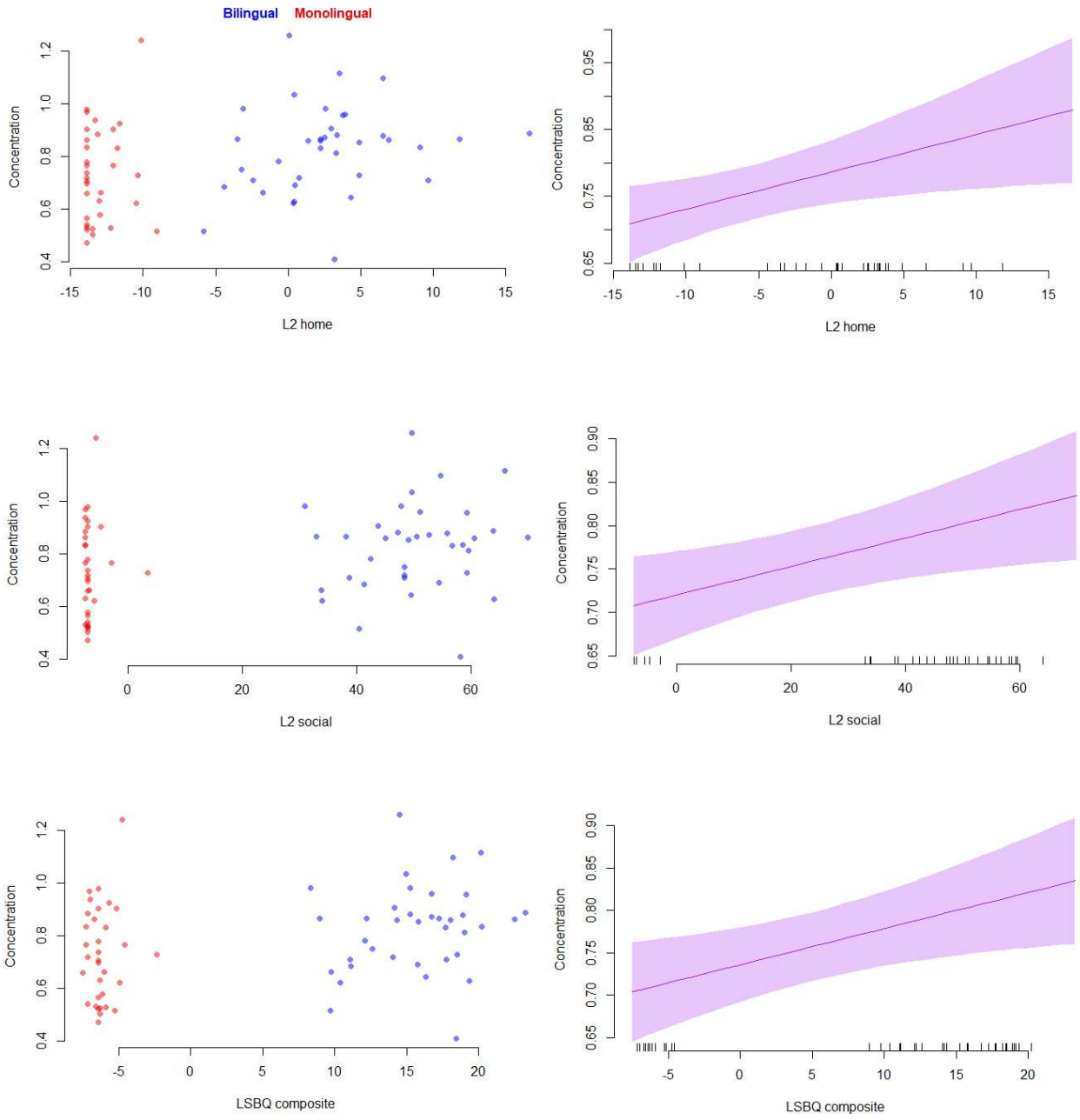

Figure 2: L2 home, L2 social and LSBQ composite scores as predictors of the relative INS concentrations. Shaded regions represent confidence bands for the smoothed effects.

