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1 **Bariatric Surgery Restores Visual Cortical Plasticity in Non-Diabetic Subjects with Obesity**

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6 Giuseppe Daniele\*<sup>1</sup>, Claudia Lunghi\*<sup>2</sup>, Angela Dardano<sup>1</sup>, Paola Binda<sup>3</sup>, Giovanni Ceccarini<sup>1</sup>, Ferruccio  
7 Santini<sup>1</sup>, Laura Giusti<sup>1</sup>, Annamaria Ciccarone<sup>4</sup>, Rosario Bellini<sup>5</sup>, Carlo Moretto<sup>5</sup>, Maria Concetta  
8 Morrone<sup>3</sup>, Stefano Del Prato<sup>1</sup>.

9  
10 <sup>1</sup>Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

11 <sup>2</sup>Laboratoire des Systèmes Perceptifs, Département d'études Cognitives, École Normale Supérieure,  
12 PSL University, CNRS, 75005 Paris, France.

13 <sup>3</sup>Department of Translational Research on New Technologies in Medicine and Surgery, University of  
14 Pisa, Via San Zeno 31, 56123 Pisa, Italy.

15 <sup>4</sup>Section of Diabetes and Metabolic Diseases, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy.

16 <sup>5</sup>Section Bariatric Surgery, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy.

17  
18 \*Authors have equal authorship

19  
20  
21  
22  
23  
24 Corresponding Author  
25 Stefano Del Prato  
26 Department of Clinical & Experimental Medicine  
27 Section of Metabolic Diseases & Diabetes  
28 Nuovo Ospedale Santa Chiara  
29 Via Paradisa 2  
30 56124 Pisa – Italy  
31 Tel +39 050 995103  
32 Fax +39 050 541521  
33 E-mail [stefano.delprato@unipi.it](mailto:stefano.delprato@unipi.it)  
34  
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45 **Abstract**

46  
47 **Background/Objectives:** Obesity leads to changes in synaptic plasticity. We aimed at investigating the  
48 impact of bariatric surgery (RYGB) on visual neural plasticity (NP) and its relationship with the main  
49 gut peptides, leptin, and brain-derived neurotrophic factor (BDNF).

50 **Subjects/Methods:** NP was assessed testing binocular rivalry before and after 2h of monocular  
51 deprivation (index of visual brain plasticity) in 15 subjects with obesity (age  $42.3 \pm 9.8$  years; BMI  
52  $46.1 \pm 4.9$  kg/m<sup>2</sup>) before and after RYGB. Gut peptides, leptin and BDNF were obtained at baseline and  
53 six months after surgery in 13 subjects.

54 **Results:** a significant reduction in BMI ( $p < 0.001$  vs baseline) and a significant increase of disposition  
55 index (DI,  $p = 0.02$  vs baseline) were observed after RYGB. Total and active GLP-1 release in response  
56 to glucose ingestion significantly increased after RYGB, while no changes occurred in VIP, GIP and  
57 BDNF levels. Fasting leptin concentration was lower after RYGB ( $p = 0.001$  vs baseline). Following  
58 RYGB, NP was progressively restored ( $p < 0.002$ ). NP was correlated with DI and fasting Glucose at  
59 baseline ( $r = 0.75$ ,  $p = 0.01$ ;  $r = -0.7$ ,  $p = 0.02$ ; respectively), but not with BMI. A positive correlation  
60 between post-pre RYGB changes in  $AUC_{\text{active GLP-1}}$  and NP was observed ( $r = 0.70$ ,  $p < 0.01$ ). Leptin was  
61 inversely correlated with NP six months after surgery ( $r = -0.63$ ,  $p = 0.02$ ). No correlation was observed  
62 between GIP, VIP, BDNF and NP.

63 **Conclusions:** Visual plasticity is altered in subjects with obesity, and it can be restored after RYGB.  
64 The improvement may be mediated by amelioration of insulin sensitivity, increased GLP-1 levels and  
65 reduced leptin levels.

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72 **Introduction**

73 The worldwide dramatic increase of the prevalence of obesity is generating major health concerns  
74 because of the severe comorbidities accompanying this condition (1). Among them, cognitive  
75 dysfunction and neurodegenerative diseases have been recently drawn growing attention (2). The  
76 negative impact of obesity on the central nervous system is supported by studies of magnetic resonance  
77 imaging (MRI) that have identified lacunar infarcts, white matter hyperintensities, and brain atrophy  
78 (3). These anatomic alterations are suggested to be a consequence of the metabolic disturbances, insulin  
79 resistance, abnormalities in the signaling of gut hormones and adipokines on the brain (4). Peripheral  
80 insulin resistance is a common feature in obesity and recent studies have suggested that impaired  
81 insulin action may occur in the brain as well (5). Insulin receptors are widespread in the hippocampal  
82 and cortical brain structures, and brain insulin resistance may contribute to the cognitive impairment  
83 and neurodegeneration observed in obesity (6). Insulin can regulate neuronal survival and metabolism  
84 through modulation of gamma-aminobutyric acid (GABA) and glutamate receptors in the hippocampus  
85 and motor cortex, structures involved in learning and memory functions (7).

