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DOTTORATO DI RICERCA TOSCANO IN NEUROSCIENZE

CICLO XXXIV

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Impact of metabolic and non-metabolic variables on human adults' visual cortical plasticity

Settore Scientifico Disciplinare BIO/09

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Summary

The main aim of my thesis research project is to investigate the mechanisms underlying visual neuroplasticity in adult humans and the impact of metabolic and non-metabolic variables on its inter-individual variability.

After a brief literature review summarized in the Introduction, in Chapter 2 I present a study investigating inter-individual variability in response to a short-term monocular deprivation. In adult humans, patching the dominant eye for two hours triggers an ocular imbalance in favor of the deprived eye, contrary to what happens during development. This effect is a form of homeostatic plasticity, and it has been linked to GABAergic inhibition in visual cortex. Lunghi et al. (Lunghi, Emir, Morrone, & Bridge, 2015), have clearly shown that the stronger the imbalance effect, the larger the GABA decrease. To investigate the mechanism underlying monocular deprivation wide variability, we focused on this GABAergic hypothesis. The main question that Chapter 2 aims to answer is, if inhibition is the key mechanism that regulates plasticity, can I predict individuals' deprivation outcome trough psychophysical tests that have previously been linked to GABA levels in visual cortex? Our results show that the individual percentage of mixed percepts – a stable binocular rivalry parameter - can explain up to 50% of the final variance of the plasticity effect. Mixed percepts have been linked to GABAergic inhibition by a recent paper by Mentch et al. (Mentch, Spiegel, Ricciardi, & Robertson, 2019). Moreover, when categorizing subjects based on their mixed percepts' percentage, other psychophysical stimuli – structure from

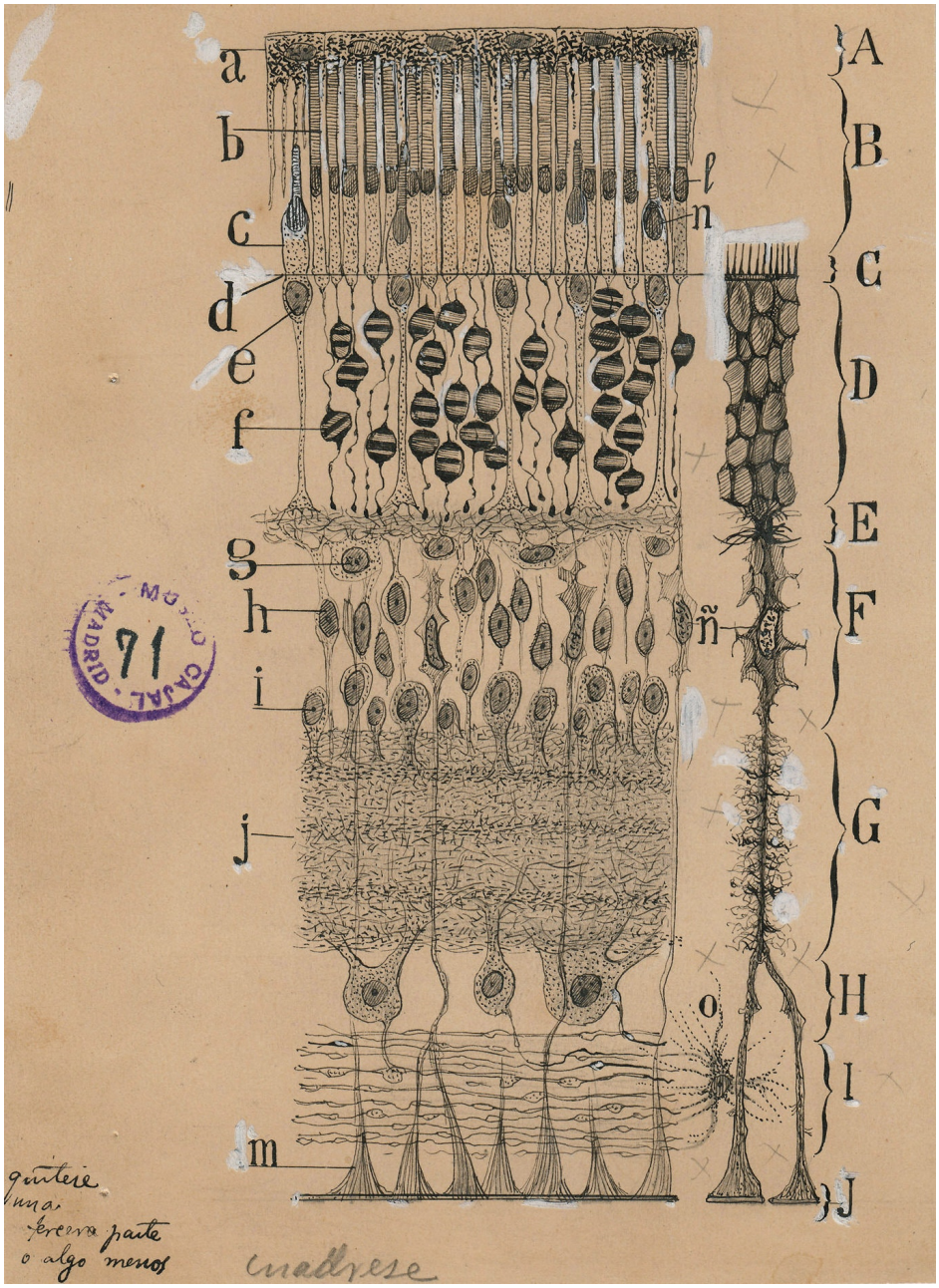
motion and tilt-illusion - do contribute to explaine the final variance of short-term monocular deprivation. Taken together, these results make a great contribution to the idea that different type of inhibitory interaction are elemental in shaping the limits of adult visual plasticity.

I then moved to a rather unexplored field, that is, the effect of state-like, metabolic variables, on short-term visual plasticity. As recent evidence is showing that metabolic manipulation can modulate visual plasticity in animal models, in Chapter 3 I present a study investigating the sensitivity of short-term monocular deprivation to simple metabolic manipulations. Our results show that plastic responses to deprivation are linked to metabolic states. We found that overnight fasting, compared to having a meal, reduces visual cortical plasticity, and this reduction is tightly linked with ketone bodies levels in blood plasma. Ketone bodies – a class of molecules physiologically produced in fasting conditions – have been shown to increase cortical inhibition in animal models, by regulating GABA synthesis and release among the possible mechanisms.

To directly test this hypothesis, we designed the study presented in Chapter 4. In cooperation with the Endocrinology team of Azienda Ospedaliera-Universitaria di Pisa, we simulated ketone bodies fasting blood plasma levels in healthy participants through a ketone-ester drink. We performed MR and MRS measurements to detect neuro-metabolites changes (especially GABA), and EEG measures sensitive to cortical excitation/inhibition ratio, before and after the drink. Our preliminary results show

EEG changes related to GABA levels in visual cortex, and brain metabolites changes possibly related to the new energetic balance in the neuronal cells.

Finally, in the last part of the thesis, I discuss the results of these three experiments, underlying their importance and limitations. Overall, the data presented in this thesis research project provide novel contributions to the understating of visual cortical plasticity, with exciting new insights on the link between brain and metabolism functioning.



"Cells in the retina of the eye" (1904), one of Santiago Ramón y Cajal's most striking drawings at the Grey Art Gallery in Manhattan (Cajal Institute, Madrid)

"Why, sometimes I've believed as many as six impossible things before breakfast." (Alice in Wonderland)

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Publications

Parts of this thesis have been included in the following peer-reviewed journal publications.

Experiment in Chapter 2 was included in the in the following publication.

Data collection was conducted together with Silvia Animali. Analysis and writing were conducted by me with editing for publication by supervisors.

Steinwurzel, C., Animali, S., Cicchini, G. M., Morrone, M. C., & Binda, P. (2020). *Using psychophysical performance to predict short-term ocular dominance plasticity in human adults*. Journal of Vision, 20(7):6, 1–13, https://doi.org/10.1167/jov.20.7.6.

Experiment in Chapter 3 was included in the following paper, that is currently under preparation.

Data collection and analysis were conducted by me together with Silvia Animali. Blood samples analysis were conducted by the Endocrinology Team of the Azienda Ospedaliera-Universitaria di Pisa. Writing was conducted by me and Silvia Animali with editing for publication by supervisors.

Silvia Animali, **Cecilia Steinwurzel**, Angela Dardano, Stefano Del Prato, Veronica Sancho-Bornez, Maria Concetta Morrone, Giuseppe Daniele, Paola Binda. *Skipping breakfast changes visual processing: incretins contribution to short-term visual plasticity*.

Experiment in Chapter 4 was included in the following paper, that is currently under preparation.

Data collection was conducted by me and Paola Binda, together with Francesca Frijia and Domenico Montanaro for the MR and MRS data.

Analysis was conducted by me together with my supervisors. Blood samples analysis were conducted by the Endocrinology Team of the Azienda Ospedaliera-Universitaria di Pisa.

Writing was conducted by me with editing for publication by supervisors.

Acknowledgments

First, I would like to thank my supervisor Maria Concetta Morrone, for having guided me through the difficult path of becoming a researcher. I feel very thankful for the great opportunity she gave me, and for all the time and effort she has spent into my development as a scientist. It has been a life-changing route.

A giant thank is also for Prof. Paola Binda; for the huge amount of time and effort she has put in helping me becoming a more conscious researcher, for all her precious advice and full support.

These experiments were honestly very tough: I am particularly grateful to all the participants that helped me in collecting these data, volunteering for hours in a MRI scan or in a hospital, sometimes without eating, sometimes with a patch over one eye.

A hearth felt thank goes to the whole Pisavisionlab, not only for being competent and helpful colleagues, but also and most importantly, for being amazing friends.

I am sincerely thankful to all my friends, scattered around the world, that helped me overcome every difficult moment with deep affection and a pinch of madness.

A special thank is for my family; I always felt surrounded by their love and support every single day I spent working in Pisa and far from home.

And of course, the last thank is for Francesco, who has illuminated every step of my path with his limitless energy and love. I wouldn't be the person I am today, without you being in my life.

Declaration

I, the author, declare that the work presented in this thesis is my own and has not been submitted for a degree at any other institution

Introduction

1.1 Visual cortical plasticity and short-term monocular deprivation

Our brain has the extraordinary capability to continuously adapt in response to a changing environment and to subsequently optimize its resources: this property is called neuroplasticity. (Berardi, Pizzorusso, & Maffei, 2000; Pascual-Leone, Amedi, Fregni, & Merabet, 2005).

The intrinsic plastic potential of the brain is maximal during early development, when windows of heightened sensitivity to external stimuli - the so-called critical periods - concur in shaping the adult brain (Berardi et al., 2000). Any pathological event occurring during a critical period can have a dramatical impact on brain's normal development: sensory deprivation, for example, can cause long-term morphological and functional alterations of sensory cortices (Berardi et al., 2000; Hubel & Wiesel, 1963). Classical studies in animal models have used the visual system to investigate the potential and limits of this early sensory plasticity. It has been shown that monocularly depriving one eye, even for a few days, during this period of specific susceptibility caused a severe visual impairment that could not be recovered even after prolonged periods of restored vision (Hubel & Wiesel, 1963, 1970; Hubel, Wiesel, & LeVay, 1977). Seminal work by Nobel laureates Hubel and Wiesel focused

on ocular dominance in V1 neurons. They recorded from V1 in kittens of different ages, both normal and deprived of visual stimulation through one eye, occluded by eyelid suture (monocular deprivation). Monocular deprivation disrupted the formation of ocular dominance column in V1. Moreover, they found a loss of neurons driven by the deprived eye, a strong increment of cells driven by the open eye, and a reduced number of binocular neurons. Anatomically, the result was a reduction of the deprived eye ocular dominance columns, those layer IV regions that receive thalamic inputs driven by the closed eye, and in the expansion of the open eye's columns. (Figure 1.1)

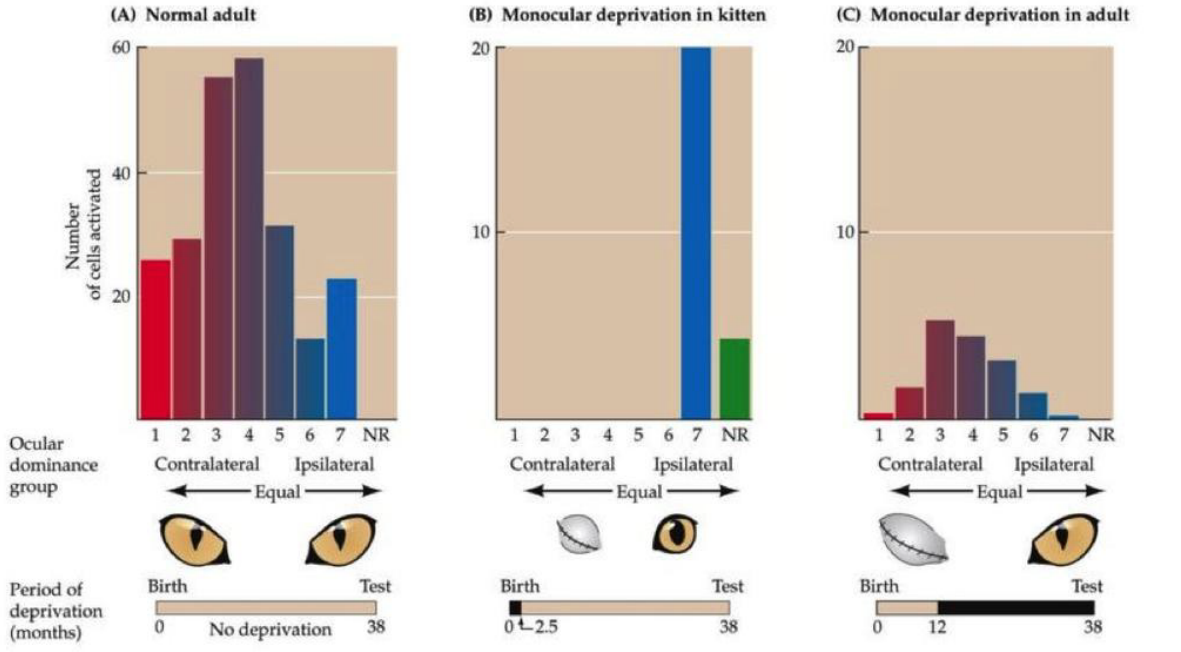

Figure 1.1 Effect of visual deprivation on Ocular Dominance. (A) Ocular dominance distribution of single unit recordings from many neurons in the primary visual cortex of normal adult cats. Cells in the group 1 are activated exclusively by the contralateral eye, cells in the group 7 by the ipsilateral eye. (B) Following the closure of one eye from 1 week after birth until 2.5 months of age, no cells could be activated by the deprived (contralateral) eye. Some cells could not be activated by either eye (NR). (C) A much longer period of monocular deprivation in an adult cat has little effect on ocular dominance (although overall cortical activity is diminished). In this case, the contralateral eye was closed from 12 to 38 months of age. (From Neuroscience. 2nd edition. Purves D., Sinauer Associates, 2001).

Similarly, early visual deprivation or abnormal early visual experience in humans (for example due to medical conditions as strabismus, untreated congenital cataracts, etc), can cause a long-lasting suppression of the suboptimal eye and a permanent reorganization of visual cortices, a condition called amblyopia (Birch, 1993; Ostrovsky, Andalman, & Sinha, 2006). Amblyopia can be treated in childhood with inverse eye-patching (Birch, 1993) but it is hardly correctable in adulthood (Braddick & Atkinson, 2011). This evidence led researchers to consider the adult visual system as hard-wired – although there was evidence of retained plasticity in the adult (Frégnac, Shulz, Thorpe, & Bienenstock, 1988).

Recent studies challenged this view, showing that the visual cortex can effectively retain a high level of residual plasticity for ocular dominance, even in adulthood. (Bai, Dong, He, & Bao, 2017; Binda et al., 2018; Binda & Lunghi, 2017; Chadnova, Reynaud, Clavagnier, & Hess, 2017; Lunghi, Berchicci, Morrone, & Di Russo, 2015; Lunghi, Burr, & Concetta Morrone, 2013; Lunghi, Burr, & Morrone, 2011a; Lunghi, Daniele, et al., 2019; Lunghi, Emir, et al., 2015; Lyu, He, Jiang, Engel, & Bao, 2020; Min, Baldwin, Reynaud, & Hess, 2018; Schwenk, VanRullen, & Bremmer, 2020; Wang, McGraw, & Ledgeway, 2020; Wang, Yao, He, Zhou, & Hess, 2017; Zhou, Baker, Simard, Saint-Amour, & Hess, 2015; Zhou, Clavagnier, & Hess, 2013). Lunghi et al 2011 (Lunghi et al., 2011a) were the first to observe that visually depriving healthy adult individuals for a short period of time, by occluding one eye with a translucent patch, profoundly altered ocular dominance balance. In this first paper, the authors

measured binocular rivalry before and after 150 minutes of monocular deprivation of the dominant eye.

Binocular rivalry is a form of bistable perception arising when two incompatible stimuli are presented separately to the eyes: conscious visual perception alternates between the two monocularly presented stimuli (Blake & Logothetis, 2002; Brascamp, Klink, & Levelt, 2015). Binocular rivalry is one of the most robust psychophysical methods to assess sensory eye dominance: the average time in which the image presented to each eye dominates the observer's perception reflects eye dominance. Furthermore, mixed percepts are those periods where neither eye clearly dominates perception, but the two images mix into a fused percept.

Lunghi et al. showed that, whilst binocular rivalry dynamics are usually stable, they change dramatically after deprivation: the deprived eye dominates twice as much as the non-deprived eye (opposite to what happens during development (Braddick & Atkinson, 2011; Maurer, Lewis, & Mondloch, 2005; Maurer, Mondloch, & Lewis, 2007) where the non-deprived eye dominates over the fellow eye). The observed ocular dominance shift progressively decays after patch removal, returning to a baseline condition after 90 to 180 min (Lunghi et al., 2013). At the end of the deprivation period there is a temporary apparent contrast increase: this suggests an up-regulation of the contrast gain-control mechanisms in the patched eye boosting the neuronal responses to compensate for the reduced incoming signal (Castaldi, Lunghi, & Morrone, 2020). The gain-control change probably occurs at early levels of visual

processing, since short-term monocular deprivation modulates the earliest component of Visual Evoked Potential (Lunghi, Berchicci, et al., 2015): boosting the C1 component of steady-state evoked potentials in response to stimulation of the deprived eye and suppressing responses to the non-deprived eye. This is supported by a study with Ultra-high field Functional Magnetic Resonance showing that short-term (2h) monocular deprivation in adult humans boosts V1 BOLD responses to the deprived eye and suppress responses to the non-deprived eye in V1 (as well as later areas, particularly in the ventral stream), leading to a shift of ocular dominance at the level of V1 (Binda et al., 2018).

It has been discussed if this effect might reflect a genuine form of plasticity rather than a temporary contrast adaption (Castaldi et al., 2020). However, the obtained ~36% boost in apparent contrast is not sufficient to explain the change in ocular dominance (Lunghi et al., 2011a) and when chromatic vision is specifically targeted (using iso-luminant visual stimuli), the effect can out-last the duration of deprivation, lasting for up to 3 h (Lunghi et al., 2013).

Furthermore, recent studies (Bai et al., 2017; Kim, Kim, & Blake, 2017; Ramamurthy & Blaser, 2018) have shown that a transient shift in ocular dominance can be triggered even without reducing monocular contrast – e.g., showing an altered image of the world instead of patching produce the same plasticity effect.

Taken together, these data suggest that binocular rivalry unbalance after deprivation is more likely to reflect a form of "homeostatic plasticity", which may consist of an

initial compensatory reaction of the visual system to deprivation, aimed at maintaining the average cortical activity constant despite the impoverished incoming visual input (Castaldi et al., 2020).

Despite the well-established replicability of the effect (Binda et al., 2018; Binda & Lunghi, 2017; Lunghi, Berchicci, et al., 2015; Lunghi et al., 2013, 2011a; Lunghi, Daniele, et al., 2019; Lunghi, Emir, et al., 2015; Y. Wang et al., 2017; Zhou et al., 2015, 2013), the response to short-term deprivation presents a high level of inter-individual variability, possibly reflecting an individual susceptibility to neuronal plasticity. Moreover, the exact mechanism underlying the whole effect still need to be completely defined. Understanding the processes underlying short-term monocular deprivation is of seminal importance for empower plasticity in pathological conditions, as, for example, amblyopia.

In the present work, I will first discuss the amount of inter-individual variability in this effect and present evidence regarding the possible mechanisms underlying the whole process. Then, I will discuss the role played by metabolic variables, in regulating this plasticity effect.

I aimed to identify 2 different classes of variables impacting each subject's level of plasticity: trait-like variables, inherently related to each subject cortical mechanisms, especially inhibitory, and state-like variables, related to the different environmental variables to which the subject is exposed.

1.2 Trait-like variability in short-term visual deprivation and binocular rivalry

Much work has been focused on investigating the cortical mechanism underlying visual plasticity (Boroojerdi, Battaglia, Muellbacher, & Cohen, 2001; Boroojerdi et al., 2000; Fierro et al., 2005; Lou et al., 2011; Lunghi, Berchicci, et al., 2015; Pitskel, Merabet, Ramos-Estebanez, Kauffman, & Pascual-Leone, 2007). As stated in the first part of the introduction, a key concept when studying neuroplasticity is that of "critical period", a moment of neuronal heightened sensitivity to external stimuli. Seminal work on animal models have clearly demonstrated that the opening and closure of such a period in visual cortex is strictly dependent on the maturation of intracortical inhibition (Fagiolini & Hensch, 2000; Hensch et al., 1998). Moreover, it has been shown that pharmacologically increasing intracortical GABAergic inhibition was able to anticipate both the opening and closure of the critical period for monocular deprivation (Fagiolini & Hensch, 2000; Hensch et al., 1998).

Since this evidence, much research has focused on the possibility to restore visual plasticity in adults by reducing levels of inhibition. Consistent with this hypothesis, it has been shown that ocular dominance plasticity can be rescued in adult animals by decreasing GABAergic inhibition, through different manipulations, e.g. pharmacologically (Vetencourt et al., 2008). Specifically, it has been reported that a chronic treatment with fluoxetine, a selective serotonin reuptake inhibitor, is able to

reinstate ocular dominance plasticity in adulthood and promote the recovery of visual functions in adult amblyopic animals. These effects are accompanied by a decrease of GABAergic intracortical inhibition in the visual cortex.

These findings suggest that the stabilization of GABAergic inhibitory circuits in adult brains represent a limiting factor for cortical plasticity – nevertheless interfering with the balance between excitation/inhibition (pharmacologically or otherwise) in the cortex can restore cortical plastic responses similar to those of the critical period. (Berardi, Pizzorusso, Ratto, & Maffei, 2003; Fagiolini et al., 2004; Fagiolini & Hensch, 2000; Harauzov et al., 2010; Hensch et al., 1998; Huang et al., 1999; Levelt, Heimel, & Van Versendaal, 2011; Spolidoro et al., 2011; Vetencourt et al., 2008).

In line with these results, a recent study by Lunghi et al 2015b (Lunghi, Emir, et al., 2015) suggests that GABAergic inhibition plays a major role in promoting ocular dominance plasticity also in adult humans. Subjects underwent the classical monocular deprivation paradigm, comprising binocular rivalry measures plus a 7 T MR spectroscopy session before and after 150 min of eye-patching. Monocular occlusion triggered an enhance dominance of the deprived eye over the non-deprived eye and the resulting perceptual changes were associated with decreased GABA concentration in V1. Participants showing the strongest perceptual effect after deprivation were those with the larger decrease in resting GABA concentration.

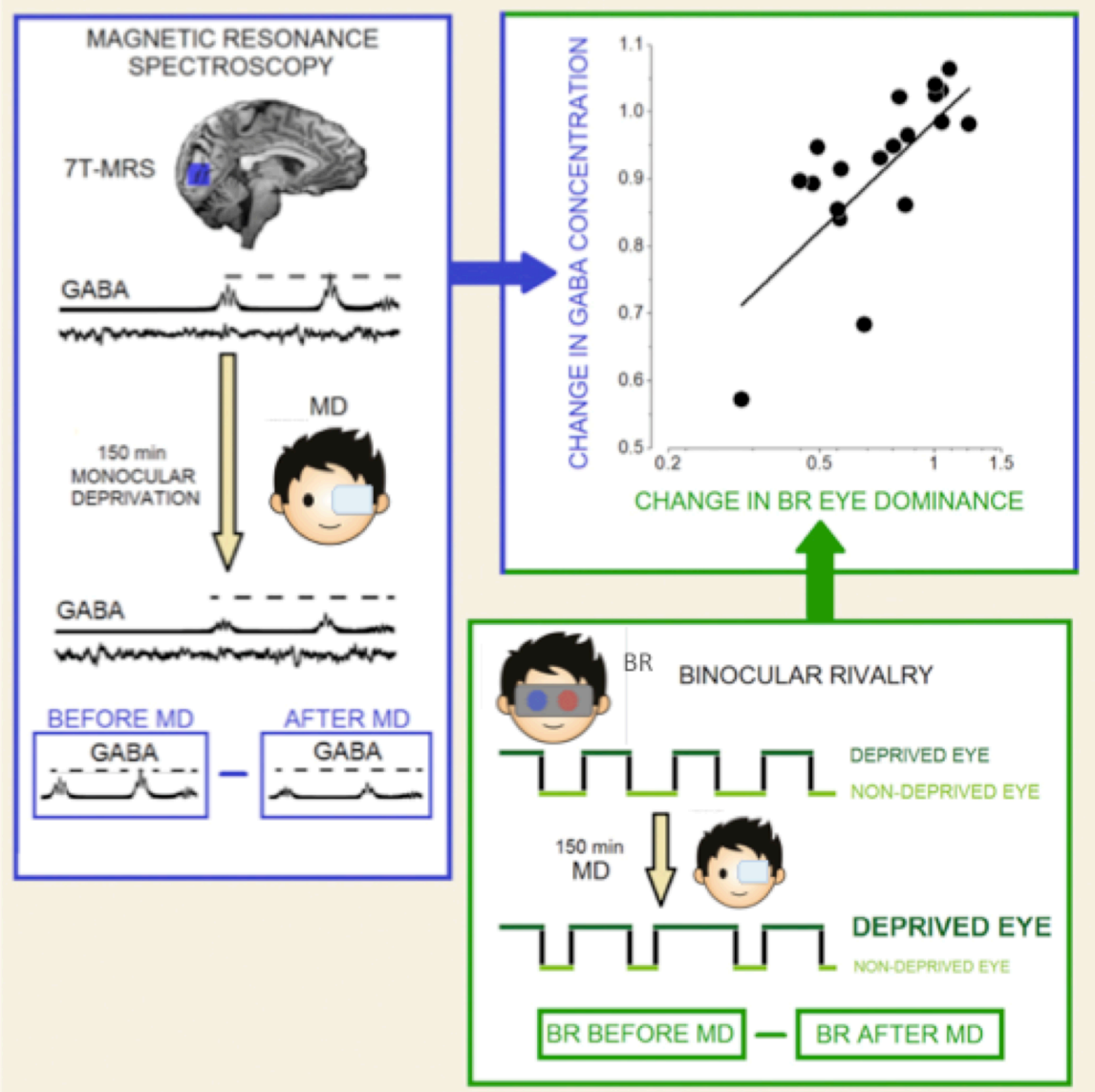

Figure 1.2. Using 7T-MR spectroscopy, is shown that resting GABA concentration decreases after deprivation and this decrease strongly correlates with the individual plastic change, measured as boosted mean phase duration for the deprived eye. (Modified from Short-Term monocular deprivation alters GABA in the adult human visual cortex. (Lunghi C. et al, Current Biology, 2015)

However, GABAergic circuits are not the only ones mediating plasticity in visual cortex (Berardi et al., 2003). In animal models, ocular dominance plasticity can be restored and modulated also by enhancing excitatory neurotransmission systems such as serotoninergic (Vetencourt et al., 2008) and cholinergic (Morishita, Miwa, Heintz, & Hensch, 2010) systems. In humans, (Boroojerdi et al., 2001) demonstrated

that GABA, NMDA and cholinergic receptors might be all involved in mediating rapid changes after visual deprivation.

If short term monocular deprivation in adult humans is mediated by a GABA reduction in visual cortex, it seems reasonable to speculate that the inter-individual plasticity variability observed in the forementioned studies might be at least partially explained by individual difference in GABA levels.

Many psychophysical tasks have been linked to each subjects' GABA levels in visual cortex. The dynamics of binocular rivalry, the most used paradigm used to assess short term monocular deprivation, have been correlated with inhibition in visual cortex. In fact, reciprocal inhibitory interactions across neuronal populations are thought to modulate each eye percepts, at multiple hierarchical levels of visual processing (Blake, 1989; Laing & Chow, 2002; Li, Rankin, Rinzel, Carrasco, & Heeger, 2017; Logothetis, Leopold, & Sheinberg, 1996; Noest, van Ee, Nijs, & van Wezel, 2007; Said & Heeger, 2013; Seely & Chow, 2011; Tong, Meng, & Blake, 2006; Van Loon et al., 2013). Similar models apply to other bistable/rivalrous phenomena, such as structure-from-motion rivalry, (Brascamp, Becker, & Hambrick, 2018; Van Loon et al., 2013) but see (Gallagher & Arnold, 2014; Sandberg et al., 2016).

In computational models of rivalrous perception, mutual inhibition simultaneously influences two distinct properties of rivalry. On the one hand, interocular inhibition is critical for instantiating the competition between eyes for visual awareness (Klink,

Brascamp, Blake, & Van Wezel, 2010; Said, Egan, Minshew, Behrmann, & Heeger, 2013) and decreased inhibition implies increased periods of nonrivalrous mixed perception, that is, periods where neither eye dominates perception but the two images mix into a fused percept (Robertson, Ratai, & Kanwisher, 2016). In contrast, increased inhibition is related to longer durations of dominance phases, i.e. periods during which the image in either eye completely dominates perception. This finding is supported by magnetic resonance spectroscopy (MRS) evidence in humans, indicating that higher levels of the inhibitory neurotransmitter gamma-amino butyric acid (GABA) in the occipital cortex is related to longer dominance phases (Pitchaimuthu et al., 2017b; Van Loon et al., 2013); but see (Gallagher & Arnold, 2014; Song, Sandberg, Andersen, Blicher, & Rees, 2017). A recent study (Mentch et al., 2019) specifically supported the hypothesis that two distinct forms of GABAergic inhibition may be differently involved in regulating these two rivalry properties: mixed percepts and dominance phase durations.

Intracortical GABAergic inhibition also affects other visual tasks besides rivalry (Cook, Hammett, & Larsson, 2016; Frangou et al., 2019; Van Loon et al., 2013). In particular, one fundamental perceptual mechanism relying on inhibition is surround suppression, whereby visual processing of one stimulus is affected—through competitive interactions—by the stimuli in the surrounding visual field. Surrounding inhibition may modify the quality of perception, for example, by inducing a shift of perceived orientation of a target grating, which is tilted away from the orientation of the

surrounding grating, the so-called tilt illusion (Clifford, 2014). Theoretical models of the tilt illusion suggest a neural mechanism (or mechanisms) that involves inhibition in V1 (Blakemore, Carpenter, & Georgeson, 1970; Blakemore, Muncey, & Ridley, 1973; Clifford, Wenderoth, & Spehar, 2000; Schwartz, Sejnowski, & Dayan, 2009; Seriès, Lorenceau, & Frégnac, 2003; Solomon & Morgan, 2006). Furthermore, this phenomenon has been linked to GABAergic inhibition through MRS in humans (Cook et al., 2016; Pitchaimuthu et al., 2017b; Schallmo, Sponheim, & Olman, 2015; Seymour, Stein, Clifford, & Sterzer, 2018; Song et al., 2017).

Given this link between homeostatic plasticity and GABA, in the first paper presented in Chapter 2, we investigated whether the interindividual variability on short-term plasticity can be explained, at least partially, by other perceptual tasks strongly mediated by intracortical inhibitory circuitry.

1.3 State-like variability in short-term visual deprivation: the role of metabolism

1.3.1 Visual Plasticity and metabolism

A growing body of literature supports a hitherto unexpected role of metabolism and gastro-intestinal activity on brain function and overall behaviour (Mattson, 2012; Mattson, Moehl, Ghena, Schmaedick, & Cheng, 2018). Many studies suggest that metabolism and diet profoundly affect brain function – not only mood regulation and

high-level cognition, but even neuronal plasticity and plasticity-dependent cognitive functions (Longo & Mattson, 2014; Mattson, 2012; Mattson et al., 2018; Murphy, Pereira-Dias & Thuret, 2014).

In animal models, metabolic manipulations have been shown to promote plasticity in primary visual cortex, where they might re-open a "critical period" for cortical reorganization in adulthood. For example, protocols of caloric restriction have been effective in restoring ocular dominance plasticity in the adult visual cortex of rats (Spolidoro et al., 2011), promoting a complete recovery from amblyopia. These effects were accompanied by a reduction of intracortical GABAergic inhibition. Further studies confirmed that dietary restrictions can affect neuronal response properties and GABA synthesis also in cats' primary visual cortex (Yang et al., 2016).

In adult humans, little is known about a possible impact of metabolic variables on visual plasticity. However, recent evidence reports that short-term monocular deprivation, eliciting homeostatic plasticity, might be dependent on subjects' metabolic state.

A recent study by Lunghi et al. (Lunghi, Daniele, et al., 2019), has shown that short-term visual plasticity is severely blunted in subjects with body mass index (BMI) > 40 kg/m2. The amount of residual plasticity presents a strong correlation with each's subject BMI, with monocular deprivation effects progressively declining with increasing BMI to a complete absence in subjects with morbid obesity. The authors discuss the possibility that intracortical excitation/inhibition balance might be altered

in obese individuals in favor of a stronger GABAergic inhibition (Lunghi, Daniele, et al., 2019) .

