JNeurosci
THE JOURNAL OF NEUROSCIENCE

Research Articles: Systems/Circuits

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https://doi.org/10.1523/JNEUROSCI.3188-18.2019

Received: 11 December 2018

Revised: 5 March 2019

Accepted: 6 March 2019

Published: 13 March 2019

Author contributions: V.C. and H.-K.L. designed research; V.C. and B.D. performed research; V.C. analyzed data; V.C. and H.-K.L. wrote the first draft of the paper; V.C., P.F.W., and H.-K.L. edited the paper; V.C. and H.-K.L. wrote the paper; P.F.W. contributed unpublished reagents/analytic tools.

Conflict of Interest: The authors declare no competing financial interests.

This work was supported by NIH grant R01-EY014882 to H.-K.L. and R01-DA010309 to P.F.W.

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Cite as: J. Neurosci 2019; 10.1523/JNEUROSCI.3188-18.2019

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Abbreviated Title: H1a is required for ocular dominance
Number of pages: 24
Number of figures: 7
Number of tables: 0
Number of words for Abstract: 247; Introduction: 557; Discussion: 948
Conflict of Interest: None.

Acknowledgement: This work was supported by NIH grant R01-EY014882 to H.-K.L. and R01-DA010309 to P.F.W.

Abstract

It is well established across many species that neurons in the primary visual cortex (V1) display preference for visual input from one eye or the other, which is termed ocular dominance (OD). In rodents, V1 neurons exhibit a strong bias towards the contralateral eye. Molecular mechanisms of how OD is established and later maintained by plastic changes are largely unknown. Here we report a novel role of an activity-dependent immediate early gene Homer1a (H1a) in these processes. Using both sexes of H1a knockout (KO) mice, we found that there is basal reduction in the OD index of V1 neurons measured using intrinsic signal imaging. This was due to a reduction in the strength of inputs from the contralateral eye, which is normally dominant in mice. The abnormal basal OD index was not dependent on visual experience and is driven by postnatal expression of H1a. Despite this, H1a KOs still exhibited normal shifts in OD index following a short-term (2-3 days) monocular deprivation (MD) of the contralateral eye with lid suture. However, unlike wildtype counterparts, H1a KOs continued to shift OD index with a longer duration (5-6 days) of MD. The same phenotype was recapitulated in a mouse model that has reduced Homer1 binding to metabotropic glutamate receptor 5 (mGluR5). Our results suggest a novel role of H1a and its interaction with mGluR5 in strengthening contralateral eye inputs during postnatal development to establish normal contralateral bias in mouse V1 without much impact on OD shift with brief MD.

Significance Statement

Visual cortical neurons display varying degree of responsiveness to visual stimuli through each eye, which determines their ocular dominance (OD). Molecular mechanisms responsible for establishing normal OD are largely unknown. Development of OD has been

shown to be largely independent of visual experience, but guided by molecular cues and spontaneous activity. We found that activity-dependent immediate early gene H1a is critical for establishing normal OD in V1 of mice, which show contralateral eye dominance. Despite the weaker contralateral bias, H1aKOs undergo largely normal OD plasticity. The basic phenotype of H1aKO was recapitulated by mGluR5 mutation that severely reduces H1a interaction. Our results suggest a novel role of mGluR5-H1a interaction in strengthening contralateral eye inputs to V1 during postnatal development.

Introduction

In higher mammals, most neurons in V1 respond to inputs from both eyes, but show varying degrees of preference toward one or the other eye. While the initial setup of OD is known to depend largely on molecular cues and spontaneous activity (Rakic, 1976; Stryker and Harris, 1986; Horton and Hocking, 1996; Crowley and Katz, 2000; Espinosa and Stryker, 2012), alterations in visual experience during the early postnatal life can shift the OD of neurons. For instance, monocular deprivation during the critical period of development (3-5 weeks postnatally) induces a shift in OD towards the open eye. Studies have demonstrated that both Hebbian and homeostatic mechanisms underlie functional changes observed during various stages of OD plasticity (Espinosa and Stryker, 2012; Cooke and Bear, 2014). It is well established that OD plasticity occurs through two stages. MD first produces depression of the deprived eye response (2-3 days MD, 2-3dMD) through homosynaptic LTD mechanisms (Dudek and Bear, 1992; Kirkwood et al., 1993; Rittenhouse et al., 1999) followed by a delayed component (5-6 days MD, 5-6dMD), which resembles homeostatic mechanisms, leading to strengthening of open eye responses (Sawtell et al., 2003; Frenkel and Bear, 2004; Mrsic-Flogel et al., 2007; Ranson et al., 2012). There is strong evidence that weakening of the deprived eye response is due to NMDAR-dependent LTD (Rittenhouse et al., 1999; Heynen et al., 2003; Sawtell et al., 2003; Crozier et al., 2007), while there are a couple of alternatives proposed as the homeostatic plasticity mechanisms triggered during the later phase of OD plasticity (5-6dMD). One mechanism proposed is synaptic scaling, which induces global cell-wide changes, hence can explain the parallel potentiation of both eye responses during the second phase of OD plasticity (Kaneko et al., 2008; Ranson et al., 2012). A second mechanism proposed is NMDAR-dependent metaplasticity, where loss of inputs coming from the deprived eye reduces the synaptic

modification threshold to allow inputs from the open eye to potentiate following prolonged MD (Sawtell et al., 2003; Frenkel and Bear, 2004; Chen and Bear, 2007; Cho et al., 2009). The latter mechanism allows for input-specific changes and can explain potentiation of previously weaker inputs, as would happen when the dominant contralateral eye is sutured.