86 Weight loss, as it can be obtained with bariatric surgery, has been shown to exert beneficial effects on  
87 both brain structure and function (8). Body weight reduction has been associated with enhanced  
88 activity in neural circuits in areas involved in executive control, such as the dorsolateral prefrontal  
89 cortex (9), the superior frontal gyri and the posterior cingulate cortex (10). Bariatric surgery does not  
90 simply lead to significant body weight reduction, but it is also associated with changes in incretins and  
91 adipokines (11). They are key elements of the gut-brain axis, a multidirectional communication system  
92 linking the enteric nervous system and the central nervous system via endocrine signals, such as  
93 Glucagon-Like Peptide 1 (GLP-1), Glucose-dependent insulintropic polypeptide (GIP) and Vasoactive  
94 Intestinal Peptide (VIP), and the activation of the enteric nervous system and vagal signaling (12). In  
95 keeping with an effect of GLP-1 on brain function, we have recently shown that exenatide  
96 administration can modulate cerebral glucose metabolic rate in several cortical and sub-cortical areas

97 (13) along with modulation of visual responses to food and non-food images in non-diabetic subjects  
98 with obesity undergoing functional MRI (fMRI) (14). White adipose tissue, the body's energy depot, is  
99 also connected with the central nervous system through leptin signaling. Leptin receptors are  
100 widespread in the brain (e.g., throughout the cortex and the hippocampus) and their activation is  
101 implicated in memory processes (15). Leptin has also been shown to exert neuroprotective functions by  
102 inhibiting apoptosis and improving cell survival through regulation of apoptotic enzymes and activation  
103 of neurotrophic factors such as the brain-derived neurotrophic factor (BDNF) (16). However, it is still  
104 unknown whether bariatric surgery can impact on one of the fundamental properties of the brain: the  
105 ability to modify its connection or rewire in response to experience, a phenomenon known as neural  
106 plasticity (17).

107 We have recently reported (18) that a particular form of neural plasticity (visual homeostatic plasticity  
108 of ocular dominance) is severely blunted in subjects with BMI >40 Kg/m<sup>2</sup>. In normal weight adult  
109 subjects, a short period of monocular deprivation transiently shifts ocular dominance in favor of the  
110 deprived eye both at the perceptual (19,20,21,22) and at the neural level (23,24) reflecting a  
111 compensatory regulation of ocular dominance in response to deprivation consistent with homeostatic  
112 plasticity (25) We have shown that a strong correlation exists between body mass index (BMI) and this  
113 form of visual plasticity (18): the effect of short-term monocular deprivation declines with increasing  
114 BMI and is completely absent in subjects with morbidly obesity. Here, we investigate the impact of  
115 bariatric surgery on early sensory plasticity and the role of main circulating gut hormones and  
116 adipokines in patients with obesity by measuring the effect of short-term monocular deprivation on  
117 ocular dominance before and after bariatric surgery.

118

## 119 **Subjects and Methods**

### 120 *Participants and Recruitment*

121 Subjects with obesity candidate for Roux-en-Y gastric bypass (RYGB) were screened for enrollment at  
122 the Obesity Centre of the Azienda-Ospedaliero Universitaria Pisana, Pisa, Italy. Subjects with age 18-

123 60 years, BMI >35 kg/m<sup>2</sup>, HbA1c <6.5% and eGFR >60 ml/min/1.73m<sup>2</sup> were considered eligible.

124 Individuals with the following conditions were excluded from the study:

- 125 • psychiatric disorders
- 126 • mental retardation
- 127 • severe cognitive impairment
- 128 • neurodegenerative diseases
- 129 • epilepsy
- 130 • steroid treatment
- 131 • traumatic brain injury over the preceding six months
- 132 • liver function enzymes higher than twice the upper limit
- 133 • heart failure (NYHA III-IV)
- 134 • overt Type 2 and Type 1 diabetes
- 135 • pregnant or breastfeeding women
- 136 • history or current evidence of any condition, therapy, laboratory abnormality or other
- 137 circumstances which, in the opinion of the investigators, were deemed to expose the patient to
- 138 an unacceptable risk or could interfere with trial procedures

139 The study has been registered at ClinicalTrials.gov (NCT03414333) and the study protocol was  
140 approved by the local Ethics Committee and conducted in accordance with the Declaration of Helsinki  
141 (2008). Each participant provided written informed consent before entering the study.

142 A total of 15 subjects (Age: 42.3 ± 9.8 years, BMI: 46.1±4.9 Kg/m<sup>2</sup>) were included in the study. All had  
143 normal or corrected to normal visual acuity and no visual deficits. Two participants chose not to  
144 complete the oral glucose level test; for these participants, only the neural plasticity results are  
145 available.

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149 ***Study Design***

150 At baseline, participants underwent two visits (V1 and V2) at 5-14-day interval. In V1 brain plasticity  
151 of the visual cortex was measured in fasting condition through a psychophysical technique based on the  
152 binocular rivalry evaluation before and after 2-hr monocular deprivation (MD) according to the  
153 standard protocol (19).

154 On V2, participants were admitted to our Research Unit in the morning after an overnight fast to  
155 undergo an oral glucose load (75 g OGTT). Blood samples were obtained at -120, 0, 15, 30, 45, 60, 75,  
156 90, 105 and 120 min for determination of plasma substrate and hormone concentrations. All  
157 participants underwent RYGB within 2 weeks upon completion of baseline evaluation. Brain plasticity  
158 evaluation was then repeated 1 (V3), 3 (V4) and 6 months (V5) after surgical intervention whereas the  
159 OGTT was repeated only after 6 months (V6).

160 ***Visual Plasticity Assessment***

161 Experimental procedures took place in a dark, quiet room after an overnight fast. Visual stimuli were  
162 generated by a VSG 2/5 stimulus generator (CRS, Cambridge Research Systems) housed in a laptop  
163 (Dell, Round Rock, TX, USA) controlled by Matlab programs (MathWorks, Natick, MA, USA). The  
164 stimuli consisted in 2 sinusoidal gratings, oriented either 45° clockwise or counterclockwise (size:  $2\sigma =$   
165  $2^\circ$ , spatial frequency: 2 cpd), presented on a uniform background (luminance: 9cd/m<sup>2</sup> CIE  $x=.311,$   
166  $y=.341$ ) with a central black fixation point and a common squared frame to facilitate dichoptic fusion.  
167 Visual stimuli were displayed on a 17-inch CRT monitor (FD Trinitron CRT multiscan G200) monitor,  
168 driven at a resolution of 1024x600 pixels, with a refresh rate of 120 Hz. Observers viewed the display  
169 at a distance of 57 cm through CRS Ferro-Magnetic shutter goggles that occluded alternately one of the  
170 two eyes each frame. Responses were recorded through the computer keyboard.