In a further study, the marked reduction in visual cortical plasticity present in obese subjects was reversed after bariatric surgery, reaching values commonly found in healthy participants (Daniele et al., 2021).

As GABA is also involved in mice eating behavior (Avena, Bocarsly, Murray, & Gold, 2014) and high-fat diet are associated with decreased GABA concentration in mice frontal cortex and hippocampus (Sandoval-Salazar, Ramírez-Emiliano, Trejo-Bahena, Oviedo-Solís, & Solís-Ortiz, 2016), the authors speculate that changes occurring with body-weight reduction can interfere with the GABAergic modulation in the visual cortex and, possibly, in other areas of the brain.

Interestingly, the improvement of insulin sensitivity brought up by body-weight reduction was strongly associated with the improvement of the brain plasticity index, and a positive correlation between post–pre-RYGB changes in AUC active GLP−1 and short-term plasticity was observed.

Taken together, these results suggest that short-term ocular dominance plasticity can be affected by metabolic states; however, studies have only considered the effects of metabolism over prolonged periods of times (dietary restrictions; obesity etc). Moreover, the mechanisms linking the observed changes in ocular dominance plasticity and the metabolic changes remain speculative. A possible way to

disentangle the complex role of metabolism on different brain functions is to include healthy participants and to use simpler paradigms.

For example, there is a limited literature examining the effects of transient reduced caloric intake, such as overnight fasting, on plasticity. Short-term metabolic manipulations are less understood compared to more complex dietary protocols or diagnosed pathologies. However, they could allow one to rule out an entire set of possible mechanisms through which diets or long-term medical conditions can modulate neuroplasticity, as anti-inflammatory, neurogenetic and neuroprotective mechanisms (Mattson et al., 2018) .

Breakfast skipping is one of the more commonly studied types of meal abstention (Benau, Orloff, Janke, Serpell, & Timko, 2014; Peña-Jorquera et al., 2021). Its effects are typically assessed in children, adolescents, and young adults and in the cognitive domains (Benau et al., 2014; Peña-Jorquera et al., 2021). A recent meta-analysis has shown that, in humans, short-term fasting has effects on the activity of brain areas involved in memory tasks (Benton & Parker, 1998; Chechko et al., 2015; Green, Elliman, & Rogers, 1997; Okauchi et al., 2020; Owen, Scholey, Finnegan, Hu, & Sünram-Lea, 2012; Sünram-Lea, Foster, Durlach, & Perez, 2001) perceptual abilities (Pender, Gilbert, & Serpell, 2014; Zitron-Emanuel & Ganel, 2018, 2020) verbal learning (Sünram-Lea et al., 2001), attention (Anderson, Hawkins, Updegraff, Gunstad, & Spitznagel, 2018; Komiyama et al., 2016; Solianik, Žlibinaitė, Drozdova-

Statkevičienė, & Sujeta, 2020), and psychomotor functions (Cherif et al., 2017). However, findings are mostly inconsistent and there is a lack of consensus regarding the possible underlying mechanisms.

In the third chapter of the present work, we present a study in which we assessed the possible impact of breakfast skipping, compared to having a meal, on short-term monocular deprivation. This paradigm also offered the opportunity to directly test the effects of incretins - one of the key players in glucose metabolism that was found to correlate with plasticity effects in morbid obese participants. Incretins (GLP1) are normally released with every meal as they take part in the regulation of insulin response (Alvarez et al., 2005). GLP-1 effects involve the modulation of high-cognitive process', as sense of satiety and food seeking behavior (De Silva et al., 2011; Gutzwiller et al., 1999; Verdich et al., 2001) and extend to basic physiological mechanisms, including hippocampal LTP (Gault & Hölscher, 2008; Ming Wang, Yoon, Song, & Jo, 2021). A very recent study (Binda et al., 2019) has further shown that GLP-1 infusion can directly modulate visual response in visual cortex, and GLP-1 levels were correlated with plasticity re-acquirement in morbidly obese subjects.

1.3.2 Brain metabolism and ketone bodies

Food intake or small fasting trigger a variety of responses, that are not limited to factors involved in glucose metabolism. Among all the possible metabolic factors

having an impact on brain function and plasticity, a key role may be played by ketone bodies.

Under normal physiological circumstances, the brain primarily relies on glucose afflux and exploits glucose oxidation to produce energy (Owen et al., 1967). Since only a limited amount of glycogen is stored in neuronal cells, a continuous intake of glucose is needed for normal brain functioning (Bak & Walls, 2018). If glucose supplies become insufficient, i.e. during prolonged fasting, other substrates support ATP production (Owen et al., 1967). Ketone bodies represent the main alternative substrate. Ketogenesis mainly occurs in hepatocytes and present a rate-limiting reaction catalyzed by the HMGCS2 enzyme, regulated by insulin and glucagon (Puchalska & Crawford, 2017). The three final substrates are Acetone, Acetoacetate and beta-hydroxybutyrate (**β**HB), the last one being the most abundant (~80%) ketone body in the human circulation (Newman & Verdin, 2017).

Once produced, Acetoacetate and **β**HB are not utilized in the liver, but released in the blood stream for extra-hepatic metabolism (Jensen, Wodschow, Nilsson, & Rungby, 2020). Plasma ketone levels are usually low after an overnight fast (<0.5 mM) and contribute to less than 5% of the brain's metabolism (Jensen et al., 2020). However, during prolonged fasting, their levels can rise dramatically and are able to contribute almost 60% of brain's energy requirement (Jensen et al., 2020).

Ketone bodies can cross the blood–brain barrier through monocarboxylate transporters (MCTs) in endothelial cells and astroglia (Pierre & Pellerin, 2005). Once in the brain, ketone bodies are metabolized in a concentration-dependent manner (Jensen et al., 2020).

Ketonemia can be achieved also without fasting, through ketogenic diets or the ingestion of supplements in the form of ketogenic medium-chain fatty acids (MCFA) or exogenous ketone esters or salts (Stubbs et al., 2017).

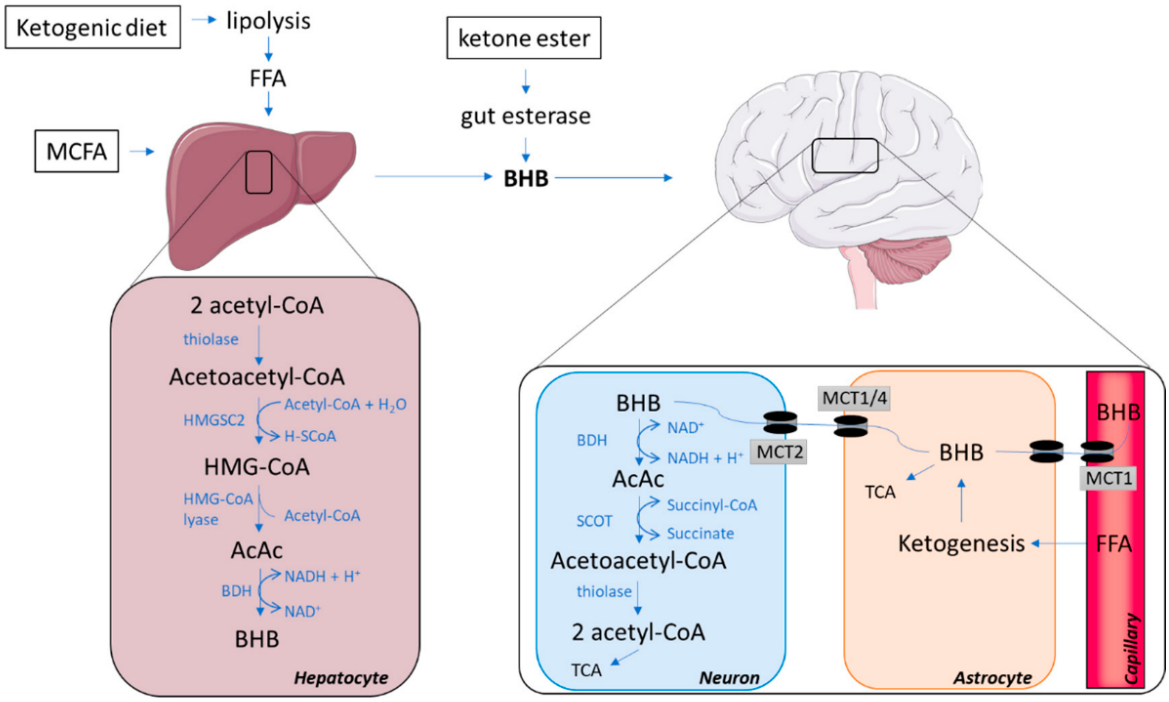

Figure 1.3. Pathways involved in synthesis and catabolism of ketone bodies. AcAc, acetoacetate; Acetyl-CoA, acetyl coenzyme A; BHB, beta-hydroxybutyrate; BHD, beta-hydroxybutyrate dehydrogenase; FFA, free fatty acids; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGCS2, 3-Hydroxy-3-Methylglutaryl-CoA Synthase 2; MCFA, medium-chain fatty acids; MCT, monocarboxylate transporter; SCOT, succinyl-CoA:3-ketoacid Coenzyme A transferase; TCA, tricarboxylic acid cycle. (Jensen et al., International Journal of Molecular Sciences, 2020)

During the last decade, the interest in ketogenic diets and other ketogenic treatments has increased rapidly, as a growing body of literature on animal models supports the

idea that ketone bodies might modulate cortical excitability, through several possible mechanisms.

A first study in rats reported that acetoacetate and **β**-hydroxybutyrate (**β**HB) increased the accumulation of GABA in presynaptic vesicles (Erecińska, Nelson, Daikhin, & Yudkoff, 1996). **β**HB was shown to alter the aspartate-to-glutamate ratio by driving the aspartate aminotransferase reaction (specifically, by decreasing the transamination of glutamate to aspartate) such that glutamate decarboxylation to GABA is increased (Daikhin & Yudkoff, 1998). Furthermore, ketotic rats exhibit lower levels of glutamate; since GABA is synthesized from glutamate, this indicates a greater proportion of glutamate may be converted to GABA in neurons, and thereby shift the total balance of these neurotransmitters towards inhibition (Melo et al., 2006). However, direct physiological evidence for this mechanism is still missing (Lund, Obel, Risa, & Sonnewald, 2011; Lund, Risa, Sonnewald, Schousboe, & Waagepetersen, 2009; Valente-Silva, Lemos, Köfalvi, Cunha, & Jones, 2015; Yudkoff, Daikhin, Nissim, Lazarow, & Nissim, 2001). This GABAergic hypothesis of ketone body action has not been reconciled with the fact that patients with epilepsy who were refractory to GABAergic medications often respond to the ketogenic diets (Freeman, Veggiotti, Lanzi, Tagliabue, & Perucca, 2006).

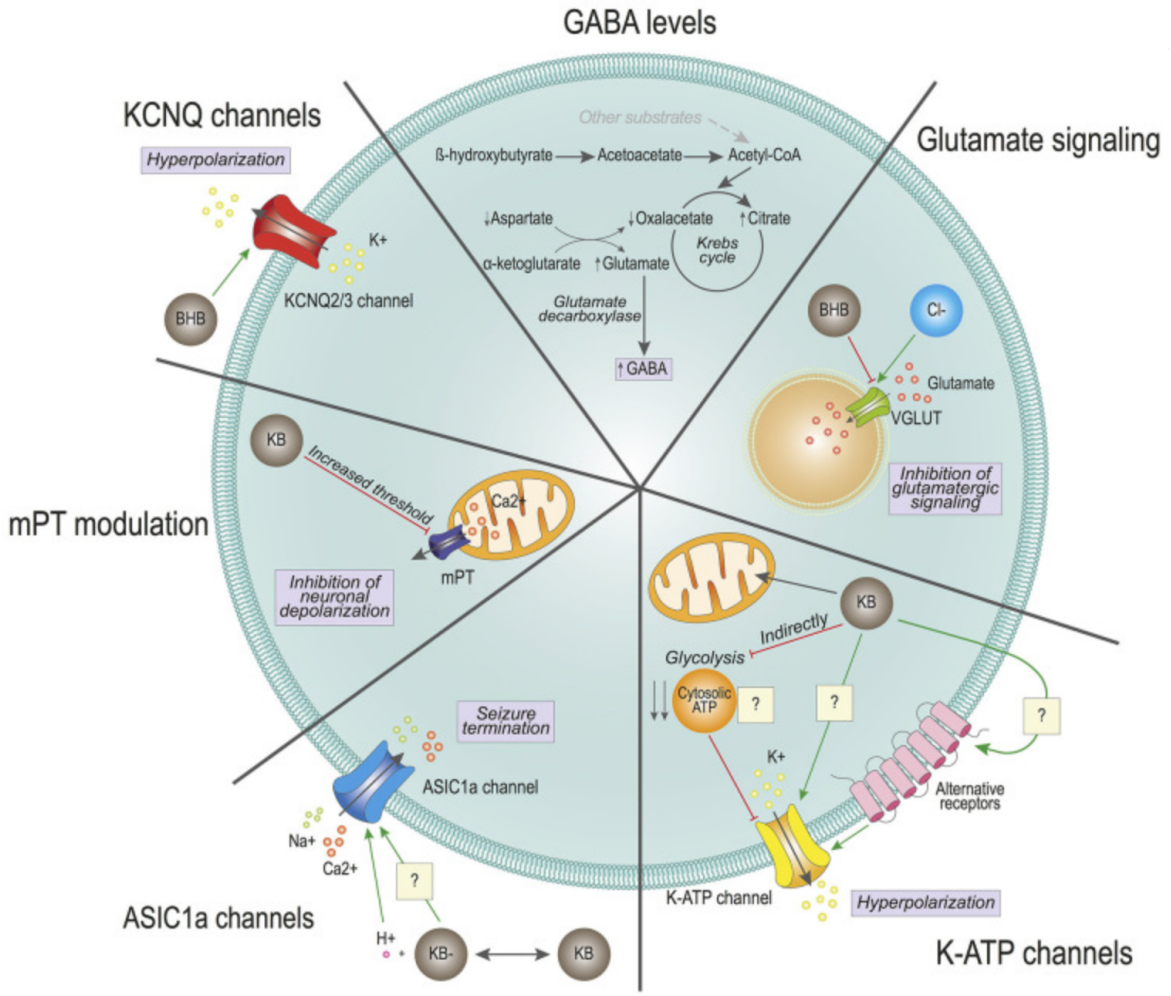

Figure 1.4. Effects of ketone bodies on cells' excitability. The proposed mechanisms for ketone bodies' (KBs) action on neuronal excitability are depicted. GABA levels: KB ?-hydroxybutyrate (BHB) and acetoacetate are converted into Acetyl-CoA at a faster rate than with other substrates, which enters the Krebs cycle reducing the levels of oxaloacetate. To replenish the Krebs cycle, aspartate is converted to oxaloacetate, generating high levels of glutamate. Through the glutamate decarboxylase of GABAergic neurons, glutamate is converted into GABA, increasing the intracellular GABA pool. Glutamate signaling: BHB competes with chloride (Cl-) for the allosteric binding site of the vesicular glutamate transporter (VGLUT). The competition reduces the levels of glutamate inside the vesicles and reduces glutamatergic signaling. K-ATP channels: Ketone bodies (KBs) enter directly into the mitochondria, without generating cytosolic ATP. The lack of cytosolic ATP could provoke the activation of potassium ATP-sensitive (K-ATP) channels, causing the hyperpolarization of the cell. K-ATP channels may also be modulated directly by KBs or indirectly through the activation of alternative receptors. ASIC1a channels: KBs generate a local decrease in pH, which activates the acid sensing ion channel (ASIC1a). These channels participate in seizure termination. KBs may also directly modulate the ASIC1a. KCNQ2/3 channels: BHB directly activates KCNQ channels, which generate a potassium current. This potassium current causes the hyperpolarization of the cell. KBs may also regulate neuronal excitability by participating in mitochondrial permeability transition (mPT) and subsequent oscillations in cytosolic calcium levels. (D.Garcia-Rodriguez et al, Frontier in Molecular Neurosciences, 2021)

Other studies reported that ketone bodies reversibly inhibit glutamate release (Juge et al., 2010). A recent study has shown increased synaptic efficiency, low-theta band oscillations, and learning consolidation during intermittent-fasting-induced ketosis linked to increased expression of the *N*-methyl-D-aspartate (NMDA) receptor for glutamate instead (Fontán-Lozano et al., 2007).

Another hypothesis is that ketone bodies might impact neuronal excitability independently from neurotransmitter production, through enhancing ATP production and cell's metabolic efficiency (Poff, Rho, & D'Agostino, 2019). Hence, the change in the ATP/ADP ratio could influence the activity of ATP-sensitive potassium (K_{ATP}) channels and thus alter neuron membrane potential. Yellen and colleagues (Ma, Berg, & Yellen, 2007) showed that ketone bodies, and especially **β**HB, were able to decrease the spontaneous firing rate of GABAergic interneurons in the substantia nigra pars reticulata trough K_{ATP} channels and $GABA_B$ receptors. More recently, Tanner et al. demonstrated that **β**-hydroxybutyrate enhances K_{ATP} channel opening in cultured mouse hippocampal neurons (Tanner, Lutas, Martínez-François, & Yellen, 2011).This evidence, together with the fact that K_{ATP} channels are a well-known mechanism for rapid coupling between metabolism and electrical excitability, give a possible and clear link between ketone bodies and the inhibition/excitation ratio in brain cortices (García-Rodríguez & Giménez-Cassina, 2021; Pflanz, Daszkowski, James, & Mihic, 2019; Yellen, 2008).

One study in humans has specifically studied the impact of ketogenic diet on brain metabolites of epileptic patients, using magnetic resonance spectroscopy (Wang et al., 2003). The authors show a GABA increase in parietal lobes and adjacent brain tissue. Despite the small sample size of the study, these results are in line with the animal literature discusses before.

On the other hand, another study on epileptic patients undergoing ketogenic diets has focused mainly on the bioenergetics hypothesis, using [^{31}P] MR spectroscopy (Pan, Bebin, Chu, & Hetherington, 1999). With both an increase in PCr/Pi and PCr/y-ATP, these data suggest that ketogenic diet induces an increase in grey-matter PCr; by virtue of the near equilibrium of the creatine kinase reaction, this implies that the $\Delta G_{ATP\ hydrolysis}$ should effectively increase. Based on this, the authors speculate that an ATP increase would manifest physiologically through a hyperpolarization of membrane resting state potentials.

Interestingly, a very recent study has investigated high energy phosphate content, that is, adenosine triphosphate (ATP) and phosphocreatine (PCr) in cerebral cortex and skeletal muscle of a conspicuous group of healthy and obese participants. Using [^{31}P] MR spectroscopy, the authors measured these metabolites at baseline and repeatedly on induction of hypoglycemia, finding a strong inverse correlation between PCr and BMI, i.e. subjects with lowest BMI are those showing higher PCr contents, and clear rise in PCr/Pi ratio (Schmoller et al., 2010).

An alternative to the ketogenic diet in raising plasma ketone levels in humans is the intake of exogenous ketone esters and salts. These supplements significantly increase ketone levels to >1 mM post ingestion, with the ketone ester being most potent in raising circulating ketones even while consuming regular meals (Stubbs et al., 2017).

Ketone esters have been found to be safe and well tolerated in humans (Clarke et al., 2012a), and offer the unique opportunity to study the effect of ketone bodies themselves, without many of the confounding factors related to a long-term dietary change. Moreover, exogenous ketosis allows researcher to accurately set and monitor blood **β**HB concentrations (Soto-mota, Norwitz, & Clarke, 2020).

Although remarkable progress in our knowledge on the biological effects and mechanisms of action of exogenous ketone supplements, their mechanisms of action on human CNS are largely unknown.

A recent human study suggests a direct effect of **β**HB ketone ester on brain function. Mujica-Parodi et al (2020), have shown that both 1 week of ketogenic diet and acute 1-shot administration of exogenous ketones had stabilizing effects on cortical networks. (Mujica-Parodi et al., 2020). We define as being part of the same networks those brain regions that present synchronous activity. The stability of a network is the degree to which this synchronous activity persists over time; a network can be unstable in many ways: for example, it can keep changing its topology (network switching). The authors hypothesized that enhanced network stability,

neurobiologically, may reflect the altered energy balance of brain's cells, that subsequently 'reroute' their paths towards networks with lower metabolic cost. Interestingly, network stability has previously been correlated with GABA levels in brain cortices through MRS studies, and GABA levels are known to modulate different brain functions, including visual cortical plasticity, in adult humans. (Stagg et al., 2014).

The rapid response to ketone ester salt effectively ruled out indirect inflammatory, antioxidant, tau/amyloid, and/or adaptive mitochondrial mechanisms of action, allowing them to isolate a more straightforward role of diet on metabolism. This rapid effect is also in line with the opposite consideration that epileptic patients in KD diets eating sweets might have a sudden seizure relapse (Yellen, 2008).

Taken together, this evidence suggest that ketone bodies might have a direct impact on cortical excitation-inhibition balance. In our last study, we used the visual cortex as a model to investigate the direct impact of acute ketosis on neural processing and neuro-metabolites, testing the hypothesis that it might directly increase GABA levels and enforcing inhibition.

1.4 Aim of and overview of the thesis

In the thesis, I aim to explore the mechanisms underlying the wide range of adult human inter-individual variability in short-term visual plasticity.

After exploring ocular dominance plasticity trait-like variability, that is, how this homeostatic effect relates with other psychophysical parameters, a large part of the focus will be on how metabolic variables, or state-like variables, can impact visual plasticity and visual processing.

The main questions are: why every subject seems to have a different response in short-term monocular deprivation? is there a way to predict, behaviorally, if a subject will be plastic? Is a short-term monocular deprivation outcome just related to every single subject visual circuit, or are there other, extra-brain factors, having an impact?

In the first chapter, I present a study about inter-individual variability response in short-term deprivation and how it relates to binocular rivalry itself and other psychophysical illusions. I focused on the pioneering work of Lunghi et al (Lunghi, Emir, et al., 2015), in which monocular deprivation reduce GABA levels in the visual cortex; for this reason, in our work we used psychophysical stimuli that have been also related to GABAergic inhibition in visual cortex. If inhibition is the key mechanism that regulates plasticity, can I predict the final patching outcome trough different tests that have singularly been linked to GABA levels in visual cortex?

I then moved to a rather unexplored field, that is, the effect of state-like, metabolic variables, on short-term visual plasticity.

In the third chapter, I investigated whether short-term monocular deprivation might be sensible to simple metabolic manipulations, as an overnight fasting compared to having a meal, and to a molecule well known for its effects on brain functioning, that

is, GLP-1. These results clearly show that part of the variance in the final plasticity effect, besides being determined by single subjects' neural circuitries, also depend on his actual metabolic state. We found that a prolonged overnight fasting, compared to having a meal, reduces plasticity, and this reduction is tightly linked with ketone bodies levels in blood plasma. Given these results, we decided to assess more precisely the role of ketone bodies on visual processing. In the fourth chapter I present the preliminary data of a study designed in cooperation with the Endocrinology team of Azienda Ospedaliera-Universitaria di Pisa. We simulated ketone bodies fasting blood plasma levels in healthy participants through a ketone-ester drink. Then, we performed MR and MRS measurements to detect neuro-metabolites changes (especially GABA), and EEG measures sensitive to cortical excitation/inhibition ratio, before and after the drink. Our preliminary results show that **β**HB levels in the blood after an oral administration of ketone ester can reliably be enhanced up to fasting levels; moreover, we show very promising EEG changes related to GABA levels in visual cortex, and brain metabolites changes possibly related to the new energetic balance in the neuronal cells'.

2 Chapter

Using psychophysical performance to predict short-term Ocular Dominance plasticity in human adults

2.1 Introduction

As mentioned in the main introduction, binocular rivalry is a classic example of bistable perception, where visual awareness oscillates between two percepts (e.g. clockwise and counter-clockwise oriented gratings) in spite of constant and incoherent stimulation of the two eyes (e.g. clockwise to the left eye and counter-clockwise to the right eye). Much research has been dedicated to identifying the mechanisms leading to the perceptual switches. A major role is typically assigned to reciprocal inhibitory interactions across neuronal populations encoding each eye's percept, at multiple stages of visual processing, (Blake, 1989; Laing & Chow, 2002; Li et al., 2017; Logothetis et al., 1996; Noest et al., 2007; Said & Heeger, 2013; Seely & Chow, 2011; Tong et al., 2006; Van Loon et al., 2013) – and similar models apply to other bistable/rivalrous phenomena, such as structure-from-motion rivalry (Brascamp et al., 2018; Van Loon et al., 2013) but see (Gallagher & Arnold, 2014; Sandberg et al., 2016).

In formal rivalry models, mutual inhibition simultaneously influences two distinct properties of rivalry. On the one hand, interocular inhibition is critical for instantiating the competition between eyes for visual awareness (Klink et al., 2010; Said et al., 2013) and decreased inhibition implies increased periods of nonrivalrous mixed perception, that is, periods where neither eye dominates perception but the two images mix into a fused percept (Robertson et al., 2016). In contrast, increased inhibition is related to longer durations of dominance phases, i.e. periods during

which the image in either eye completely dominates perception. This finding is supported by magnetic resonance spectroscopy (MRS) evidence in humans, indicating that higher levels of the inhibitory neurotransmitter gamma-amino butyric acid (GABA) in the occipital cortex is related to longer dominance phases (Pitchaimuthu et al., 2017b; Van Loon et al., 2013); but see (Gallagher & Arnold, 2014; Song et al., 2017). A recent study (Mentch et al., 2019) specifically supported the hypothesis that two distinct forms of GABAergic inhibition may be differently involved in regulating these two rivalry properties: mixed percepts and dominance phase durations.

Intracortical GABAergic inhibition is not only instrumental in modulating interocular suppression or summation, but it also affects selectivity in many other dimensions (Cook et al., 2016; Frangou et al., 2019; Van Loon et al., 2013). In particular, one fundamental perceptual mechanism relying on inhibition is surround suppression, whereby visual processing of one stimulus is affected—through competitive interactions—by the stimuli in the surrounding visual field. Surrounding inhibition may modify the quality of perception, for example, by inducing a shift of perceived orientation of a target grating, which is tilted away from the orientation of the surrounding grating, the so-called tilt illusion (Clifford, 2014). Theoretical models of the tilt illusion suggest a neural mechanism (or mechanisms) that involves inhibition in V1 (Blakemore et al., 1970, 1973; Clifford et al., 2000; Schwartz et al., 2009; Seriès et al., 2003; Solomon & Morgan, 2006). Furthermore, this phenomenon has been

linked to GABAergic inhibition through MRS in humans (Cook et al., 2016; Pitchaimuthu et al., 2017b; Schallmo et al., 2015; Seymour et al., 2018; Song et al., 2017).

Recent evidence indicates that inhibitory GABAergic signaling also plays a critical role in setting the level of neuroplasticity in the visual cortex, both during development and in the adult brain. Neuroplasticity refers to the capability of neural networks to change and adapt to the external environment (Berardi et al., 2000; Scheyltjens & Arckens, 2016). This property is maximal during early childhood, when sensory systems gradually organize and fine tune in response to environmental inputs (Berardi et al., 2000; Hubel & Wiesel, 1970; Hubel et al., 1977; Wiesel & Hubel, 1963) Abnormal visual experience in this early critical period can produce long-lasting structural and functional changes. For example, monocular deprivation during the critical period leads to long-lasting suppression of the deprived eye, a medical condition called amblyopia (Berardi et al., 2000; Levi, McKee, & Movshon, 2011; Maurer et al., 2005; Ostrovsky et al., 2006) These forms of neuroplasticity decrease with age, in parallel with the development of intracortical inhibitory signaling, particularly GABAergic (Bono & Clopath, 2019; Spolidoro, Sale, Berardi, & Maffei, 2009). Consequently, when deprivation occurs in the adult individual, long-lasting effects are not usually seen.

However, mounting evidence suggests that the adult sensory cortex does retain a degree of plasticity (Baseler et al., 2002; Wandell & Smirnakis, 2009). Functional changes have been observed with perceptual learning (Dosher & Lu, 2017; Karni & Sagi, 1991, 1993; Watanabe & Sasaki, 2015), and short-term visual deprivation (Binda & Lunghi, 2017; Kwon, Legge, Fang, Cheong, & He, 2009; Lunghi, Berchicci, et al., 2015; Lunghi et al., 2013, 2011a; Zhang, Bao, Kwon, He, & Engel, 2009; Zhou et al., 2013; Zhou, Reynaud, & Hess, 2014). Studies on adult human plasticity have used a variety of visual manipulations; (Zhang et al., 2009) showed that a few hours deprivation of one cardinal orientation leads to enhanced sensitivity to the deprived orientation—the opposite effect observed during development when the deprived orientation response is suppressed (Blakemore & Campbell, 1969). Similarly, 2 hours of monocular contrast deprivation is followed by a transient boost of the deprived eye (Binda & Lunghi, 2017; Lunghi, Berchicci, et al., 2015; Lunghi et al., 2013, 2011a; Lunghi, Emir, et al., 2015; Zhou et al., 2013, 2014) —opposite to the amblyopia induced by monocular deprivation during the critical period. Importantly, in amblyopic patients, the transient boost is accompanied by an improvement in visual acuity that persists for months after patching (Lunghi, Sframeli, et al., 2019), suggesting that this form of homeostatic plasticity can be conducive to other more permanent system rearrangements.

Despite the well-established replicability of the effect (Binda et al., 2018; Binda & Lunghi, 2017; Lunghi, Berchicci, et al., 2015; Lunghi et al., 2013, 2011a; Lunghi, Emir,

et al., 2015; Wang et al., 2017; Zhou et al., 2013, 2014) the response to short-term deprivation presents a high level of interindividual variability, possibly reflecting an individual susceptibility to neuronal plasticity. Interestingly, this variability has been related to cortical inhibitory mechanisms, given the strong correlation between the transient boost of the deprived eye and the decrease in GABA concentration in occipital cortex measured by MRS (Lunghi, Emir, et al., 2015).

Given this link between homeostatic plasticity and GABA, we investigate here whether the interindividual variability on short-term plasticity can be explained, at least partially, by other perceptual tasks strongly mediated by intracortical inhibitory circuitry. We measured interindividual variability in a relatively large group of participants, ocular dominance short-term plasticity, binocular rivalry, structure from motion rivalry, and tilt illusion induced by surround context.

2.2 Methods

2.2.1 Human participants

Experimental procedures were approved by the regional ethics committee (Comitato Etico Pediatrico Regionale—Azienda Ospedaliero-Universitaria Meyer—Firenze; protocol "Plasticita' del Sistema visivo") and are in line with the declaration of Helsinki. Written informed consent was obtained from each participant, which included consent to process and preserve the data and publish them in anonymous form.

We recruited 54 volunteers (40 females, 14 males; mean age, 26.5 ± 3.5 years) who were selected to have (i) normal or corrected-to-normal vision and normal color vision, (ii) no known history of amblyopia, eye surgery, or other active eye disease (such as keratoconus), (iii) typical ocular dominance balance measured with binocular rivalry (before deprivation). We included all participants with a ratio of mean phase durations of the dominant over the nondominant eye smaller than 0.2 log units.

2.2.2 Experimental design and procedure

Each participant reported to our laboratory at 9.00 a.m. (after breakfast) and performed three psychophysical tasks: tilt illusion, structure from motion, and binocular rivalry. Owing to a technical failure, data from four participants in the structure from motion and tilt illusion tasks are not available. Immediately after these baseline measures, a subset of 34 participants agreed to undergo a procedure of short-term monocular deprivation described elsewhere in this article.

2.2.2.1 Stimuli and apparatus

Tilt illusion: The tilt illusion stimulus was generated with PsychoPhysics Toolbox routines for MATLAB and presented at a distance of 57 cm on a Sony Trinitron Multiscan E230 linearized monitor (1024 × 768 pixels, 100 Hz) by a Macbook Pro (OSX El Capitan, 10.11.6). The stimulus comprised a centrally presented target grating (diameter: 1.2° of visual angle, or deg) of variable orientation, surrounded by an annular grating (diameter 2.88°) with the same spatial frequency (2 cpd) and contrast

(21% Michelson). The target grating and the surround annulus differed in orientation by either –15° or +15° and could be presented around ±45°. To minimize the impact of vertical/horizontal edges on orientation judgments, the stimulus was shown at the center of a mid-level grey disk (diameter 21.3°), overlaid with a small white fixation point (diameter 0.15°).

The target and surround were presented simultaneously within a temporal Gaussian window of 100 ms full width at half maximum. Three seconds after the stimulus offset, observers performed a two-alternative forced choice task, reporting the orientation of the target (more vertical or horizontal, ignoring the surround). Participants completed 120 trials, divided into four blocks. For the first 12 trials, target orientation was chosen between 33° and 57° (where smaller values indicate more vertical stimuli). For the rest of the trials, the target orientation was determined by fitting two independent Gaussian functions (one per surround orientation) and homing in on the median of each curve using two independent adaptive procedures.

The median of the Gaussian fit to the complete dataset (60 trials per surround orientation) estimated the point of subjective equality to 45 deg, that is, the target orientation that is reported as closer to vertical on 50% of trials. The distance between the point of subjective equality to 45 deg for the two surround orientations was taken as the magnitude index of the tilt illusion. Positive values indicate a repulsive effect of the surround orientation over the perceived target orientation.

Structure from motion: The apparatus for generating the structure from motion stimulus was the same as for the tilt illusion stimulus. The structure from motion stimulus was made of 300 dots (diameter 0.3°), randomly placed within a rectangular aperture (8° wide and 14° tall) shown at screen center and overlaid with a red 0.15° fixation mark. One-half of the dots were black (0.08 cd/m^2) and moved rightwards; the rest were white (68 cd/m^2) and moved leftwards, both with a linear velocity profile that followed a cosine function, peaking at 3.9°/s at screen center.