Homer1a (H1a) is an immediate early gene expressed with increase in neuronal activity (Brakeman et al., 1997; Hu et al., 2010), and its expression is increased in V1 upon light exposure following a period of dark adaptation (Brakeman et al., 1997). Hence H1a is well posed to respond to changes in visual experience. In addition, H1a is involved in homeostatic synaptic scaling observed in neuronal cultures upon chronic increase in activity (Hu et al., 2010) suggesting a role in homeostatic control of excitatory synaptic transmission. In this study, we aimed to study whether activity-dependent H1a expression is required for any of the processes mediating OD plasticity. Our initial hypothesis was that it would be involved in the homeostatic maintenance phase of OD with prolonged MD. Using optical imaging of intrinsic signals in V1, we report two novel mechanisms of H1a in OD plasticity: (1) H1a expression is required for the establishment of normal contralateral bias by strengthening contralateral eye inputs during postnatal development, which is independent of visual experience, and (2) H1a is not necessary for OD shift with short-term MD (2-3d) but is involved in the later phase of OD (5-6d MD).

Materials and methods

Animals

Male and female mice were reared in a 12hr light/12hr dark cycle. The knockout for H1a (H1aKO), knock-in on the F1128R amino acid in the C-terminus of mGluR5 (FRKI), and floxed-Homer1 (Homer1$^{fl/fl}$) line were obtained from Dr. Paul Worley (Johns Hopkins School of

105 Medicine, Baltimore). Wildtype strains for H1aKO and FRKI were generated by breeding
106 mutant mice with C57BL/6 (The Jackson Laboratory) mice, yielding H1aWT and FRWT,
107 respectively. To create a conditional knockout for H1a with the normal long form of Homer1, we
108 created a H1aKOflox line (H1b/c$^{fl/+}$;H1a$^{fl/-}$) by crossing Homer1$^{fl/fl}$ mice with H1aKO mice.
109 H1aWTflox mice (H1b/c$^{f/+}$;H1a$^{f/+}$) were generated by breeding Homer1$^{fl/fl}$ mice with H1aWT
110 mice. All the animals were handled according to protocols approved by the Institutional Animal
111 Care and Use Committee (IACUC) and guidelines provided by the Animal Care Act and
112 National Institutes of Health (NIH).
113
114 *Visual deprivation by dark rearing*
115 Pregnant females and new born pups were dark reared (DR) in a dark room and were
116 cared for with infrared vision goggles using dim infrared light. DR animals (Figure 3) were
117 anesthetized in the dark room and brought to the imaging setup in a light tight box to minimize
118 exposure to light.
119
120 *Monocular deprivation by eye lid suture*
121 Animals were monocularly deprived after each control imaging session by performing a
122 monocular lid suture. During the procedure, animals were maintained at a deep anesthetic level
123 using 1.5-2% isoflurane in oxygen (flow rate 1.0 l/min). Lid suturing was performed on the eye
124 contralateral to the imaged V1. The upper and lower lids were slightly trimmed and then sutured
125 together (Prolene P-6; Ethicon, Cat# 8648G). Animals recovered on a heating pad and then were
126 returned to the animal colony. Each animal was housed individually until the next imaging
127 session.

128

129 *Bolus injection in new born pups*

130 The home cage, containing pups and their mother, was brought to the injection setup.
131 Pups (P0-2) were anesthetized by placing them between a wet paper towel surrounded by ice for
132 5 mins (Phifer and Terry, 1986). Under a dissection scope, V1 was located and viral injection
133 was performed using an injection pump at 100 nl/s. The left hemisphere of each pup was injected
134 with virus (200 µl). Pups recovered at 37°C before being returned to their homecage. H1aKOflox
135 or H1aWTflox (Worley lab, Johns Hopkins School of Medicine) were injected in V1 with either
136 a Cre-expressing virus (AAV9.CamKII.HI.eGFP-Cre.WPRE.SV40) to knockout the floxed
137 Homer1 hemi-gene or with a control enhanced GFP virus (AAV9.CamKII0.4.eGFP.WPRE.rBG)
138 to provide a control group.