Conclusions

Building on findings that the cognitively challenging experience of bilingualism can have knock-on consequences for the structure and function of brain regions related to language acquisition and control, and the (functional and structural) connectivity between them[6,31,46], the present study used MRS to investigate metabolic correlates of these adaptations. Recall that we focused on the concentrations of several well-understood metabolites in the ventral striatum, a region crucial for language selection and control in bilinguals[28]. Because brain effects of bilingualism can vary as a function of engagement with relevant experience, our sample included a considerable group of individuals varying in degree of bilingual engagement factors. Given that age is a comorbid factor, our sample also included a considerable range, enabling us to tease apart the effects of ageing from bilingualism. Results revealed age effects that largely corroborate previous findings in this particular brain region[21]; specifically, while the INS concentration increased and CHO remained stable, NAA concentration decreased, and a similar pattern was observed for GLX. Notably, the effects of age on INS and GLX concentrations emerged as non-linear, a finding that might explain the inconsistencies in the literature, and which should be considered in future studies. Crucially, our results also revealed that our Bilingual subgroup had higher INS levels than the Monolingual one. Notably, when we examined the unified group accounting for age, the INS concentration was positively correlated with the amount of bilingual experience.

We now turn to the effects on INS, interpreting them within the framework of bilingualism-induced neuroplasticity. INS is typically linked to increased activity of glial cells like astrocytes and microglia[47,48]. Increased levels of INS have been reported in the brains of blind individuals, interpreted to indicate repurposing of visual regions[23,24], as well as for several diseases, including HD[12], MCI/AD[13] and MS[14]. In the cases of patient studies, increases are typically attributed to *astrogliosis,* a manifestation of abnormal proliferation of astrocytes due to the destruction of nearby brain cells due to trauma or neurodegeneration. This interpretation explains our finding of increased INS levels with increased age: ageing brains suffer neuronal loss, a process which might be accompanied with or followed by astrogliosis[49]. However, this does not account for the higher INS concentrations observed in bilinguals, who had a comparable age-range to that of monolinguals. A distinct mechanism might be at play in this case.

Recall that elevated INS levels can also relate to increased microglia activation. Among other functions, microglia are thought to be involved in regulating synaptic activity, and notably in synaptic pruning[49]. This function might be key to interpreting the present findings. Recall that bilingualism, similar to other types of cognitively complex skill acquisition[4], can induce temporal volumetric grey matter increases followed by renormalisation over increased experience of using the new skill[6]. In the case of bilingualism, this process particularly affects the ventral striatum, which is key to language control for bilinguals[28], with the caudate nucleus and the putamen undergoing dynamic structural changes[6]. Crucially, this expansion-renormalisation process has been (at least tentatively) attributed to pruning of superfluous synapses that were formed during the skill acquisition, in order for the more efficient ones to be utilised[4]. Therefore, the increased INS concentration in the present study might be a marker of a bilingualism-specific process of expansion and renormalisation of regions related to controlling

two languages. Since our participants were all residents of the UK at the time of testing, thus active users of their L2 (English), they all had substantial experience in language switching and control, which should have contributed to optimisation of the language control system via processes like synaptic pruning. This interpretation is further supported by the finding that the INS concentration is positively correlated with the amount of bilingual experiences; higher levels of bilingual experience lead to more extensive renormalisations of the previously expanded structures of the ventral striatum, observed via higher INS concentrations as a function of more experience. Finally, this effect appears to be independent of age, as the interaction in our main analyses did not emerge significant. In other words, the significant effects of age in INS concentration are not affected by the language status of the individual (and vice versa); while both groups show the expected INS increases, bilinguals have elevated INS concentration across all ages. This suggests that the two effects (of age and of bilingualism) might be underpinned by different mechanisms. It is also worth noting that, contrary to our predictions, bilingualism did not emerge as a significant predictor of CHO levels in the ventral striatum. Effects in CHO concentrations related to neuroplasticity have been reported mainly in occipital regions[23,24], so our finding suggests that similar mechanisms may also apply to the ventral striatum; nevertheless, we remain cautious to overinterpreting this trend.