The resulting stimulus could be perceived as a cylinder rotating about its vertical axis at 60°/s (taking 6 seconds to complete a revolution), with bistable direction: clockwise (with white dots forming the front surface, and black dots forming the rear one) and counter-clockwise (with black dots forming the front surface). A third possibility was a mixed percept, where neither clockwise nor counter-clockwise coherent percept dominated perception exclusively. The stimulus was played for 10 trials of 59s each (and 1-second intertrial intervals) and observers continuously reported their perception by pressing one of the three keys, corresponding with clockwise, counter clockwise, and mixed percepts.

Binocular rivalry: Stimuli for binocular rivalry were two luminance contrast modulated Gaussian-vignetted sinusoidal gratings (orientation: ±45°, **σ**: 2°, spatial frequency: 2 cpd, contrast: 50%), presented dichoptically on a uniform equiluminant background, with a binocular central black fixation point and a squared black frame to facilitate fusion. Visual stimuli were generated by the ViSaGe (CRS, Cambridge Research

Systems, Rochester, UK) housed in a PC (Dell) controlled by MATLAB programs and displayed on a linearized monitor (Barco CDCT 6551, 800 × 600 pixels, 140 Hz). Dichoptic presentation was achieved through CRS ferromagnetic shutter goggles (Cambridge Research Systems) that alternated the two gratings at each frame. Observers had their head stabilized with a chin rest and viewed the display at a distance of 57 cm.

Binocular rivalry was measured in two short sessions (each comprising two runs of 3 minutes each), immediately before and after the short-term monocular deprivation. During each trial, participants continuously reported their perception (clockwise, or +45° oriented grating, counter-clockwise, or –45° oriented grating, and mixed) by pressing one of three keys. Participants were instructed to classify the stimuli as mixed when none of the two gratings appeared to clearly dominate perception. Each participant was given ample opportunity to practice the task (associating the appropriate response key to each monocularly presented stimulus, and familiarizing with the binocular rivalry phenomenon) before starting the experiment.

Monocular deprivation: Monocular deprivation was achieved by patching the dominant eye, defined as the eye with longer mean phase duration in binocular rivalry, for 2 hours. Dominance was also confirmed using the Porta test (Lunghi, Berchicci, et al., 2015).

As in previous studies (Binda & Lunghi, 2017; Lunghi et al., 2013, 2011a) the eye patch was made of a translucent plastic material that allowed light to reach the retina (attenuation 15%), but no pattern information, as assessed by the Fourier transform of a natural world image seen through the eye patch. During the 2 hours of monocular deprivation, participants were free to read, work at the computer, or walk around the laboratory (but not to eat or sleep).

To quantify the plasticity effect elicited by monocular deprivation, we computed an ocular dominance index, calculated as in Equation 1, and examined it before versus after deprivation

$$OD = \frac{p_d}{(p_d + p_{nd})}$$

Equation 1

where *p* is the ratio between the time spent seeing with either the deprived (*d*) or nondeprived (*nd*) eye over the total dominance time (i.e., mixed excluded) and *OD* is ocular dominance. In previous work, the plasticity effect was evaluated by comparing the dominance of the deprived eye after deprivation versus the same value before deprivation (Lunghi, Sframeli, et al., 2019). Here we chose to assess it using only data acquired after deprivation to have an index that is statistically independent from measurements of the predeprivation sessions. This difference is important, given that we aimed to correlate the plasticity effect with the predeprivation dynamic parameters, like mixed percept and phase duration. Given that we selected participants with balanced eyes before deprivation, the ocular

dominance index of Equation 1 is very similar to that used in previous research (Lunghi, Daniele, et al., 2019).

2.2.3 Reliability analysis

To assess the reliability and consistency of our dataset, we used a split-half approach. For the tilt illusion, we randomly assigned trials to two subsets and correlated them across participants. For binocular rivalry, we correlated the parameters estimated in the first one-half of the data acquisition with that of the second half. Similarly, for the structure from motion test, we split the ten 60-second-long trials into two sets (the first five in one set, the rest in the other) and correlated them across participants. These correlation coefficients are reported in Table 1, with their associated *p* values and lgBF values, i.e. the base-10 logarithm of the JZS Bayes Factor. For this and the other analyses in the paper, lgBF quantifies the evidence for or against the null hypothesis, with |lgBF| > 0.5 indicating substantial evidence for (negative lgBF) or against (positive lgBF) the null hypothesis.

In addition, we used the Spearman-Brown prediction formula (Spearman, 1904; Spearman, 1910) to transform these coefficients into reliability estimates, reported in the last column of the Table 2.1.

Parameter:	*rho*	*p-value*	*lgBF*	*reliability*
BR mpd pre	*0.85*	*<0.001*	*13.39*	*0.92*
BR % mixed pre	*0.59*	*<0.001*	*3.84*	*0.75*
BR mpd post	*0.81*	*<0.001*	*6.3*	*0.89*
BR % mixed post	*0.72*	*<0.001*	*3.98*	*0.83*
SFM mpd	*0.80*	*<0.001*	*9.41*	*0.89*
Tilt illusion size	*0.64*	*<0.001*	*4.54*	*0.78*

Table 2.1. Reliability analysis

2.3 Results

We measured interindividual variability across 50 healthy adult observers, in three tasks: tilt illusion, structure from motion, and binocular rivalry. Our primary aim was to establish whether performance in any of these tasks could predict the homeostatic plasticity induced by monocular deprivation. In addition, we investigated the associations between the three tasks, measured before the monocular deprivation procedure.

Figures 2.1A and 2.1B show example results for the tilt illusion task: psychometric curves obtained for one participant with a continuously changing target and surround stimuli oriented at ±15° from the target. The perceived orientation of the target was repulsed away from the surround orientation. The repulsive illusory effect was present in all tested participants. The mean orientation difference, 14.7 ± 5.53° (mean ± standard error across participants), was highly significant, $t(49) = -18.92$, $p < 0.001$, lgBF = 21.0. This finding was not associated with any change of steepness of the psychometric functions, $t(49) = 0.67$, $p = 0.508$, lgBF = –0.7. A split-half reliability test

(see Methods and Table 2.1) showed that the internal consistency of the tilt illusion magnitude is 0.78, moderately high.

Figures 2.1C and 2.1D show results for the structure from motion task. For each participant, we normalized phase durations for the two main percepts (clockwise or counter-clockwise rotation) to their mean, then computed a probability density function, which was finally averaged across participants. The mean phase durations were 10.6 ± 1.15 seconds (mean ± standard error of the mean), for the clockwise percept, and 10.6 ± 1.12 seconds for the counter-clockwise percept.

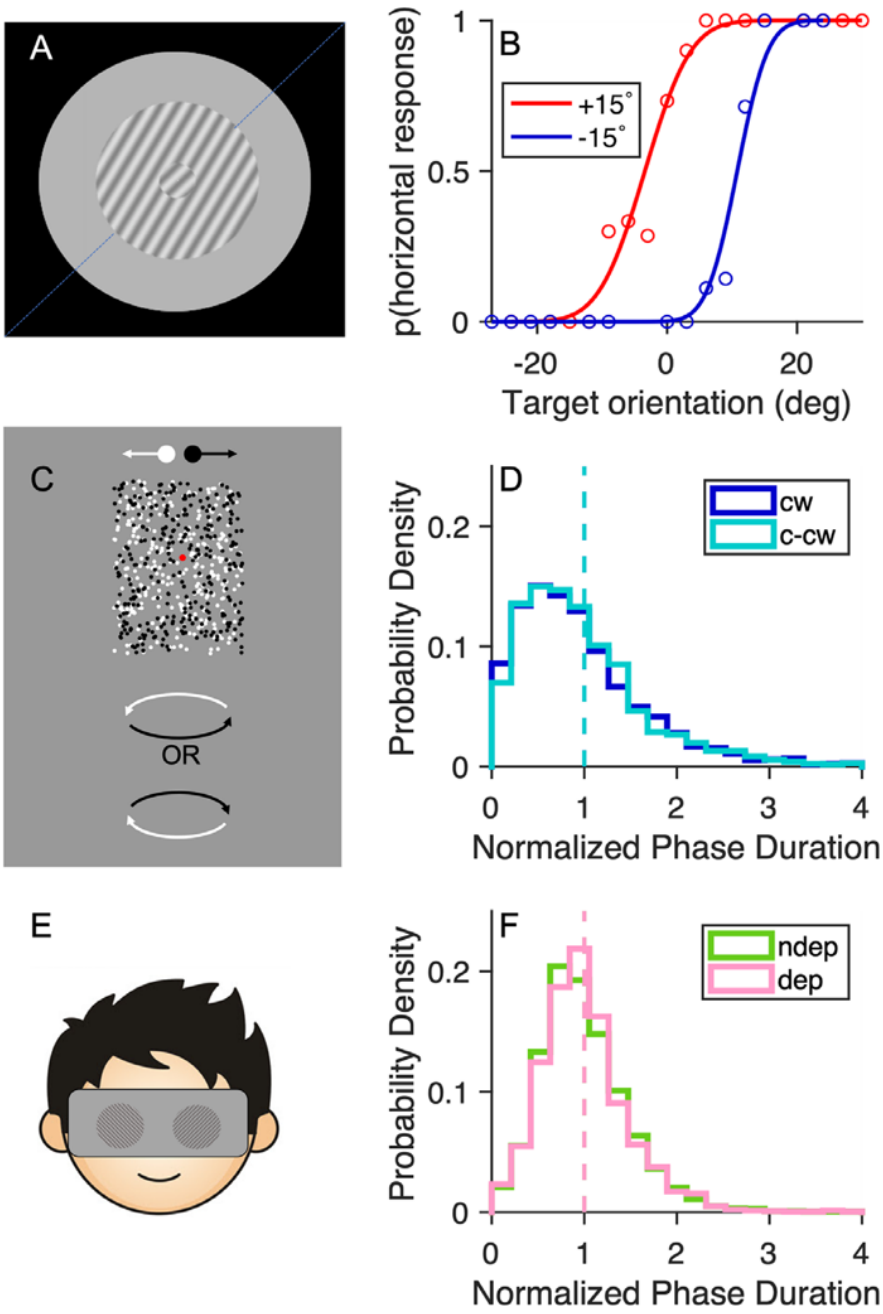

Figure 2.1 The three psychophysical tasks. A, B) Tilt illusion. (A) Stimulus arrangement, showing an example case where the target is oriented at 45° (dashed line) but appears as tilted clockwise owing to the surround (tilted counter-clockwise from 45°). (B) Example of psychometric curves for trials with surround tilted ±15° from the target; the separation of the curves (measured by the difference in their points of subjective equality [PSEs] to 45°) estimates the magnitude of the tilt illusion. (C, D) Structure from motion. (C) Two clouds of white and black dots moving rightwards or leftwards, respectively, could be perceived as a cylinder rotating about its vertical axis, with bistable direction: clockwise or counter-clockwise. (D): probability density function of the normalized phase durations for each percept. The dashed line shows the mean of the distributions, which by definition is equal to 1. E-F) Binocular rivalry. E): stimulus display, made of two orthogonal Gabors presented dichoptically. (F): probability density function of the normalized phase durations for each eye (deprived and non-deprived). Same format as in panel D

Mixed precepts amounted to a small proportion of the total viewing time, 10.81 ± 1.09%. The split-half analysis revealed that mean phase durations had a reliability of 0.89, moderately high.

Finally, Figures 2.1E and 2.1F show the results for the binocular rivalry task. For each observer, we normalized the phase duration for the deprived (pink) and nondeprived eye (green) to their mean; the probability density functions were then averaged across participants, yielding very similar curves for the two eyes. The average phase durations were 4.43 ± 0.23 seconds (mean ± standard error of the mean) for the dominant eye and of 4.2 ± 0.22 seconds for the nondominant one. The proportion of viewing time during which each eye dominated percept was 0.47 ± 0.05% for the dominant eye, and 0.43 ± 0.05% for the nondominant, corresponding with a mean ocular dominance index of 0.52 ± 0.05. The mixed percepts dominated for a small proportion of the total viewing time (9.97 ± 0.78%). We used the split-half analysis based on the temporal order of the participant's reports to compute the reliability of both these parameters, separately for sessions before and after deprivation. In all cases, reliability indices are 0.75 or greater (see Table 2.1).

We found systematic associations between performance in the three tasks (all performed before deprivation).

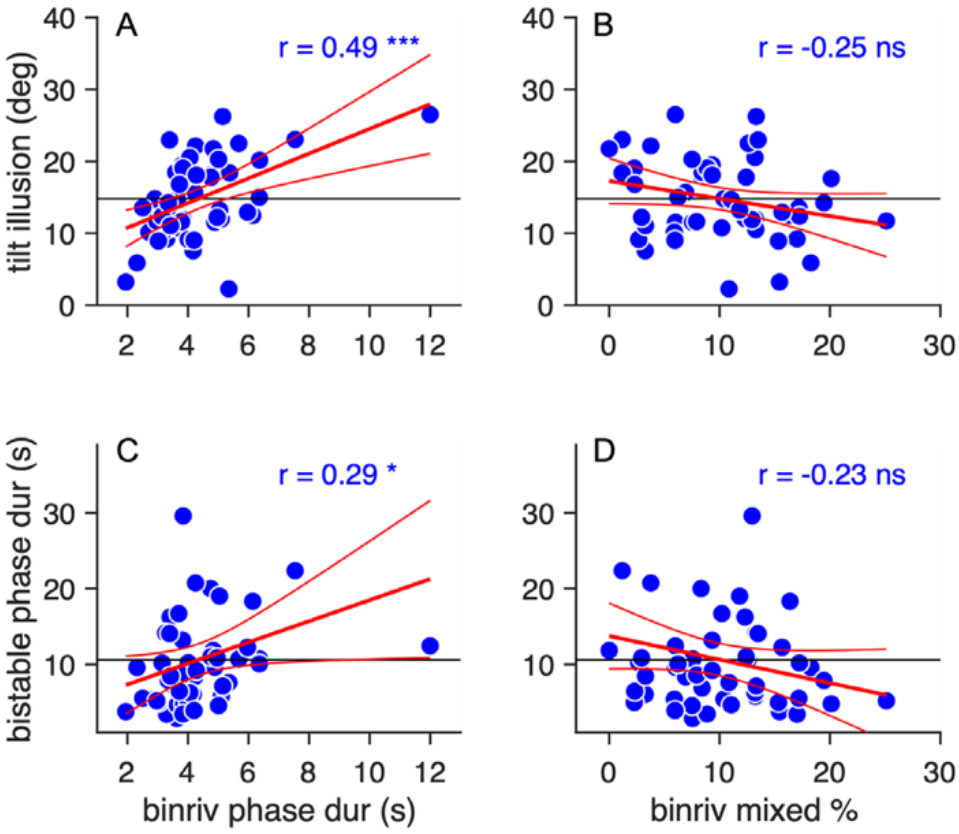

Figure 2.2 Associations between task performances. Associations between predeprivation performance in binocular rivalry, measured as mean phase duration (A and C) or proportion of mixed percepts (B and D), and magnitude of the tilt illusion (A and B) or mean phase durations in the structure from motion (C and D). Each symbol is one participant; the continuous horizontal line shows the mean of the values on the ordinate; the red lines show the best fit linear function with CIs. Text insets give the Pearson's correlation with associated sample size and p value.

Figure 2.2 explores the two main parameters describing binocular rivalry: mean phase durations (averaged for the two eyes) and proportion of mixed percepts, which are themselves not correlated: r(34) = –0.21, p = 0.23, lgBF = –0.6. We found a tight correlation between binocular rivalry mean phase durations and the magnitude of the tilt illusion, r(50) = 0.49, p <0.001, lgBF = 1.9, across participants, those with slower binocular rivalry dynamics are those showing stronger tilt illusion effects (Figure 2.2A). The correlation remains significant, r(49) = 0.41, p = 0.003, lgBF = 0.89, after excluding the outlier in the top right of Figure 2.2A.

We also found a mild positive correlation between mean phase durations in binocular rivalry and structure from motion, r(50) = 0.29, p <0.05, lgBF = –0.1, implying a portion of shared variance in the rate of perceptual alternation in the two bistable phenomena (Figure 2.2C). The correlation remains significant, either excluding the outlier in the bottom right, r(49) = 0,37, p = 0.009, lgBF = 0.54, or the one at top left, r(49) = 0,33, p <0.05, lgBF = 0.2 of Figure 2.2C.

The other main parameter of binocular rivalry, the percentage of mixed percept, was not significantly correlated with either tilt illusion magnitude, r(50) = –0.25, p = 0.07, lgBF = –0.3, Figure 2.2B,or structure from motion mean phase durations, r(50) = –0.23, p = 0.22, lgBF = –0.6, Figure 2.2D. Also, the magnitude of the tilt illusion was not significantly correlated with the structure from motion mean phase durations, r(50) = 0.21, p = 0.138, lgBF = –0.48; not shown.

Having established the pattern of correlations at baseline, we investigated the ability of these measures to predict the effect of monocular deprivation on the dynamics of binocular rivalry.

In line with previous studies, we found that monocular deprivation affected ocular dominance, shifting it in favor of the deprived eye, t(33) = –9.07, p < 0.001, Figure 2.3B. This was primarily due to an increase in phase durations for the deprived eye, t(33) = –7.095, p < 0.001, and also to a smaller and nonsignificant decrease for the nondeprived eye, t(33) = 1.792, p = 0.082, resulting in a significant Time × Eye

interaction in a two-way ANOVA for repeated measures, $F(1,33) = 56.35235$, $p < 0.001$, Figure 2.3A. Figure 2.3C and 2.3D show that deprivation affected the distribution of phase durations for each eye, normalized to its pre-deprivation mean. The distribution of phase durations for the deprived eye became broader and shifted toward the longer durations (the mean moving from 4.43 ± 0.23 seconds to 5.67 ± 0.25 seconds), whereas the distribution of phase durations for the non-deprived eye was only marginally shifted in the opposite direction (mean before, 4.20 ± 0.22 seconds; mean after, 3.77 ± 0.15 seconds). Despite these changes, distributions maintained their typical gamma-like characteristics.

Finally, Figure 2.3E plots the proportion of mixed percepts before and after deprivation, which were unaffected, $t(33) = 0.97$, $p = 0.33$, lgBF = –0.5. Note that this measurement was tightly correlated before/after deprivation, $r(34) = 0.66$, $p < 0.001$, lgBF = 3.1, suggesting that the failure to measure a change cannot be due to its unreliability (see also the split-half reliability reported in Table 2.1).

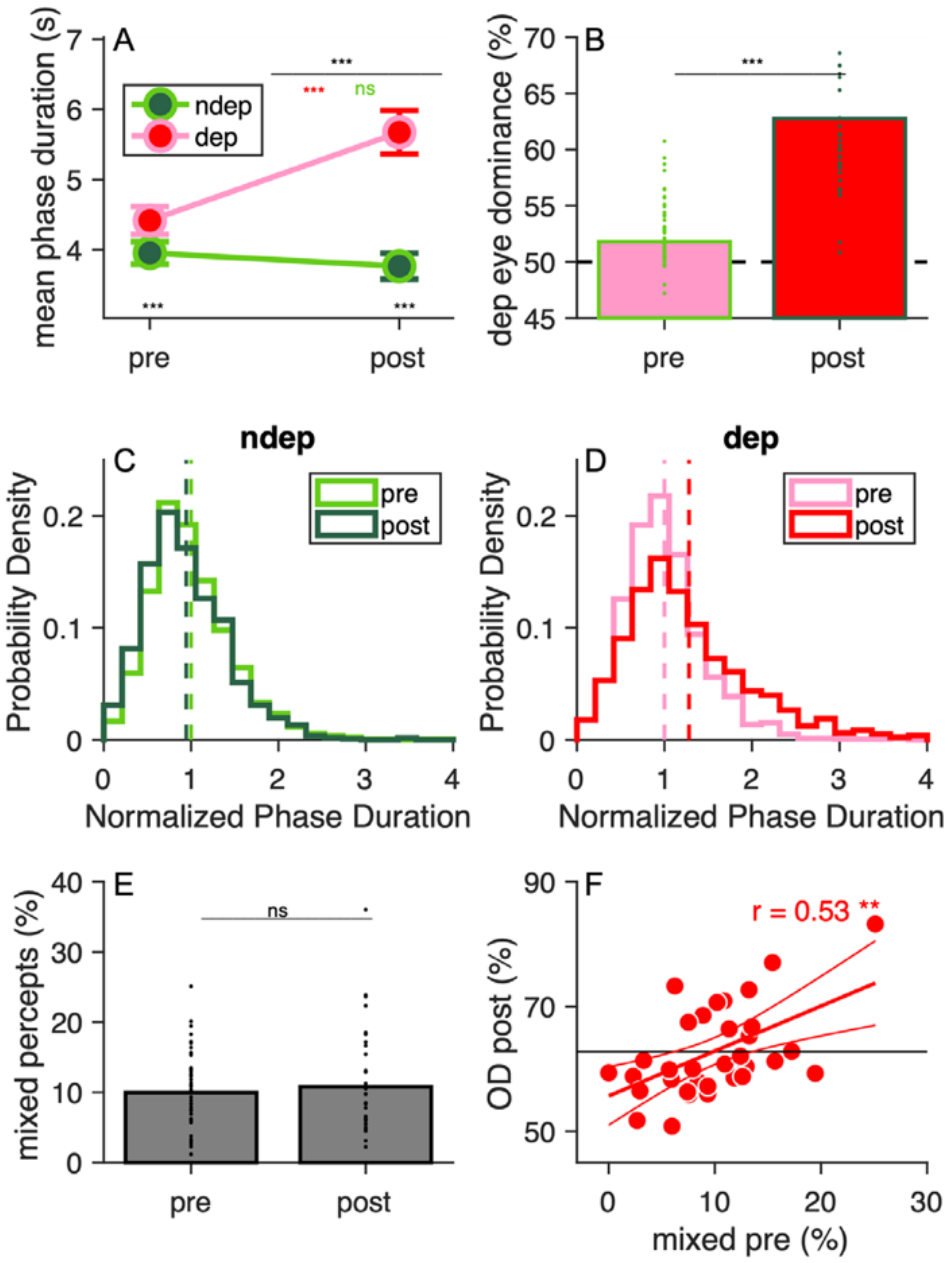

Figure 2.3 Effects of monocular deprivation (A) Mean phase durations for each eye (deprived and nondeprived), before and after deprivation. Error bars are standard errors across participants; the top black stars indicate the significance of the Time × Eye interaction; red and green stars give the significance of the change across deprivation for the deprived and nondeprived eye respectively; black stars near the x-axis give the significance of the difference across eyes, before and after deprivation. (B) Dominance of the deprived eye (Equation 1), measured before and after deprivation. Each dot is one participant; bars give the mean across participants and black stars show the significance of the paired t test. (C, D) Probability density functions of the mean phase durations for each eye (nondeprived and deprived, respectively), normalized to the respective pre-deprivation mean. Dashed lines show the means, which by definition are equal to 1 for before deprivation, but shift right or left for the after deprivation, reflecting the plasticity effect. (E) Percentage of mixed percepts, before and after deprivation. Each dot is one participant; bars give the mean across participants and text shows the significance of the paired t test. (F) Association between postdeprivation ocular dominance (OD post) and the percentage of mixed percepts before deprivation. Each symbol is one participant; the continuous horizontal line shows the mean OD post; the red line shows the best fit linear function with

*CI s. Text insets give the Pearson's correlation with associated sample size and p-value. ns, nonsignificant. *p < 0.05; **p < 0.01; ***p < 0.001 .*

As shown in Figure 2.3B, ocular dominance shows considerable interindividual variability. However, this is unrelated before/after deprivation, r(34) = 0.13, p = 0.45, lgBF = –0.8: our participants were selected to have balanced eyes, and a slight ocular preference before deprivation is not predictive of a larger (or smaller) ocular dominance imbalance after deprivation.

Although the baseline ocular dominance is not predictive of the plasticity effect, we found that the prominence of mixed percepts does predict the effect of monocular deprivation. Specifically, we found a positive correlation between the percentage of mixed percepts before deprivation and the ocular imbalance after deprivation, r(34) = 0.53, p < 0.01, lgBF = 1.3. This robust association implies that participants who experienced more mixed percepts before deprivation tend to show a stronger dominance of the deprived eye, after deprivation (Figure 2.3F).

Ocular dominance after deprivation was not correlated with the other parameters describing perceptual performance before deprivation (Figures 2.4A–4C): the mean duration of binocular rivalry phases, averaged for the two eyes, r(34) = –0.06, p = 0.750, lgBF = –0.85, the magnitude of the tilt illusion, r(30) = –0.09, p = 0.619, lgBF = –0.80, or the mean duration of structure from motion phases, r(30) = –0.15, p = 0.418, lgBF = –0.71. However, entering any of these parameters in a multiple regression model significantly increased its ability to capture interindividual variability

in ocular dominance after deprivation compared with a single-regressor model including only the prominence of mixed percepts (Equation 2). The single-regressor model explains 27.6% of postdeprivation ocular dominance variance, R^2 = 0.276, adjusted R^2 = 0.254, F (32,1) = 12.22, $p < 0.001$.

$$OD_{post} = \beta 0 + \beta 1 Mixed + err$$

Equation 2

A more complex model (Equation 3) considering both mixed percepts and the mean duration of binocular rivalry phases (averaged for the two eyes), both measured before deprivation, allows for explaining 43.8% of variability in postdeprivation ocular dominance, R^2 = 0.43, adjusted R^2 = 0.38, F(30,2) = 7.788 , p = 0.001.

$$OD_{post} = \beta 0 + \beta 1 Mixed + \beta 2 PhaseDur + \beta 3 Mixed * PhaseDur + err$$

Equation 3

The multiple regression analysis reveals a significant interaction term ($\beta 3_{Mixed*PhaseDur}$ = –0.581, confidence interval [CI] = [–0.989 to –0.173]), implying that the effect of mean phase duration varies depending on the percentage of mixed percepts. We explored this with Figures 2.4D and 2.4G, by plotting the postdeprivation ocular imbalance against the mean duration of binocular rivalry phases, separately for participants with higher or lower prominence of mixed percepts before deprivation (median split). Longer phase durations predict a reduced effect of deprivation, but only for individuals experiencing a relatively high

percentage of mixed percepts (Figure 2.4G), not for the other half of the sample (Figure 2.4D). A similar picture emerges when entering the model with the magnitude of the tilt illusion (Figures 2.4B, 2.4E, and 2.4H) and the mean duration of structure from motion phases (Figures 2.4C, 2.4F, and 2.4I). Both parameters are correlated with binocular rivalry mean phase durations (see Figure 2.2); consequently, they both play a similar role in the multiple regression model.

The model including magnitude of the tilt illusion, Equation 4, R^2 = 0.391, adjusted R^2 = 0.33, F(30,2) = 5.560, p = 0.004, reveals a significant interaction between tilt illusion magnitude and the percentage of mixed percepts in pre-deprivation binocular rivalry, $\beta 3_{Mixed*TiltIll}$ = –0.11, CI = [–0.21 to –0.01].

$$OD_{post} = \beta 0 + \beta 1 Mixed + \beta 2 TiltIll + \beta 3 Mixed * TiltIll + err$$

Equation 4

Similarly, the model including the mean duration of structure from motion phases (Equation 5), R^2 = 0.41, adjusted R^2 = 0.35, F(30,2) = 6.032, p = 0.003, reveals a significant interaction between structure from motion phase durations and the percentage of mixed percepts in pre-deprivation binocular rivalry, $\beta 3_{Mixed*SFM}$ = –0.144, CI = [–0.280 to –0.008].

$$OD_{post} = \beta 0 + \beta 1 Mixed + \beta 2 SFM + \beta 3 Mixed * SFM + err$$

Equation 5

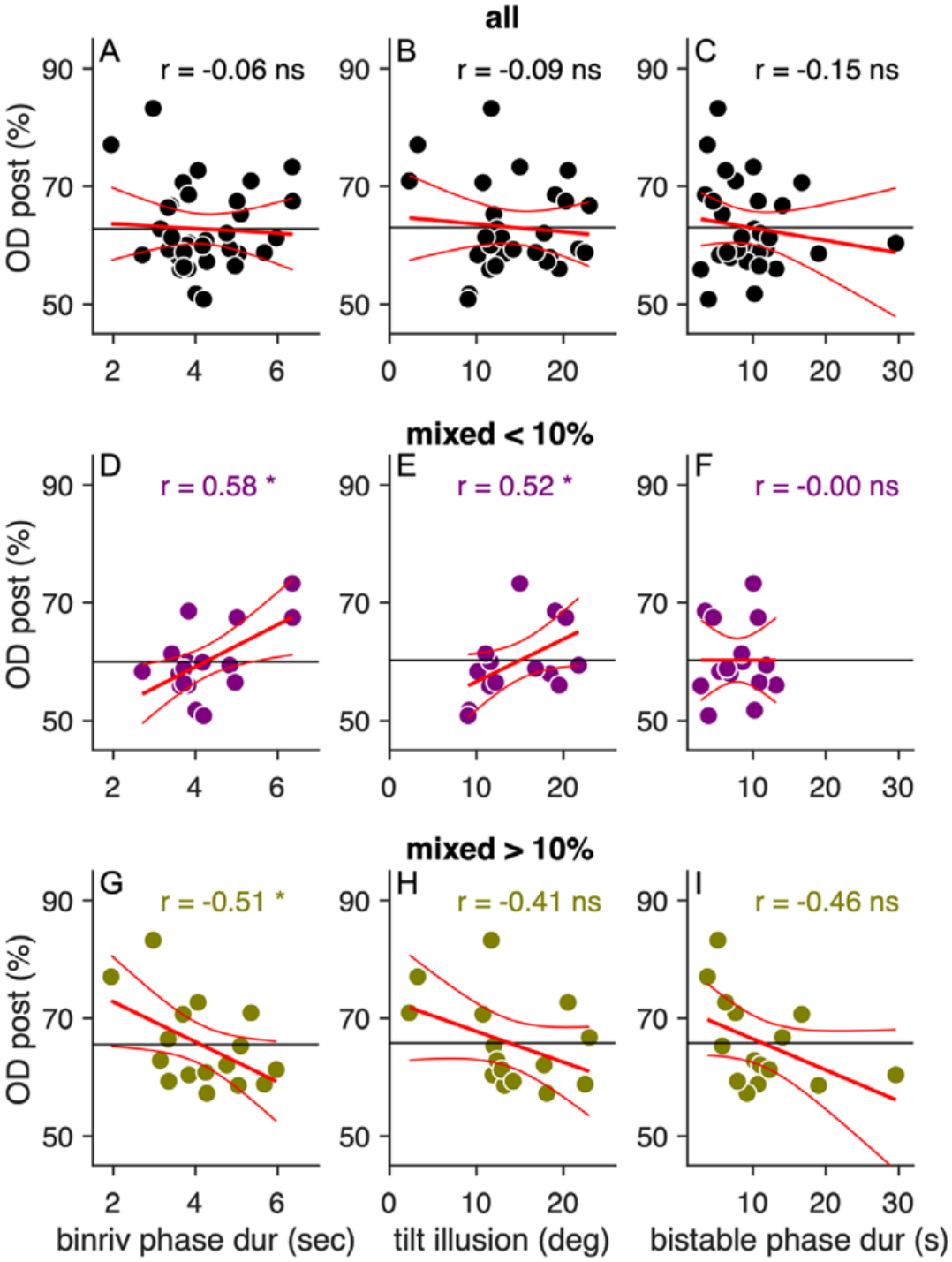

Figure 2.4 Associations between post deprivation ocular dominance and pre deprivation psychophysical performance. (A–C) Association between postdeprivation ocular dominance and the three main parameters of predeprivation performance, besides mixed percepts in binocular rivalry (shown in Figure 2.3F): the mean duration of binocular rivalry phases (A), magnitude of the tilt illusion (B), and mean duration of structure from motion phases (C). None of these parameters is directly related to the plasticity effect. However, all three interact significantly with binocular rivalry mixed percepts in explaining postdeprivation ocular dominance, as shown by the multiple regression analysis. This is visualized in D through I, which display the same correlations as in A through C, measured in two subsamples of participants: those with mixed percepts lower (D–F) or higher (G–I) than the median value (10%). Same conventions as in Figure 2.3F.

Equations 3 to 5 have similar adjusted R^2, and they all represent better models than the single-predictor model (Equation 2). We also tested a more complex model including all these parameters and their interaction, but this did not further increase the adjusted R^2, $R^2 = 0.499$, adjusted $R^2 = 0.339$, $F(30,2) = 2.619$, $p = 0.037$.

A similar pattern of results was obtained when measuring plasticity as the change of ocular dominance before versus after deprivation (instead of ocular dominance after deprivation). In particular, the interaction indices for all three models described in Equations 3 to 5 are significant, $\beta 3_{Mixed*PhaseDur} = -51.992$, CI = [–91.215 to –12.769], $p = 0.003$; $\beta 3_{Mixed*TiltIll} = -12.237$, CI = [–21.026 to –3.448], $p = 0.003$; $\beta 3_{Mixed*SFM} = -13.624$, CI = [–25.931 to –1.317], $p = 0.004$.

2.4 Discussion

The main aim of our research was to test the possibility of predicting the strength of homeostatic sensory plasticity, induced through short-term monocular deprivation, based on performance in standard, well-known psychophysical tests: binocular rivalry, structure from motion, and tilt illusion. As a preliminary step toward this goal, we also investigated interindividual variability in each task and checked for potential associations between tasks at baseline (before the monocular deprivation procedure).