139

140 *Optical imaging of intrinsic signals*

141 Mice were brought to the imaging setup in a fresh cage and anesthetized with 3%
142 isoflurane in O_2 (flow rate: 1.0 l/min). The animals were head-fixed using a stereotaxic apparatus
143 (Kopf Instruments) and maintained with constant level of isoflurane (~0.75%) in O_2 (flow rate:
144 1.0 l/min) with single injection of chlorprothixene hydrochloride (Sigma, C1671, 2 mg/kg in
145 saline). Heart rate was monitored and recorded during the imaging session using an EKG
146 instrument (EKG, Harald Stauss Scientific). Temperature was maintained at 37°C with a heating
147 pad. The imaging method developed in the Stryker lab (Kalatsky and Stryker, 2003; Cang et al.,
148 2005) was used to measure the ocular dominance index in juvenile mice (P25-P35). A visual
149 stimulus consisting of a horizontal bar moving in a vertical direction (upward and downward)
150 was displayed in the binocular visual field (-5° to 15° azimuth) to elicit responses in the

151 binocular zone of V1. Vasculature was visualized by illuminating the surface of the brain with
152 555 nm light. Elicited responses were measured as changes in the reflectance of 610 nm light and
153 were imaged transcranially with a Dalsa CCD camera (Dalsa, Waterloo, Canada) through a
154 coverslip fixed with 3% agarose. For each eye, cortical activity elicited at the stimulus frequency
155 was calculated by Fourier analysis and presented as ΔR/R (reflectance of 610 nm light). The
156 ocular dominance index was calculated as an average of (Contra-Ipsi)/(Contra+Ipsi) from each
157 pixel in a region of interest (ROI), which was manually selected on the basis of raw signal from
158 ipsi eye responses with a threshold at 40% of the peak magnitude.
159
160 *Confirmation of viral transfection by immunohistochemistry*
161 Animals were deeply anesthetized with isoflurane after the final imaging session and
162 were then transcardially perfused with 10 ml of 0.1M PBS followed by 10 ml of 10% formalin in
163 0.1M PBS. Brains were then harvested and fixed overnight in 10% formalin. V1 was isolated and
164 40-µm thick slices were collected using a vibrotome (Ted Pella). Free floating sections were
165 rinsed with PBS and permeabilized with 2%Triton-X100 buffered in PBS solution. Following
166 another wash, the slices were incubated in blocking buffer (10% Normal donkey serum, 0.2%
167 Triton X-100, 4% Bovine Serum Albumin). Primary antibody treatment for NeuN (1:100 in
168 blocking buffer, Millipore, Cat# MAB377) was carried out for 2 days at 4°C. Afterwards, slices
169 were washed with PBS twice and treated with secondary antibody (Rabbit anti-mouse Alexa 633,
170 ThermoFisher Scientific). Slices were then washed, mounted on glass slides and dried at room
171 temperature for 20 mins. Cover slips were affixed using mounting media (Prolong gold antifade,
172 Invitrogen) and sealed with nail polish. Slices were later imaged for NeuN (633) and Cre (GFP)
173 expression with an LSM 510 confocal microscope.

174

175 *Experimental Design and Statistical Analysis*

176 Experiments were designed to determine either changes associated with experimental
177 manipulation (e.g. MD or DR) and/or genotype. All data are displayed as Mean ± S.E.M.
178 ANOVAs, 2-way ANOVAs, and unpaired t-tests were performed using Graphpad Prism, as
179 mentioned in each figure legend. Newman-Keuls multiple comparison *posthoc* test was used
180 following ANOVA to determine statistically significant difference between multiple groups as
181 noted in each figure legend. Raw data are available on request.

182

183 **Results**

184 *H1aKO exhibit abnormal contralateral bias and fails to maintain homeostatic OD plasticity*

185 The role of H1a in OD plasticity was assessed by imaging of intrinsic signals produced in
186 V1 by presentation of visual stimuli in the binocular visual field (-5° to 15° azimuth) to each eye
187 (Figure 1). Visually evoked intrinsic signals were analyzed using previously reported method to
188 calculate the ocular dominance index (ODI), which is basically a measure of response ratio
189 between visual stimuli to the contralateral eye compared to that of the ipsilateral eye (Kalatsky
190 and Stryker, 2003; Cang et al., 2005) (See methods) (Figure 1B). After a control imaging
191 session, the contralateral eye was sutured for MD during the postnatal sensitive age for OD
192 plasticity (P25 - P35, Figure 1A) (Gordon and Stryker, 1996), and were re-imaged either after 2-
193 3 days (2-3dMD) or 5-6 days (5-6dMD) to assess changes to OD in V1. Vasculature imaging
194 was done in parallel to confirm the imaging location (Figure 1C).

195 In wild-type control, there was a basal contralateral bias in V1 indicating dominance of
196 contralateral (contra) eye responses over ipsilateral (ipsi) eye (Figure 2A), which was due to a

197 greater response magnitude from the contralateral eye stimulation in comparison to that of the
198 ipsi eye stimulation (Figure 2B; Response magnitude in the order of 10^{-4} H1aWT Ctrl: Contra
199 =2.62 ± 0.10, Ipsi = 1.28 ± 0.05, Student's paired t-test $p < 10^{-13}$). Unexpectedly, H1aKO mice
200 had significantly lower basal ODI than the wild-type controls (Figure 2A). This decrease in
201 contralateral bias in H1aKO mice was due to a reduction in the contra eye responses (Figure 2B).
202 The ipsi eye responses were comparable to that of H1aWT (Figure 2B). These results suggest an
203 unexpected novel role of H1a in strengthening the contralateral eye inputs to V1.