171 For binocular rivalry assessment, experimental blocks of 125 seconds were used. After an acoustic  
172 signal (beep), the binocular rivalry stimuli appeared. Subjects reported their perception (clockwise,

173 counterclockwise or mixed) by continuously pressing with the right hand one of three keys (left, right  
174 and down arrows) of the computer keyboard. At each experimental block, the orientation associated to  
175 each eye was randomly varied so that neither subject nor the investigators knew which stimulus was  
176 associated with which eye until the end of the session. Two binocular rivalry experimental blocks were  
177 acquired before monocular deprivation and four blocks after eye-patch removal.

178 Monocular deprivation was performed using an eye-patch of a translucent plastic material that allowed  
179 light to reach the retina (luminance attenuation 0.07 logUnits) but completely prevented pattern vision,  
180 as assessed by the Fourier transform of a natural world image seen through the eye-patch. The dominant  
181 eye (the eye showing longer perceptual predominance in binocular rivalry) was patched for 120-min.  
182 During the 2h of monocular occlusion patients were free to perform normal quiet activities (walking,  
183 reading, using a computer).

184

#### 185 ***Laboratory assays***

186 Plasma glucose concentration was measured using the glucose oxidase method (Beckman, Fullerton,  
187 CA, USA). Samples for GIP and GLP-1 were drawn in tube containing protease inhibitors, specifically  
188 optimized for stabilization of metabolic markers (BD P800, BD Biosciences, USA). Total GLP-1,  
189 active GLP-1 and GIP were measured by ELISA (EDM Millipore, St. Charles, Missouri, USA). Insulin  
190 and C-peptide were assessed by radioimmunoassay (IRMA, PANTEC srl Turin, Italy). VIP was  
191 measured using an Enzyme Immunoassay (RayBiotech, Norcross, GA, USA). Brain-derived  
192 neurotrophic factor (BDNF) and leptin were measured using a Sandwich Enzyme-Linked  
193 Immunosorbent Assay (RayBiotech, Norcross, GA, USA).

194 Insulin sensitivity was calculated as ISIcomp according to the formula:

195  $10.000/\sqrt{[fasting\ plasma\ glucose\ (mg/dl) \times fasting\ plasma\ insulin\ (\mu UI/ml) \times glucose\ at\ 120\ min$   
196  $(mg/dl) \times insulin\ at\ 120\ min\ (\mu UI/ml)]}$

197 The oral disposition index was calculated as ISSI-2 equal to ISIcomp x AUCins/AUCgluc as previously

198 described (26). The area under the curve of hormones and plasma glucose were computed using the  
199 trapezoidal rule.

200

### 201 *Analyses of visual plasticity*

202 Perceptual reports during binocular rivalry were analyzed using Matlab. We computed the total time (T)  
203 during which the observer reported each of the three percepts: the oriented grating presented to either  
204 eye (deprived and non-deprived) and a mixture of the two (mixed percepts). To quantify ocular  
205 dominance, we defined an index (Ocular Dominance Index, ODI) ranging from 0 (complete dominance  
206 of the non-deprived eye) to 1 (complete dominance of the deprived eye), given by:

$$207 \text{ ODI} = T_{\text{DepEye}} / (T_{\text{DepEye}} + T_{\text{NonDep Eye}})$$

208 Eq.1

209 The Ocular Dominance Index does not consider the mixed percepts that do not change after  
210 deprivation. The effect of monocular deprivation (Neural Plasticity index, NP) was computed as the  
211 difference between the ODI measured after and before the 2h of monocular deprivation.

212

### 213 *Statistical Analysis*

214 Statistical analyses were performed using SPSS-2.0 (Statistical Package for Social Sciences, Chicago,  
215 IL, USA) and Matlab software. Values are presented as mean±SEM or as median (interquartile range)  
216 for variables with a skewed distribution; categorical data are expressed as percentages. Variables that  
217 were not normally distributed were log-transformed before analysis. Treatment-induced changes were  
218 examined by Wilcoxon's signed rank test and a p<0.05 (two-tailed analysis) was considered to be  
219 statistically significant.

220 Correlations were assessed by using a robust correlation method (27), the skipped Pearson's correlation  
221 coefficient (r) and statistical significance determined by a bootstrap t-test and p-values were corrected  
222 for multiple comparisons using the Bonferroni method.

223 To assess the effect of bariatric surgery on visual plasticity, the change in the visual plasticity index  
224 (NP) measured before and after surgery was tested with a linear mixed model analysis performed using  
225 the fitlme function in Matlab in which time (baseline, 1 month, 3 months and 6 months after surgery)  
226 was used as fixed effect with random slope and intercept, with the model: “ $y \sim \text{time} + (1 + \text{time} | \text{ID})$ .”  
227 Post-hoc tests between the visual plasticity index measured before and after surgery were performed  
228 using a Wilcoxon signed rank test, p-values were corrected for multiple comparisons using the  
229 Bonferroni method. To determine whether, before surgery, participants with obesity showed a response  
230 to monocular deprivation, the plasticity index measured at baseline was compared against the value 0  
231 using a Wilcoxon signed rank test.