We found a surprising and strong association between the dynamics of binocular rivalry and the magnitude of the tilt illusion. These tasks differ on a number of levels (one requires continuous reporting of percept, the other relies on a standard two-alternative forced choice task; one is strictly foveal, the other measures the influence of a parafoveal region) and their association has never been hypothesized. However, inhibitory mechanisms are assumed to underlay both phenomena. The tilt illusion may be mediated by inhibitory and disinhibitory interactions between spatial frequency and orientation-selective mechanisms at multiple levels of the cortical processing hierarchy. Theoretical models of the tilt illusion suggest a neural mechanism (or mechanisms) that involves inhibition in V1; (Blakemore et al., 1970, 1973; Clifford et al., 2000; Schwartz et al., 2009; Seriès et al., 2003; Solomon & Morgan, 2006). In line with this, recent MRS studies in humans have linked occipital GABA levels with the magnitude of tilt illusion (Cook et al., 2016; Seymour et al., 2018; Song et al., 2017). Combining MRS studies on tilt illusion and binocular rivalry, showing that occipital GABA is positively correlated with tilt illusion magnitude (Song et al., 2017) and positively correlated with the duration of mean phases of binocular rivalry (Van Loon et al., 2013), one would predict a positive correlation between tilt illusion magnitude and binocular rivalry phase durations. This pattern of results is precisely that we report.

We also found that the dynamics of the two bistable phenomena (duration of perceptual phases in binocular rivalry and structure from motion) were mildly

correlated—participants with longer phase durations in binocular rivalry, tended to experience longer phases in structure from motion. There is considerable evidence that different bistable phenomena share at least partially some underlying mechanisms (Leopold & Logothetis, 1999), generally involving reciprocal inhibition between competing neural representations (Blake, Brascamp, & Heeger, 2014; Blake & Wilson, 2011; Brascamp et al., 2015). However, previous studies find weak (nonsignificant) correlations between binocular rivalry and bistable structure from motion dynamics (Brascamp et al., 2018; Cao, Wang, Sun, Engel, & He, 2018), attributing this lack of correlation to the possibility that the two sets of stimuli may be processed by very different brain mechanisms. Here we did observe a correlation between these two widely different stimuli; however, the correlation is weak and hence not inconsistent with previous reports. Moreover, one factor potentially playing an important role in explaining this correlation is motor noise, shared between the two forms of bistability (both reported by continuous key presses) (Gallagher & Arnold, 2014) but see also (Brascamp et al., 2018). Lacking a rivalry replay control condition to isolate the impact of motor noise on rivalry reports, we cannot exclude that this phenomenon contributes to the correlations we observed.

Although the main parameter used to describe the dynamics of binocular rivalry is the duration of perceptual phases (or the rate of perceptual switches), a second parameter is gaining interest in the literature: the proportion of mixed percepts, where images in the two eyes, rather than competing, fuse in a mixed percept. A

recent meta-analysis (Brascamp et al., 2018) reported a lack of consensus on the way mixed percepts are quantified and analyzed, as well as on the importance that is attached to parameter. In many binocular rivalry experiments, participants are not given the option to report mixed percepts—they can only report which eye is relatively dominant (Brascamp et al., 2018; Pitchaimuthu et al., 2017a). In contrast, several recent studies have specifically examined individual differences in the predominance of mixed perception, either focusing on different mixed percepts subtypes (Sheynin, Proulx, & Hess, 2019) or, for example, relating it to autistic spectrum disorders and the alterations of cortical inhibition that may accompany them (Robertson, Kravitz, Freyberg, Baron-Cohen, & Baker, 2013; Said et al., 2013). Mentch et al. (Mentch et al., 2019) went further, suggesting that the proportion of mixed percepts and the duration of exclusive-dominance phases are independently regulated by two distinct inhibitory pathways. Specifically, they show how the duration of mixed percepts and that of exclusive dominance during binocular rivalry are modulated differently by GABA-A (less mixed, no change in percepts durations) and GABA-B (mainly more percepts durations, and slightly less mixed) agonists. In line with this finding, we find that binocular rivalry mean phase durations and mixed percepts are not significantly correlated, despite a nonsignificant negative trend that may be a byproduct of mixed percepts usually occurring at the transition between phases, implying that participants with shorter phases will tend to have more mixed percepts. We speculate that mixed percepts may indicate interocular summation, the

logic opposite of interocular inhibition, which would be consistent with (Mentch et al., 2019) observation that pharmacologically increasing GABA leads to a decrease of mixed percepts, but only if the manipulation occurs at the GABA-A receptor. This implies that mixed percepts are a stimulus-specific index of bistability—explaining its lack of correlation with any parameter of structure from motion and tilt illusion. It may also explain its importance in predicting the change of binocular rivalry with monocular deprivation, which is thought to modulate the balance between eyes by interfering with interocular inhibition, and has been correlated with MRS estimates of occipital GABA levels (Lunghi, Emir, et al., 2015). Our results regarding mixed percepts are in contrast with those of (Sheynin et al., 2019): we found no change in mixed percepts while they found an increase both in proportion and phase duration after deprivation. This difference could be due to different methodologic procedures used; in fact, they (Sheynin et al., 2019) instructed their participants to differentiate between different kinds of mixed percepts (piecemeal vs superimposition), whereas we decided to avoid this as a possible confusion for our participant.

We measured the effects of monocular deprivation on binocular rivalry on a sample of 34 participants, a larger sample than those used in previous studies from our laboratory (Binda & Lunghi, 2017; Lunghi et al., 2011a) and others' (Zhou et al., 2013). Besides confirming the robustness and replicability of the monocular deprivation effect, these figures give us a chance to leverage the substantial interindividual variability of the effect to assess the predictive power of several perceptual indices

measured before monocular deprivation. The proportion of mixed percepts was found to be stable before and after monocular deprivation, and to have the greatest predictive power on the monocular deprivation effect. However, including binocular rivalry mean phase durations as a second predictor allowed for the substantially larger portion (almost 50%) of variance in the monocular deprivation effect. Similar, although lower, predictive power was achieved by substituting binocular rivalry mean phase durations with structure from motion phase durations or tilt illusion magnitude (as may be expected, given the positive correlation among these three variables, discussed elsewhere in this article). In all models, a key component was the interaction between mixed percepts and phase durations (or tilt illusion magnitude), which may be taken to suggest that at least two different mechanisms may influence the effect of monocular deprivation, possibly related to two (or more) distinct inhibitory mechanisms acting at the level of the visual cortex.

In fact, anatomophysiologic evidence indicates that the population of GABAergic interneurons in V1 is extremely heterogeneous (Trachtenberg, 2015), comprising at least two very distinct classes of cells (Maffei, Lambo, & Turrigiano, 2010; Priya et al., 2019; Scheyltjens & Arckens, 2016). Parvalbumin cells (Scholl, Pattadkal, Dilly, Priebe, & Zemelman, 2015) mediate local inhibitory interactions and could mediate the reciprocal inhibition between neighboring ocular dominance cells (Hensch & Quinlan, 2018; Saiepour et al., 2015; Trachtenberg, 2015). Somatostatin expressing inhibitory

neurons, in contrast, act at a more global level, mediating long-range interactions across remote visual field regions and have been specifically associated with center–surround interactions (Adesnik, Bruns, Taniguchi, Huang, & Scanziani, 2012; Vangeneugden et al., 2019; Yazdani, Serrano-Pedraza, Whittaker, Trevelyan, & Read, 2015). Somatostatin expressing inhibitory neurons have been suggested to play a role in short-term plasticity, proposing that their modulation during physical exercise (Lunghi, Sframeli, et al., 2019) could be responsible for the enhanced plasticity reported in (Lunghi & Sale, 2015). Interestingly, our findings show that variability in short-term plasticity is explained by at least two independent factors related, possibly, to GABAergic inhibition. This finding may suggest an influence of parvalbumin cells inhibitory mechanisms that modulates interocular inhibition revealed by mixed percepts measures and of long-range mechanisms through somatostatin expressing inhibitory neurons that modulate center–surround inhibition (Clifford, 2014) and dynamics of bistability. The interaction between the two factors observed in our results is reminiscent of a normalization model (Reynolds & Heeger, 2009), whereby relatively low local inhibition (high prevalence of mixed percepts) is permissive for long-range inhibition to drive the plasticity effect: slow bistable dynamics or strong center–surround interactions predict smaller plasticity, but high local inhibition (low prevalence of mixed percepts) obscure the effects of the other inhibitory input. We acknowledge that this model is entirely speculative and, although indirectly supported by evidence of multiple sources, requires further research. In

conclusion, we find that a significant portion of interindividual variability in short-term plasticity may be predicted based on performance on standard psychophysical tasks. However, the key predictive parameters are derived from binocular rivalry measurements. This could be a simple consequence of rivalry being our probe for short-term plasticity, or it could be indicative of binocular rivalry being highly informative of the functional properties of early visual cortex.

3 Chapter

Skipping breakfast changes visual processing: incretins contribution to short-term visual plasticity

3.1 Introduction

Metabolic abnormalities underlying obesity and diabetes affects plasticity-dependent cognitive function and are associated with poorer performance on tests of global cognitive function, memory, language (Dore, Elias, Robbins, Budge, & Elias, 2008; Gunstad, Paul, Cohen, Tate, & Gordon, 2006; Gunstad, Lhotsky, Wendell, Ferrucci, & Zonderman, 2010; Nilsson & Nilsson, 2009) and motor skills (Etou et al., 1989) independently of socioeconomic factors, depression, and cardiovascular factors. Previous studies have demonstrated that obesity is associated with decreased grey matter volume in the orbitofrontal cortex (Walther, Birdsill, Glisky, & Ryan, 2010). The reduced plasticity in obese individuals may be due to changes in structural and functional brain characteristics because of the underlying pathophysiological consequences of obesity (Sui, Ridding, & Hordacre, 2020). Recent evidence from our laboratories indicates that the visual homeostatic plasticity of ocular dominance, a particular form of brain plasticity valued in vivo in an ambulatory setting, is inversely correlated with body mass index (BMI) and severely impaired in individuals with BMI >40 kg/m^2 (Lunghi, Daniele, et al., 2019). The changes in visual cortex activity consistent with the deprived eye dominance boost observed at the perceptual level have been observed with different techniques (Binda et al., 2018; Lunghi, Berchicci, et al., 2015; Lunghi, Emir, et al., 2015; Zhou et al., 2015), and it is thought to reflect transient changes in neuronal circuitry in primary visual cortex providing a marker of brain plasticity. Moreover, previous studies from our laboratory demonstrated that

massive weight loss brought by Roux-en-Y gastric bypass (RYGB) can progressively reverse blunted brain plasticity achieving normal values 6 months after surgery (Daniele et al., 2021). The improvement in brain plasticity was strongly correlated with glucagon-like peptide-1 (GLP-1) increase in fasting and postprandial condition observed after bariatric surgery (Daniele et al., 2021), suggesting a potential role of gut-brain axis. Nonetheless, mechanisms behind gut-brain axis are complex and bariatric surgery modulates multiple hormonal axes and energy metabolism, therefore correlations between metabolic parameters (e.g. GLP-1) and brain plasticity index changes after surgery do not imply causality. GLP-1 is released with every meal as it regulates glucose metabolism and besides the peripheral targets, there is growing evidence that GLP-1 has central effects with receptors in multiple brain areas (Alvarez et al., 2005). Previous studies from our laboratory have shown that the administration of exenatide, a GLP-1 receptor agonist, to subjects with obesity in fasting condition can modulate brain responses to food images as monitored by fMRI (Binda et al., 2019), participate in high brain functions and affect the food intake. GLP-1 effects are not limited to modulation of the sense of satiety and food seeking behavior (De Silva et al., 2011; Gutzwiller et al., 1999; Verdich et al., 2001) but extend to basic physiological mechanisms, including hippocampal long-term potentiation (Gault & Hölscher, 2008; Wang et al., 2021) and glucose metabolism modulation (Daniele et al., 2015) confirming the extensive literature that supports the neuroprotective and synaptic plasticity promoting effects of GLP-1(McClean, Gault,

Harriott, & Hölscher, 2010). Previous studies of our laboratory also observed that the reduction of leptin concentration after bariatric surgery was inversely associated to improvement in brain plasticity (Daniele et al., 2021). Leptin is a cytokine and a satiety hormone involved in appetite regulation and energy expenditure modulation (Lehr, Hartwig, & Sell, 2012), suggesting another pathway of signal integration between the central nervous system and the energy balance through signals generated in the gut and adipocytes. The impact of metabolic status, such as fasting and fed state on brain plasticity has not been fully explored. In humans, during fasting conditions, a metabolic switch occurs, and liver glycogen stores are broken down to produce glucose via glycogenolysis. However, 12 to 24 hours of food deprivation result in depletion of the hepatic glycogen, therefore, lipolysis of triacylglycerols in the adipose tissue generate free fatty acids, that are released into the blood and then transported into hepatocytes, where they are metabolized via **β**-oxidation to acetyl-CoA, that is used to sustain the production of the ketones **β**-hydroxybutyrate (**β**HB) and acetoacetate. Thus, ketones represent the preferred source of energy for body and brain during periods of fasting as they are transported from the blood into cells. Neurons of adult brain present all the enzymes necessary for using ketones to produce energy. Recent evidence shows that the metabolic changes, consequent to a fasted state, may enhance brain function in terms of better cognitive performance, increased neuroplasticity and resistance to injury and disease. (Longo & Mattson, 2014; Mattson, 2012). Moreover, the effect of GLP-1 on brain plasticity independently

of metabolic rearrangements that occur during fasting or mixed meal ingestion has not been investigated. Here we aimed to further explore the impact of metabolic status on brain plasticity in three different conditions: 1. Overnight 12 hours fasting 2. Mixed meal ingestion 3. Overnight 12 hours fasting and GLP-1 intravenous administration to match GLP-1 concentrations observed during a mixed meal. We also aimed to explore whether the key metabolic parameters that are modulated in the post-prandial state can explain plasticity variations across conditions and across individuals.

3.2 Methods

3.2.1 Human Participants

14 young and heathy volunteers (8 females) participated in the experiment, 12 of which completed the whole study. All participants were naive to the study, had normal or corrected-to-normal vision, no strong eye preference, normal weight, and good state of health without chronic or acute illnesses in progress. Moreover, all included participants had been pre-tested and trained with the binocular rivalry tasks and we checked that they had typical binocular rivalry dynamics, with low percentage (<20%) of mixed percepts (i.e. periods of fusion or superimposition of the two rivalrous stimuli).

Baseline characteristics of study participants are reported in Table 3.1. All participants gave written informed consent before starting the study.

Characteristic **(mean ± SD)**	**Baseline** **(n=14)**
Age (years)	26.86 ± 4.96
Gender (M/F)	6/14
BMI (kg/m^2)	22.49±3.03
SBP (mmHg)	117±12
DBP (mmHg)	75±9
Hb glicata (mmol/mol)	32±3
Total Cholesterol (mg/dl)	167±26
HDL Cholesterol (mg/dl)	59±15
LDL Cholesterol (mg/dl)	90.4±26
Triglycerides (mg/dl)	82±45
Creatinine (mg/dl)	0.8±0.2
AST (UI/l)	19±5
ALT (UI/l)	17±12
GGT (UI/l)	14±7
Hb% (g/dL)	14±2

Table 3.1 Characteristics of the participants.

Experimental procedures were approved by the local Ethics committee (Project approval n. 1031 of April 15th, 2016).

3.2.2 Procedure

Tests were performed in a dark and quiet hospital room, with an attending physician monitoring participants over the course of the experiment. We measured binocular rivalry before and after two hours of monocular deprivation. Before deprivation, we tested the participants with two 180 sec experimental blocks for a total of 6 minutes. Immediately after eye-patch removal, we measured binocular rivalry in four separate 180 sec blocks for a total of 12 minutes. A diagram of the experimental procedure is shown in (Figure 3.1A). During the two hours of monocular deprivation, participants set in an experimental room and were engaged in additional visual tests.

Testing was performed three times over the course of about 90 days. The first phase of the experiment was common to the three conditions, in that participants reported to the hospital at 8AM after overnight fasting (about 12h fasting and no breakfast); following cannulation, baseline binocular rivalry measurements were acquired.

At this point, the monocular patch was applied, and the metabolic manipulation started by choosing one of the following three options (with order pseudo-randomized across participants):

- No manipulation, so that the small fasting regime was maintained throughout the 2h of monocular deprivation

- Administration of a standardized meal (Nutridrink Nutricia, 300 Kcal) immediately before application of the monocular patch
- Intravenous infusion of GLP-1 (0.9 pmol/Kg/min), starting immediately before application of the monocular patch and continuing over the 2h of monocular deprivation. Blood glucose concentration was measured every 10 minutes and 20% D-glucose (Braun, Melsungen AG, Germany) was intravenously infused at variable rate to maintain blood glucose concentration at fasting levels.

3.2.2.1 Monocular Deprivation

Monocular deprivation was achieved by patching the dominant eye for two hours. We patched the dominant eye, as previous studies have shown that monocular deprivation induces stronger shifts in eye-dominance when the dominant eye is patched compared to the non-dominant eye. Eye-dominance was assessed using binocular rivalry: the dominant eye was defined as the eye showing the longer mean phase duration in the baseline measurements. The eye-patch was made of a translucent plastic material that allowed light to reach the retina (attenuation 15%) but completely prevented pattern vision.

3.2.2.2 Apparatus and Procedure: Binocular Rivalry

Visual stimuli for binocular rivalry were two oblique red and blue gratings (orientation: ± 45°, size: 3°, spatial frequency: 2 cpd, contrast 50%), surrounded by a white smoothed circle, presented on a black uniform background in central vision. Participants viewed the visual stimuli presented on a monitor (IPS LED 24EA53,

1920*1080, 60Hz) through anaglyph red-blue goggles (right lens blue, left lens red). Seen through the anaglyph goggles, each of these stimuli was presented to one eye (and the orientation was swapped randomly across recording blocks). The peak luminance of the red grating was reduced to match the peak luminance of the blue one using photometric measures. Participants sat in front of the monitor, at 57 cm, wearing the goggles. After an acoustic signal (beep), the binocular rivalry stimuli appeared. Participants reported their perception (clockwise, counterclockwise, or mixed) by continuously pressing with the right hand one of the keys (right, left, and down arrows) of the computer keyboard. Another acoustic signal (three beeps) signaled the end of each 180 sec experimental block. At each experimental block, the orientation presented to each eye was randomly varied so that the participants did not know which stimulus was associated with which eye.

3.2.2.3 Analyses: Binocular Rivalry

The perceptual reports recorded through the computer keyboard were analyzed with custom MATLAB scripts. During binocular rivalry, visual perception oscillates between the monocular images with periods of exclusive dominance of one of the two rivalrous stimuli, sometimes interleaved with periods of mixed percepts – in which the observer perceived a mixture of the two images. We checked that in all cases phase durations followed the typical gamma distribution: for each subject we normalized phase-duration for the deprived eye (purple) and non-deprived eye (light blue) to their

mean. Probability density functions were then averaged across participants, yielding similar curves for the two eyes, as shown in Figure 3.1B. We checked also that the proportion of mixed perceived dominates only for a small proportion of total viewing (<20%) and that this measurement was not affected by monocular deprivation (Meal t(12) = -0.71, p = 0.50, lgBF = -0.46, Fasting t(11) = -2.13, p = 0.06, lgBF= 0.18, GLP-1 Infusion t(10) = -0.47, p = 0.64, lgBF = -0.48).

To quantify ocular dominance, for each subject and each experimental block, we computed the proportion of dominance for either eye, i.e. the total time spent seeing with either eye over the total time. To index the effect of deprivation, we computed the Plasticity Index (PI) which summarizes the change in eye-dominance (defined as the ratio between the deprived and non-deprived eye proportions) before/after deprivation. (Equation 6)

$$\text{Plasticity Index} = \log10\left(\frac{DepEyepost}{DepEyepre} / \frac{NdepEyepost}{NdepEyepre}\right)$$

Equation 6

As a result:

Log10(PI) > 0 increased dominance of the deprived eye,

Log10(PI) < 0 increased dominance of the non-deprived eye.

3.2.2.4 Analyses: Blood samples

Blood samples were collected with a standardized timing and analyzed to quantify the following parameters and their variation within and across sessions: Glucagon-

like-peptide1 (GLP-1), glucose, insulin, c-peptide, leptin and Beta-Hydroxybutyrate (**β**HB), shown in Figure 3.2.

Samples were stored on ice, centrifuged (3000 crf, 15 minutes at 4 Celsius degrees) and duplicate plasma aliquots stored at −80◦C.

Plasma glucose was measured by the glucose oxidase method (Analox GM9 Analyzer; Analox Instruments, London, UK). Plasma insulin and C-peptide (Pantec, Turin, Italy) were measured by RIA. Leptin was measured using a Sandwich Enzyme-linked Immunosorbent Assay (RayBiotech, Norcross, GA, USA). Beta-Hydroxybutyrate was measured by in-house automated spectrophotometric enzymatic methods on a Beckman UniCel DXC600 Synchron Analyser (Fullerton, CA, USA). Intra- assay and between-assay coefficients of variation were <1% and <5%, respectively, for all hormone and substrate measurements. The area under the curve (AUC) for glucose, **β**HB and active GLP-1 was computed using the trapezoidal rule.

3.2.2.5 Statistical analysis

Statistical analyses were performed using Matlab software (MATLAB_R2019a) and JASP software (JASP Team (2020). JASP (Version 0.14.1) [Computer software]). For t-tests and ANOVAs we complemented the standard inferential approach using p-value to define significance with Bayes factors, which quantifies the evidence for or against the null hypothesis as the ratio of their likelihoods given the observed data.

We expressed it as the base-10 logarithm of the ratio (Log Bayes Factors (lgBF)), where negative numbers indicate that the experimental hypothesis is likely to be false. Conventionally, absolute lgBF greater than 0.5 are considered substantial evidence for or against the experimental hypothesis. For correlation analyses, we applied a robust correlation approach using the option "skipped" which automatically identifies outliers and discounts their effects on the correlation coefficient and its significance at the standard threshold of $p < 0.05$ (Pernet, Wilcox, & Rousselet, 2013).

3.2.3 Internal replication

As an internal replication of the study, we recruited additional 10 adult volunteers (all females, mean age 25.70 ± 1.63), all naïve to the purposes of the study, with normal or corrected-to-normal vision, no strong eye preference and normal weight. Participants gave their written informed consent. We repeated the binocular rivalry measurement before and after two hours of monocular deprivation over two days, in two conditions (in pseudorandom order): at 9 AM, either after overnight fasting or after having had their usual breakfast. Procedures and analyses were the same as in the main experiment, except for the following details. We did not draw blood samples but simply estimated glycemic levels at the beginning of each session with a portable glucometer (Countour®next). During deprivation, participants did not undergo additional tests, they were free to read, work at the computer or walk around the

laboratory (but not to eat or sleep). We measured binocular rivalry with ferromagnetic shutter goggles (rather than with anaglyphic glasses), with monocromatic stimuli (Gaussian-vignetted sinusoidal gratings, oriented 45° clockwise or counterclockwise, with size: 2**σ**=2°, spatial frequency: 2 cycles/degree of visual angle, and contrast: 50%). Stimuli were generated by the VSG 2/5 stimulus generator (CRS, Cambridge Research Systems), housed in a PC (Dell) controlled by MATLAB (2019a) scripts. They were displayed on a linearized monitor (Barco CDCT 6551, Barco Federal System, LLC, Duluth, GA) driven at a resolution of 987 x 777 pixels with a refresh rate of 120 Hz.

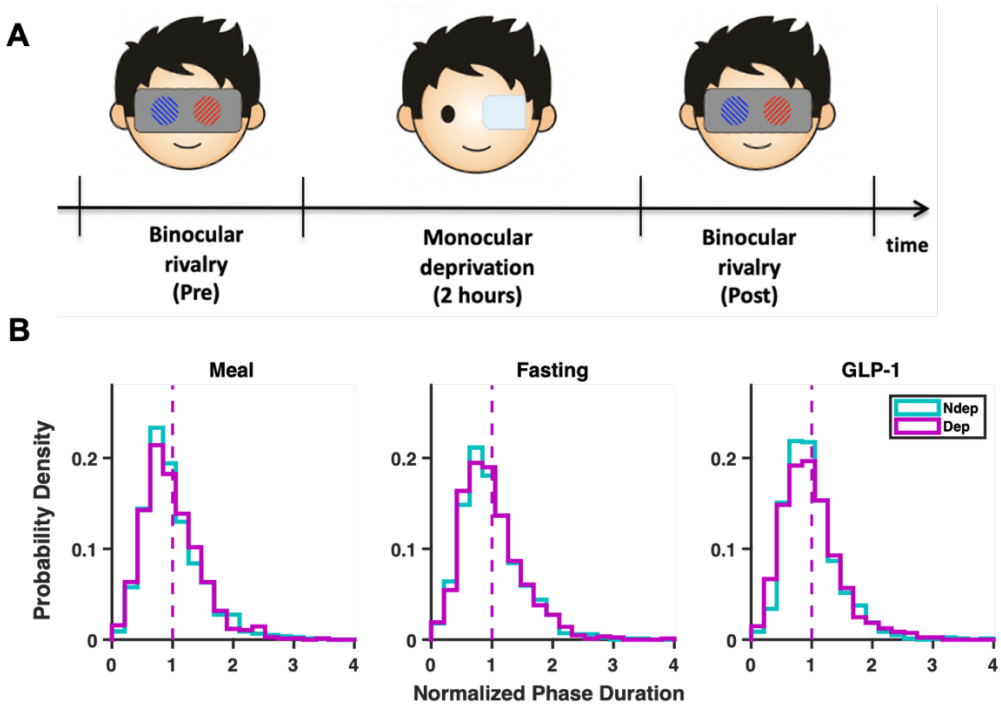

Figure 3.1 Experimental procedure and baseline binocular rivalry measurements. A) time course of the experiment, where binocular rivalry dynamics were measured before deprivation (2 x 180 sec binocular rivalry blocks) and after deprivation (4 x 180 sec binocular rivalry blocks). Short-term monocular deprivation was achieved by having the subject wear a translucent eye-patch over the dominant eye for two hours. B) probability density function of the normalized phase durations for each eye (deprived and non-deprived), before deprivation and in the three metabolic states, all conforming to the typical gamma distribution.

3.3 Results

We measured a form of short-term sensory plasticity (ocular dominance plasticity) in a group of healthy adult volunteers experiencing different metabolic states: after a controlled standardized meal; in a small fasting regime (overnight fasting of 12h); in a small fasting regime plus a continuous intra-venous infusion of GLP-1. Plasticity was measured by comparing the dynamics of binocular rivalry before and after 2 hours of monocular deprivation, during which participants wore a translucent eye patch over the dominant eye (Figure 3.1 A) while blood samples were drawn at regular intervals to quantify the variation of key metabolic parameters (Figure 3.2).

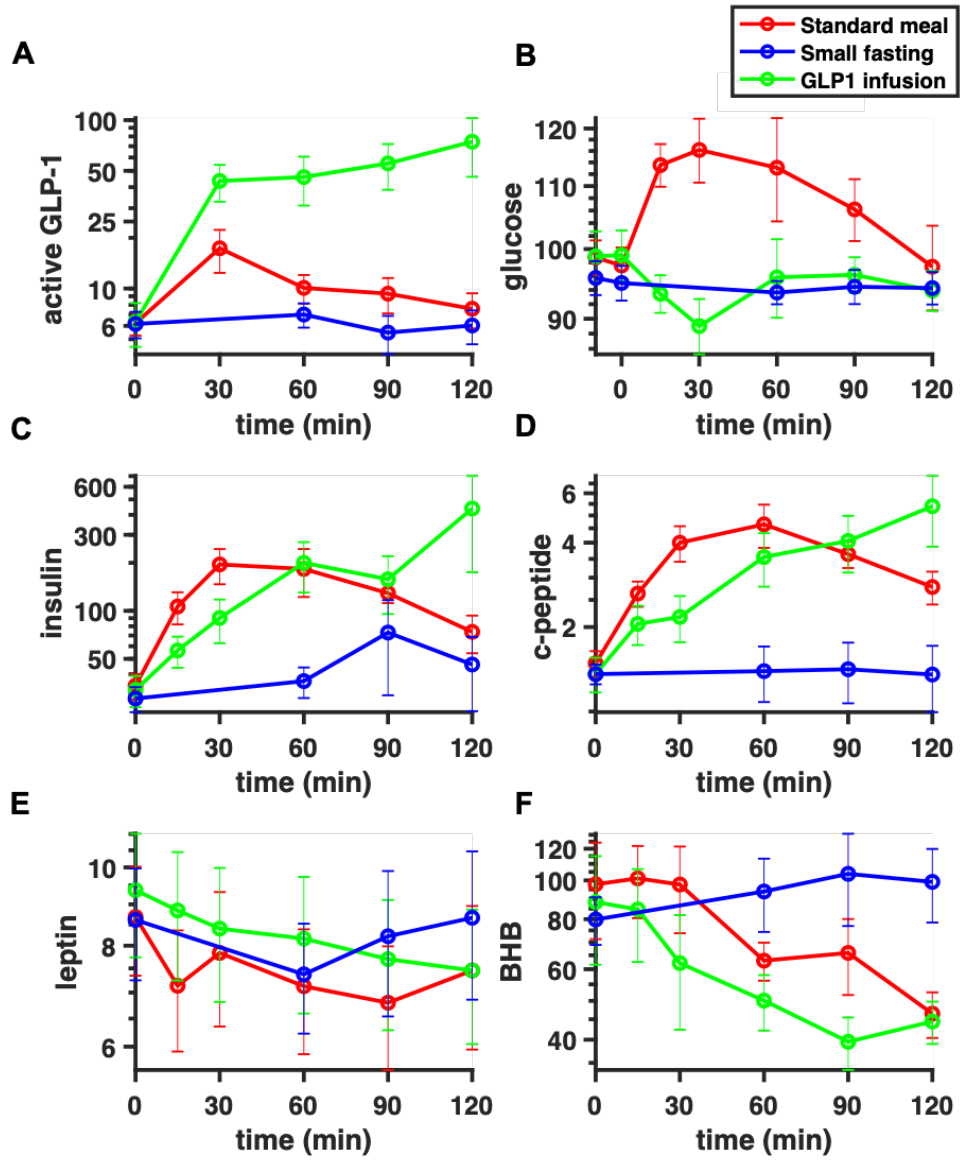

Figure 3.2 Metabolic parameters. Plasma concentrations of the active fraction of Glucagon-like-peptide1, glucose, insulin, c-peptide, leptin, and BHB, over the two hours of deprivation, separately for the three metabolic states (standard meal, small fasting, small fasting plus GLP1 infusion).

In line with previous studies, we found that a 2-h monocular deprivation was sufficient to reliably affect rivalry dynamics, boosting the deprived eye signal. Collapsing data from the three metabolic conditions, Figure 3.3A shows the mean dominance durations (average periods of time when perception was dominated by the deprived eye, in purple, or non-deprived eye, in light blue), before and after deprivation. A

two-way ANOVA for repeated measures shows a significant interaction between the factors time (pre vs. post deprivation) and eye (deprived vs. non-deprived) ($F(1,12) = 6.09$, $p = 0.03$, lgBF = 0.11). Post-hoc t-tests confirmed the significant increase of deprived eye dominance durations after deprivation (pre vs. post: $t(12) = -2.78$, $p = 0.02$, lgBF = 0.58), with no significant changes for the non-deprived eye ($t(12) = -0.93$, $p = 0.37$, lgBF = -0.40).

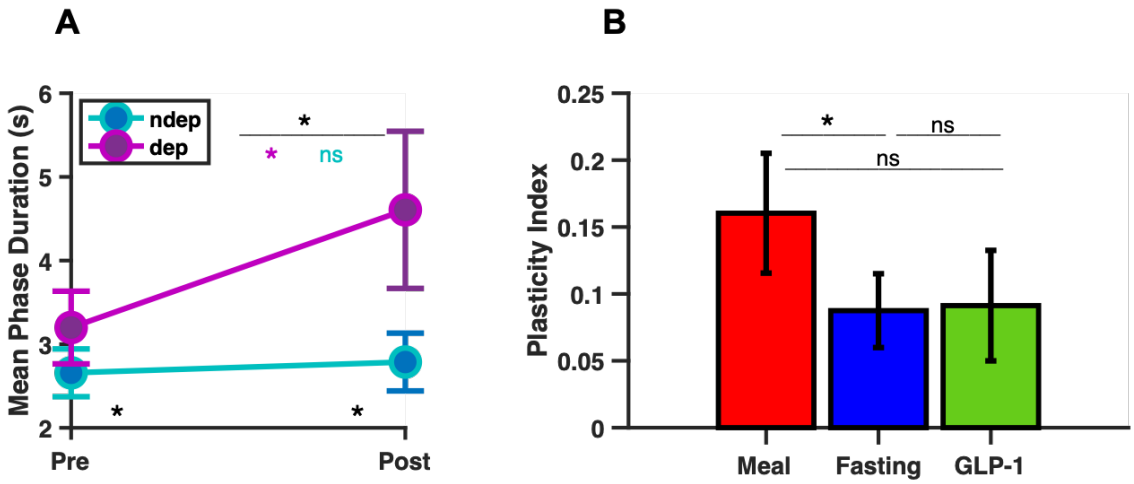

Figure 3.3 Short term plasticity effect. A) Average phase duration for the deprived and non-deprived eye, before (Pre) and after (Post) a 2h monocular deprivation; data averaged across the three metabolic conditions. Error bars are s.e.m. (standard error of the mean) across participants. The black star on the top indicates the significance of the interaction between factors time (Pre, Post deprivation) and eye (Deprived, Non-deprived); purple and light blue stars give the significance of the change across deprivation for the deprived and non-deprived eye respectively (for p <0.05, ns otherwise). Black stars near the x-axis give the significance of the difference across eyes, before and after deprivation. B) Plasticity Index (computed with Equation 1) in the three metabolic states. Bars and error bars give the average and s.e.m. across participants and symbols above horizontal lines reports the statistical significance of paired t-tests (* for p <0.05, ns otherwise).*

This effect is adequately summarized by a plasticity index, defined based on previous studies as the log transformed post- to pre-deprivation dominance ratios (see Methods, Eq. 6). Figure 3.3B (red, blue, and green bars) shows the average Plasticity

Index, separately for the three metabolic conditions. In spite of a tight correlation of individual plasticity indices in the two conditions (Pearson's Skipped Rho=0.81, 95% CI [0.67, 0.95]), which supports the robustness of this psychophysical measure, we found that the fasting regime was reliably associated with a reduction in short-term ocular dominance plasticity compared to the standard meal condition (red vs. blue bar, $t(11) = -2.41$, $p = 0.03$, lgBF = 0.34).