204 To determine whether H1a is involved in ODP, we next performed varying durations of
205 MD by suturing the contralateral eye (Figure 3). In wildtype mice, 2-3dMD significantly
206 depressed deprived eye (contra) responses leading to a shift in ODI towards the open eye (ipsi)
207 (Figure 3A). As reported earlier (Frenkel and Bear, 2004; Sato and Stryker, 2008; Ranson et al.,
208 2012), this short duration MD (2-3dMD) did not produce significant changes in the open eye
209 responses (Figure 3B). However, a longer duration MD (5-6dMD) led to strengthening of the
210 open eye (ipsi) responses along with a slight increase in deprived eye responses (Figure 3B).
211 This led to a maintenance of ODI with longer MD to a similar level as that observed with shorter
212 MD (Figure 3A). Such maintenance of ODI with longer MD has been attributed to homeostatic
213 plasticity mechanisms triggered by prolonged deprivation of visually-driven activity (Ranson et
214 al., 2012).

215 Despite the reduced contralateral bias under normal conditions, upon 2-3dMD H1aKO
216 mice were able to produce normal depression of deprived-eye responses without affecting open
217 eye responses (Figure 3D) resulting in a normal shift of ODI towards the open eye (Figure 3C).
218 However, ODI further shifted towards the open eye (ipsi) with 5-6dMD (Figure 3C). We
219 observed normal open eye potentiation during this phase of MD in H1a KOs (Figure 3D), which

220 suggests that H1a is not involved in this process. Our results indicated that H1a is not necessary
221 for OD plasticity, but is required for the maintenance of ODI during longer periods of MD. In
222 addition, we found a novel role of H1a in setting up normal contralateral bias in rodent V1.
223
224 *H1a is necessary for postnatal establishment of contralateral bias*
225 Postnatal development of visual cortex involves refinement of connections that confers
226 the cells with ocular selectivity. This phase occurs before eye opening in mice (Rakic, 1976; Li
227 et al., 2008; Rochefort et al., 2011). However, visual experience after eye opening is necessary
228 for maturation of individual eye responses in mammals including mice (Hubel and Wiesel, 1963;
229 Wiesel and Hubel, 1974; Smith and Trachtenberg, 2007). Based on our observation of a lower
230 basal ODI in H1aKO, we examined whether this is due to lacking visual experience-dependent
231 changes. To do this, we examined whether normal visual experience is needed to establish ODI
232 in H1aWT and H1aKO mice by dark rearing them from birth (Figure 4A). ODI value in both
233 H1aWT and H1aKO mice after dark rearing were comparable to their respective age-matched
234 normal reared groups (Figure 4B,D), suggesting that visual experience is not necessary to
235 establish normal ODI values in either genotype. However, responses from individual eyes were
236 significantly weaker as compared to normal reared animals in both genotypes (Figure 4C,E). Our
237 results suggest that basal ODI measured in critical period mice is setup independent of visual
238 experience. Based on this, we conclude that the lower basal ODI values seen in H1aKO are not
239 due to abnormal responses to visual experience during development but are due to a novel role of
240 H1a in establishing contralateral eye input dominance in a visual experience-independent
241 manner.

242 H1aKO is a constitutive KO; hence we cannot rule out abnormalities in prenatal
243 development that could have contributed to lower basal ODI. Prenatal development of visual
244 cortex in mice involve genetic mechanisms that lay the chemo-architecture to drive correct
245 connections from the eye through thalamus (O'Leary et al., 2007; Kanold and Luhmann, 2010).
246 To determine if H1a knockout during this phase of development was affecting the establishment
247 of ODI, a conditional knockout was required. However, H1a is a splice variant of a constitutive
248 form of Homer1 (Homer1b/c; H1b/c) and exists on the same gene. Hence generation of Cre-
249 inducible conditional H1a knockout mice (H1aKOflox, H1b/c$^{fl/+}$;H1a$^{fl/-}$) was performed by cross-
250 breeding floxed-Homer1 mice (H1b/c$^{fl/fl}$;H1a$^{fl/fl}$) with H1aKO mice (Figure 5B). This allows
251 temporal control for knockout of Homer1 variants postnatally. Cre was expressed postnatally by
252 injection of CaMKII promoter driven Cre adeno-associated virus system
253 (AAV9.CamKII.Cre.GFP) in new born pups (P0-1) (Figure 5A). This method produced
254 widespread expression of Cre in the postnatal brain as visualized by the GFP tag (Figure 5C,D).
255 In this system, Cre expressing neurons are conditional knockouts of H1a (H1b/c$^{+/-}$;H1a$^{-/-}$). To
256 control for viral injections, control mice were injected with only GFP-expressing virus
257 (AAV9.CamKII.eGFP) driven by CaMKII promoter to generate H1b/c$^{+/+}$;H1a$^{+/-}$ neurons. Under
258 this scheme, Cre injected conditional KO of H1a is also heterozygous for H1b/c (H1b/c$^{+/-}$;H1a$^{-/-}$),
259 but the GFP only group is wildtype for H1b/c but heterozygous of H1a (H1b/c$^{+/+}$;H1a$^{+/-}$).
260 Therefore, to control for the differences in H1b/c and H1a gene dosage, we also generated a set
261 of control animals by cross-breeding floxed-Homer1 mice (H1b/c$^{fl/fl}$;H1a$^{fl/fl}$) with H1aWT,
262 which produced H1aWTflox mice (H1b/c$^{fl/+}$;H1a$^{fl/+}$). Without any viral injections these mice are
263 wildtype for both H1b/c and H1a (H1b/c$^{+/+}$; H1a$^{+/+}$), and with Cre-GFP injection they produce
264 neurons which are heterozygous for both H1b/c and H1a (H1b/c$^{+/-}$;H1a$^{+/-}$). As summarized in