232

## 233 **Results**

### 234 *Participants and Metabolic Parameters*

235 15 subjects with severe obesity completed the neural plasticity assessment, 13 of which also completed  
236 the metabolic assessment. Two patients withdrew the consent to repeat post RYGB assessment. The  
237 characteristics of the 13 participants who completed the whole study are summarized in Table 1.  
238 Participants were relatively young with a baseline BMI of  $46.1 \pm 4.9 \text{ Kg/m}^2$ . Four subjects had impaired  
239 fasting glucose and all the remaining had normal glucose tolerance and HbA1c levels (Table 1). No  
240 patient was on any medication known to affect glucose metabolism and brain function.

241 RYGB was followed by a significant reduction in body weight ( $-30.5 \pm 0.2 \text{ Kg}$ ) with a BMI at month 6  
242 of  $35.3 \pm 5.0 \text{ Kg/m}^2$  ( $p=0.001$  vs. baseline). Figure 1 shows the plasma concentrations of glucose, insulin  
243 and gut hormones during OGTT. Overall, 6 months after RYGB, plasma glucose and insulin levels  
244 showed an earlier peak but lower 2-hour concentration as compared to baseline. Accordingly, ISIcomp  
245 (from  $2.4 \pm 0.5$  to  $8.2 \pm 1.9$ ;  $p=0.004$ ) and the Disposition Index (from  $1.1 \pm 0.2$  to  $2.9 \pm 1.1$  ISSI2;  $p=0.02$ )  
246 improved in a significant manner. Total and active GLP1 release in response to glucose ingestion

247 increased after bariatric surgery, while no changes occurred with GIP, VIP, and BDNF. Fasting plasma  
248 leptin concentration was significantly lower after RYGB as compared to baseline (Table 1).

249

### 250 *Effect of Bariatric Surgery on Visual Cortical Plasticity*

251 Consistently with our previous observations (18), at baseline the neural plasticity index obtained by  
252 monocular deprivation was  $0.03 \pm 0.03$ ,  $p = 0.45$ , implying impaired visual cortical plasticity (20).  
253 Following RYGB, a progressive increase of the neural plasticity index (Linear Mixed Model Analysis:  
254  $t(57)=3.35$ ,  $p=0.0014$ ) was observed, with ocular dominance shifting in favour of the deprived eye  
255 following deprivation, consistently with previous studies on normal-weight subjects (19,20,21). This  
256 increase was already statistically significant 3 months after surgery ( $p=0.008$ ) (Figure 2A-B) when an  
257 average 25% weight loss was achieved (BMI from  $46.1 \pm 4.9$  to  $35.3 \pm 5.0$ ;  $p < 0.01$ ). Thereafter, the  
258 improvement of visual plasticity was constant at 6 months after surgery ( $p=0.0036$ ).

259

### 260 *Visual Cortical Plasticity and Glucose Tolerance*

261 Baseline BMI was not correlated with plasticity index (Figure 2C). An inverse correlation was apparent  
262 between fasting plasma glucose and the plasticity index ( $r = -0.71$ ;  $p=0.02$ ) (Figure 2D) but not with  
263 plasma glucose and insulin concentrations during OGTT before surgery. The plasticity index strongly  
264 correlated also with the Disposition Index (and  $r=0.75$ ,  $p=0.01$ ; Figure 2E). No correlation was found  
265 between change of BMI, of fasting plasma glucose, of HbA1c and change of plasticity index after  
266 surgery.

267

### 268 *Brain Plasticity, Gut Peptides, Leptin and BDNF*

269 At baseline plasticity index showed a trend for a negative correlation with total and active GLP1 as well  
270 as with GIP, VIP, leptin and BDNF (data not shown). After surgery we found a positive correlation  
271 between changes of incremental  $AUC_{\text{active GLP-1}}$  and change of plasticity index ( $r=0.70$ ,  $p=0.008$ ; Figure  
272 3A). Moreover, six months after surgery leptin was inversely and significantly correlated with

273 plasticity index ( $r = -0.63$ ;  $p = 0.02$ )(Figure 3B). GIP, VIP and BDNF did not correlate with the plasticity  
274 index (data not shown).

275 A multiple linear regression analysis was performed using the change in the different hormones tested  
276 as predictors of the change in NP observed 6 months after surgery. The analysis revealed that only the  
277 change in active GLP1 was a significant predictor of the plasticity effect ( $t = 2.6$ ,  $p = 0.04$ ), while the  
278 other hormones had no significant predictive value (all  $p > 0.25$ ).

279

## 280 **Discussion**

281

282 This study explored the impact of weight loss obtained by bariatric surgery on brain plasticity and its  
283 relationship with circulating gut peptides, leptin, and BDNF. To the best of our knowledge this is the  
284 first study exploring visual cortex plasticity (17) during massive weight loss following RYGB and  
285 contemporary changes in gut hormones and glucose handling in non-diabetic subjects. The results of  
286 our study confirm our earlier finding that morbid obesity is associated with a marked reduction of brain  
287 plasticity (18) and reveal for the first time, that this abnormal accommodation of the brain to external  
288 stimuli can be progressively reversed by body weight reduction. In fact, the plasticity index improved 1  
289 month after surgery to approach values commonly found in healthy subjects after 6 months with a  
290 200% increase above the plasticity levels assessed before surgery. The response to monocular  
291 deprivation is thought to reflect transient changes in neuronal circuitry in primary visual cortex  
292 providing a marker of neural plasticity: changes in visual cortex activity consistent with the deprived  
293 eye dominance boost observed at the perceptual level have been observed with different techniques  
294 (23,24,28,29). Importantly, it has been shown that the effect of monocular deprivation is also  
295 accompanied by a decrease of gamma-aminobutyric acid (GABA) concentration in the primary visual  
296 cortex of adult humans (24). GABA is the main inhibitory neurotransmitter in mammalian brain and  
297 has been implicated in the control of excitability, information processing, synchronization of neuronal  
298 activity and, ultimately, neuroplasticity (30). GABA is also involved in eating behaviour as suggested

299 by experiments in rodents using GABA receptor agonists and antagonists (31). In the animal model,  
300 high-fat diet was associated with decreased GABA concentration in the frontal cortex and hippocampus  
301 (32). We therefore speculate that changes occurring with body weight reduction can interfere with the  
302 GABAergic modulation in the visual cortex and, possibly, in other areas of the brain.