Figure 3.4 shows the results from an internal replication, where we confirmed the effect of fasting in a separate set of participants and a more usual set-up (in the laboratory, with participants reporting at 9 AM with or without having had their usual breakfast). Even in this less controlled situation, fasting was associated with a reliable reduction of the plasticity index.

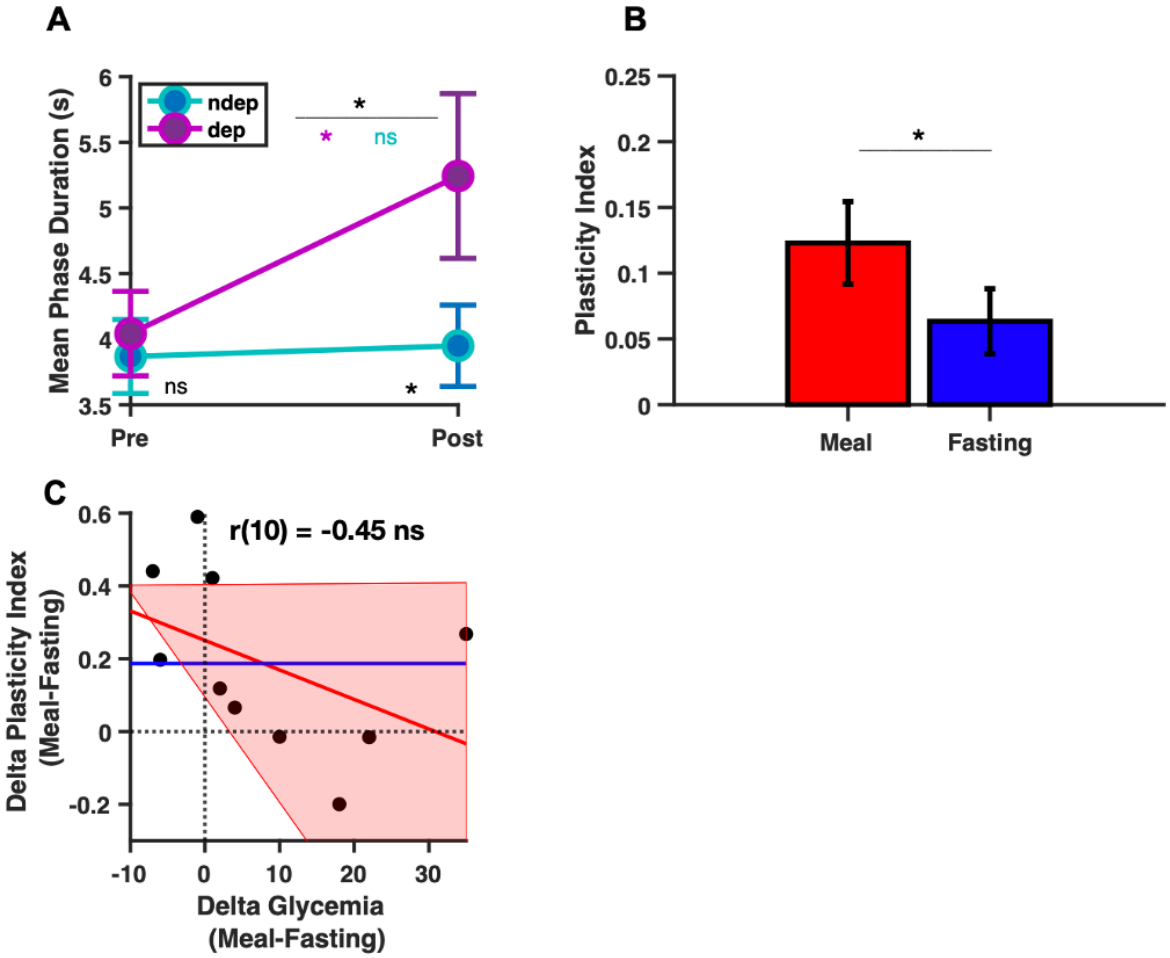

*Figure 3.4 Internal replication A) Effect of a 2h monocular deprivation on binocular rivalry dynamics, with data averaged across metabolic states. B) Plasticity indices (Eq.1) separately for the meal and fasting conditions, with the asterisk indicating the significance of the paired t-test at p<0.05. C) lack of association between the difference in plasticity and the difference in glycemic levels measured in the two conditions, estimated with the robust correlation approach. Black dots give individual participants results; red points mark participants that were marked as outliers by the automatic robust regression routine. Red thick links with red shading the robust regression lines with associated 95% confidence interval. Text insets give the Pearson's correlation with associated sample size and p-value. ns, nonsignificant. *p < 0.05; **p < 0.01; ***p < 0.001 .*

We sought to investigate the associations between variations in short-term plasticity and metabolic parameters, estimated through analyses of the blood samples collected during psychophysical testing. Contrary to our expectations, we found no association between plasticity indices and any of the parameters related to glucose metabolism. Figure 3.5 A-B plots the difference of plasticity indices in the

standardized meal vs. fasting regime as function of the difference in blood glycemia or GLP-1 release in the two conditions. Although both differences are positive on average (fasting being associated with reduced plasticity and reduced glycemia, as well as with reduced GLP-1 release over the 2h of the experiment compared to the standard meal condition), and in spite of considerable variability across participants, there was no hint to an association between the variables (non-significant Pearson's Skipped Rho = 0.24, 95% CI [-0.49, 0.67] for glycemia, Figure 3.5 A; non-significant Pearson's Skipped Rho= -0.28, 95% CI [-0.87, 0.22] for GLP-1, Figure 3.5 B). Similarly uncorrelated to plasticity were insulin and c-peptide values (not shown). Also in the replication study, where only glycemic levels could be estimated through a portable device (Countour®next glucometer), we found no association with plasticity values (non-significant Pearson's skipped Rho = -0.45, 95% CI [- 0.89, 0.02], Figure 3.4 C).

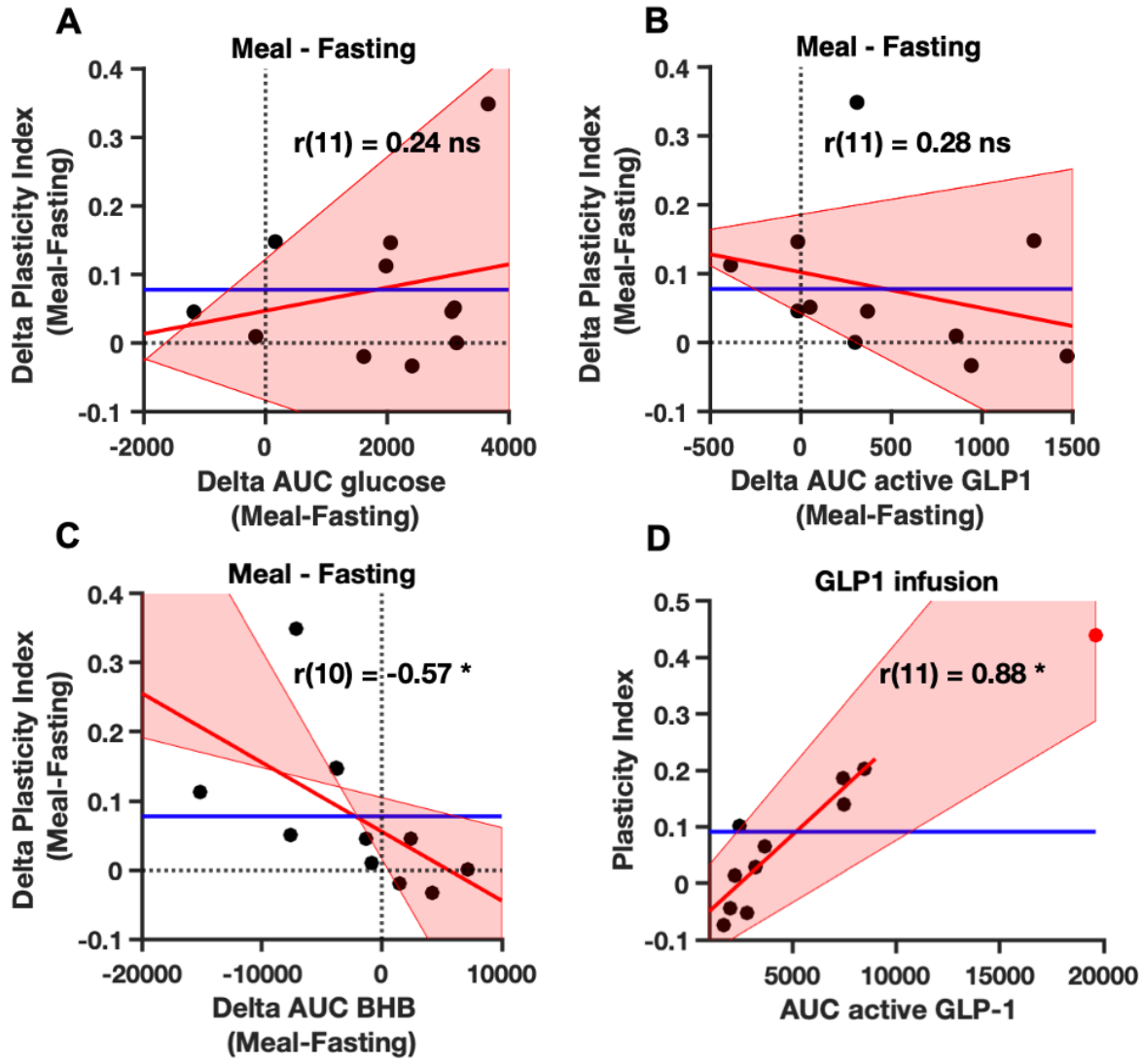

*Figure 3.5 Associations between Plasticity and metabolic variables estimated with the robust correlation approach. A-C: Association (or lack thereof) of the plasticity difference in the standardized meal versus the small fasting regime (y-axis) with the difference in plasma concentrations of glucose, active GLP1 and BHB between the two conditions. Dotted black lines mark y=0 and x=0. D: association of the plasticity value in the GLP1 infusion condition and the plasma concentration of active GLP1 in the same condition. In all panels, black dots give individual participants results; red points mark participants that were marked as outliers by the automatic robust regression routine. Red thick links with red shading the robust regression lines with associated 95% confidence interval. The horizontal blue line marks the mean of y-axis values. Text insets give the Pearson's correlation with associated sample size and p-value. ns, nonsignificant. *p < 0.05; **p < 0.01; ***p < 0.001 .*

In line with the lack of association between plasticity and glucose metabolism, and again in contrast with our predictions, we found that the GLP-1 infusion did not rescue plasticity to the levels associated with a standard meal (green bar in Figure 3.3B), with

a plasticity index that was not reliably distinguishable from the fasting condition ($t(10) = 0.05$, $p = 0.96$, lgBF= - 0.53). Note the negative lgBF value, indicating strong evidence against the presence of a difference between these conditions and implying that the lack of a significant effect cannot be put down to lack of statistical power.

Having found that plasticity could not be predicted by parameters of the glucose metabolism, we turned to indices of alternative pathways, namely ketone metabolism (**β**HB) and functioning of adipose tissue (leptin). While leptin was never associated with any of our plasticity measures (not shown), we found that a reliable inverse relationship did emerge between the plasticity reduction and the **β**HB increase in the fasting condition, revealing a potential negative relationship between ketone metabolism and short-term plasticity (significant Pearson's Skipped Rho = -0.57, 95% CI [-0.91, -0.35], Figure 3.5 C). The GLP-1 infusion modulated the blood concentration of ketone bodies (Figure 3.2F), and this apparently disrupted the relationship between **β**HB and plasticity (no correlation between the plasticity and **β**HB variation in the GLP-1 infusion vs. fasting conditions, non-significant Pearson's Skipped Rho = - 0.13, 95% CI [-0.75, 0.79], not shown in Figure). At the same time, there was an exceptionally tight relationship between the blood concentration reached through GLP-1 infusion and the levels of plasticity measured in that condition (significant Pearson's Skipped Rho = 0.88, 95% CI [0.62, 0.98], Figure 3.5 D), which could not be explained away by regressing out any of the other factors we could quantify (**β**HB, glycemia or infused glucose). This counterintuitive finding suggests that, while

glucose metabolism does not physiologically impact short-term plasticity, it might nevertheless interact with cortical function when its activity is driven to uncharacteristically high and sustained values through a pharmacological manipulation.

3.4 Discussion

The study demonstrates that 12 hours fasting was associated with a reduced sensory plasticity as assessed by short-term monocular deprivation. A significant boost of sensory plasticity could be observed in response to mixed meal ingestion suggesting a potential direct role of metabolic factors on neural dynamics even in low-level sensory cortices, and in short-term.

The direct impact of transient reduced caloric intake (as an overnight fasting) is less understood compared to more complex dietary protocols, and there is limited extant literature examining these effects. Breakfast skipping is one of the more commonly studied types of meal abstention, and its effects are typically assessed in children, adolescents, and young adults and in the cognitive domains (Benau et al., 2014; Peña-Jorquera et al., 2021). A recent meta-analysis has shown that, in humans, short-term fasting has effects on the activity of brain areas involved in memory tasks (Benton & Parker, 1998; Chechko et al., 2015; Green et al., 1997; Okauchi et al., 2020; Owen et al., 2012; Sünram-Lea et al., 2001), perceptual abilities (Pender et al., 2014;

Zitron-Emanuel & Ganel, 2018, 2020), verbal learning (Sünram-Lea et al., 2001), attention (Anderson et al., 2018; Komiyama et al., 2016; Solianik et al., 2020), and psychomotor functions (Cherif et al., 2017). However, findings are mostly inconsistent and there is a lack of consensus regarding the possible underlying mechanisms.

The plasticity indexes in our three different experimental conditions were uncorrelated to any of the parameters of glucose metabolism; however, ketone metabolism does associate with short term plasticity in the fasting condition. This may converge with recent work in animal models in indicating a role of ketone metabolism in regulating multiple cortical functions (Jensen et al., 2020), including plasticity (Achanta & Rae, 2017; Jensen et al., 2020; Mattson et al., 2018). This association is potentially consistent with the hypothesis linking ketosis and cortical inhibition; in fact, ketone bodies, of which **β**-hydroxybutyrate (**β**HB) represents the 80%, are thought to modulate cortical activity by impacting GABA storing and release (Daikhin & Yudkoff, 1998; Yudkoff et al., 2001), beyond regulating cell metabolic efficiency and ATP availability (Achanta & Rae, 2017). The response to monocular deprivation after patching is thought to reflect transient changes in neuronal circuitry in primary visual cortex providing a marker of neuronal plasticity: changes in visual cortex activity consistent with the deprived eye dominance boost observed at the perceptual level have been observed with different techniques (Binda et al., 2018; Lunghi, Berchicci, et al., 2015; Lunghi, Burr, & Morrone, 2011b). Importantly, it has been shown that the effect of short-term monocular deprivation in humans is also accompanied by a

decrease of GABA concentration in the primary visual cortex (Lunghi, Emir, et al., 2015). Moreover, βHB supplements have been shown to promote brain network stability (Mujica-Parodi et al., 2020), that in turns has been correlated with GABA levels in multiple brain regions (Stagg et al., 2014); hence, we speculate that the reduced plasticity we observe in the fasting condition might result from ketone bodies promoting stability over change, possibly through a modulation of the excitation-inhibition balance in the visual cortex. This last hypothesis needs verifying in a larger sample and perhaps with direct intervention upon ketosis.

GLP-1 intravenous administration during fasting condition, albeit mimicked GLP-1 concentrations obtained during mixed meal, did not rescue plasticity levels. Somewhat counterintuitively, however, GLP-1 levels, in particular active GLP-1, which is biologically active form, varied markedly across participants (even though the infusion was regulated based on each participant's weight) reaching non-physiologically high and sustained level at the end of experimental procedure. This phenomenon may be in part due to dipeptidyl peptidase-4 (DPPIV) activity variability across participants. DPPIV is an enzyme produced in multiple tissues and is known to cleave a broad range of substrates including GLP-1. Active GLP-1 circulates as GLP-1 [7–37] and GLP-1[7–36]NH2 these peptides are cleaved by DPPIV to generate GLP-1 [9–37] and GLP-1[9–36]NH2, respectively (Deacon, Johnsen, & Holst, 1995). DPPIV cleavage eliminates the classical glucoregulatory actions of GLP-1 and generates peptide(s) with 100-fold less receptor affinity (Knudsen & Pridal, 1996). There are

several potential mechanisms whereby the activity of DPPIV could be modulated, including biosynthesis, release into plasma, or breakdown and clearance of the circulating form. In the present study DPPIV activity could not be measured therefore it would be of interest to further explore the potential relationship between DPPIV activity, active GLP-1, and net effect on brain plasticity. Nonetheless, GLP-1 levels were tightly and positively associated with the plasticity level observed in the corresponding condition, suggesting that GLP-1, when reaching a non-physiologically high and sustained level, can disrupt the physiological regulation of plasticity. GLP-1 receptors are expressed in many areas of the human brain and widely present in visual cortex (Grieco et al., 2019) and several studies have shown the influence of GLP-1 on different neuronal function such as neurogenesis, neurodegeneration, retinal repair, control of satiety, water intake, and stress reaction (Katsurada & Yada, 2016). It would be of interest to evaluate the impact of GLP-1 on brain plasticity on long-term basis exploring the effect of a chronic administration of GLP-1 analogues, which are resistant to DPPIV cleavage and may maximize the GLP-1 receptor activation at pharmacological level.

Many studies have investigated the role of dietary protocols as caloric restriction and intermittent fasting on brain health (Longo & Mattson, 2014), showing promising results in promoting neuroplasticity (Murphy et al., 2014), even in primary visual cortex (Spolidoro et al., 2011). Here we report that short-term fasting seems instead to reduce plasticity, as measured by monocular deprivation. This suggests that short-

term fasting and long-term fasting protocols may rely on different mechanisms, difficult to disentangle.

The main limitations of our work are the small size of the study population and the limited range of metabolic parameters defined a priori based on study design. The plasticity effects we quantify are based on sensory parameters (i.e. visual ocular dominance plasticity): these might perhaps be less relevant to cognitive function than standard neuropsychological tests, but are instead insensitive to such confounding factors as motivation or arousal which is an important strength of our approach.

In fact, short-term monocular deprivation has been proven to be a reliable, robust, and quantitative measure of cortical plasticity; in our data, we have shown that this form of plasticity systematically co-varies with physiological and short-term modifications of the metabolic state. We are totally aware that correlations between metabolic parameters and plasticity index do not imply causality, yet our results set the basis for future investigation on the outstanding complex relationship between brain plasticity and brain–gut axis.

In summary, we have shown that sensory plasticity can be modulated by short-term metabolic manipulations, suggesting that skipping breakfast makes us less capable to adapting to the stimuli we receive, and favoring stability over plasticity of cortical function.

4 Chapter

Short-term ketosis impact on visual processing and brain metabolism

4.1 Introduction

There is growing evidence for an unexpected role of different metabolic variables on brain function and behavior – both in healthy and pathological human adults (Longo & Mattson, 2014; Mattson, 2012; Mattson et al., 2018; Murphy et al., 2014).

Under normal circumstances, the brain primarily relies on glucose afflux and glucose oxidation to produce energy (Owen et al., 1967). In fasting or strenuous physical exercise, ketone bodies represent the main alternative metabolic fuel for brain's cells (Jensen et al., 2020). Endogenous ketogenesis occurs mostly in hepatocytes, where free fatty acids undergo a process of beta-oxidation, regulated by insulin and glucagon (Puchalska & Crawford, 2017). The three final substrates (Acetone, Acetoacetate and beta-hydroxybutyrate (**β**HB)(Newman & Verdin, 2017) are released in the blood stream and then transported across the blood brain barrier (BBB) via monocarboxylic acid transporters (MCT 1–4) to meet the brain's metabolic demands (Jensen et al., 2020) through concentration-dependent metabolic processes. (Jensen et al., 2020). Beside physiological conditions, ketone bodies plasma levels can also be raised by dietary approaches as ketogenic diets or exogenous supplements in form of ketone salts or ester (Clarke et al., 2012a; Soto-Mota, Norwitz, & Clarke, 2020; Stubbs et al., 2017).

Ketone ester (KE) drinks have been proven to be safe and well tolerated in humans (Clarke et al., 2012a), and this provides an opportunity to accurately set and monitor blood **β**HB concentrations (Soto-Mota et al., 2020).

The interest in how ketone bodies affect brain function dates back to the 1920s, when a low-carb and high-fat diet was successful in treating pediatric epilepsy (Helmholz & Keith, 1933; McQuarrie & Keith, 1927). With the advent of anticonvulsant drugs, the ketogenic diet was used less frequently, although it has regained interest in the last 2–3 decades due to its capacity to reduce seizures in pharmacoresistant epilepsy (Thiele, 2003). Since then, a wide number of studies have tried to disentangle the complex effects of ketone bodies on brain function and there is now compelling evidence that these molecules play a critical role in shaping neural circuits, through a variety of mechanisms (García-Rodríguez & Giménez-Cassina, 2021). They have been proven to 1) influence intracellular signaling post- translationally 2) directly as extracellular receptor ligand, 3) modulate cells' energy balance and expenditure 4) modulate cortical excitability through enhancing or lowering neurotransmitter synthesis and release (Jensen et al., 2020).

In particular, it has been shown that ketone bodies can favor the biochemical synthesis of GABA (Daikhin & Yudkoff, 1998; Erecińska et al., 1996; Yudkoff et al., 2001). A recent human study (Mujica-Parodi et al., 2020) has shown an effect of a **β**HB ketone ester in stabilizing cortical networks, a phenomenon previously linked to an increase of inhibition (Stagg et al., 2014). In a previous study from our laboratory, we have shown that overnight fasting reduces homeostatic ocular dominance plasticity, which has also been previously correlated with reduced GABAergic inhibition in primary visual cortex as measured by Magnetic Resonance Spectroscopy (1H-MRS)

(Lunghi, Emir, et al., 2015). We then speculate that a short-term ketosis might enhance GABA levels in visual cortex, boosting inhibition (and reducing cortical plasticity). To test this hypothesis, we designed a study that 1) directly measured metabolites levels in visual cortex after a ketone ester drink using MRS, 2) measured visual evoked responses changes known to be sensitive to level of excitation-inhibition cortical balance.

4.2 Methods

4.2.1 Participants

We recruited 12 healthy volunteers (2 females, 10 males; mean age 27.2 ± 3.4 years). Participants provided written informed consent prior to inclusion and completed a confidential medical screening questionnaire to determine eligibility. Anthropometric and screening blood tests were also taken before including participants in the study. Moreover, they were selected to have normal or corrected-to-normal vision and normal color vision, and no known history of amblyopia, eye surgery, or other active eye disease. The first participant recruited for the experiment was then discarded from the analysis due to a protocol change occurred later. One female subject was further excluded due to an anatomical abnormality encountered in the MR anatomical acquisition. One subject was later discarded due to an abnormal fasting glycemia levels found after analyzing his blood samples (>140 mg/dl).

4.2.2 Experimental design and procedure

Each participant reported to our laboratory at 8.00 a.m, after an overnight fasting of 12 hours. Blood samples were collected immediately upon arrival, and then a structural standard MRI (Magnetic Resonance Imaging) and MRS (Magnetic Resonance Spectroscopy) acquisition were performed. Immediately after the MR acquisitions, the participants underwent an EEG recording, including visual stimulations (Steady State Visual Evoked Potentials and Cross-Orientation Inhibition stimuli). After completing EEG recording, a ketone ester drink (HVMN®) was orally administrated. One hour later, EEG recordings, anatomical MRI and MRS acquisitions were performed again. Venous blood samples for pharmacokinetic analysis were collected before starting the whole experimental procedure and at 60-, 120-, and 180-minutes post ketone ester drink.

4.2.3 βHB -ester and blood samples collection and analysis

Participants received a single standard oral dose of the ketone ester drink (HVMN®), containing 25 grams of D-β-Hydroxybutyrate. Samples were stored on ice, centrifuged (3000 crf, 15 minutes at 4 Celsius degrees) and duplicate plasma aliquots stored at −80◦C.

D-b-hydroxybutyrate was stored in a K2E (EDTA) 7,2mg BD Vacutainer test tube and assayed using the commercially available ß-Hydroxybutyrate LiquiColor® (EKF Diagnostics). Plasma glucose was stored in a FX 10mg 8mg, BD Vacutainer and was assessed using a commercial Glucometer (YSI 2300 STAT Plus). Insulin and c-peptide

were stored in Litio/Heparina 102 IU BD Vacutainer and measured using a commercially available ELISA assay (Mercodia, DBA Italia S.r.l.). Glucagon, GLP-1 total and active were stored using a BD™ P800 Blood Collection System test tube. Glucagon was then assayed using a commercially available ELISA assay (Mercodia, DBA Italia S.r.l.), whilst GLP-1 levels were assessed with dedicated commercially available ELISA kit (Merck Life Science S.r.l.). Blood levels of D-b-hydroxybutyrate, glycemia, insulin, c-peptide, glucagon, glp-1 total were assessed, and their plasma concentration–time profiles and both area-under-the-curve (AUC) and incremental area-under-the-curve (iAUC) further calculated for the analysis.

4.2.4 EEG recordings

Apparatus and stimuli

EEG was recorded using a wireless g.Nautilus system, with a sampling rate of 250 Hz. The scalp electrodes were positioned according to the 10-20 international system and the reference electrode on the right earlobe. The impedance was checked before each recording and kept below 50 kΩ. For the present study, we recorded 8 electrodes, including central electrodes FZ, CZ and the posterior and occipital electrodes PZ, OZ, PO3, PO7, PO4, PO8.

The visual stimuli were generated with Psychtoolbox for Matlab (Matlab r2017b, The Mathworks, inc.) and displayed on a gamma-calibrated Barco monitor (Barco CDCT 6551, 800 × 600 pixels, 100 Hz) through a ViSaGe (CRS, Cambridge Research Systems, Rochester, UK). A custom-made trigger connected the ViSaGe with the

g.Nautilus system. Participants were positioned at 118 cm from the screen. 2 conditions were tested: steady-state visual evoked potentials, and cross-orientation inhibition.

For steady state visual evoked potentials, the stimulus consisted in a whole-screen horizontal sinusoidal grating (spatial frequency = 1 c/°, nominal contrast 48%), following a sinusoidal luminance modulation (temporal frequency) of 8.33 Hz.

For cross-orientation inhibition the stimulus used in experiment was superimposed with vertical sinusoidal grating (spatial frequency = 0.8 c/°, nominal contrast 60%, temporal frequency = 7.1 Hz).

Analysis

Offline analyses were performed using EEGlab (14.1.2b) and custom-made MATLAB scripts (MATLAB 2019a; MathWorks). The EEG signal was segmented into 240 ms epochs (2 cycles of stimulation), synchronized with the phase of the of the visual stimulus. Raw EEG signal was visually inspected, and epochs with gross artifacts were discarded from further analyses. Then, a fast Fourier transform (FFT) was applied to the average of the retained epochs. Second (H2) and fourth (H4) harmonics resulting from the FFT were further analysed.

4.2.5 MRI and MRS – acquisition

All participants underwent an anatomical MRI brain scans with 3.0 T system (GE Excite HDx, GE Medical Systems, Wisconsin, Milwaukee, USA) equipped with TwinSpeed gradients in the Foundation CNR/Regione Toscana G. Monasterio, Pisa, Italy.

3D SPGR

The scanning protocol included a three-dimensional (3D) fast inversion-recovery prepared gradient echo acquisition covering all the brain (TR/ TE = 10.7/4.9 msec, FOV= 25.6cm, acquisition matrix= 256x256, thickness 1mm, slice gap 0mm, BW 15.6kHz, NEX 1) used to obtain high resolution three-dimensional images.

1H-MRS

Proton magnetic resonance spectroscopy (1H-MRS) was performed by sampling for occipital grey matter, using a single-voxel technique (SVS) with the short TE PRESS (Point Resolved Spectroscopy Sequence) sequence (TR= 2000 ms, TE= 35, FOV=240 mm, voxel= 20x20x20mm, acquisition time 5.04 min), to get metabolic NAA, Cho, Cr and ml peaks.

Orthogonal images (axial, coronal, and sagittal FLAIR) were used to locate volumes of interest. Partial volume effects have the potential to cause inaccuracies when quantifying metabolites using H-MRS. For LC model, we corrected the spectroscopy results for partial volume cerebrospinal fluid (CSF) contamination. The volumetric T1-

weighted images were segmented into grey matter (GM), white matter (WM), and CSF maps using FSL (www.fmrib.ox.ac.uk/fsl).

SAGE PROCESSING for 1H-MRS

The 1H-MRS were reconstructed with SAGE software 7.0 (Spectroscopy Analysis by General Electric) a spectroscopic processing and display software tool made by GE Healthcare, using a dedicated semi-automated program (PROBE/SVQ).

Residual water was removed in the time domain from the raw data. Data were zero-filled once, apodised with a 2.5-Hz Gaussian filter, Fourier transformed, and phase and baseline corrected. Spectra were then fitted to Lorentzian line shapes using the Levenberg-Marquardt method of nonlinear least-squares minimization.

Spectral quantification was performed by manually selecting (in the frequency domain) the detected resonances (NAA, mI, Cho and Cr).

A table of the estimated peak amplitude, linewidth and frequency was then automatically generated.

LCMODEL PROCESSING for 1H-MRS

LCModel (LCMODEL Inc., Oakville, Ontario, Canada) was used to calculate GABA and Glutamate concentrations based on model spectra derived from in vivo recordings.

Data quality and reliability were assessed by reporting the Cramer-Rao value. Glutamate and glutamine (Glugln) and GABA had Cramer-Rao values of less than 26 % and 9% in the first session, and of less than less than 25 % and 10% in the second session.

MEGAPRESS – **β**HB

βHB concentrations were estimated with a separate MEGA-PRESS (Mescher et al., 1998) editing sequence at 3T. A total of 128 spectral averages were acquired for each spectrum with TR of 1800 ms, echo time of 68 ms, and an eight-step phase cycle, resulting in an acquisition time of approximately 8 min. The voxel size used in MEGA-PRESS is large when compared to H-MRS, this is necessary to offset the inherent low signal to noise ratio (SNR) for **β**HB. We used a voxel of 25×25×25 mm^3 in occipital localization.

GANNET PROCESSING for MEGAPRESS

Coil-combination, phasing, apodization, and frequency correction were performed automatically. **β**HB was modelled as a single Gaussian superimposed on a linear baseline, and both the water scaled **β**HB concentration, and the **β**HB /Cr ratio were calculated.

Quantification—Water-scaled **β**HB concentrations were calculated considering the editing efficiency and approximate macromolecular contributions to the **β**HB+ peak.

4.3 Results

Pharmacokinetic analyses were conducted on the blood samples drawn at regular intervals to quantify the variation of key metabolic parameters (Table 4.1).

Characteristic (mean ± SD)	Baseline	T60	T120	T180
Age (y)	26.67 ± 3.54	-	-	-
BMI (kg/m^2)	22.06 ± 1.89	-	-	-
βHB (μM/dl)	118.47 ± 63.41	3199.18 ± 685.31	2340.56 ± 403.96	1786.49 ± 402.53
Glycemia (mg/dl)	96 ± 12	77.22 ±12.35	78.89 ±10.89	83.22 ±9.51
Insulin (pmol/l)	29.01 ± 15.14	33.68 ± 11.89	24.56 ± 16.48	15.71 ± 9.15
C-peptide (ng/ml)	1.15 ± 0.51	1.3 ± 0.46	1.08 ± 0.38	0.79 ± 0.35
GLP-1 total (pM)	32.72 ±10.39	31.71 ±11.98	34.23 ±11.21	-
GLP-1 active (pM)	4.68 ± 1.72	2.10 ± 2.17	3.36 ± 2.41	-
Glucagon (pM)	6.58 ± 4	6.62 ± 3.08	7.82 ± 3.15	-
Homa-IR	1.08 ± 0.58	1.3 ± 0.58	0.78 ± 0.57	0.66 ± 0.51

Table 4.1. Characteristics of the participants.

Blood D-βHb concentrations (Figure 4.1A) raised from a baseline level of 118.47 ± 63.41 μM/dl (mean and SD) to a maximum of 3199.18 ± 685.31 μM/dl (mean and SD)

at 60 minutes after oral administration of ketone ester, in line with previous reports (Clarke et al., 2012b; Mikkelsen, Seifert, Secher, Grøndal, & Van Hall, 2015; Stubbs et al., 2017) After the peak was reached, blood D-βHb levels decreased non-linearly (Stubbs et al., 2017). After 3 hours from the ingestion, blood D-βHb remained significantly elevated compared to baseline ($t(8) = 12.41$, $p<0.001$, logBF10 = 4). Variability within individual D-βHB time courses was very low, and likely due to normal daily changes in GI function, including gastric emptying, portal blood flow or intestinal transit time, which may alter KE hydrolysis and absorption (Clarke et al., 2012b). Glucose plasma levels (Figure 4.1B) decreased slightly but significantly over time, from 96 ± 12 mg/dl to 83.22 ±9.51 mg/dl at the last measurement, with a minimum of 77.22 ±12.35 mg/dl at 60 minutes.