The importance of these findings is, at least, twofold. First, the data shows that the well documented effects of bilingualism on brain structure and function have their correlates in changes in brain metabolism. Crucially, we report markers of metabolic activity which are compatible with experience-based approaches, arguing for bilingualism-induced dynamic brain adaptations[6]. Given the important implications these adaptations may have for healthy and pathological ageing of the bilingual brain[50], future studies should pay particular attention to these

indices of neuroplasticity and how they interact with brain decline in key areas related to language processing and control. Second, and more generally, we show here that sustained, long-term cognitively challenging experiences, such as controlling two languages, might also have persisting effects on metabolite concentrations in the brain. Therefore, it is possible that similar long-term findings could be reported for other types of skill learning and experiences that have shown to restructure the brain (e.g. music, driving, exercise), and such effects are not limited to short-term training. Insofar as cognitively challenging experiences have a direct impact on metabolite concentrations in the healthy brain; they are useful in furthering our theoretical understanding of the mechanisms underlying skill acquisition and use, and the accompanying neural adaptations.

Online methods

Participants

In total, 99 adults were recruited. Inclusion criteria for the study included being right-handed (self-reported), no history of speech and language disorders and no contraindication to MRI scanning. The participants were divided into two groups. The Bilingual group consisted of participants who spoke English as their second language (L2) and were resident in the UK at the time of testing, i.e. they were immersed in the L2-speaking environment. Importantly, there were no inclusion criteria relating to their native language or other language factors in order to recruit the widest possible range of linguistic experiences [38,39]. The English native comparison group (henceforth called "Monolingual" group for convenience) included individuals born and raised in

the UK who had minimal or no exposure to additional languages, a typical and representative demographic in the UK. Of the participants that were recruited to the study, 33 were removed from the final cohort for several reasons[2]. The final sample consisted of 70 participants (age range 19-83), including 33 monolinguals (20 female) and 37 bilinguals (28 female). See Table 3 for full descriptors of the final sample. This research was approved by the University of Reading Research Ethics Committee. Informed consent was obtained from all participants.

Table 3. Mean (SD) of group demographics and relative metabolite concentrations

	Age	**L2_Home**	**L2_Social**	**Composite**	**INS/CRE**	**NAA/CRE**	**CHO/CRE**	**GLX/CRE**
Bilingual	42.6 (15.54)	2.59 (4.56)	50.60 (9.79)	15.64 (3.78)	0.83 (0.17)	1.30 (0.18)	0.30 (0.04)	2.11 (0.44)
Monolingual	40.45 (21)	-13 (1.33)	-6.53 (2.02)	-6.19 (1)	0.73 (0.18)	1.33 (0.23)	0.29 (0.04)	2.03 (0.34)

Materials

Both participant groups completed the Language and Social Background Questionnaire (LSBQ)[51] which documents the participants' language use from childhood to the present day and across several settings and dimensions. The LSBQ yields two scores related to the amount of (bilingual) language use within specific communicative settings, which have been shown to predict bilingualism-induced changes in brain structure and function[38,39]. Specifically, L2 social corresponds to the degree of L2 exposure and use in societal and community settings whereas L2

[2] For sixteen participants, several metabolite levels exceeded 2 standard deviations from the group mean; for another six participants visual inspection revealed that the manual voxel placement for the MRS scanning sequence was poor; four participants were outside the cramer-ratio; two anticipated native speakers of English wound up being too highly exposed to a second language, therefore constituting outliers to their group, and one participant presented a non-readable MRS spectrum.

home corresponds to the extent of L2 proficiency and use in home settings. Moreover, LSBQ outputs a composite score accounting for the overall bilingual experience. For all three scores, a higher value indicates increased bilingual engagement, i.e. increased (balance in) use of, and switching between, the two languages.