303 The mechanisms responsible for this improvement are not completely clear. However, it is of interest  
304 that a number of changes occurring after bariatric surgery showed a relationship with changes in the  
305 plasticity index. Thus, plasticity was strongly associated to the metabolic status as indicated by the  
306 correlation with plasma glucose and beta-cell function (Disposition Index). Therefore, the worse the  
307 metabolic status, the worse the neuronal plasticity although we cannot exclude that similar association  
308 may occur with other conditions associated with insulin resistance. In all case, this association is not  
309 surprising since it has been shown that insulin signaling can enhance synaptic plasticity by increasing  
310 synapsis density in brain regions involved in the processing of visual inputs (33). The well-known  
311 peripheral insulin resistance characterizing obesity may well reflect insulin resistance in the central  
312 nervous system as previously suggested (34). Therefore, it is tempting to postulate that brain insulin  
313 resistance may contribute to impaired activation of synaptic plasticity in the visual cortex of our  
314 subjects with obesity. In keeping with this hypothesis, the improvement of insulin sensitivity brought  
315 up by body weight reduction was strongly associated with the improvement of the brain plasticity  
316 index. These findings also are in keeping with previous studies demonstrating a partial reversibility of  
317 impaired insulin-stimulated hypothalamic response (35) after massive reduction of body weight in  
318 subjects with obesity (36). The improvement of the plasticity index may be supported not only by  
319 amelioration of insulin action but also by changes of other hormones known to exert an effect in the  
320 brain. Bariatric surgery is associated with increased GLP-1 response to meal ingestion (37), a finding  
321 confirmed in our subjects as well in response to oral glucose load. Much literature supports the central  
322 effect of GLP1, so it was not surprising to find an association between changes in GLP-1 levels ensuing  
323 RYGB and concomitant changes in the plasticity index. These results can be taken as an additional

324 support to the proposed neuroprotective and synaptic plasticity promoting effects of GLP-1 (38). This  
325 relationship is also of interest because of the potential implication of the effect of this hormone in the  
326 modulation of satiety and appetite. We have previously shown that the administration of exenatide, a  
327 GLP-1 analogue, to subjects with obesity can modify brain responses to food images as monitored by  
328 fMRI (14). GLP-1 receptors are expressed in many areas of the human brain (39) where they can  
329 participate in high brain functions related to food responses. An effect of GIP is not apparent in our  
330 study. Similarly, no association between VIP levels and plasticity index was found. Finally, we have  
331 also explored whether signals that are generated by the adipose tissue, the main energy depot in the  
332 body, could be linked to changes in brain plasticity that occurred with body weight reduction (i.e. loss  
333 of fat mass). To this aim we have measured baseline concentration of leptin, which dropped  
334 dramatically after surgery. Leptin is a cytokine and a satiety hormone involved in appetite regulation  
335 and energy expenditure modulation. It can cross the blood brain barrier and bind to presynaptic  
336 GABAergic neurons to produce its effects and its concentrations are typically increased in human  
337 obesity (40). We now report a strong and inverse correlation between reduction in leptin concentration  
338 after RYGB and the increase in the plasticity index pointing to another pathway of signal integration  
339 between the central nervous system (here probed by the plasticity index) and the energy balance  
340 through signals generated in the gut (GLP-1) and adipocytes (leptin). BDNF is widely expressed in the  
341 central nervous system, gut and other tissues but was not affected by body weight reduction and showed  
342 no association with changes of the plasticity index.

343 The main limitations of our work are the size of the study population and the lack of a control group.  
344 However, this was an exploratory study and our results set the basis for future investigation on the  
345 relationship between brain plasticity and disturbances of energy metabolism. Similarly, we are totally  
346 aware that correlations between metabolic parameters and plasticity index change after surgery do not  
347 imply causality, yet they are hypothesis generating and as such our findings can help designing future  
348 studies to unravel the complexity of the brain-gut axis.

349 In summary, we have shown that severe impairment of visual brain plasticity occurring in subjects with  
350 morbidly obesity can be recovered by reduction in body weight. These improvements may be mediated  
351 by amelioration of (brain) insulin sensitivity, increased availability of GLP-1 and strong reduction of  
352 leptin resistance, thus supporting the fine integration between the CNS and the periphery.

353

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357 **Competing Interest.** No potential conflicts of interest relevant to this article were reported.

358 **Author Contributions.** GD, MCM, and SDP conceived the objective and designed the study. GD, AD,  
359 GC, FS and AC have screened subjects with obesity. GD and AD collected the study data, analyzed the  
360 result and wrote the first version of the manuscript. CL, PB and MCM designed, measured and  
361 analyzed the visual performance to asses visual plasticity. RB and CM performed RYGB. LG  
362 conducted the laboratory analysis. All authors have reviewed and approved the final version of the  
363 manuscript. SDP is the guarantor of this work and, as such, had full access to all the data in the study  
364 and takes responsibility for the integrity of the data and the accuracy of the data analysis.