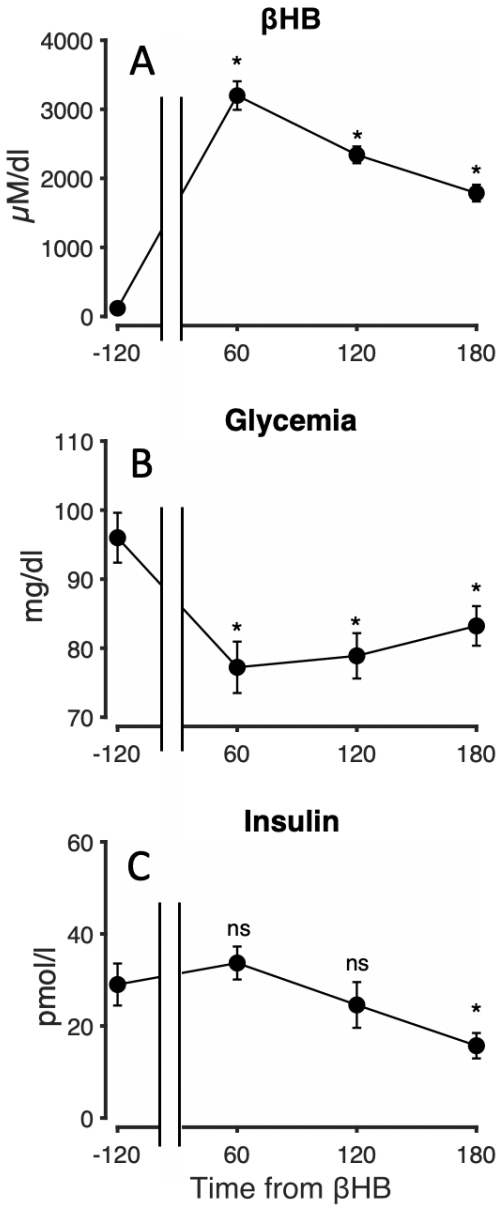

Figure 4.1. Metabolic parameters. Plasma concentrations of D-b-hydroxybutyrate, glucose and insulin. Black dots and error bars give the average and s.e.m. across participants, text insets report statistical significance of paired t-tests of every time point with respect to baseline (for p <0.05, ns otherwise).*

Glucose lowering remained significant compared to baseline until our last measurement 3 hours later ($t(8) = -6.48$, $p<0.001$, $logBF10 = 2.2$). There was a small and non-significant ($t(8) = 0.82$, $p=0.43$, $logBF10 = 0.4$) rise in insulin concentration 1 hour after the oral administration, followed by a progressive decrease (Figure 4.1C), with the last measurement significantly lower compared to baseline ($t(8) = -2.75$,

$p<0.05$, logBF10 = 0.5). C-peptide levels were similar to those of insulin, showing a significant decrease in the last measurement ($t(8) = -2.66$, $p<0.05$, logBF10 = 0.4). Glucagon and GLP-1 total (*not shown*) mean plasma levels measured 60 and 120 minutes after KE drink did not significantly differ from baseline; however, GLP-1 active (*not shown*) showed a significant lowering 1 hour after KE drink ingestion($t(8) = -3.12$, $p<0.05$, logBF10 = 0.7). We also assessed Insulin Resistance through the (HOMA-IR) index. (HOMA-IR = 1.08 ± 0.58). HOMA-IR was calculated as fasting insulin (μU/mL) X fasting glucose (mg/dL)/405. Insulin Resistance, after a small and non-significant enhancement, progressively decreased (*not shown*) throughout the experiment, becoming significantly lower than baseline 3 hours after the oral administration of ketone ester ($t(8) = -3.22$, $p<0.05$, logBF10 = 0.8).

We investigated the associations between variations in D-βHB levels and the other metabolic parameters. We found no direct correlation between D-βHB levels reached after KE drink and any of the parameters related to glucose metabolism.

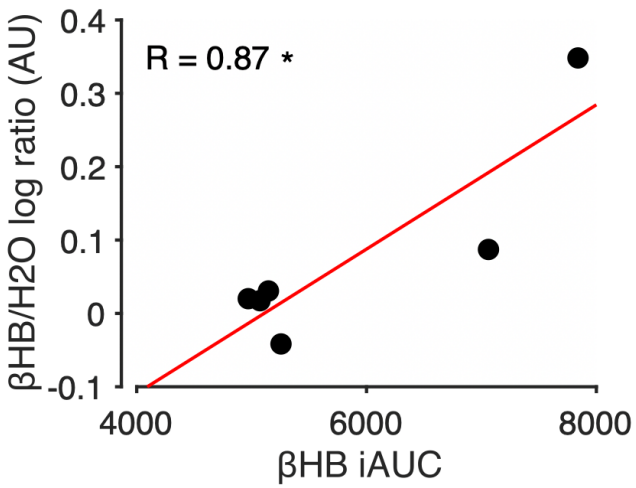

*Figure 4.2. Association between βHb plasma iAUC (incremental Area Under the Curve) and βHb/h20 log ratio (post/pre) measured with MEGAPRESS. Black dots represent single participants, and the thick red line the regression line. Text insets give the Pearson's correlation with associated p value. *p < 0.05; **p < 0.01; ***p < 0.001.*

We assessed βHB cerebral levels with a dedicated MEGAPRESS sequence on a subsample of 6 participants in the occipital region. Although the difference in βHB/H_2O or βHB/Cr levels pre and post the KE drink was not significant (**β**HB /H_2O, t(5)=-1.29,p=0.25, logBF10 =-0.2; **β**HB /Cr, t(5)= -1.38,p=0.22, logBF10 =-0.1), expected given the small sample size, we observed a significant correlation between the log10(post/pre) ratio of βHB spectra and plasma **β**HB iAUC (**β**HB /H_2O, r(6)=0.87,p<0.05, logBF10 =0.63; **β**HB /Cr, r(6)=0.86,p<0.05, logBF10 = 0.53, Figure 4.2). Moreover, D-βHB plasma peak 60 minutes after KE drink was the single best value relating to occipital βHB log10(post/pre) ratio (**β**HB /H_2O, r(6)=0.87,p<0.05, logBF10 =0.6; **β**HB /Cr, r(6)=0.83, p<0.05, logBF10 =0.4).

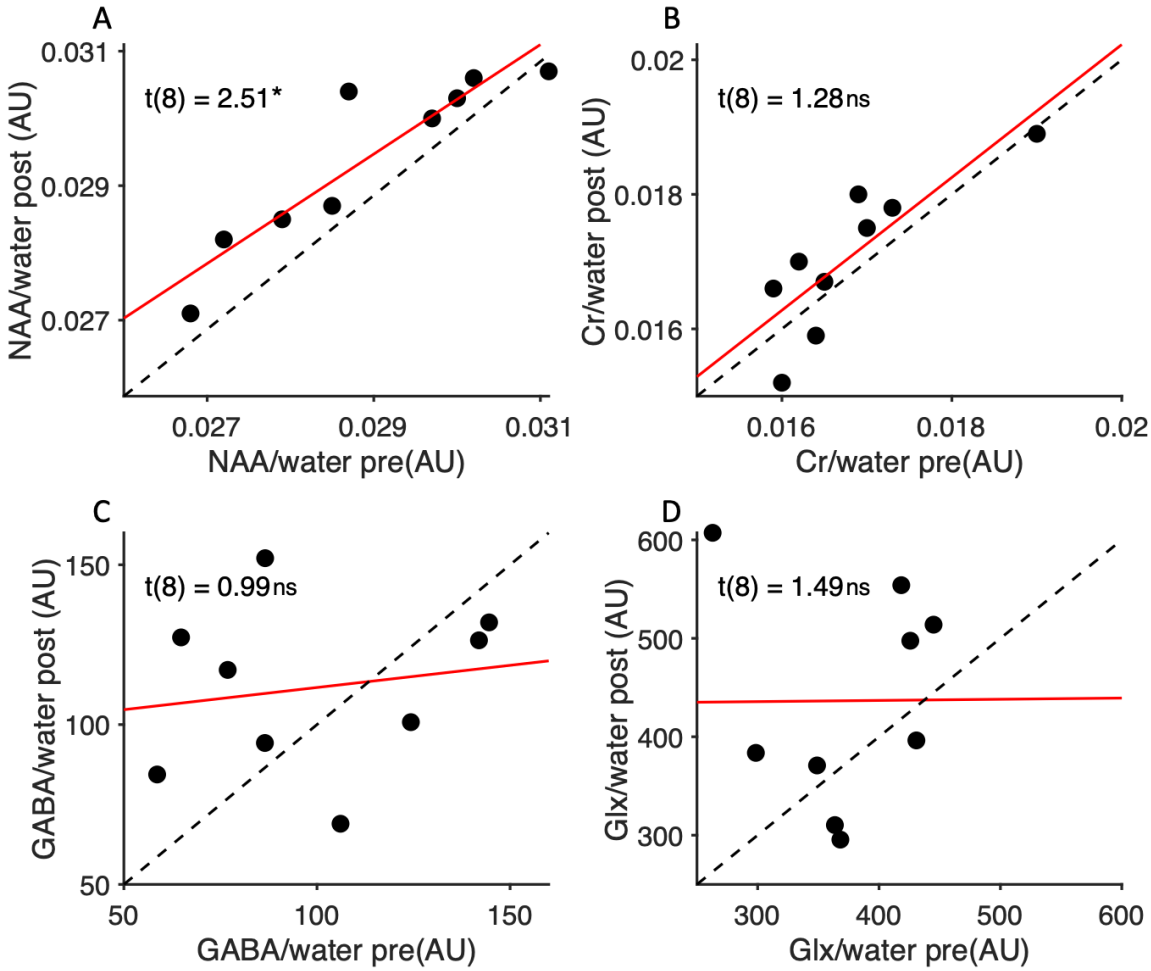

*Figure 4.3. MRS main metabolites levels pre and post KE ester drink. A) Increase of NAA/water measured by SAGE-MRS post KE-drink B) Cr/ water measured by SAGE-MRS post KE-drink remains stable C) GABA/water measured by LC model pre and post a KE drink. D) Glx/water measured by LC model pre and post a KE drink. For all figures the dotted black line represents the bisector of the graph. Text insets give the Student's T with associated p value. Black dots represent single participants, and the thick red line the regression line. *p < 0.05; **p < 0.01; ***p < 0.001.*

Neurochemicals levels assessed with SAGE shown that NAA/H_2O, but not NAA/Cr, increased in the occipital region (t(8)=-2.51,p<0.05, logBF10 =0.4, Figure 4.3A). We could not find a mean main effect for other neurochemical in 1H-MRS data analyzed with SAGE (Cr, t(8)=1.28,p=0.23, logBF10 =-0.2, Figure 4.3 B; mI, t(8)=-1.03,p=0.33, logBF10 =-0.3, *not shown*; Cho, t(8)=-1.16,p=0.27, logBF10 =-0.3, *not shown*). Also GABA and Glugln levels measured with LC model did not show any mean main effect

(GABA, t(8)=0.99,p=0.34, logBF10 =-0.3, Figure 4.3 C; GluGln, t(8)=1.49,p=0.17, logBF10 =-0.1, Figure 4.3 D). Figure 3.5 shows two examples MRS spectra before and after the KE ester drink.

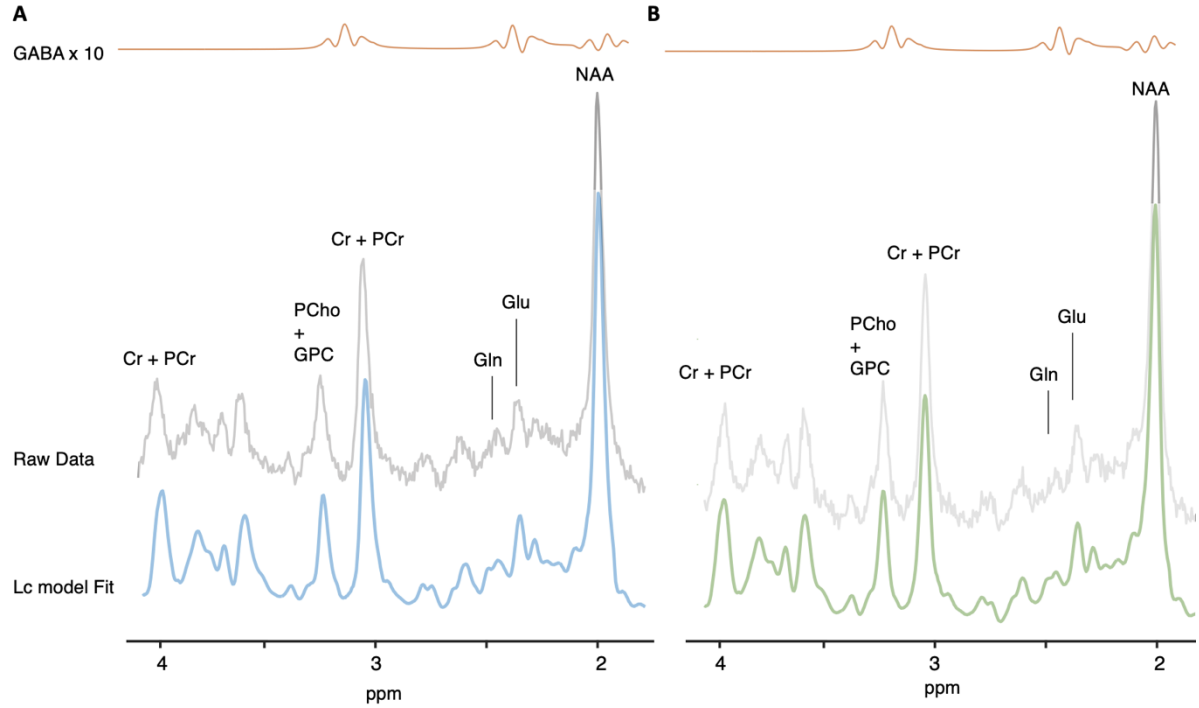

Figure 4.4. MRS spectra examples. A) A representative MRS spectrum pre–KE ester. From bottom to top: blue spectrum shows LC Model fit to raw data. Gray spectrum shows raw data. Topmost line shows the LC Model fit to GABA signal. For illustrative purposes, the GABA signal was multiplied by 10. B) A representative MRS spectrum post–KE ester. From bottom to top: green spectrum shows LC Model fit to raw data. Gray spectrum shows raw data. Topmost line shows the LC Model fit to GABA signal. For illustrative purposes, the GABA signal was multiplied by 10.

We observed a significant correlations between occipital NAA/H_2O log10(post/pre) ratio and D-βHB delta between t180 plasma level and baseline (r(9)= -0.81, p<0.01, logBF10 =0.92); note that t180 is the closest time point to the 1H-MRS acquisition (Figure 4.5 A).

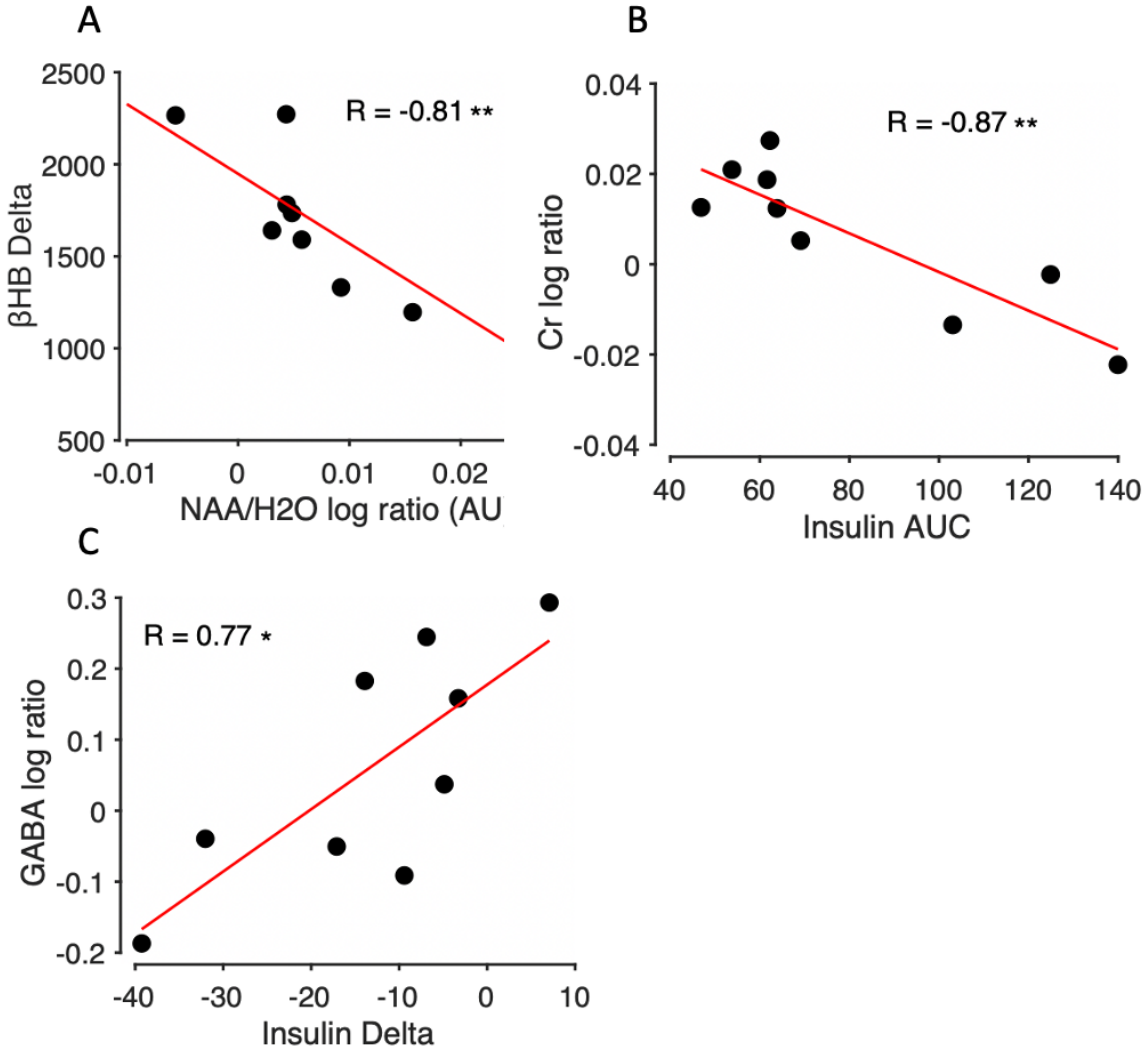

*Figure 4.5. Spectra change and their associations with BHB and plasma Insulin. A) NAA/H_2O log10 ratio (post/pre) change measured in Arbitrary Units with SAGE, is inversely related to BHB change, measured as the difference between its value at 180 minutes after KE drink and baseline. B) Cr/H_2O log10 ratio (post/pre) change measured in Arbitrary Units with SAGE, is inversely related to Insulin AUC (Area under the Curve). C) GABA/H_2O log10 ratio (post/pre) change measured in Arbitrary Units with LC model, relates to Insulin change, measured as the difference between its value at 180 minutes after KE drink and baseline. Black dots represent single participants, and the thick red line the regression line. Text insets give the Pearson's correlation with associated p value. *p < 0.05; **p < 0.01; ***p < 0.001.*

In addition, we found a strong inverse correlation between Cr/H_2O log10(post/pre) ratio (calculated with SAGE) and insulin AUC (Area Under the curve) (r(9)= -0.87, p<0.01, logBF10 =1.39, Figure 4.5B). Blood plasma insulin measured at 3 hours after oral administration of ketone ester was the single best predictor of Creatine ratio. The

opposite picture emerges when considering LC model GABA/H_2O log10(post/pre) ratio; we found a strong correlation between GABA/H_2O log10(post/pre) ratio and the delta between baseline and t180 insulin values ($r(9)= 0.77$, $p<0.05$, logBF10 =0.66), meaning that subjects with more GABA in the pre condition had the largest decrease of insulin plasma levels after ketone ester administration (Figure 4.5C).

We then explored the possibility that **β**HB changes in visual processing might be detectable through EEG recordings of Visual Evoked Potentials.

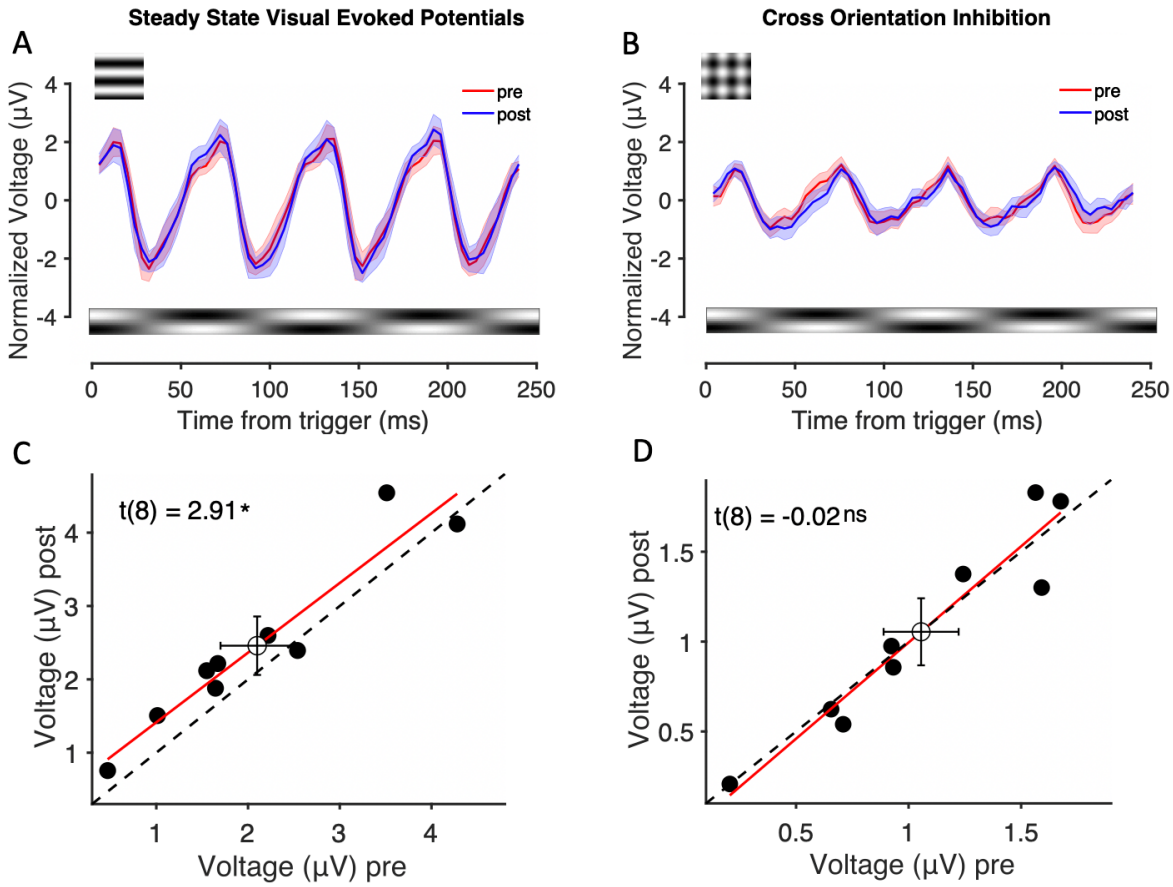

Figure 4.6. SSVEP (Steady State Visual Evoked Potentials) and XOR (Cross Orientation Inhibition). A) SSVEP mean trace pre (red solid line) and post (blue solid line), averaged on 2 cycles (250 ms). Shaded areas indicate the s.e.m. The solid square on the up left is the stimulus used. B) XOR mean trace pre (red solid line) and post (blue solid line), averaged on 2 cycles (250 ms). Shaded areas indicate the s.e.m. The solid square on the up left is the stimulus used. C) Increase of SSVEP 2nd harmonic amplitude post KE-drink. D) Lack of increase of XOR 2nd harmonic amplitude. For both C

*and D, black dots represent single participants, and the thick red line the regression line. The dashed black line represents the x = y line. Text insets give the Student's T with associated p value. *p < 0.05; **p < 0.01; ***p < 0.001.*

We found a small but significant increase of the amplitude of the second harmonics of the Steady State Visual Evoked Potentials (t(8)= 2.91, p<0.05, logBF10 = 0.6, Figure 4.6C), while the amplitude of the second harmonics for Cross-Orientation Inhibition (XOR) remained constant (t(8)=-0.02, p=0.9, logBF10 = -0.5, Figure 4.6 D). Fourth harmonics of both SSVEP and XOR stimuli remained stable (H4, t(8)=-0.21, p=0.83, lo logBF10 logBF10 = -0.5; XOR H4, t(8)=0.75, p=0.47, logBF10 = -0.4, *not shown*).

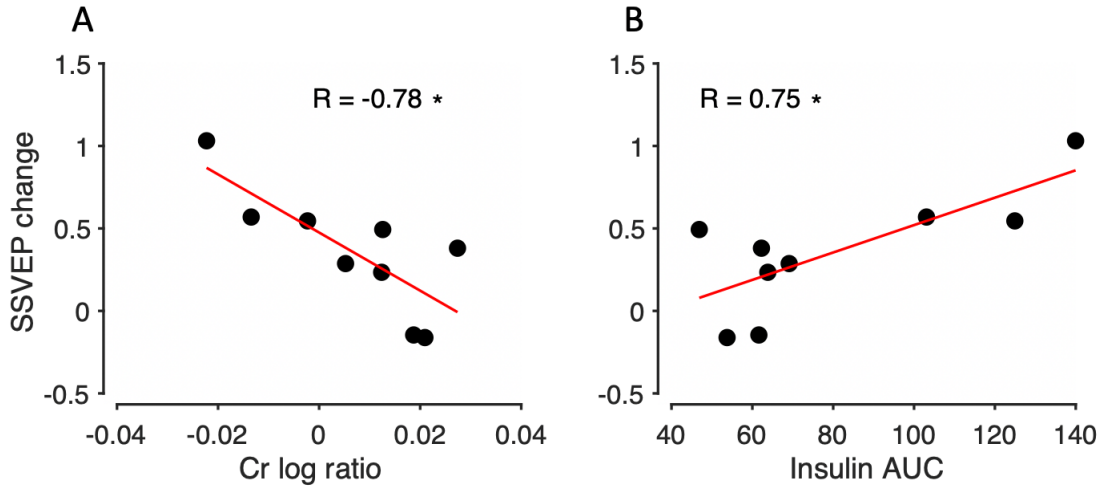

Figure 4.7. *SSVEP (Steady State Visual Evoked Potentials) change and their associations with plasma Insulin and spectra change. A) SSVEP change (post-pre) is inversely related to Cr/h20 log10 ratio (post/pre) change measured in Arbitrary Units with SAGE. B) SSVEP change (post-pre) relates to Insulin AUC (Area under the Curve). Black dots represent single participants, and the thick red line the regression line. Text insets give the Pearson's correlation with associated p value. *p < 0.05; **p < 0.01; ***p < 0.001 .*

Steady state visual evoked potentials (SSVEP) amplitude change (post-pre) was correlated with Creatine log10(post/pre) ratio ($r(9)= -0.78$, $p<0.05$, logBF10 =0.75) and with Insulin AUC ($r(9)= 0.75$, $p<0.05$, logBF10 =0.58, Figure 4.7 A-B).

We then considered the SSVEP/XOR ratio for pre and post conditions, as this may be considered and index of inhibition (D. C. Burr & Morrone, 1987); this SSVEP/XOR ratio was significantly increased in the post KE-drink condition ($t(8)= 2.72$, $p<0.05$, logBF10 = 0.5, Figure 4.8A).

When computing the difference between SSVEP/XOR ratio in pre and post condition, we found a significant correlation with LC model GABA/H_2O log10(post/pre) ratio ($r(9)= 0.71$, $p<0.05$, logBF10 =0.39, Figure 4.8B).

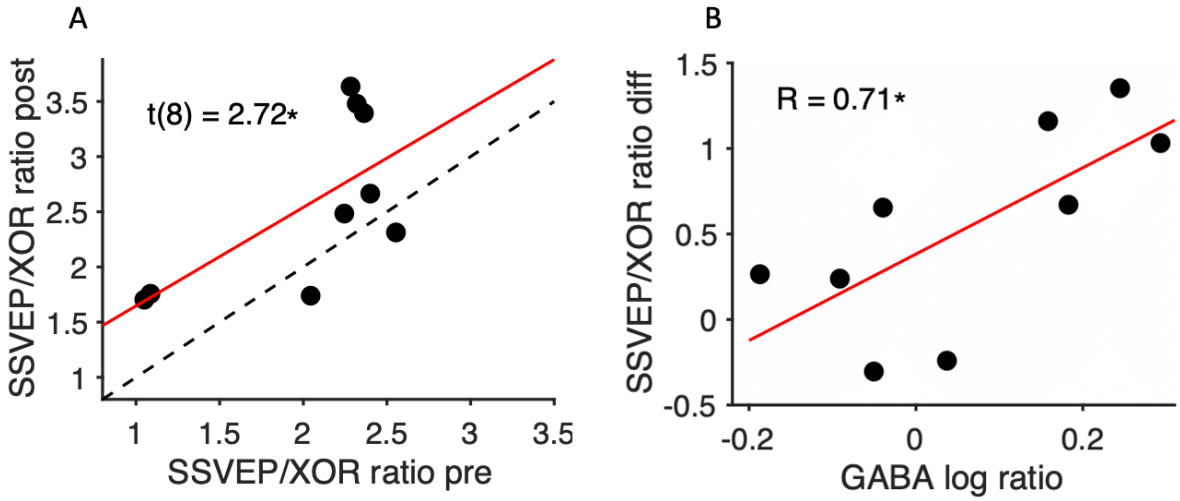

*Figure 4.8. ERP ratio change and their association with GABA. SSVEP 2nd harmonic amplitude normalized on XOR 2nd harmonic amplitude difference (post-pre) correlates with GABA log10(post/pre) ratio, measured with LC model. Black dots represent single participants, and the thick red line the regression line. Text insets give the Pearson's correlation with associated p value. *p < 0.05; **p < 0.01; ***p < 0.001.*

4.4 Discussion

Main Findings

In the present study, we tested the hypothesis that short-term ketosis enhances GABA levels in visual cortex, thus enforcing neural inhibition. We found that Steady State Visual Evoked Potentials amplitude was enhanced after ketone ester administration; moreover, SSVEP/XOR ratio was increased, being this change positively correlated with enhanced GABA levels in visual cortex. Ketone ester administration elevated blood βHB, and this enhancement correlated with a significant change in NAA and βHB levels in occipital cortex. Finally, ketone ester drinks lowered glucose, HOMA-IR and insulin, the latter being significantly related to Creatine and GABA balance (post/pre) in the occipital cortex.

Effects of exogenous ketones on Steady State Visual Evoked Potentials and XOR

We found a significant increase of the second harmonics amplitude of the Steady State Visual Evoked Potentials, while the amplitude of the second harmonics for Cross-Orientation Inhibition (XOR) remained constant. Moreover, SSVEP/XOR ratio was significantly increased in the post ketone ester-drink condition.

The enhancement of the SSVEP/XOR ratio seems consistent with enhanced inhibition found in previous works (Burr & Morrone, 1987); in fact, when computing the difference between SSVEP/XOR ratio in pre and post condition, we found a significant correlation with GABA/H20 log10(post/pre) ratio.

Steady state visual evoked potentials were elicited by contrast reversed gratings; in this presentation mode, the mean luminance of the changing pattern is constant, and only the contrast changes at the stimulation rate. The two spatial alternations comprising the pattern reversal stimuli evoke equivalent neural populations response; because the two phases of the stimulus activate the same number and type of neurons, the response to each reversal is the same, and this leads to an EEG spectrum that contains only even harmonics (2*f*, 4*f*, 6*f*) (Norcia, Appelbaum, Ales, Cottereaur, & Rossion, 2015). The second harmonic (the evoked amplitude at double the stimulation frequency) is the main response at the stimulation frequencies range used in this experiment (Norcia et al., 2015).

Both for Steady State Visual Evoked Potentials and Cross orientation inhibition the target grating (i.e the horizontal one) is equally designed; however, in XOR, the target grating is masked with a superimposed vertical grating having a different temporal frequency. Seminal studies have demonstrated inhibition between cortical neurones of differing orientation selectivity (Burr, Morrone, & Maffei, 1981; Morrone, Burr, & Maffei, 1982; Ramoa, Shadlen, Skottun, & Freeman, 1986). Mask stimuli oriented away from the preferred orientation of a neurone attenuate the response of the neurone to an optimally oriented conditioning, or test grating. (Burr & Morrone, 1987). Inhibition plays an important role in shaping this phenomenon. In fact, blocking the action of GABA almost completely destroys the orientation selectivity of most cortical cells (Sillito, 1979; Sillito, Kemp, Milson, & Berardi, 1980).

Finally, the difference (post-pre) of the SSVEP amplitude is positively correlated with insulin AUC. As stated before, insulin receptors are found throughout the brain, and previous studies have shown that insulin infusion can affect amplitude and latency of visual evoked potentials (Benedict, Nelson, Schunk, Sullwold, & Seaquist, 2006). The inverse correlations observed between the difference (post-pre) of the SSVEP amplitude and Cr log10 (post/pre) ratio, is of difficult interpretation. We speculate that a change in the neuronal energetic balance, represented by Cr change, might impact neuronal excitability, and thus, the amplitude of the evoked visual response.

Effects of exogenous ketosis on neuro-metabolites

Then, we verified how brain βHB spectroscopy relate with βHB blood levels in a subset of 6 subjects. Ketone bodies are known to accumulate in the brain in low millimolar concentrations during ketosis, and prior studies indicate that they are detectable by MR spectroscopy (Berrington et al., 2019; Mujica-Parodi et al., 2020; Schreck et al., 2021; Wright, Saneto, & Friedman, 2018).

There is only a single study assessing the precise cerebral time course of a single βHB KE drink bolus with MRS spectroscopy (Mujica-Parodi et al., 2020), showing that after reaching its peak, βHB maintains a plateau up to 90 minutes after its ingestion. We observed a correlation between βHB iAUC and βHB cerebral levels measured 2 hours after ketone ester drink administration; as βHB is known to pass the BBB (blood brain barrier) in proportion to its plasma concentration (Jensen et al., 2020) the observed

correlation of cerebral βHB with systemic measures is likely the result of its effective cerebral increase. The impossibility of detecting a main βHB change by MR spectroscopy might be the results of the small sample size, resulting in fewer available data points and greater variability, and is not necessarily indicative of a true lack of a cerebral mean **β**HB increase.