MRI data acquisition

Neuroimaging data were acquired on a 3T Siemens MAGNETOM Prisma_fit MRI scanner, with a 32-channel Head Matrix coil and Syngo software. A high-resolution T1-weighted MPRAGE sequence was acquired (256 sagittal slices, 0.7 mm slice thickness, in-plane resolution 250 x 250, acquisition matrix of 246 x 256 mm, echo time (TE) = 2.41 ms, repetition time (TR) = 2400ms, inversion time = 1140ms, flip angle = 8°). For purposes of voxel placement an isometric T2-weighted (HASTE) scan was also run (15 coronal slices, 3mm slice thickness, acquisition matrix of 263 x 250 mm, TR = 1500ms, TE=82ms, flip angle= 150°). Finally, an MRS sequence was run, involving a single voxel placed at the left ventral striatum (Figure 3). (10x10x15mm, 128 averages, transversal orientation, TR=2000ms, TE=30ms, flip angle= 90°).

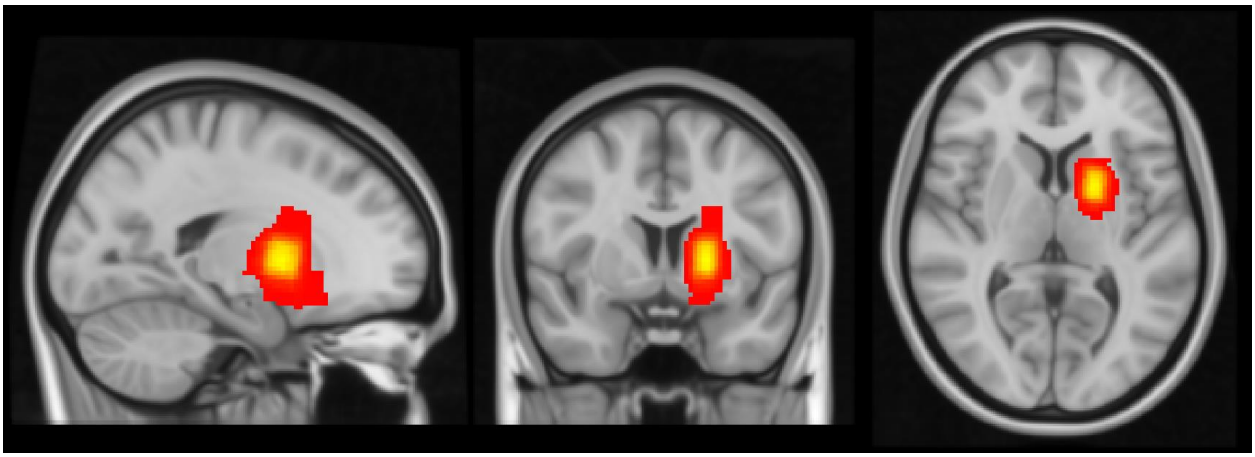

Figure 3. Location of the MRS voxels of the entire group in the left ventral striatum, shown in standard space. Warmer colours represent greater overlap between participants