365 **Prior Presentation.** Parts of this study were presented at the 79<sup>th</sup> and 80<sup>th</sup> Scientific Sessions of  
366 American Diabetes Association and the 54<sup>th</sup> and 55<sup>th</sup> Annual Meeting of the European Association for  
367 the Study of Diabetes

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## 370 References

- 371 1. Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. *Pharmacoeconomics* 2015
- 372 2. Geijselaers SLC, Sep SJS, Stehouwer CDA, Biessels GJ. Glucose regulation, cognition, and
- 373 brain MRI in type 2 diabetes: a systematic review. *Lancet Diabetes Endocrinol* 2015;3:75-89
- 374 3. Karlsson HK, Tuulari JJ, Hirvonen J, Lepomaki V, Parkkola R, Hiltunen J et al. Obesity is
- 375 associated with white matter atrophy: a combined diffusion tensor imaging and voxel-based
- 376 morphometric study. *Obesity (Silver Spring)* 2013;21:2530-2537
- 377 4. Balistreri CR, Caruso C, Candore G. The role of adipose tissue and adipokines in obesity-related
- 378 inflammatory diseases. *Mediators Inflamm* 2010;2010:802078
- 379 5. Heni M, Kullmann S, Preissl H, Fritsche A, Häring HU. Impaired insulin action in the human
- 380 brain: causes and metabolic consequences. *Nat Rev Endocrinol* 2015;11:701-711
- 381 6. Sripetchwandee J, Chattipakorn N, Chattipakorn SC. Links Between Obesity-Induced Brain
- 382 Insulin Resistance, Brain Mitochondrial Dysfunction, and Dementia. *Front Endocrinol*
- 383 (Lausanne) 2018;9:496
- 384 7. Stagg CJ, Bachtiar V, Johansen-Berg H. The role of GABA in human motor learning. *Curr Biol*
- 385 2011;21:480-484
- 386 8. Tuulari JJ, Karlsson HK, Antikainen O, Hirvonen J, Pham T, Salminen P et al. Bariatric surgery
- 387 induces white and grey matter density recovery in the morbidly obese. *Hum Brain Mapp*
- 388 2016;37:3745-3756
- 389 9. Goldman RL, Canterberry M, Borckardt JJ, Madan A, Byrne TK, George MS et al. Executive
- 390 control circuitry differentiates degree of success in weight loss following gastric-bypass surgery.
- 391 *Obesity (Silver Spring)* 2013;21:2189-2196
- 392 10. Ness A, Bruce J, Bruce A, Aupperle R, Lepping R, Martin L et al. Pre-surgical cortical
- 393 activation to food pictures is associated with weight loss following bariatric surgery. *Surg Obes*
- 394 *Relat Dis* 2014;10:1188-1195
- 395 11. Karra E, Yousseif A, Batterham RL. Mechanisms Facilitating Weight Loss and Resolution of
- 396 Type 2 Diabetes Following Bariatric Surgery. *Trends Endocrinol Metab* 2010;21:337-344
- 397 12. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut- brain axis: interactions between
- 398 enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* 2015;28:203-209
- 399 13. Daniele G, Iozzo P, Molina-Carrion M, Lancaster J, Ciociaro D, Cersosimo E et al. Exenatide
- 400 Regulates Cerebral Glucose Metabolism in Brain Areas Associated With Glucose Homeostasis
- 401 and Reward System. *Diabetes* 2015;64:3406-3412
- 402 14. Binda P, Eldor R, Huerta C, Adams J, Lancaster J, Fox P et al. Exenatide modulates visual
- 403 cortex responses. *Diabetes Metab Res Rev* 2019;35:e3167
- 404 15. Farr OM, Tsoukas MA, Mantzoros CS. Leptin and the brain: influences on brain development,
- 405 cognitive functioning and psychiatric disorders. *Metabolism* 2015;64:114-130
- 406 16. Teryaeva NB. Leptin as a neuroprotector and a central nervous system functional stability
- 407 factor. *Neurosci Behav Physiol* 2015; 45. 612–618
- 408 17. Castaldi E, Lunghi C, Morrone, M. C. (2020). Neuroplasticity in adult human visual cortex.
- 409 *Neurosci Biobehav Rev* 2020;112:542-552
- 410 18. Lunghi C, Daniele G, Binda P, Dardano A, Ceccarini G, Santini F et al. Altered Visual Plasticity
- 411 in Morbidly Obese Subjects. *iScience* 2019;22:206-213
- 412 19. Lunghi C, Burr DC, Morrone MC. Brief periods of monocular deprivation disrupt ocular
- 413 balance in human adult visual cortex. *Curr Biol* 2011;21:R538-539
- 414 20. Lunghi C, Burr DC, Morrone, MC. Long-term effects of monocular deprivation revealed with
- 415 binocular rivalry gratings modulated in luminance and in color. *J Vis* 2013; 13(6):1
- 416 21. Lunghi C, Sale A. A cycling lane for brain rewiring. *Curr Biol* 2015; 25(23):R1122-3