An important finding is that the ingestion of βHB supplement seems to directly impact neuro-metabolites routinely assessed with 1H-MRS spectroscopy. We found a main increase of NAA/H_2O in the occipital voxel. NAA (n-acetyl aspartate) is considered to be a marker of neuronal metabolism, used in a wide range of pathological conditions (Blüml & Ross, 1999; Martin, 2007; Yan, Ishihara, Serikawa, & Sasa, 2003)

The higher concentrations of n-acetyl aspartate observed in the present study might consistent with an increased availability of the TCA cycle-derived acetyl-coA molecule and carbon units from aspartate via oxaloacetate (Yudkoff et al., 2005). Interestingly, a very recent study also observed an increase in hippocampal NAA in mice fed with a ketogenic diet (Pawlosky, Kashiwaya, King, & Veech, 2020). We then found an inverse relationship between blood levels of **β**HB and brain NAA ratios (Wright et al., 2018). Our finding may reflect in vivo changes in amino acid handling that occur with ketosis after a ketone ester drink. As mentioned before, an increased acetyl-coenzyme A as a by-product of ketone body metabolism in the brain drives increased tricarboxylic acid cycling; this might secondarily result in increased conversion of

cytosolic aspartate to glutamate (Yudkoff et al., 2005). Aspartate serves as the precursor molecule for NAA, while glutamate is converted to the inhibitory neurotransmitter GABA. Thus, depending on the amount of ketone bodies reaching the brain, reaction balances might change their equilibrium point (Yudkoff et al., 2005).

Even tough total Creatine (Cr+PCr) levels do not change considering the mean across subjects, the log10 post/pre ratio is related to the observed cumulative insulin levels (AUC).

Creatine (Cr) and phosphocreatine (PCr) play a central role in energy metabolism, buffering rapid changes in ADP/ATP ratios (Blüml & Ross, 1999).

Perhaps the most widely used approach in H-MRS is to express data as a ratio between the metabolite of interest and the endogenous metabolite total Creatine, that is presumed to remain constant. The accuracy of these ratio-based methods is therefore directly related to the accuracy of the assumption that the chosen standard is appropriate. However, total Cr levels are known to change in different pathological conditions as trauma, strokes, genetic diseases, hypoxia and hyperosmolar condition (Blüml & Ross, 1999). Even more strikingly, creatine cerebral can be controlled by distant events, due to the complex biosynthetic pathway through liver and kidney enzymes (Blüml & Ross, 1999).

Recent studies also reported that lower level of total creatine was found in the hippocampus of rats fed with high fat diet (Lizarbe, Soares, Larsson, & Duarte, 2019;

Raider et al., 2016), consistent with earlier work in high fat ketogenic diet (Devivo, Leckie, Ferrendelli, & McDougal, 1978).

Importantly, creatine is taken up into brain by a sodium dependent transporter and inward movement is enhanced by the presence of insulin (Beal, 2011; Sora et al., 1994; Steenge, Lambourne, Casey, Macdonald, & Greenhaff, 1998).

Taken together, these results suggest that insulin might impact brain balance of Cr, and further studies are needed to elucidate the possible biochemical pathways underlying this correlation.

Beside its well-established role in regulating glucose metabolism, many studies are starting to emerge providing insight into the mechanisms through which insulin might impact brain activities. Insulin and insulin-like growth factor-1 receptors are widespread expressed on all cell types in the central nervous system, and insulin is able to freely cross the blood–brain barrier from the circulation (Ferrario & Reagan, 2018; Kleinridders, Ferris, Cai, & Kahn, 2014). Noteworthy, insulin is known to support cognition, enhance the outgrowth of neurons, modulate the release and uptake of catecholamine, and, more importantly, to regulate the expression and localization of gamma-aminobutyric acid (GABA) (Ferrario & Reagan, 2018; Hammoud et al., 2021; Kleinridders et al., 2014).

In the present work, we show that insulin change, expressed as the difference between baseline levels and our last measurement, are related to GABA log10(post/pre) ratio in the visual cortex. The exact mechanism underlying such a

correlation is still to be understood in humans but might be correlated to animal models showing that insulin signaling usually works through GABAergic neurons, playing an important role not only in the central regulation of energy homeostasis, but also in memory and plasticity (Ferrario & Reagan, 2018). As expected, this correlation is not very strong, and many other factors are known to impact GABA levels in visual cortex (Ip & Bridge, 2021).

Pharmacokinetics results and effects of exogenous ketones on blood metabolites

Finally, pharmacokinetic parameters of the synthetic (KE) drink were determined in our healthy volunteers. Maximum plasma levels of D-β-hydroxybutyrate were achieved within 1 h, in line with previous studies showing a similar peak timing after ingestion (Clarke et al., 2012b; Soto-Mota et al., 2020).

Glycemia levels were lowered by ketone ester administration and remained low throughout the whole experiment. This reduction in glycaemic response following the ingestion of KE was accompanied by a decrease in HOMA-IR index, insulin, and c-peptide after 3 hours, and by a decrease of GLP-1 active after one hour. The hypoglycaemic action of ketone bodies has been shown using both orally administered ketone ester supplements and **β**-OHB infusion, in animal and humans (Balasse & Ooms, 1968; Mikkelsen et al., 2015; Miles, Haymond, & Gerich, 1981; Myette-Côté, Neudorf, Rafiei, Clarke, & Little, 2018; Stubbs et al., 2017).

Among these previous studies, (Myette-Côté et al., 2018; Stubbs et al., 2017) and (Mikkelsen et al., 2015) showed that **β**HB significantly decreased circulating glucose, suppressing endogenous glucose production by ~15-20% in healthy males. In exogenous ketosis, carbohydrate stores are plentiful, yet the glucose-lowering action of βHB may be the result of a direct effect on hepatic gluconeogenesis and peripheral uptake, because it has been shown to occur in the absence of changes in insulin or glucagon (Balasse & Ooms, 1968; Stubbs et al., 2017). The effects of ketone supplements on endogenous insulin secretion are still unclear: previous studies have observed a two-fold increase in insulin levels during a post-exercise hyperglycaemic clamp (Holdsworth et al., 2017) and a small increase in the fasting state (Stubbs et al., 2017), whereas Myette-Cote (Myette-Côté et al., 2018) found a decrease in C-peptide iAUC following KE drinks and during an OGTT. In line with this latter study, we observed a decreased of both insulin and c-peptide in our very last observation, accompanied by a lowering of HOMA-IR index, compatible with enhanced insulin-sensitivity after 3 hours from KE ingestion. Moreover, insulin levels decreasing rather than increasing, also support the idea that glucose lowering might be due to a direct effect of βHB on glucose hepatic production. As a side note, the forementioned studies reporting an increase in insulin levels, speculate that insulin secretion might have been driven by small quantity of dextrose in the ketone ester drink diluent, even though it has also been proposed that ketones could potentiate or even stimulate

insulin secretion (Biden & Taylor, 1983; Holdsworth et al., 2017; Madison, Mebane, Unger, & Lochner, 1964; Miles et al., 1981).

We also found a decrease in GLP-1 active levels (but not total) after 1 hours from ketone ester ingestion. This observation agrees both with Myette-Cote (Myette-Côté et al., 2018), and (Stubbs et al., 2017) who also reported a decrease in GLP-1 during an OGTT and in a small fasting state, respectively. GLP-1 is produced by the gut in response to food (or carbohydrate) ingestion and acts to potentiate insulin secretion (Seino, Fukushima, & Yabe, 2010). Thus, even if not directly correlated, the observed decrease in GLP-1 at 1 hour might be linked to the subsequent decrease in insulin and C-peptide following the ketone ester drink.

The present study was not able to directly test the underlying mechanisms responsible for the reduced glycaemic response after ketone ester ingestion, and the insulin and c-peptide decrease. However, based on the observations of several previous studies performed in both human and animal models (Balasse & Ooms, 1968; Mikkelsen et al., 2015; Miles et al., 1981; Myette-Côté et al., 2018; Stubbs et al., 2017), it could be speculated that the reduced glycaemic response is mainly attributable to **β**HB-mediated reduction in hepatic glucose output, while the decrease in HOMA-IR might supports the notion that exogenous ketone supplementation may improve insulin sensitivity and thus modulate insulin and c-peptide levels.

In conclusion, in the present study we have shown short-term ketosis seems to enhance GABA levels in visual cortex, proportionally to the insulin levels reached by each subject. Visual cortical inhibition – expressed as the ratio between GABA levels pre and post the ketone ester drink – was related to the observed changes in Steady State Visual Evoked Potentials / Cross- orientation inhibition ratio. Ketone ester administration elevated blood D-βHB, and this enhancement correlated with a significant change in NAA in occipital cortex. Finally, ketone ester drinks lowered glucose, HOMA-IR and insulin, the latter being significantly related to Creatine and GABA balance (post/pre) in the occipital cortex.

These results suggest that neurons' switch to a ketotic states drive a variety of changes difficult to disentangle. Most of the present work is based on a correlative approach and correlations between metabolic parameters and either EEG or MRS measures do not imply causality. Beside these limitations, our study expands the literature available suggesting an important impact of ketone bodies on human visual processing and neuro-metabolites balance, providing new understanding on the complex relationship between metabolism and brain functioning.

5 General discussion and Conclusions

In brief, in this thesis I have shown that visual plasticity in adult humans, as measured by monocular deprivation, is dependent both on trait-like and state-like variables. Trait-like variables are those inherently related to relatively stable inter-individual differences in cortical processing, while state-like variables refer to the different environmental variables to which individuals are exposed during their everyday life.

In the 2nd Chapter we have shown that individuals have their own profile of psychophysical performance, which give us clues on inhibitory local and long-large interaction between different neuronal classes. The pattern of these complex visual networks is shaped by the maturation of inhibitory GABAergic circuits during development (Hensch & Fagiolini, 2005; Levelt et al., 2011) and presents a wide range of inter-individual variability that can be reliably quantified.

We have shown that the outcome of a manipulation such as monocular patching is dependent on each baseline subject's pattern, which remains sensitive to external stimuli in adult life and can be continuously and dynamically re-arranged to reach a new homeostatic balance. Binocular rivalry mixed percepts – that do not change before and after deprivation – represent a reliable index of each participant's strength of inhibitory interactions between cells with different ocular preference (Mentch et al., 2019; Robertson et al., 2016). Thus, knowing each individual's mixed percepts

percentage allow us to predict how plastic he or she will be. However, this balance is not univocally pre-determined, and other factors continually modulate it.

In the third Chapter we have shown that the ocular dominance shift that follows a short-term monocular deprivation is reduced when participants skip breakfast instead of having a meal. These metabolic states seem to operate on each individual's cortical substrate to modulate its function, as supported by work on animal models (Spolidoro et al., 2011); this might suggest that the visual system homeostatic response to a challenge as a brief monocular patching is moment-to-moment modulated by relatively stable inhibitory circuits (as represented by mixed percepts) and by a variety of other factors that cooperate in shaping the final ocular dominance shift. The extent to which this can happen is a whole field still to be explored.

Our results may converge with those of Lunghi and Sale (Lunghi & Sale, 2015), showing that moderate physical exercise performed during a monocular deprivation results in a larger dominance boost of the deprived eye - compared to when monocular deprivation was applied while subjects sat passively in front of a TV. In both cases in fact, the metabolic manipulations studied resulted effective in modulating the patching outcome compared to a control condition.

Much previous work has focused on studying the impact of metabolic variables on cognitive functions, rather than sensory ones; our results show that state-like variables can impact brain functioning at a very basic level, unbeknownst to the subject. This

suggest that the effect of short-term manipulations, i.e breakfast skipping, in the cognitive domain might also be affected by unexpected changes in sensory processing.

Interestingly, we found a correlation between the reduced plasticity effect observed after an overnight fasting and ketone bodies levels in the same condition. This may be important to disentangle the mechanisms through which metabolic variables can effectively act on ocular dominance circuits. As introduced before, ketone bodies are thought to modulate GABA production, store, and release, (Daikhin & Yudkoff, 1998; Erecińska et al., 1996) and are gaining much attention for their ability to modulate cortical excitability (Jensen et al., 2020). Thus, it is tempting to speculate that, in our experiment, they might have reduced the ocular dominance shift after deprivation by strengthening inhibition. This would be consistent with previous work showing a correlation between GABA reduction in visual cortex and ocular dominance shift (Lunghi, Emir, et al., 2015).

As discussed in the introduction, the developmental maturation of intracortical inhibitory circuitries leads to the closure of the critical period in the visual system (Hensch & Fagiolini, 2005; Levelt et al., 2011); thus, much research has focused on the possibility to restore plasticity in adults by reducing levels of inhibition. Consistent with this hypothesis, it has been shown that ocular dominance plasticity can be rescued in adult animals by decreasing GABAergic inhibition, through different manipulations, e.g. pharmacologically (Vetencourt et al., 2008). This is a direct

demonstration that GABAergic signalling is crucial in limiting visual cortex plasticity, and that we can interfere with the balance between excitation/inhibition in the cortex, to reinstate a juvenile-like form of plasticity. In that, metabolic manipulations emerge as particularly interesting, because they are non-invasive and then suitable for potential clinical application in humans.

In the last part of the thesis, I present a third study where we attempted to assess the impact of ketone metabolism on brain function by using the visual system as a model. Our results depict a complex image, in which the switch from glucose to ketones in neuronal metabolism triggers a variety of responses. Visual cortex evoked responses change their amplitude, and this change is related to GABA balance in visual cortex. Although of course correlation does not imply causation, this result is in line with our hypothesis: that ketone metabolism may affect GABAergic inhibition. Moreover, ketosis changed the concentration of neuro-metabolites related to the cell energy balance and efficiency, enhancing NAA and modulating creatine levels; this latter effect is related to circulating blood plasma insulin. Taken together, these results suggest that metabolic states can effectively modulate visual responses and that GABAergic inhibition might be involved, but the exact mechanism is far from established. More studies are needed to elucidate the contribution of every single variable in modulating cortical inhibition, with larger sample size and multi-disciplinary efforts.

To conclude, the data presented in this thesis provide novel contributions to the understating of visual cortical plasticity, with exciting new insights on the link between brain and metabolism functioning.

6 References

Achanta, L. B., & Rae, C. D. (2017). β-Hydroxybutyrate in the Brain: One Molecule, Multiple Mechanisms. *Neurochemical Research*. https://doi.org/10.1007/s11064-016-2099-2

Adesnik, H., Bruns, W., Taniguchi, H., Huang, Z. J., & Scanziani, M. (2012). A neural circuit for spatial summation in visual cortex. *Nature*. https://doi.org/10.1038/nature11526

Alvarez, E., Martínez, M. D., Roncero, I., Chowen, J. A., García-Cuartero, B., Gispert, J. D., … Blázquez, E. (2005). The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *Journal of Neurochemistry*, *92*(4), 798–806. https://doi.org/10.1111/j.1471-4159.2004.02914.x

Anderson, J. R., Hawkins, M. A. W., Updegraff, J., Gunstad, J., & Spitznagel, M. B. (2018). Baseline glucoregulatory function moderates the effect of dairy milk and fruit juice on postprandial cognition in healthy young adults. *European Journal of Nutrition*. https://doi.org/10.1007/s00394-017-1505-0

Avena, N. M., Bocarsly, M. E., Murray, S., & Gold, M. S. (2014). Effects of baclofen and naltrexone, alone and in combination, on the consumption of palatable food in male rats. *Experimental and Clinical Psychopharmacology*. https://doi.org/10.1037/a0037223

Bai, J., Dong, X., He, S., & Bao, M. (2017). Monocular deprivation of Fourier phase information boosts the deprived eye's dominance during interocular competition but not interocular phase combination. *Neuroscience*, *352*, 122–130. https://doi.org/10.1016/j.neuroscience.2017.03.053

Bak, L. K., & Walls, A. B. (2018). Astrocytic glycogen metabolism in the healthy and diseased brain. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.R117.803239

Balasse, E., & Ooms, H. A. (1968). Changes in the concentrations of glucose, free fatty acids, insulin and ketone bodies in the blood during sodium \-hydroxybutyrate infusions in man. *Diabetologia*. https://doi.org/10.1007/BF01219433

Baseler, H. A., Brewer, A. A., Sharpe, L. T., Morland, A. B., Jaägle, H., & Wandell, B. A. (2002). Reorganization of human cortical maps caused by inherited photoreceptor abnormalities. *Nature Neuroscience*. https://doi.org/10.1038/nn817

Beal, M. F. (2011). Neuroprotective effects of creatine. *Amino Acids*. https://doi.org/10.1007/s00726-011-0851-0

Benau, E. M., Orloff, N. C., Janke, E. A., Serpell, L., & Timko, C. A. (2014). A systematic review of the effects of experimental fasting on cognition. *Appetite*. https://doi.org/10.1016/j.appet.2014.02.014

Benedict, L., Nelson, C. A., Schunk, E., Sullwold, K., & Seaquist, E. R. (2006). Effect of insulin on the brain activity obtained during visual and memory tasks in healthy human subjects. *Neuroendocrinology*. https://doi.org/10.1159/000093338

Benton, D., & Parker, P. Y. (1998). Breakfast, blood glucose, and cognition. *American Journal of Clinical Nutrition*. https://doi.org/10.1093/ajcn/67.4.772S

Berardi, N., Pizzorusso, T., & Maffei, L. (2000). Critical periods during sensory development. *Current Opinion in Neurobiology*. https://doi.org/10.1016/S0959-4388(99)00047-1

Berardi, N., Pizzorusso, T., Ratto, G. M., & Maffei, L. (2003). Molecular basis of plasticity in the visual cortex. *Trends in Neurosciences*. https://doi.org/10.1016/S0166-2236(03)00168-1

Berrington, A., Schreck, K. C., Barron, B. J., Blair, L., Lin, D. D. M., Hartman, A. L., … Strowd, R. E. (2019). Cerebral ketones detected by 3T MR spectroscopy in patients with high-grade glioma on an Atkins-based diet. *American Journal of Neuroradiology*. https://doi.org/10.3174/ajnr.A6287

Biden, T. J., & Taylor, K. W. (1983). Effects of ketone bodies on insulin release and islet-cell metabolism in the rat. *Biochemical Journal*. https://doi.org/10.1042/bj2120371

Binda, P., Eldor, R., Huerta, C., Adams, J., Lancaster, J., Fox, P., … Daniele, G. (2019). Exenatide modulates visual cortex responses. *Diabetes/Metabolism Research and Reviews*. https://doi.org/10.1002/dmrr.3167

Binda, P., Kurzawski, J. W., Lunghi, C., Biagi, L., Tosetti, M., & Morrone, M. C. (2018). Response to short-term deprivation of the human adult visual cortex measured with 7T bold. *ELife*. https://doi.org/10.7554/eLife.40014

Binda, P., & Lunghi, C. (2017). Short-Term Monocular Deprivation Enhances Physiological Pupillary Oscillations. *Neural Plasticity*. https://doi.org/10.1155/2017/6724631

Birch, E. E. (1993). Stereopsis in infants and its developmental relation to visual acuity. . In *Early Visual Development: Normal and Abnormal*.

Blake, R. (1989). A Neural Theory of Binocular Rivalry. *Psychological Review*. https://doi.org/10.1037/0033-295X.96.1.145

Blake, R., Brascamp, J., & Heeger, D. J. (2014). Can binocular rivalry reveal neural correlates of consciousness? *Philosophical Transactions of the Royal Society B: Biological Sciences*. https://doi.org/10.1098/rstb.2013.0211

Blake, R., & Logothetis, N. K. (2002). Visual competition. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn701

Blake, R., & Wilson, H. (2011). Binocular vision. *Vision Research*. https://doi.org/10.1016/j.visres.2010.10.009

Blakemore, C., & Campbell, F. W. (1969). On the existence of neurones in the human visual system selectively sensitive to the orientation and size of retinal images. *The Journal of Physiology*. https://doi.org/10.1113/jphysiol.1969.sp008862

Blakemore, Colin, Carpenter, R. H. S., & Georgeson, M. A. (1970). Lateral inhibition between orientation detectors in the human visual system. *Nature*. https://doi.org/10.1038/228037a0

Blakemore, Colin, Muncey, J. P. J., & Ridley, R. M. (1973). Stimulus specificity in the human visual system. *Vision Research*. https://doi.org/10.1016/0042-6989(73)90063-1

Blüml, S., & Ross, B. (1999). Magnetic Resonance Spectroscopy of the Human Brain. In *Modern Techniques in Neuroscience Research*. https://doi.org/10.1007/978-3-642-58552-4_40

Bono, J., & Clopath, C. (2019). Synaptic plasticity onto inhibitory neurons as a mechanism for

ocular dominance plasticity. *PLoS Computational Biology*. https://doi.org/10.1371/journal.pcbi.1006834

Boroojerdi, B., Battaglia, F., Muellbacher, W., & Cohen, L. G. (2001). Mechanisms underlying rapid experience-dependent plasticity in the human visual cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 14698–14701. https://doi.org/10.1073/pnas.251357198

Boroojerdi, B., Bushara, K. O., Corwell, B., Immisch, I., Battaglia, F., Muellbacher, W., & Cohen, L. G. (2000). Enhanced excitability of the human visual cortex induced by short-term light deprivation. *Cerebral Cortex*. https://doi.org/10.1093/cercor/10.5.529

Braddick, O., & Atkinson, J. (2011). Development of human visual function. *Vision Research*. https://doi.org/10.1016/j.visres.2011.02.018

Brascamp, J. W., Klink, P. C., & Levelt, W. J. M. (2015). The "laws" of binocular rivalry: 50 years of Levelt's propositions. *Vision Research*, *109*(Part A), 20–37. https://doi.org/10.1016/j.visres.2015.02.019

Brascamp, Jan W., Becker, M. W., & Hambrick, D. Z. (2018). Revisiting individual differences in the time course of binocular rivalry. *Journal of Vision*. https://doi.org/10.1167/18.7.3

Burr, D. C., & Morrone, M. C. (1987). Inhibitory interactions in the human vision system revealed in pattern-evoked potentials. *The Journal of Physiology*. https://doi.org/10.1113/jphysiol.1987.sp016643

Burr, D., Morrone, C., & Maffei, L. (1981). Intra-cortical inhibition prevents simple cells from responding to textured visual patterns. *Experimental Brain Research*. https://doi.org/10.1007/BF00238391

Cao, T., Wang, L., Sun, Z., Engel, S. A., & He, S. (2018). The independent and shared mechanisms of intrinsic brain dynamics: Insights from bistable perception. *Frontiers in Psychology*, *9*(APR), 1–11. https://doi.org/10.3389/fpsyg.2018.00589

Castaldi, E., Lunghi, C., & Morrone, M. C. (2020). Neuroplasticity in adult human visual cortex. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2020.02.028

Chadnova, E., Reynaud, A., Clavagnier, S., & Hess, R. F. (2017). Short-term monocular occlusion produces changes in ocular dominance by a reciprocal modulation of interocular inhibition. *Scientific Reports*. https://doi.org/10.1038/srep41747

Chechko, N., Vocke, S., Habel, U., Toygar, T., Kuckartz, L., Berthold-Losleben, M., … Kohn, N. (2015). Effects of overnight fasting on working memory-related brain network: An fMRI study. *Human Brain Mapping*. https://doi.org/10.1002/hbm.22668

Cherif, A., Meeusen, R., Farooq, A., Briki, W., Fenneni, M. A., Chamari, K., & Roelands, B. (2017). Repeated Sprints in Fasted State Impair Reaction Time Performance. *Journal of the American College of Nutrition*. https://doi.org/10.1080/07315724.2016.1256795

Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., Todd King, M., Musa-Veloso, K., … Veech, R. L. (2012a). Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regulatory Toxicology and Pharmacology*, *63*(3), 401–408. https://doi.org/10.1016/j.yrtph.2012.04.008

Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., Todd King, M., Musa-Veloso, K., …

Veech, R. L. (2012b). Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regulatory Toxicology and Pharmacology*. https://doi.org/10.1016/j.yrtph.2012.04.008

Clifford, C. W.G., Wenderoth, P., & Spehar, B. (2000). A functional angle on some after-effects in cortical vision. *Proceedings of the Royal Society B: Biological Sciences*. https://doi.org/10.1098/rspb.2000.1198

Clifford, Colin W.G. (2014). The tilt illusion: Phenomenology and functional implications. *Vision Research*, *104*, 3–11. https://doi.org/10.1016/j.visres.2014.06.009

Cook, E., Hammett, S. T., & Larsson, J. (2016). GABA predicts visual intelligence. *Neuroscience Letters*, *632*, 50–54. https://doi.org/10.1016/j.neulet.2016.07.053

Daikhin, Y., & Yudkoff, M. (1998). Ketone bodies and brain glutamate and GABA metabolism. *Developmental Neuroscience*. https://doi.org/10.1159/000017331

Daniele, G., Iozzo, P., Molina-Carrion, M., Lancaster, J., Ciociaro, D., Cersosimo, E., … Gastaldelli, A. (2015). Exenatide regulates cerebral glucose metabolism in brain areas associated with glucose homeostasis and reward system. *Diabetes*. https://doi.org/10.2337/db14-1718

Daniele, G., Lunghi, C., Dardano, A., Binda, P., Ceccarini, G., Santini, F., … Del Prato, S. (2021). Bariatric surgery restores visual cortical plasticity in nondiabetic subjects with obesity. *International Journal of Obesity*. https://doi.org/10.1038/s41366-021-00851-0

De Silva, A., Salem, V., Long, C. J., Makwana, A., Newbould, R. D., Rabiner, E. A., … Dhillo, W. S. (2011). The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2011.09.010

Deacon, C. F., Johnsen, A. H., & Holst, J. J. (1995). Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jcem.80.3.7883856

Devivo, D. C., Leckie, M. P., Ferrendelli, J. S., & McDougal, D. B. (1978). Chronic ketosis and cerebral metabolism. *Annals of Neurology*. https://doi.org/10.1002/ana.410030410

Dore, G. A., Elias, M. F., Robbins, M. A., Budge, M. M., & Elias, P. K. (2008). Relation between central adiposity and cognitive function in the Maine-Syracuse study: Attenuation by physical activity. *Annals of Behavioral Medicine*. https://doi.org/10.1007/s12160-008-9038-7

Dosher, B., & Lu, Z. L. (2017). Visual Perceptual Learning and Models. *Annual Review of Vision Science*. https://doi.org/10.1146/annurev-vision-102016-061249

Erecińska, M., Nelson, D., Daikhin, Y., & Yudkoff, M. (1996). Regulation of GABA level in rat brain synaptosomes: Fluxes through enzymes of the GABA shunt and effects of glutamate, calcium, and ketone bodies. *Journal of Neurochemistry*. https://doi.org/10.1046/j.1471-4159.1996.67062325.x

Etou, H., Sakata, T., Fujimoto, K., Kurata, K., Terada, K., Fukagawa, K., … Miller, R. E. (1989). Characteristics of psychomotor performance and time cognition in moderately obese patients. *Physiology and Behavior*. https://doi.org/10.1016/0031-9384(89)90225-4

Fagiolini, M., Fritschy, J. M., Löw, K., Möhler, H., Rudolph, U., & Hensch, T. K. (2004). Specific GABAA Circuits for Visual Cortical Plasticity. *Science*, *303*(5664), 1681–1683. https://doi.org/10.1126/science.1091032

Fagiolini, M., & Hensch, T. K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. *Nature*. https://doi.org/10.1038/35004582

Ferrario, C. R., & Reagan, L. P. (2018). Insulin-mediated synaptic plasticity in the CNS: Anatomical, functional and temporal contexts. *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2017.12.001

Fierro, B., Brighina, F., Vitello, G., Piazza, A., Scalia, S., Giglia, G., … Pascual-Leone, A. (2005). Modulatory effects of low- and high-frequency repetitive transcranial magnetic stimulation on visual cortex of healthy subjects undergoing light deprivation. *Journal of Physiology*. https://doi.org/10.1113/jphysiol.2004.080184

Fontán-Lozano, Á., Sáez-Cassanelli, J. L., Inda, M. C., De Los Santos-Arteaga, M., Sierra-Domínguez, S. A., López-Lluch, G., … Carrión, Á. M. (2007). Caloric restriction increases learning consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.2757-07.2007

Frangou, P., Emir, U. E., Karlaftis, V. M., Nettekoven, C., Hinson, E. L., Larcombe, S., … Kourtzi, Z. (2019). Learning to optimize perceptual decisions through suppressive interactions in the human brain. *Nature Communications*, *10*(1), 1–12. https://doi.org/10.1038/s41467-019-08313-y

Freeman, J., Veggiotti, P., Lanzi, G., Tagliabue, A., & Perucca, E. (2006). The ketogenic diet: From molecular mechanisms to clinical effects. *Epilepsy Research*. https://doi.org/10.1016/j.eplepsyres.2005.10.003

Frégnac, Y., Shulz, D., Thorpe, S., & Bienenstock, E. (1988). A cellular analogue of visual cortical plasticity. *Nature*. https://doi.org/10.1038/333367a0

Gallagher, R. M., & Arnold, D. H. (2014). Interpreting the temporal dynamics of perceptual rivalries. *Perception*. https://doi.org/10.1068/p7648

García-Rodríguez, D., & Giménez-Cassina, A. (2021). Ketone Bodies in the Brain Beyond Fuel Metabolism: From Excitability to Gene Expression and Cell Signaling. *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2021.732120

Gault, V. A., & Hölscher, C. (2008). GLP-1 agonists facilitate hippocampal LTP and reverse the impairment of LTP induced by beta-amyloid. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2008.03.025

Green, M. W., Elliman, N. A., & Rogers, P. J. (1997). The effects of food deprivation and incentive motivation on blood glucose levels and cognitive function. *Psychopharmacology*. https://doi.org/10.1007/s002130050429

Grieco, M., Giorgi, A., Gentile, M. C., d'Erme, M., Morano, S., Maras, B., & Filardi, T. (2019). Glucagon-Like Peptide-1: A Focus on Neurodegenerative Diseases. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2019.01112

Gunstad, J., Paul, R. H., Cohen, R. A., Tate, D. F., & Gordon, E. (2006). Obesity is associated with memory deficits in young and middle-aged adults. *Eating and Weight Disorders*. https://doi.org/10.1007/BF03327747

Gunstad, John, Lhotsky, A., Wendell, C. R., Ferrucci, L., & Zonderman, A. B. (2010). Longitudinal examination of obesity and cognitive function: Results from the baltimore longitudinal study of aging. *Neuroepidemiology*. https://doi.org/10.1159/000297742

Gutzwiller, J. P., Drewe, J., Göke, B., Schmidt, H., Rohrer, B., Lareida, J., & Beglinger, C. (1999). Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*. https://doi.org/10.1152/ajpregu.1999.276.5.r1541

Hammoud, H., Netsyk, O., Tafreshiha, A. S., Korol, S. V., Jin, Z., Li, J. P., & Birnir, B. (2021). Insulin differentially modulates GABA signalling in hippocampal neurons and, in an age-dependent manner, normalizes GABA-activated currents in the tg-APPSwe mouse model of Alzheimer's disease. *Acta Physiologica*. https://doi.org/10.1111/apha.13623

Harauzov, A., Spolidoro, M., DiCristo, G., De Pasquale, R., Cancedda, L., Pizzorusso, T., … Maffei, L. (2010). Reducing intracortical inhibition in the adult visual cortex promotes ocular dominance plasticity. *Journal of Neuroscience*, *30*(1), 361–371. https://doi.org/10.1523/JNEUROSCI.2233-09.2010

Helmholz, H. F., & Keith, H. M. (1933). Ten years' experience in the treatment of epilepsy with ketogenic diet. *Archives of Neurology & Psychiatry*, *29*(4), 808–812. https://doi.org/10.1001/archneurpsyc.1933.02240100127010

Hensch, T. K., & Fagiolini, M. (2005). Excitatory-inhibitory balance and critical period plasticity in developing visual cortex. *Progress in Brain Research*. https://doi.org/10.1016/S0079-6123(04)47009-5

Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S., & Kash, S. F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science*. https://doi.org/10.1126/science.282.5393.1504

Hensch, T. K., & Quinlan, E. M. (2018). Critical periods in amblyopia. *Visual Neuroscience*. https://doi.org/10.1017/S0952523817000219

Holdsworth, D. A., Cox, P. J., Kirk, T., Stradling, H., Impey, S. G., & Clarke, K. (2017). A Ketone Ester Drink Increases Postexercise Muscle Glycogen Synthesis in Humans. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/MSS.0000000000001292

Huang, Z. J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M. F., … Tonegawa, S. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell*, *98*(6), 739–755. https://doi.org/10.1016/S0092-8674(00)81509-3

Hubel, D. H., & Wiesel, T. N. (1963). Shape and arrangement of columns in cat's striate cortex. *The Journal of Physiology*. https://doi.org/10.1113/jphysiol.1963.sp007079

Hubel, D. H., & Wiesel, T. N. (1970). The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *The Journal of Physiology*. https://doi.org/10.1113/jphysiol.1970.sp009022

Hubel, D. H., Wiesel, T. N., & LeVay, S. (1977). Plasticity of ocular dominance columns in monkey striate cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. https://doi.org/10.1098/rstb.1977.0050

Ip, I. B., & Bridge, H. (2021). Investigating the neurochemistry of the human visual system using magnetic resonance spectroscopy. *Brain Structure and Function*.

https://doi.org/10.1007/s00429-021-02273-0

Jensen, N. J., Wodschow, H. Z., Nilsson, M., & Rungby, J. (2020). Effects of ketone bodies on brain metabolism and function in neurodegenerative diseases. *International Journal of Molecular Sciences*, *21*(22), 1–17. https://doi.org/10.3390/ijms21228767