265 Figure 6, by comparing the 4 groups (H1b/c$^{+/+}$;H1a$^{+/+}$, H1b/c$^{+/+}$;H1a$^{+/-}$, H1b/c$^{+/-}$;H1a$^{+/-}$, and
266 H1b/c$^{+/-}$;H1a$^{-/-}$) we were able to compare dose-dependent effects of H1b/c and H1a expression.
267 ODI measurement was done during the critical period of OD plasticity (P28-P35, Figure 6). All
268 the animals were confirmed for expression of virus after the imaging session (Figure 5C,D). The
269 transfection efficiency in groups receiving Cre-GFP virus (H1b/c$^{+/-}$;H1a$^{+/-}$ and H1b/c$^{+/-}$;H1a$^{-/-}$)
270 was about 90%.

271 Conditional H1a knockout neurons (H1b/c$^{+/-}$;H1a$^{-/-}$) had significantly lower contralateral
272 bias than the Homer1 wild-type group (H1b/c$^{+/+}$;H1a$^{+/+}$) (Figure 6A). However, heterozygous
273 H1a controls (H1b/c$^{+/+}$;H1a$^{+/-}$ and H1b/c$^{+/-}$;H1a$^{+/-}$) were not significantly different than either of
274 the groups, which suggests that there might be H1a gene dose-dependent regulation of ODI
275 during postnatal development. While there was a trend of a decrease in contralateral eye
276 responses and a slight increase in ipsilateral eye responses in the H1b/c$^{+/-}$;H1a$^{-/-}$ group, this did
277 not reach statistical significance when compared with other groups (Figure 6B). These
278 observations support the premise that H1a expression during postnatal development is required
279 to establish normal contralateral bias in V1.
280

281 *Homer1 interaction with mGluR5 is required for establishing contralateral bias and OD*
282 *maintenance*

283 H1a acts by displacing long-forms of Homer1 from their binding partners, one of which
284 is mGluR5. To determine if the function of H1a in establishing basal ODI and maintaining OD
285 shift is due to its interaction with mGluR5, OD plasticity was assessed in transgenic mice with
286 mutations on mGluR5 that severely reduces Homer1 binding (F1128R mutation; FRKI) (Park et
287 al., 2013). Similar to H1aKOs, FRKI mice had significantly lower contralateral bias than FRWT

288 mice under basal control conditions (Figure 7A,C; ODI – FRWT: Ctrl = 0.32 ± 0.03, FRKI: Ctrl
289 = 0.21 ± 0.03, unpaired t-test $p < 0.05$). Furthermore, long term MD (5-6dMD) produced
290 significantly greater decrease in ODI than the wild-type animals similar to what we observed in
291 H1aKO (2-way ANOVA for ODI between FRWT versus FRKI shows statistically significant
292 interaction *p = 0.0471, $F(1,12) = 4.892$; Figure 7). Our results suggest that mGluR5 interaction
293 with Homer1 is required for establishing basal ODI and for maintenance of OD during longer
294 durations of MD (5-6dMD). While we cannot exclude the possibility that FRKI phenotype is due
295 to reduced binding of mGluR5 to long-forms of Homer1, our observation that FRKI phenocopies
296 H1aKO can be explained most parsimoniously by the loss of mGluR5-H1a interaction.
297 Furthermore, long-forms of Homer1 are expressed in greater abundance compared to its splice
298 variant H1a, hence reduced affinity of mGluR5 to these two splice variants is expected to more
299 severely affect the formation of mGluR5-H1a complexes.
300

301 **Discussion**

302 The main finding of this study is that H1a and its interaction with mGluR5 are required
303 for establishing the normal contralateral bias in V1 and maintenance of ocular dominance
304 following longer periods of MD. Even with the lower basal OD index, H1aKO displayed OD
305 shifts with short-term MD, which suggests that the initial phase of MD, which is mainly driven
306 by weakening of the closed eye inputs, is not dependent on H1a expression. Moreover, we found
307 evidence that H1a and mGluR5 interaction is critical for establishing the basal ODI and for
308 preventing a further decrease in ODI during longer term MD.
309