- 417 22. Zhou J, Clavagnier S, Hess, RF. Short-term monocular deprivation strengthens the patched  
418 eye's contribution to binocular combination. *J Vis* 2013; 13(5):12
- 419 23. Lunghi C, Berchicci M, Morrone MC, Di Russo F. Short-term monocular deprivation alters  
420 early components of visual evoked potentials. *J Physiol* 2015; 593(19):4361–4372
- 421 24. Lunghi C, Emir UE, Morrone MC, Bridge H. Short-term monocular deprivation alters GABA in  
422 the adult human visual cortex. *Curr Biol* 2015;25:1496-1501
- 423 25. Turrigiano G. Homeostatic synaptic plasticity: local and global mechanisms for stabilizing  
424 neuronal function. *Cold Spring Harb Perspect Biol* 2012; 4(1):a005736
- 425 26. Retnakaran R, Qi Y, Goran MI, Hamilton JK. Evaluation of proposed oral disposition index  
426 measures in relation to the actual disposition index. *Diabet Med* 2009;26:1198-1203
- 427 27. Pernet CR, Wilcox R, Rousselet GA. Robust correlation analyses: false positive and power  
428 validation using a new open source matlab toolbox. *Front Psychol* 2013;3:606
- 429 28. Binda P, Kurzawski JW, Lunghi C, Biagi L, Tosetti M, Morrone MC. Response to short-term  
430 deprivation of the human adult visual cortex measured with 7T BOLD. *ELife* 2018, 7:e40014
- 431 29. Zhou J, Baker DH, Simard M, Saint-Amour D, Hess RF. Short-term monocular patching boosts  
432 the patched eye's response in visual cortex. *Restor Neurol Neurosci* 2015;33(3):381-7
- 433 30. Owens DF, Kriegstein AR. Is there more to GABA than synaptic inhibition? *Nat Rev Neurosci*  
434 2002;3:715–727
- 435 31. Avena NM, Bocarsly ME, Murray S, Gold MS. Effects of baclofen and naltrexone, alone and in  
436 combination, on the consumption of palatable food in male rats. *Exp Clin Psychopharmacol*  
437 2014;22:460-467
- 438 32. Sandoval-Salazar C, Ramirez-Emiliano J, Trejo-Bahena A, Oviedo-Solis CI, Solis-Ortiz MS. A  
439 high-fat diet decreases GABA concentration in the frontal cortex and hippocampus of rats. *Biol*  
440 *Res* 2016; 49:15
- 441 33. Chiu SL, Chen CM, Cline HT. Insulin receptor signaling regulates synapse number, dendritic  
442 plasticity, and circuit function in vivo. *Neuron* 2008;58:708-719
- 443 34. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Häring HU. Brain Insulin Resistance  
444 at the Crossroads of Metabolic and Cognitive Disorders in Humans. *Physiol Rev* 2016;96:1169-  
445 1209
- 446 35. Matsuda M, Liu Y, Mahankali S, Pu Y, Mahankali A, Wang J et al. Altered hypothalamic  
447 function in response to glucose ingestion in obese humans. *Diabetes* 1999;48:1801-1806
- 448 36. Van de Sande-Lee S, Pereira FRS, Cintra DE, Fernandes PT, Cardoso AR, Garlipp CR et al.  
449 Partial reversibility of hypothalamic dysfunction and changes in brain activity after body mass  
450 reduction in obese subjects. *Diabetes* 2011;60:1699-1704
- 451 37. Salehi M, Prigeon RL, D'Alessio DA. Gastric Bypass Surgery Enhances Glucagon-Like Peptide  
452 1–Stimulated Postprandial Insulin Secretion in Humans. *Diabetes* 2011; 60: 2308-2314
- 453 38. McClean PL, Gault VA, Harriott P, Holscher C. Glucagon-like peptide-1 analogues enhance  
454 synaptic plasticity in the brain: a link between diabetes and Alzheimer's disease. *Eur J*  
455 *Pharmacol* 2010;630:158-162
- 456 39. Alvarez E, Martínez MD, Roncero I, Chowen JA, García-Cuartero B, Gispert JD et al. The  
457 Expression of GLP-1 Receptor mRNA and Protein Allows the Effect of GLP-1 on Glucose  
458 Metabolism in the Human Hypothalamus and Brainstem. *J Neurochem* 2005;92:798-806
- 459 40. Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for  
460 metabolic disorders. *Proteomics Clin Appl* 2012;6:91-101
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467 **Figures and Table Legend**

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469 **Figure 1-** Plasma concentrations of gut hormones during OGTT. **A:** Plasma Glucose, **B:** Plasma  
470 Insulin, **C:** Total GLP-1, **D:** Active GLP-1, **E:** GIP, **F:** VIP. White circle: before RYGB, Black circle:  
471 after RYGB. \*:p<0.05.

472 **Figure 2- A:** Effect of monocular deprivation (plasticity index) at baseline and 1, 3 and 6 months after  
473 surgery. Error bars represent  $1 \pm \text{SEM}$  **B:** Scatter plot reporting the effect of monocular deprivation  
474 measured for each participant before and at two different time points after surgery: 1 month (light grey  
475 symbols) and 6 months (dark grey symbols). The dashed lines on the 0 value indicate the absence of  
476 plasticity (no effect of monocular deprivation). **C:** Correlations before surgery of Neural Plasticity  
477 Index and baseline BMI **D:** Correlations before surgery of Neural Plasticity Index and baseline fasting  
478 plasma glucose **E:** Correlations before surgery of Neural Plasticity Index Baseline and Disposition  
479 Index. All robust correlations are computed by a skipped Pearson correlation (27).

480 **Figure 3- A:** Correlation between 6 months post-pre surgery change in incremental  $\text{AUC}_{\text{active GLP-1}}$   
481 ( $\text{iAUC}_{\text{active GLP-1}}$ ) and plasticity index. **B:** Correlation between leptin and plasticity index 6 months after  
482 surgery. All robust correlations are computed by a skipped Pearson correlation (27).

483 **Table 1-** Anthropometric and metabolic characteristics of study participants at baseline and 6 months  
484 after RYGB.