Juge, N., Gray, J. A., Omote, H., Miyaji, T., Inoue, T., Hara, C., … Moriyama, Y. (2010). Metabolic Control of Vesicular Glutamate Transport and Release. *Neuron*. https://doi.org/10.1016/j.neuron.2010.09.002

Karni, A., & Sagi, D. (1991). Where practice makes perfect in texture discrimination: Evidence for primary visual cortex plasticity. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.88.11.4966

Karni, A., & Sagi, D. (1993). The time course of learning a visual skill. *Nature*. https://doi.org/10.1038/365250a0

Katsurada, K., & Yada, T. (2016). Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist. *Journal of Diabetes Investigation*, *7*, 64–69. https://doi.org/10.1111/jdi.12464

Kim, H. W., Kim, C. Y., & Blake, R. (2017). Monocular Perceptual Deprivation from Interocular Suppression Temporarily Imbalances Ocular Dominance. *Current Biology*, *27*(6), 884–889. https://doi.org/10.1016/j.cub.2017.01.063

Kleinridders, A., Ferris, H. A., Cai, W., & Kahn, C. R. (2014). Insulin action in brain regulates systemic metabolism and brain function. *Diabetes*. https://doi.org/10.2337/db14-0568

Klink, P. C., Brascamp, J. W., Blake, R., & Van Wezel, R. J. A. (2010). Experience-driven plasticity in binocular vision. *Current Biology*. https://doi.org/10.1016/j.cub.2010.06.057

Knudsen, L. B., & Pridal, L. (1996). Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36) amide after in vivo administration to dogs, and it acts as an antagonist on the pancreatic receptor. *European Journal of Pharmacology*. https://doi.org/10.1016/S0014-2999(96)00795-9

Komiyama, T., Sudo, M., Okuda, N., Yasuno, T., Kiyonaga, A., Tanaka, H., … Ando, S. (2016). Cognitive function at rest and during exercise following breakfast omission. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2016.02.013

Kwon, M. Y., Legge, G. E., Fang, F., Cheong, A. M. Y., & He, S. (2009). Adaptive changes in visual cortex following prolonged contrast reduction. *Journal of Vision*. https://doi.org/10.1167/9.2.20

Laing, C. R., & Chow, C. C. (2002). A spiking neuron model for binocular rivalry. *Journal of Computational Neuroscience*. https://doi.org/10.1023/A:1014942129705

Lehr, S., Hartwig, S., & Sell, H. (2012). Adipokines: A treasure trove for the discovery of biomarkers for metabolic disorders. *Proteomics - Clinical Applications*. https://doi.org/10.1002/prca.201100052

Leopold, D. A., & Logothetis, N. K. (1999). Multistable phenomena: Changing views in perception. *Trends in Cognitive Sciences*. https://doi.org/10.1016/S1364-6613(99)01332-7

Levelt, C. N., Heimel, J. A., & Van Versendaal, D. (2011). The role of GABAergic inhibition in

ocular dominance plasticity. *Neural Plasticity*, *2011*. https://doi.org/10.1155/2011/391763

Levi, D. M., McKee, S. P., & Movshon, J. A. (2011). Visual deficits in anisometropia. *Vision Research*. https://doi.org/10.1016/j.visres.2010.09.029

Li, H. H., Rankin, J., Rinzel, J., Carrasco, M., & Heeger, D. J. (2017). Attention model of binocular rivalry. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1620475114

Lizarbe, B., Soares, A. F., Larsson, S., & Duarte, J. M. N. (2019). Neurochemical modifications in the hippocampus, cortex and hypothalamus of mice exposed to long-term high-fat diet. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2018.00985

Logothetis, N. K., Leopold, D. A., & Sheinberg, D. L. (1996). What is rivalling during binocular rivalry? *Nature*. https://doi.org/10.1038/380621a0

Longo, V. D., & Mattson, M. P. (2014). Fasting: Molecular mechanisms and clinical applications. *Cell Metabolism*, Vol. 19. https://doi.org/10.1016/j.cmet.2013.12.008

Lou, A. R., Madsen, K. H., Paulson, O. B., Julian, H. O., Prause, J. U., Siebner, H. R., & Kjaer, T. W. (2011). Monocular visual deprivation suppresses excitability in adult human visual cortex. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhr082

Lund, T. M., Obel, L. F., Risa, Ø., & Sonnewald, U. (2011). β-Hydroxybutyrate is the preferred substrate for GABA and glutamate synthesis while glucose is indispensable during depolarization in cultured GABAergic neurons. *Neurochemistry International*. https://doi.org/10.1016/j.neuint.2011.06.002

Lund, T. M., Risa, Ø., Sonnewald, U., Schousboe, A., & Waagepetersen, H. S. (2009). Availability of neurotransmitter glutamate is diminished when β-hydroxybutyrate replaces glucose in cultured neurons. *Journal of Neurochemistry*. https://doi.org/10.1111/j.1471-4159.2009.06115.x

Lunghi, C., Berchicci, M., Morrone, M. C., & Di Russo, F. (2015). Short-term monocular deprivation alters early components of visual evoked potentials. *Journal of Physiology*, *593*(19), 4361–4372. https://doi.org/10.1113/JP270950

Lunghi, C., Burr, D. C., & Concetta Morrone, M. (2013). Long-term effects of monocular deprivation revealed with binocular rivalry gratings modulated in luminance and in color. *Journal of Vision*, *13*(6), 1–15. https://doi.org/10.1167/13.6.1

Lunghi, C., Burr, D. C., & Morrone, C. (2011a). Brief periods of monocular deprivation disrupt ocular balance in human adult visual cortex. *Current Biology*, Vol. 21, pp. R538–R539. https://doi.org/10.1016/j.cub.2011.06.004

Lunghi, C., Burr, D. C., & Morrone, C. (2011b). Brief periods of monocular deprivation disrupt ocular balance in human adult visual cortex. *Current Biology*. https://doi.org/10.1016/j.cub.2011.06.004

Lunghi, C., Daniele, G., Binda, P., Dardano, A., Ceccarini, G., Santini, F., … Morrone, M. C. (2019). Altered Visual Plasticity in Morbidly Obese Subjects. *IScience*, *22*, 206–213. https://doi.org/10.1016/j.isci.2019.11.027

Lunghi, C., Emir, U. E., Morrone, M. C., & Bridge, H. (2015). Short-Term monocular deprivation alters GABA in the adult human visual cortex. *Current Biology*, *25*(11),

1496–1501. https://doi.org/10.1016/j.cub.2015.04.021

Lunghi, C., & Sale, A. (2015). A cycling lane for brain rewiring. *Current Biology*, *25*(23), R1122–R1123. https://doi.org/10.1016/j.cub.2015.10.026

Lunghi, C., Sframeli, A. T., Lepri, A., Lepri, M., Lisi, D., Sale, A., & Morrone, M. C. (2019). A new counterintuitive training for adult amblyopia. *Annals of Clinical and Translational Neurology*. https://doi.org/10.1002/acn3.698

Lyu, L., He, S., Jiang, Y., Engel, S. A., & Bao, M. (2020). Natural-scene-based Steady-state Visual Evoked Potentials Reveal Effects of Short-term Monocular Deprivation. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2020.03.039

Artzi,M., Liberman,G., Vaisman,N., Bokstein,F., Vitinshtein, F., Aizenstein, O., & Bashat, D.B. (2017). Changes in cerebral metabolism during ketogenic diet in patients with primary brain tumors: (1)H-MRS study. *Journal of Neuro-Oncology*.

Ma, W., Berg, J., & Yellen, G. (2007). Ketogenic diet metabolites reduce firing in central neurons by opening KATP channels. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.0132-07.2007

Madison, L. L., Mebane, D., Unger, R. H., & Lochner, A. (1964). The Hypoglycemic Action of Ketones. II. Evidence for a Stimulatory Feedback of Ketones on the Pancreatic Beta Cells *. *Journal of Clinical Investigation*. https://doi.org/10.1172/jci104925

Maffei, A., Lambo, M. E., & Turrigiano, G. G. (2010). Critical period for inhibitory plasticity in rodent binocular V1. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.5340-09.2010

Martin, W. R. W. (2007). MR spectroscopy in neurodegenerative disease. *Molecular Imaging and Biology*. https://doi.org/10.1007/s11307-007-0087-2

Mattson, M. P. (2012). Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2012.08.012

Mattson, M. P., Moehl, K., Ghena, N., Schmaedick, M., & Cheng, A. (2018). Intermittent metabolic switching, neuroplasticity and brain health. *Nature Reviews Neuroscience*, *19*(2), 81–94. https://doi.org/10.1038/nrn.2017.156

Maurer, D., Lewis, T. L., & Mondloch, C. J. (2005). Missing sights: Consequences for visual cognitive development. *Trends in Cognitive Sciences*. https://doi.org/10.1016/j.tics.2005.01.006

Maurer, D., Mondloch, C. J., & Lewis, T. L. (2007). Effects of early visual deprivation on perceptual and cognitive development. *Progress in Brain Research*. https://doi.org/10.1016/S0079-6123(07)64005-9

McClean, P. L., Gault, V. A., Harriott, P., & Hölscher, C. (2010). Glucagon-like peptide-1 analogues enhance synaptic plasticity in the brain: A link between diabetes and Alzheimer's disease. *European Journal of Pharmacology*. https://doi.org/10.1016/j.ejphar.2009.12.023

McQuarrie, I., & Keith, H. M. (1927). Epilepsy in children: relationship of variations in the degree of ketonuria to occurrence of convulsions in epileptic children on ketogenic diets. *American Journal of Diseases of Children*, *34*(6), 1013–1029.

https://doi.org/10.1001/archpedi.1927.04130240092013

Mentch, J., Spiegel, A., Ricciardi, C., & Robertson, C. E. (2019). GABAergic inhibition gates perceptual awareness during binocular rivalry. *Journal of Neuroscience*, *39*(42), 8398–8407. https://doi.org/10.1523/JNEUROSCI.0836-19.2019

Mikkelsen, K. H., Seifert, T., Secher, N. H., Grøndal, T., & Van Hall, G. (2015). Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-β-hydroxybutyratemia in post-absorptive healthy males. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jc.2014-2608

Miles, J. M., Haymond, M. W., & Gerich, J. E. (1981). Suppression of glucose production and stimulation of insulin secretion by physiological concentrations of ketone bodies in man. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jcem-52-1-34

Min, S. H., Baldwin, A. S., Reynaud, A., & Hess, R. F. (2018). The shift in ocular dominance from short-term monocular deprivation exhibits no dependence on duration of deprivation. *Scientific Reports*, *8*(1), 17083. https://doi.org/10.1038/s41598-018-35084-1

Morishita, H., Miwa, J. M., Heintz, N., & Hensch, T. K. (2010). Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science*. https://doi.org/10.1126/science.1195320

Morrone, M. C., Burr, D. C., & Maffei, L. (1982). Functional implications of cross-orientation inhibition of cortical visual cells. I. Neurophysiological evidence. *Proceedings of the Royal Society of London - Biological Sciences*, *216*(1204), 335–354. https://doi.org/10.1098/rspb.1982.0078

Mujica-Parodi, L. R., Amgalan, A., Sultan, S. F., Antal, B., Sun, X., Skiena, S., … Clarke, K. (2020). Diet modulates brain network stability, a biomarker for brain aging, in young adults. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(11), 6170–6177. https://doi.org/10.1073/pnas.1913042117

Myette-Côté, É., Neudorf, H., Rafiei, H., Clarke, K., & Little, J. P. (2018). Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals. *Journal of Physiology*. https://doi.org/10.1113/JP275709

Newman, J. C., & Verdin, E. (2017). β-Hydroxybutyrate: A Signaling Metabolite. *Annual Review of Nutrition*. https://doi.org/10.1146/annurev-nutr-071816-064916

Nilsson, L. G., & Nilsson, E. (2009). Overweight and cognition. *Scandinavian Journal of Psychology*. https://doi.org/10.1111/j.1467-9450.2009.00777.x

Noest, A. J., van Ee, R., Nijs, M. M., & van Wezel, R. J. A. (2007). Percept-choice sequences driven by interrupted ambiguous stimuli: A low-level neural model. *Journal of Vision*. https://doi.org/10.1167/7.8.10

Norcia, A. M., Appelbaum, L. G., Ales, J. M., Cottereaur, B. R., & Rossion, B. (2015). The steady state VEP in research. *Journal of Vision*, *15*(6), 1–46. https://doi.org/10.1167/15.6.4.doi

Okauchi, H., Higo-Yamamoto, S., Sowa, T., Oike, H., Yamamoto, S., Wada, N., … Oishi, K. (2020). Chronically skipping breakfast impairs hippocampal memory-related gene expression and memory function accompanied by reduced wakefulness and body

temperature in mice. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2020.01.077

Ostrovsky, Y., Andalman, A., & Sinha, P. (2006). Vision following extended congenital blindness. *Psychological Science*. https://doi.org/10.1111/j.1467-9280.2006.01827.x

Owen, L., Scholey, A. B., Finnegan, Y., Hu, H., & Sünram-Lea, S. I. (2012). The effect of glucose dose and fasting interval on cognitive function: A double-blind, placebo-controlled, six-way crossover study. *Psychopharmacology*. https://doi.org/10.1007/s00213-011-2510-2

Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G., & Cahill, G. F. (1967). Brain metabolism during fasting. *The Journal of Clinical Investigation*. https://doi.org/10.1172/JCI105650

Pan, J. W., Bebin, E. M., Chu, W. J., & Hetherington, H. P. (1999). Ketosis and epilepsy: 31P spectroscopic imaging at 4.1 T. *Epilepsia*. https://doi.org/10.1111/j.1528-1157.1999.tb00766.x

Pascual-Leone, A., Amedi, A., Fregni, F., & Merabet, L. B. (2005). The plastic human brain cortex. *Annual Review of Neuroscience*. https://doi.org/10.1146/annurev.neuro.27.070203.144216

Pawlosky, R. J., Kashiwaya, Y., King, M. T., & Veech, R. L. (2020). A dietary ketone ester normalizes abnormal behavior in a mouse model of Alzheimer's disease. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms21031044

Peña-Jorquera, H., Campos-Núñez, V., Sadarangani, K. P., Ferrari, G., Jorquera-Aguilera, C., & Cristi-Montero, C. (2021). Breakfast: A crucial meal for adolescents' cognitive performance according to their nutritional status. the cogni-action project. *Nutrients*. https://doi.org/10.3390/nu13041320

Pender, S., Gilbert, S. J., & Serpell, L. (2014). The neuropsychology of starvation: Set-shifting and central coherence in a fasted nonclinical sample. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0110743

Pernet, C. R., Wilcox, R., & Rousselet, G. A. (2013). Robust correlation analyses: False positive and power validation using a new open source matlab toolbox. *Frontiers in Psychology*. https://doi.org/10.3389/fpsyg.2012.00606

Pflanz, N. C., Daszkowski, A. W., James, K. A., & Mihic, S. J. (2019). Ketone body modulation of ligand-gated ion channels. *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2018.12.013

Pierre, K., & Pellerin, L. (2005). Monocarboxylate transporters in the central nervous system: Distribution, regulation and function. *Journal of Neurochemistry*. https://doi.org/10.1111/j.1471-4159.2005.03168.x

Pitchaimuthu, K., Wu, Q., Carter, O., Nguyen, B. N., Ahn, S., Egan, G. F., & McKendrick, A. M. (2017a). Occipital GABA levels in older adults and their relationship to visual perceptual suppression. *Scientific Reports*, *7*(1), 14231. https://doi.org/10.1038/s41598-017-14577-5

Pitchaimuthu, K., Wu, Q. Z., Carter, O., Nguyen, B. N., Ahn, S., Egan, G. F., & McKendrick, A. M. (2017b). Occipital GABA levels in older adults and their relationship to visual perceptual suppression. *Scientific Reports*. https://doi.org/10.1038/s41598-017-14577-

Pitskel, N. B., Merabet, L. B., Ramos-Estebanez, C., Kauffman, T., & Pascual-Leone, A. (2007). Time-dependent changes in cortical excitability after prolonged visual deprivation. *NeuroReport*. https://doi.org/10.1097/WNR.0b013e3282f0d2c1

Poff, A. M., Rho, J. M., & D'Agostino, D. P. (2019). Ketone Administration for Seizure Disorders: History and Rationale for Ketone Esters and Metabolic Alternatives. *Frontiers in Neuroscience*, *13*(October), 1–13. https://doi.org/10.3389/fnins.2019.01041

Priya, R., Rakela, B., Kaneko, M., Spatazza, J., Larimer, P., Hoseini, M. S., … Stryker, M. P. (2019). Vesicular GABA transporter is necessary for transplant- induced critical period plasticity in mouse visual cortex. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.1253-18.2019

Puchalska, P., & Crawford, P. A. (2017). Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2016.12.022

Raider, K., Ma, D., Harris, J. L., Fuentes, I., Rogers, R. S., Wheatley, J. L., … Stanford, J. A. (2016). A high fat diet alters metabolic and bioenergetic function in the brain: A magnetic resonance spectroscopy study. *Neurochemistry International*. https://doi.org/10.1016/j.neuint.2016.04.008

Ramamurthy, M., & Blaser, E. (2018). Assessing the kaleidoscope of monocular deprivation effects. *Journal of Vision*, *18*(13), 1–16. https://doi.org/10.1167/18.13.14

Ramoa, A. S., Shadlen, M., Skottun, B. C., & Freeman, R. D. (1986). A comparison of inhibition in orientation and spatial frequency selectivity of cat visual cortex. *Nature*. https://doi.org/10.1038/321237a0

Reynolds, J. H., & Heeger, D. J. (2009). The Normalization Model of Attention. *Neuron*. https://doi.org/10.1016/j.neuron.2009.01.002

Robertson, C. E., Kravitz, D. J., Freyberg, J., Baron-Cohen, S., & Baker, C. I. (2013). Slower Rate of binocular rivalry in autism. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.0448-13.2013

Robertson, C. E., Ratai, E. M., & Kanwisher, N. (2016). Reduced GABAergic Action in the Autistic Brain. *Current Biology*, *26*(1), 80–85. https://doi.org/10.1016/j.cub.2015.11.019

Said, C. P., Egan, R. D., Minshew, N. J., Behrmann, M., & Heeger, D. J. (2013). Normal binocular rivalry in autism: Implications for the excitation/inhibition imbalance hypothesis. *Vision Research*, *77*, 59–66. https://doi.org/10.1016/j.visres.2012.11.002

Said, C. P., & Heeger, D. J. (2013). A Model of Binocular Rivalry and Cross-orientation Suppression. *PLoS Computational Biology*. https://doi.org/10.1371/journal.pcbi.1002991

Saiepour, M. H., Rajendran, R., Omrani, A., Ma, W. P., Tao, H. W., Heimel, J. A., & Levelt, C. N. (2015). Ocular dominance plasticity disrupts binocular inhibition-excitation matching in visual cortex. *Current Biology*. https://doi.org/10.1016/j.cub.2015.01.024

Sandberg, K., Blicher, J. U., Del Pin, S. H., Andersen, L. M., Rees, G., & Kanai, R. (2016).

Improved estimates for the role of grey matter volume and GABA in bistable perception. *Cortex*. https://doi.org/10.1016/j.cortex.2016.08.006

Sandoval-Salazar, C., Ramírez-Emiliano, J., Trejo-Bahena, A., Oviedo-Solís, C. I., & Solís-Ortiz, M. S. (2016). A high-fat diet decreases GABA concentration in the frontal cortex and hippocampus of rats. *Biological Research*. https://doi.org/10.1186/s40659-016-0075-6

Schallmo, M. P., Sponheim, S. R., & Olman, C. A. (2015). Reduced contextual effects on visual contrast perception in schizophrenia and bipolar affective disorder. *Psychological Medicine*. https://doi.org/10.1017/S0033291715001439

Scheyltjens, I., & Arckens, L. (2016). The current status of somatostatin-interneurons in inhibitory control of brain function and plasticity. *Neural Plasticity*. https://doi.org/10.1155/2016/8723623

Schmoller, A., Hass, T., Strugovshchikova, O., Melchert, U. H., Scholand-Engler, H. G., Peters, A., … Oltmanns, K. M. (2010). Evidence for a relationship between body mass and energy metabolism in the human brain. *Journal of Cerebral Blood Flow and Metabolism*, *30*(7), 1403–1410. https://doi.org/10.1038/jcbfm.2010.48

Scholl, B., Pattadkal, J. J., Dilly, G. A., Priebe, N. J., & Zemelman, B. V. (2015). Local Integration Accounts for Weak Selectivity of Mouse Neocortical Parvalbumin Interneurons. *Neuron*. https://doi.org/10.1016/j.neuron.2015.06.030

Schreck, K. C., Hsu, F.-C., Berrington, A., Henry-Barron, B., Vizthum, D., Blair, L., … Strowd, R. E. (2021). Feasibility and Biological Activity of a Ketogenic/Intermittent-Fasting Diet in Patients With Glioma. *Neurology*. https://doi.org/10.1212/wnl.0000000000012386

Schwartz, O., Sejnowski, T. J., & Dayan, P. (2009). Perceptual organization in the tilt illusion. *Journal of Vision*. https://doi.org/10.1167/9.4.19

Schwenk, J. C. B., VanRullen, R., & Bremmer, F. (2020). Dynamics of Visual Perceptual Echoes Following Short-Term Visual Deprivation. *Cerebral Cortex Communications*. https://doi.org/10.1093/texcom/tgaa012

Seely, J., & Chow, C. C. (2011). Role of mutual inhibition in binocular rivalry. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.00228.2011

Seino, Y., Fukushima, M., & Yabe, D. (2010). GIP and GLP-1, the two incretin hormones: Similarities and differences. *Journal of Diabetes Investigation*. https://doi.org/10.1111/j.2040-1124.2010.00022.x

Seriès, P., Lorenceau, J., & Frégnac, Y. (2003). The “silent” surround of V1 receptive fields: Theory and experiments. *Journal of Physiology Paris*. https://doi.org/10.1016/j.jphysparis.2004.01.023

Seymour, K. J., Stein, T., Clifford, C. W. G., & Sterzer, P. (2018). Cortical suppression in human primary visual cortex predicts individual differences in illusory tilt perception. *Journal of Vision*. https://doi.org/10.1167/18.11.3

Sheynin, Y., Proulx, S., & Hess, R. F. (2019). Temporary monocular occlusion facilitates binocular fusion during rivalry. *Journal of Vision*. https://doi.org/10.1167/19.5.23

Sillito, A. M. (1979). Inhibitory mechanisms influencing complex cell orientation selectivity and their modification at high resting discharge levels. *The Journal of Physiology*. https://doi.org/10.1113/jphysiol.1979.sp012723

Sillito, Adam M., Kemp, J. A., Milson, J. A., & Berardi, N. (1980). A re-evaluation of the mechanisms underlying simple cell orientation selectivity. *Brain Research*. https://doi.org/10.1016/0006-8993(80)91234-2

Solianik, R., Žlibinaitė, L., Drozdova-Statkevičienė, M., & Sujeta, A. (2020). Forty-eight-hour fasting declines mental flexibility but improves balance in overweight and obese older women. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2020.112995

Solomon, J. A., & Morgan, M. J. (2006). Stochastic re-calibration: Contextual effects on perceived tilt. *Proceedings of the Royal Society B: Biological Sciences*. https://doi.org/10.1098/rspb.2006.3634

Song, C., Sandberg, K., Andersen, L. M., Blicher, J. U., & Rees, G. (2017). Human occipital and parietal GABA selectively influence visual perception of orientation and size. *The Journal of Neuroscience*, *37*(37), 3945–16. https://doi.org/10.1523/JNEUROSCI.3945-16.2017

Sora, I., Richman, J., Santoro, G., Wei, H., Wang, Y., Vanderah, T., … Yamamura, H. I. (1994). The cloning and expression of a human creatine transporter. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1006/bbrc.1994.2475

Soto-mota, A., Norwitz, N. G., & Clarke, K. (2020). *Why a D - β -hydroxybutyrate monoester ? 0*(February), 51–59.

Soto-Mota, A., Norwitz, N. G., & Clarke, K. (2020). Why a D-β-hydroxybutyrate monoester? *Biochemical Society Transactions*. https://doi.org/10.1042/BST20190240

Spearman, C. (1904). The Proof and Measurement of Association between Two Things. *The American Journal of Psychology*. https://doi.org/10.2307/1412159

Spearman, C. (1910). Correlation calculated from faulty data. *British Journal of Psychology, 1904-1920*. https://doi.org/10.1111/j.2044-8295.1910.tb00206.x

Spolidoro, M., Baroncelli, L., Putignano, E., Maya-Vetencourt, J. F., Viegi, A., & Maffei, L. (2011). Food restriction enhances visual cortex plasticity in adulthood. *Nature Communications*, *2*(1). https://doi.org/10.1038/ncomms1323

Spolidoro, M., Sale, A., Berardi, N., & Maffei, L. (2009). Plasticity in the adult brain: Lessons from the visual system. *Experimental Brain Research*, *192*(3), 335–341. https://doi.org/10.1007/s00221-008-1509-3

Stagg, C. J., Bachtiar, V., Amadi, U., Gudberg, C. A., Ilie, A. S., Sampaio-Baptista, C., … Johansen-Berg, H. (2014). Local GABA concentration is related to network-level resting functional connectivity. *ELife*, *2014*(3), 1–9. https://doi.org/10.7554/eLife.01465

Steenge, G. R., Lambourne, J., Casey, A., Macdonald, I. A., & Greenhaff, P. L. (1998). Stimulatory effect of insulin on creatine accumulation in human skeletal muscle. *American Journal of Physiology - Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.1998.275.6.e974

Stubbs, B. J., Cox, P. J., Evans, R. D., Santer, P., Miller, J. J., Faull, O. K., … Clarke, K. (2017). On the metabolism of exogenous ketones in humans. *Frontiers in Physiology*, *8*(OCT), 1–13. https://doi.org/10.3389/fphys.2017.00848

Sui, S. X., Ridding, M. C., & Hordacre, B. (2020). Obesity is associated with reduced plasticity of the human motor cortex. *Brain Sciences*. https://doi.org/10.3390/brainsci10090579

Sünram-Lea, S. I., Foster, J. K., Durlach, P., & Perez, C. (2001). Glucose facilitation of cognitive performance in healthy young adults: Examination of the influence of fast-duration, time of day and pre-consumption plasma glucose levels. *Psychopharmacology*. https://doi.org/10.1007/s002130100771

Murphy,T., Pereira-Dias, G., & Thuret, S. (2014). Effects of diet on brain plasticity in animal and human studies: Mind the gap. *Neural Plasticity*, *2014*. https://doi.org/dx.doi.org/10.1155/2014/563160

Tanner, G. R., Lutas, A., Martínez-François, J. R., & Yellen, G. (2011). Single KATP channel opening in response to action potential firing in mouse dentate granule neurons. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.5951-10.2011

Thiele, E. A. (2003). Assessing the efficacy of antiepileptic treatments: The Ketogenic diet. *Epilepsia*. https://doi.org/10.1046/j.1528-1157.44.s7.4.x

Tong, F., Meng, M., & Blake, R. (2006). Neural bases of binocular rivalry. *Trends in Cognitive Sciences*, *10*(11), 502–511. https://doi.org/10.1016/j.tics.2006.09.003

Trachtenberg, J. T. (2015). Parvalbumin Interneurons: All Forest, No Trees. *Neuron*, *87*(2), 247–248. https://doi.org/10.1016/j.neuron.2015.06.041

Valente-Silva, P., Lemos, C., Köfalvi, A., Cunha, R. A., & Jones, J. G. (2015). Ketone bodies effectively compete with glucose for neuronal acetyl-CoA generation in rat hippocampal slices. *NMR in Biomedicine*. https://doi.org/10.1002/nbm.3355

Van Loon, A. M., Knapen, T., Scholte, H. S., St. John-Saaltink, E., Donner, T. H., & Lamme, V. A. F. (2013). GABA shapes the dynamics of bistable perception. *Current Biology*, *23*(9), 823–827. https://doi.org/10.1016/j.cub.2013.03.067

Vangeneugden, J., van Beest, E. H., Cohen, M. X., Lorteije, J. A. M., Mukherjee, S., Kirchberger, L., … Heimel, J. A. (2019). Activity in Lateral Visual Areas Contributes to Surround Suppression in Awake Mouse V1. *Current Biology*. https://doi.org/10.1016/j.cub.2019.10.037

Verdich, C., Flint, A., Gutzwiller, J.-P., Näslund, E., Beglinger, C., Hellström, P. M., … Astrup, A. (2001). A Meta-Analysis of the Effect of Glucagon-Like Peptide-1 (7–36) Amide on Ad Libitum Energy Intake in Humans. *The Journal of Clinical Endocrinology & Metabolism*. https://doi.org/10.1210/jcem.86.9.7877

Vetencourt, J. F. M., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O'Leary, O. F., … Maffei, L. (2008). The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science*. https://doi.org/10.1126/science.1150516

Walther, K., Birdsill, A. C., Glisky, E. L., & Ryan, L. (2010). Structural brain differences and cognitive functioning related to body mass index in older females. *Human Brain Mapping*. https://doi.org/10.1002/hbm.20916

Wandell, B. A., & Smirnakis, S. M. (2009). Plasticity and stability of visual field maps in adult primary visual cortex. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn2741

Wang, Mengxin, McGraw, P., & Ledgeway, T. (2020). Short-term monocular deprivation reduces inter-ocular suppression of the deprived eye. *Vision Research*. https://doi.org/10.1016/j.visres.2020.05.001

Wang, Ming, Yoon, G., Song, J., & Jo, J. (2021). Exendin-4 improves long-term potentiation

and neuronal dendritic growth in vivo and in vitro obesity condition. *Scientific Reports*. https://doi.org/10.1038/s41598-021-87809-4

Wang, Y., Yao, Z., He, Z., Zhou, J., & Hess, R. F. (2017). The Cortical Mechanisms Underlying Ocular Dominance Plasticity in Adults are Not Orientationally Selective. *Neuroscience*, *367*, 121–126. https://doi.org/10.1016/j.neuroscience.2017.10.030

Wang, Z. J., Bergqvist, C., Hunter, J. V., Jin, D., Wang, D. J., Wehrli, S., & Zimmerman, R. A. (2003). In vivo measurement of brain metabolites using two-dimensional double-quantum MR spectroscopy - Exploration of GABA levels in a ketogenic diet. *Magnetic Resonance in Medicine*. https://doi.org/10.1002/mrm.10429

Watanabe, T., & Sasaki, Y. (2015). Perceptual learning: Toward a comprehensive theory. *Annual Review of Psychology*. https://doi.org/10.1146/annurev-psych-010814-015214

Wiesel, T. N., & Hubel, D. H. (1963). Single-cell responses in striate cortex of kittens deprived of vision in one eye. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.1963.26.6.1003

Wright, J. N., Saneto, R. P., & Friedman, S. D. (2018). Hydroxybutyrate detection with proton MR spectroscopy in children with drug-resistant epilepsy on the ketogenic diet. *American Journal of Neuroradiology*. https://doi.org/10.3174/ajnr.A5648

Yan, H. D., Ishihara, K., Serikawa, T., & Sasa, M. (2003). Activation by N-acetyl-L-aspartate of acutely dissociated hippocampal neurons in rats via metabotropic glutamate receptors. *Epilepsia*. https://doi.org/10.1046/j.1528-1157.2003.49402.x

Yang, J., Wang, Q., He, F., Ding, Y., Sun, Q., Hua, T., & Xi, M. (2016). Dietary restriction affects neuronal response property and GABA synthesis in the primary visual cortex. *PLoS ONE*, *11*(2), 1–21. https://doi.org/10.1371/journal.pone.0149004

Yazdani, P., Serrano-Pedraza, I., Whittaker, R. G., Trevelyan, A., & Read, J. C. A. (2015). Two common psychophysical measures of surround suppression reflect independent neuronal mechanisms. *Journal of Vision*. https://doi.org/10.1167/15.13.21

Yellen, G. (2008). Ketone bodies, glycolysis, and KATP channels in the mechanism of the ketogenic diet. *Epilepsia*. https://doi.org/10.1111/j.1528-1167.2008.01843.x

Yudkoff, M., Daikhin, Y., Nissim, I., Horyn, O., Lazarow, A., Luhovyy, B., … Nissim, I. (2005). Response of brain amino acid metabolism to ketosis. *Neurochemistry International*. https://doi.org/10.1016/j.neuint.2005.04.014

Yudkoff, M., Daikhin, Y., Nissim, I., Lazarow, A., & Nissim, I. (2001). Brain amino acid metabolism and ketosis. *Journal of Neuroscience Research*. https://doi.org/10.1002/jnr.1221

Zhang, P., Bao, M., Kwon, M., He, S., & Engel, S. A. (2009). Effects of Orientation-Specific Visual Deprivation Induced with Altered Reality. *Current Biology*, *19*(22), 1956–1960. https://doi.org/10.1016/j.cub.2009.10.018

Zhou, J., Baker, D. H., Simard, M., Saint-Amour, D., & Hess, R. F. (2015). Short-term monocular patching boosts the patched eye's response in visual cortex. *Restorative Neurology and Neuroscience*, *33*(3), 381–387. https://doi.org/10.3233/RNN-140472

Zhou, J., Clavagnier, S., & Hess, R. F. (2013). Short-term monocular deprivation strengthens the patched eye's contribution to binocular combination. *Journal of Vision*, *13*(5), 1–10.

https://doi.org/10.1167/13.5.12

Zhou, J., Reynaud, A., & Hess, R. F. (2014). Real-time modulation of perceptual eye dominance in humans. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1795). https://doi.org/10.1098/rspb.2014.1717

Zitron-Emanuel, N., & Ganel, T. (2018). Food deprivation reduces the susceptibility to size-contrast illusions. *Appetite*. https://doi.org/10.1016/j.appet.2018.06.006

Zitron-Emanuel, N., & Ganel, T. (2020). Does food deprivation affect perceived size? *Appetite*. https://doi.org/10.1016/j.appet.2020.104829