310 *H1a is not necessary for OD plasticity with short-term MD, but needed for preventing a further*
311 *shift in ODI with long-term MD*

312 Short-term MD (2-3d MD) predominantly drives weakening of the closed eye inputs via
313 NMDAR-dependent LTD mechanisms (Rittenhouse et al., 1999; Heynen et al., 2003; Sawtell et
314 al., 2003; Crozier et al., 2007). Longer duration MD (5-6d MD) has a delayed additional
315 component that is thought to be produce by homeostatic plasticity that involves potentiation of
316 open eye inputs (Sawtell et al., 2003; Frenkel and Bear, 2004; Chen and Bear, 2007; Kaneko et
317 al., 2008; Cho et al., 2009; Ranson et al., 2012). Recently it was reported that global synaptic
318 scaling mechanisms through glial-derived tumor necrosis factor (TNFα) (Kaneko et al., 2008)
319 are required for this later phase of ODP. TNFα has been shown to be specifically involved in
320 homeostatic plasticity and is not necessary for Hebbian forms of plasticity (Beattie, 2002;
321 Kaneko et al., 2008; Steinmetz and Turrigiano, 2010; Pribiag and Stellwagen, 2014). Kaneko and
322 colleagues observed blockade of open eye potentiation in TNFα knockout mice. However, their
323 contralateral bias was maintained during the later phase of MD.

324 We found that H1a KOs, despite having basally low contralateral bias, undergo normal
325 OD shift to favor the open eye inputs with short-term MD (2-3d), which was mediated by
326 depression of the deprived contralateral eye inputs (Figure 3). This suggests that H1a is not
327 critical for mediating this process, and that the lower contralateral eye responses under basal
328 conditions does not preclude further weakening. This finding is consistent with the idea that
329 activity-dependent synaptic weakening process does not require H1a, and supports the idea that
330 NMDA receptor dependent LTD-like processes are involved (Rittenhouse et al., 1999; Heynen et
331 al., 2003). However, we found that H1a KOs display a further reduction in ODI upon longer
332 duration of MD (5-6d) (Figure 3). In wildtypes, ODI does not shift further due to a delayed

333 potentiation of both the deprived eye and the open eye inputs (Figure 3). The delayed open eye
334 potentiation was still intact in H1a KOs suggesting that H1a is not involved in this process. The
335 larger reduction in OD index seen with longer term MD (5-6d) was recapitulated in mGluR5
336 knockin mice that have severely reduced affinity to Homer1 (FRKI) (Figure 7). This suggests
337 that H1a interaction with mGluR5 plays a role in preventing a further decline in ODI during the
338 later phase of MD.
339
340 *Development of ocular dominance*
341 Development of OD of V1 neurons happens before eye opening in mice and kittens.
342 Establishment of OD is dependent on retinal spontaneous activity rather than visual experience
343 (Hubel and Wiesel, 1963; Stryker and Harris, 1986; Crair et al., 1998). Blockade of spontaneous
344 retinal activity after eye opening prevents the normal developmental pruning of thalamocortical
345 arbors in V1 layer 4 (Antonini and Stryker, 1993). In addition, correlated spontaneous activity in
346 the visual thalamus (LGNd) is sufficient to produce ocular dominance columns in ferret V1
347 (Weliky and Katz, 1999; Chiu and Weliky, 2001). Consistent with these studies, we found that
348 normal contralateral bias is established even when mice are dark reared from birth with no visual
349 experience (Figure 4B). However, molecular mechanisms underlying the normal establishment
350 of OD are not well understood. We found that establishment of contralateral bias is severely
351 impacted in H1aKO mice, which persisted even when H1aKO are raised in the dark from birth
352 (Figure 4D). This was due to abnormally weak contralateral responses in V1. Ipsilateral eye
353 responses were similar to what is observed in WTs. Furthermore, we demonstrated that postnatal
354 expression of H1a is important for this contralateral bias, because inducing conditional knockout
355 of H1a postnatally recapitulated the lower ODI (Figure 6A). This suggests that spontaneous

activity in the absence of vision, such as retinal waves or spontaneous LGN and/or cortical activity, may be sufficient to trigger H1a activation to support the development of contralateral bias in V1. The exact mechanism in which H1a regulates the strength of contralateral eye inputs will require further studies, but we demonstrated that its interaction with mGluR5 is critical for this process using mice carrying mutations on the mGluR5 that lack H1a binding (FRKI) (Figure 7). While the role of H1a in homeostatic scaling down of synaptic strength has been shown in neuronal cultures (Hu et al., 2010), its role in strengthening inputs has not been reported. Furthermore, our results would suggest that H1a rather selectively works on contralateral eye inputs to establish the normal contralateral bias. The exact mechanism as to how H1a can achieve input specific control of contralateral eye inputs would require further investigation. On the surface, our results differ from a proposed role of H1a in global homeostatic downregulation of synaptic strength as reported in cultured neurons (Hu et al., 2010), which may be due to differences in preparation. One potential explanation that could reconcile this apparent contradiction is that mGluR5-H1a interaction enables plasticity, but does not determine the polarity of synaptic plasticity, and whether it results in global or input-specific plasticity may be determined by the nature of neural activity received via distinct inputs.