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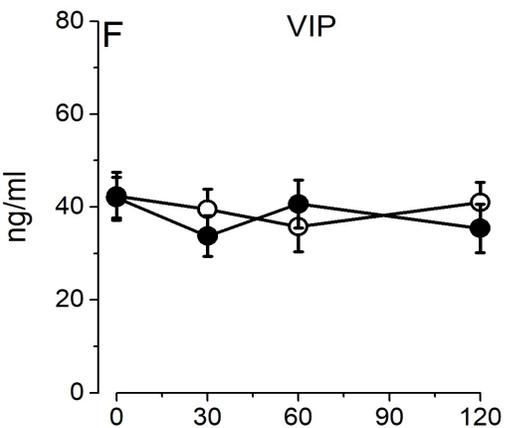
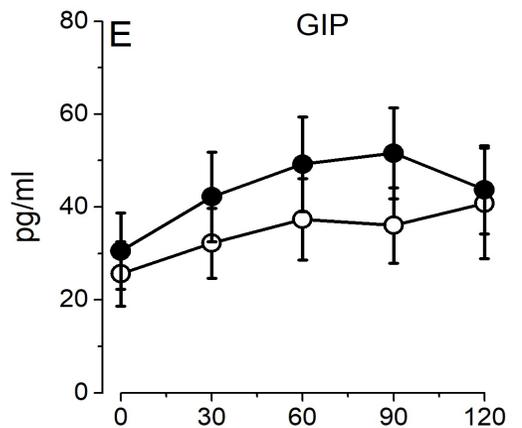
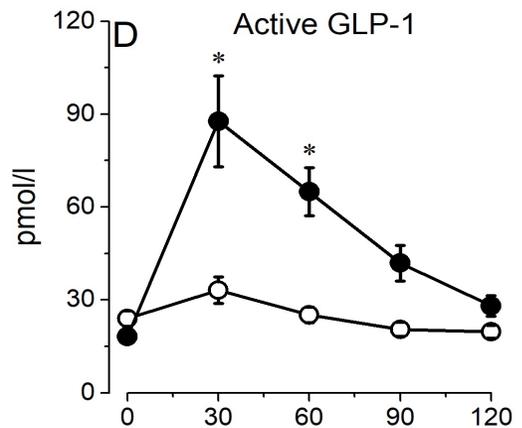
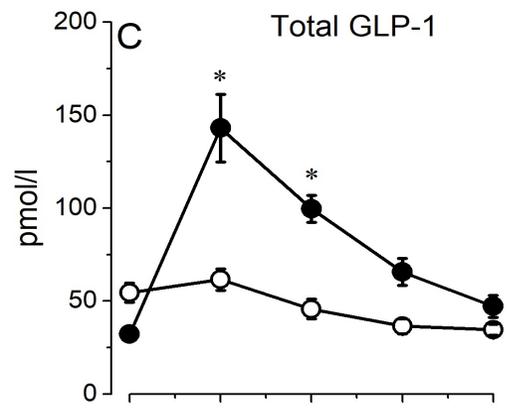
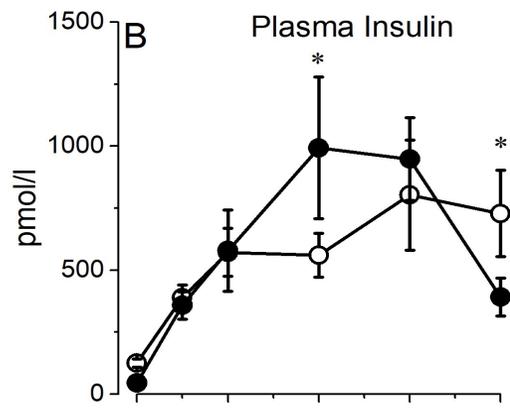
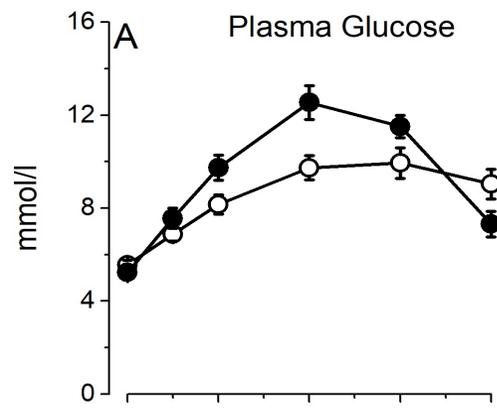
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Characteristic (mean ± SD)	Baseline (n=13)	6-months after RYGB (n=13)	p
Age (years)	42.3 ± 9.8	-	497
Sex (M/F)	2/11		498
BMI (kg/m <sup>2</sup> )	46.1±4.9	35.3±5.0	0.001
SBP (mmHg)	129±12	114±5	0.001
DBP (mmHg)	85±9	75±5	0.001
Plasma Glucose (mmol/l)	5.6±0.7	5.2±0.4	ns
HbA1c (mmol/mol)	41±6	38±4	0.01
Total Cholesterol (mg/dl)	192±32	166±21	0.001
HDL Cholesterol (mg/dl)	48±9	54±14	0.02
LDL Cholesterol (mg/dl)	126±31	96±16	0.001
Triglycerides (mg/dl)	111±48	81±29	0.001
Creatinine (mg/dl)	0.7±0.1	0.7±0.1	0.509
AST (UI/l)	20±9	19±5	0.510
ALT (UI/l)	24±19	20±9	0.511
GGT (UI/l)	28±21	14±8	0.01
Matsuda Index (ISIcomp)	2.4±0.5	8.2±1.9	0.004
Disposition Index (ISSI2)	1.1±0.2	2.9±1.1	0.02
Total GLP-1 (pmol/l)	56.6±18.6	32.1±9.9	0.001
Active GLP-1 (pmol/l)	23.9±9.1	18.2±1.1	0.01
GIP (pg/l)	25.6±6.9	30.5±8.1	0.02
VIP (ng/ml)	42.3±17.0	42.0±15.7	0.517
Leptin (ng/dl)	80.4±13.0	16.1±2.4	0.001
BDNF (ng/ml)	196.9±24.5	214.5±52	0.519

520 **Table 1**



Time (min)