MRS data quantification

^{1}H-MRS spectra were processed in the time domain within the software Java-Based Magnetic Resonance User Interface (jMRUI) software (version 5.2)[52]. Following literature in the field[20,45] we focused on the concentrations of NAA, CHO, CRE, INS and GLX. In the preprocessing phase, the water spectrum acquired closest to the measurement was used to perform phase correction. Afterwards, a Gaussian filter of 3 Hz was applied on each spectrum to improve signal quality, decrease noise and reduce signal truncation effects[53]. Residual water peaks were removed with the Hankel-Lanczos Singular Value Decomposition (HLSVD) filter tool[54]. In the quantification phase, the same metabolite models as in[8] were employed for NAA, CRE, INS and GLX; exceptionally, and following from recent literature[20,45], CHO was modelled as a single peak derived from the sum of choline, phosphocholine and glycerophosphocholine peaks. Accurate Quantitation of Short Echo time domain signals (AQSES) was applied using the method described in[55]. The spectrum and the model were shifted and aligned so that the NAA peak was at 2.02 ppm in order to correct for any chemical shift displacement. Limitation of frequency range for processing was selected at 0-8.6 ppm [equal phase for all metabolites, begin time fixed, delta damping (-10 to 25 Hz), delta frequency (-5 to 5 Hz), no background handling, 0 truncated points, 2048 points in AQSES and normalization turned on]. To account for differences in grey matter volume (GMV) in the voxel, partial volume correction was performed[56]. Metabolite values then were corrected to account for differences in gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) within the voxel. Moreover, T1 and T2

values from GM and WM at 3T were used as base to compute attenuation factors for both water and metabolites. These factors were in turn used to correct the reported values for relaxation effects (TR) dependent on voxel's tissue proportion. Last, and following standard practice in the field, we used a relative quantification method on our metabolite levels in order to avoid assumptions that the metabolite levels remained constant, and also to reduce individual variability among subjects[23,27,57]. For this study, CRE was chosen as the reference metabolite, as a preliminary analysis showed that its levels were not affected by age, bilingualism or the interaction between the two (all ps>0.3). Table 3 illustrates the mean relative concentrations for the remaining four metabolites.

Data analysis

Rejection criteria included one or more of the participants metabolite concentrations being outside the Cramer-Rao lower bound (CRLB)[58] and/or exceeding two standard deviations (2SD) from the group mean metabolite concentration (calculated separately for every metabolite). Only 3.85% exceeded the CRLB threshold (< 50% = acceptable reliability) and 15.39% the 2SD.

The corrected and quantified MRS data were analysed in R[59] with generalised additive models (GAMs), by using the bam() function of the mcgv package[60]. Since our sample covers a large age range (19-83 yrs), and the potential effects of age on metabolite concentrations are not well understood, GAMs were selected as a method that can account for potential non-linear effects of age on brain measures[61–63]. Specifically, GAMs fit a nonlinear regression spline consisting of the sum of simpler nonlinear functions, but this is only included where there is sufficient evidence for a particular curve. GAMs report the nonlinearity of the effect in the form of estimated degrees of freedom (edf), where edf=1 denotes a linear term and edf>1 a nonlinear

term. Separate GAMs were run for each metabolite of interest and in two sets of analyses (all tests two-sided) (see Supplemental Material for the full analysis code).

The first set of analyses looked at the effects of age on metabolite concentrations, and how these might interact with bilingualism. In a first-level model (Model 1) we applied a GAM in which we fit a regression spline for the effects of Age, along with random effects of Participant and Sex. In the second-level model (Model 2) we included a term for Bilingualism as an ordered factor (which allows estimating interactions), a regression spline term for Age, a smoothed term for the Age x Bilingualism interaction, as well as the random effects of Participant and Sex. This model was run twice, once for each level of Bilingualism as the reference level, following an analytical procedure akin to using a "vibration of effects" approach[63,64]. In cases were the Age x Bilingualism interaction emerged reliably significant (i.e. in both versions of Model 2), a third-level model (Model 3) would be run, in order to unpack the interaction. This model would include a main effect of Bilingualism and smooth terms for Age for each level of Bilingualism, along with random effects of Participant and Sex.

The second set of analyses looked at the effects of language experiences on the concentrations of the metabolites of interest across the entire group. These analyses were run separately for each of the three metrics of the LSBQ, namely L2 Home, L2 Social and Composite scores. Similarly to the first set of analyses, in this model (Model 4) we applied a GAM in which we fit a regression spline for the effect of the metric, a regression spline for the effect of Age, along with random effects of Participant and Sex. The entire sample of participants was included in this analysis without splitting them by their bilingual status. This was because we collected LSBQ scores from our native speakers too, and most of them reported some limited experience with other languages.