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460

461

462 **Figure Legend**

463 **Figure 1. Experimental design and technique used for ODP measurement**

464 (A) Experimental paradigm for ODI measurement. P: postnatal day, Ctrl: Control, MD:
465 monocular deprivation.

466 (B) Left: Schematic for imaging of intrinsic signals. Right: Top, Ocular dominance index
467 calculation. Bottom, Example azimuth maps for stimulation in the binocular visual field.
468 Contra: Contralateral eye, Ipsi: Ipsilateral eye.

469 (C) Example figure for repetitive imaging before and after MD. Scale bar: 1 mm.

470

471 **Figure 2. H1aKO mice display reduced contralateral bias**

472 Data shown as mean ± S.E.M. Response magnitude is of the order $x10^{-4}$. ODI: Ocular dominance
473 index, Contra: Contralateral eye, Ipsi: Ipsilateral eye. Statistics: Un-paired t-test. *$p<0.05$,
474 **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

475 (A) Left: Comparision of ODI measured from normal reared H1aWT and H1aKO. Average ODI
476 is shown as bars (mean ± S.E.M: ODI of H1aWT = 0.34 ± 0.02, ODI of H1aKO = 0.14 ±
477 0.02, unpaired t-test $p < 0.0001$, $t = 7.268$, $dF = 45$). ODI value from individual mice are
478 plotted as gray circles. Number of animals, n: H1aWT = 22, H1aKO = 25. Right: Example
479 intrinsic signal images from H1aWT and H1aKO. Scale bar: 1 mm.

480 (B) Comparison of response magnitude of intrinsic optical signals obtained by stimulating the
481 contralateral eye (left) and ipsilateral eye (right) of normal reared mice. H1aKO showed a
482 significantly lower contralateral eye responses compared to H1aWT (Contra: H1aWT = 2.62
483 ± 0.02; H1aKO = 1.65 ± 0.09; unpaired t-test $p < 0.0001$, $t = 7.123$, $dF = 45$). There was no
484 significant difference in ipsilateral eye responses between the two genotypes (Ipsi: H1aWT =

485 1.28 ± 0.05, H1aKO = 1.18 ± 0.06; unpaired t-test p > 0.25, ns – not statistically significant, ,
486 t = 1.134, dF = 45). Number of animals, n: the same as in (A).
487
488 **Figure 3. H1aKO mice show normal ODP with short-term MD, but fail to maintain ODI**
489 **with longer term MD**
490 Data shown as mean ± S.E.M. Response magnitude is of the order $x10^{-4}$. ODI: Ocular dominance
491 index, Contra: Contralateral eye, Ipsi: Ipsilateral eye. Statistics: ANOVA with Newman-Keuls
492 multiple comparison test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
493 (A) Left: Average ODI data measure in H1aWT with 2-3 days MD (2-3dMD) and 5-6 days MD
494 (5-6dMD). ODI: Ctrl = 0.34 ± 0.02, 2-3dMD = 0.22 ± 0.03. 5-6dMD ODI = 0.19 ± 0.04,
495 ANOVA, F(2,41) = 9.401, p = 0.0004; Newman-Keuls multiple comparison test.
496 Right: Example signals for each eye stimulation for corresponding MD and Ctrl groups.
497 Scale Bar: 1 mm. Number of animals, n: Ctrl = 22, 2-3dMD = 11, 5-6dMD = 11.
498 (B) Contralateral and ipsilateral eye response magnitude measured in H1aWT mice during MD
499 paradigms. Contra: Ctrl = 2.62 ± 0.10, 2-3dMD = 2.14 ± 0.17, 5-6dMD = 2.48 ± 0.12,
500 ANOVA, F(2,41) = 3.498, p = 0.0396; Newman-Keuls multiple comparison test.; Ipsi: Ctrl =
501 1.28 ± 0.05, 2-3dMD= 1.30 ± 0.12, 5-6dMD = 1.65 ± 0.12, ANOVA, F(2,41) = 5.194, p =
502 0.0098; Newman-Keuls multiple comparison test.. n: same as in (A).
503 (C) Left: Average ODI data measure in H1aKO with 2-3dMD and 5-6dMD. Right: Example
504 signals for each eye stimulation for corresponding MD and Ctrl groups. ODI: Ctrl = 0.14 ±
505 0.02, 2-3dMD = 0.01 ± 0.03, 5-6dMD = -0.08 ± 0.04, ANOVA, F(2,47) = 18.200, p <
506 0.0001; Newman-Keuls multiple comparison test. Right: Example signals for each eye

stimulation for corresponding MD and Ctrl groups. Scale Bar: 1 mm. Number of animals, n: Ctrl = 25, 2-3dMD = 16, 5-6dMD = 9.