Ethical compliance statement

This research was performed in accordance with the Declaration of Helsinki. It was approved by the University of Reading Research Ethics Committee. Informed consent was obtained from all participants.

Data availability statement

The data analysed for this study are included in this published article as a supplementary information file.

Code availability statement

The code used for data analysis in this study is included in this published article as a supplementary information file.

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Figures

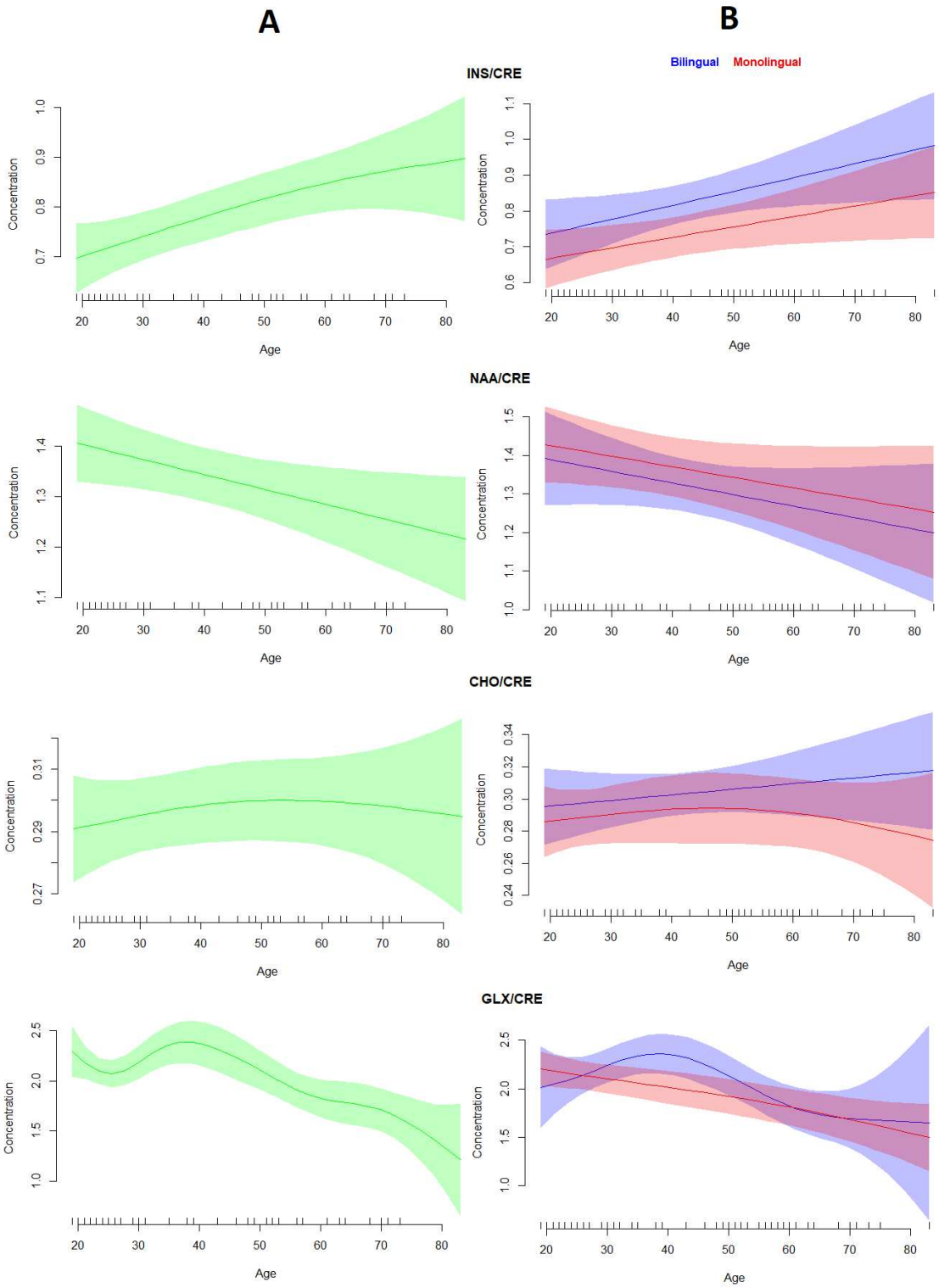

Figure 1

(A) The effects of Age on the concentrations of the metabolites of interest; (B) the effects of Age split by group. Shaded regions represent confidence bands for the smoothed effects.

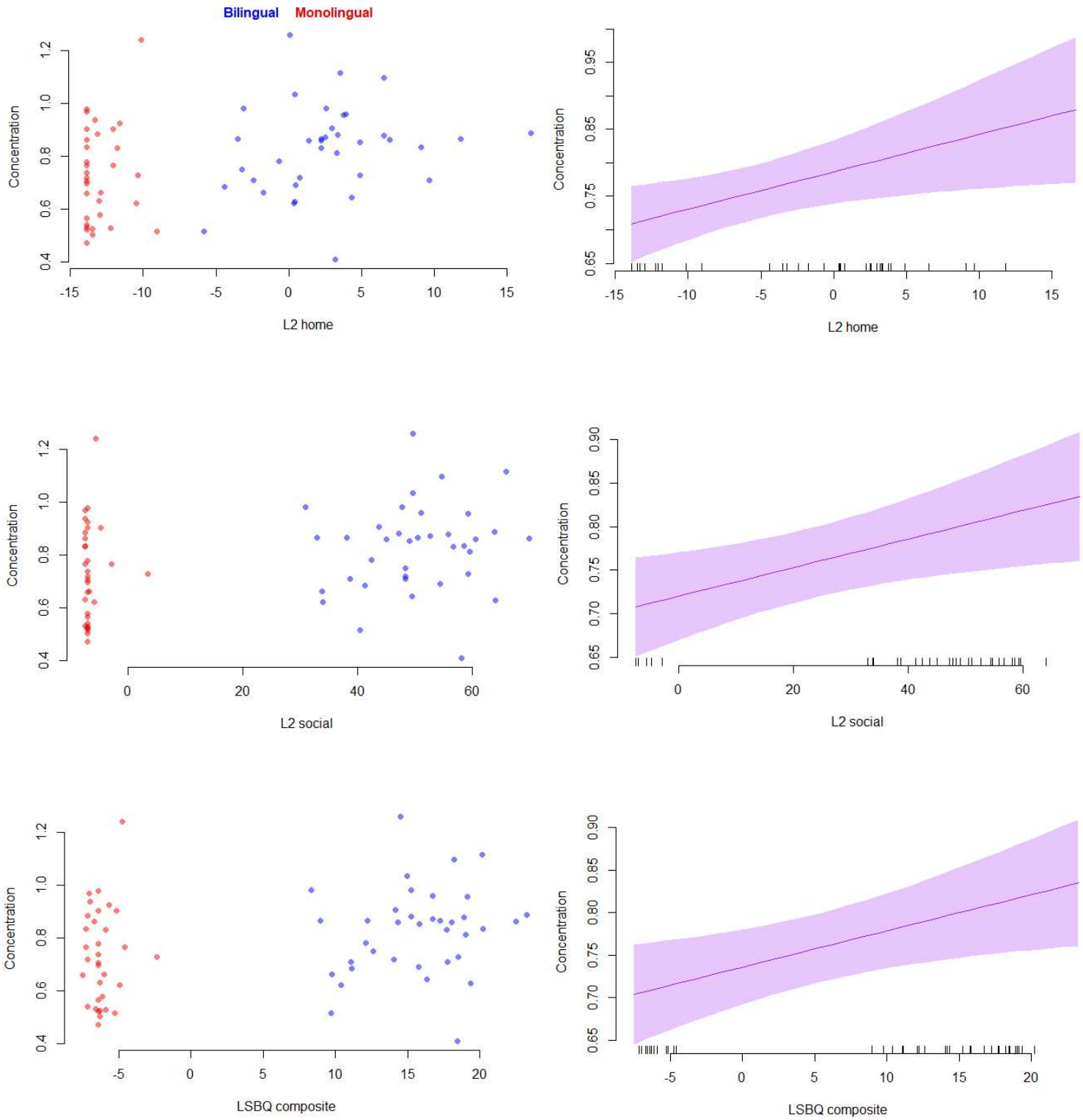

Figure 2

L2 home, L2 social and LSBQ composite scores as predictors of the relative INS concentrations. Shaded regions represent confidence bands for the smoothed effects.

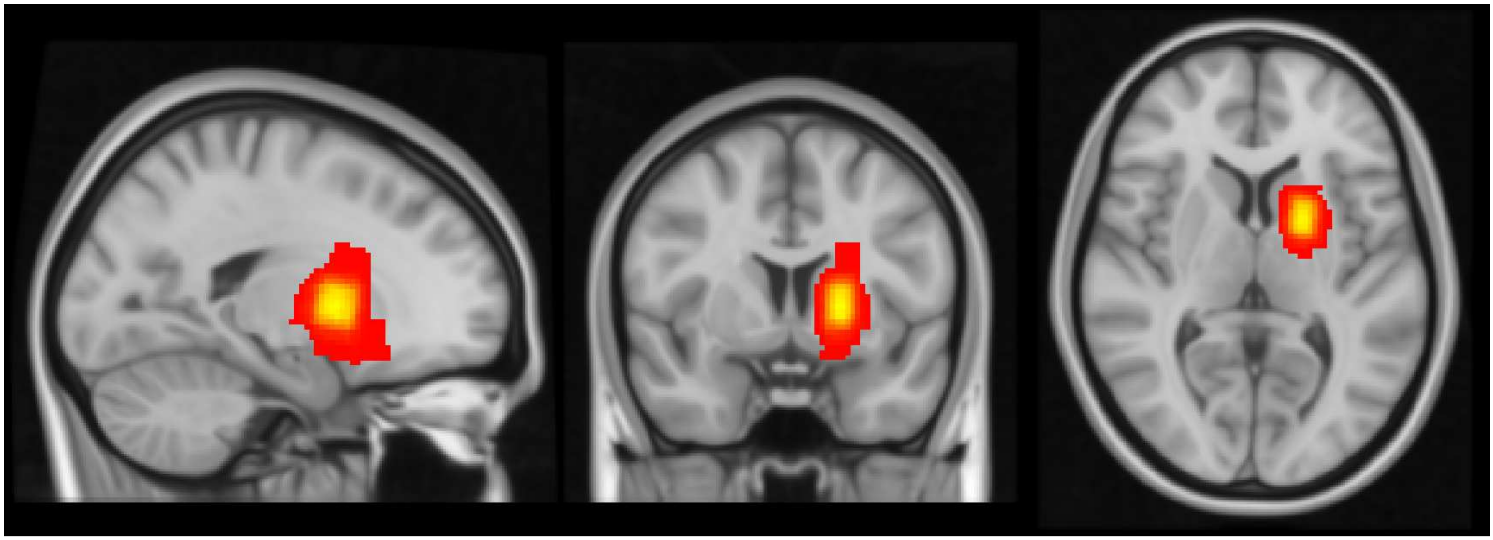

Figure 3

Location of the MRS voxels of the entire group in the left ventral striatum, shown in standard space. Warmer colours represent greater overlap between participants

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MRSanalysiscode.pdf