(D) Contralateral and ipsilateral eye response magnitude in H1aKO mice. Contra: Ctrl = 1.65 ± 0.09, 2-3dMD = 1.28 ± 0.11, 5-6dMD = 1.49 ± 0.10, ANOVA, $F(2,47) = 3.479$, $p = 0.0390$; Newman-Keuls multiple comparison test. Ipsi: Ctrl = 1.18 ± 0.06, 2-3dMD = 1.21 ± 0.09, 5-6dMD = 1.65 ± 0.12, ANOVA, $F(2,47) = 7.241$, $p = 0.0018$; Newman-Keuls multiple comparison test. n: same as in (C).

Figure 4. Developmental establishment of contralateral bias is not visual experience-dependent in both H1aWT and H1aKO

Data shown as mean ± S.E.M. Response magnitude is of the order $x10^{-4}$. Statistics: Unpaired two-tailed Student's t-test. #p=0.06, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, #p=0.06.

(A) Left: Average ODI values measured in H1aWT, which were normal reared (NR) or dark reared from birth (DR). H1aWT NR = 0.34 ± 0.02, H1aWT DR = 0.34 ± 0.05, unpaired t-test $p = 0.9430$, $t = 0.0721$, $dF = 27$. Right: Example intrinsic optical signals seen in V1 for each eye stimulation. Scale Bar: 1 mm. Number of animals, n: NR = 22 DR = 7.

(B) Contralateral and ipsilateral raw response magnitude comparison between DR and NR in H1aWT mice. Contra: H1aWT NR = 2.62 ± 0.10, H1aWT DR = 2.12 ± 0.12, unpaired t-test $p = 0.0137$, $t = 2.638$, $dF = 27$; Ipsi: H1aWT NR = 1.28 ± 0.05, H1aWT DR = 1.05 ± 0.13, unpaired t-test $p = 0.0614$, $t = 1.952$, $dF = 27$. Number of animals, n: Same as (A).

(C) Left: Average ODI data measure in H1aKO in DR and NR mice. H1aKO NR = 0.14 ± 0.02, H1aKO DR = 0.19 ± 0.05, unpaired t-test $p = 0.3262$, $t = 0.9982$, $dF = 30$. Right: Example signals for each eye stimulation. Scale bar: 1mm. Number of animals, n: NR = 25 DR = 7.

530 (D) Contralateral and ipsilateral raw response magnitude comparison between DR and NR in
531 Contra: H1aKO NR = 1.65 ± 0.10, H1a KO DR = 1.27 ± 0.06, unpaired t-test p = 0.0469, t =
532 2.072, dF = 30; Ipsi: H1aKO NR = 1.18 ± 0.06, H1aKO DR = 0.88 ± 0.08, unpaired t-test p =
533 0.0199, t = 2.460, dF = 30. Number of animals, n: Same as (C).
534

535 **Figure 5. Generation of H1a conditional KO neurons in V1**
536 (A) Experimental design for bolus injection of AAV virus at birth and ODI measurement during
537 critical age for ODP.
538 (B) Genetic design to produce H1a conditional knockout neurons in cortex. Inj: Injection. For
539 virus information see methods.
540 (C) Example slices showing transfection levels of virus in V1 L2/3 in all the three conditions.
541 NeuN (Red): Neuronal marker, GFP (Green): marker for viral infection.
542 (D) Quantification for viral transfection efficiency as percentage of GFP expressing cells per
543 NeuN expressing neurons.
544

545 **Figure 6. Postnatal H1a expression is required for establishing normal contralateral bias**
546 Data shown as mean ± S.E.M. Response magnitude is of the order $x10^{-4}$. Number of animals, n,
547 is noted on the bar of each group. Statistics: ANOVA with Newman-Keuls multiple comparison
548 test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
549 (A) Left: Average basal ODI data measured in normal reared mice with conditional KO of H1a.
550 ODI: $H1b/c^{+/+};H1a^{+/+}$ = 0.25 ± 0.02, $H1b/c^{+/+};H1a^{+/-}$ = 0.18 ± 0.03, $H1b/c^{+/-};H1a^{+/-}$ = 0.20 ±
551 0.02, $H1b/c^{+/-};H1a^{-/-}$ = 0.17 ± 0.02, ANOVA, F(3,44) = 3.084, p < 0.0369; Newman-Keuls

552 multiple comparison test. Right: Example intrinsic optical signals for each eye stimulation.
553 Scale bar: 1 mm. Number of animals noted at the base of the bar for each condition.
554 (B) Contralateral and ipsilateral eye response magnitude for each condition. Contra:
555 H1b/c$^{+/+}$;H1a$^{+/+}$ = 2.29 ± 0.13, H1b/c$^{+/+}$;H1a$^{+/-}$ = 2.10 ± 0.08, H1b/c$^{+/-}$;H1a$^{+/-}$ = 2.22 ± 0.22,
556 H1b/c$^{+/-}$;H1a$^{-/-}$ = 1.98 ± 0.07, ANOVA, F(3,44) = 0.966, p = 0.4173; Newman-Keuls
557 multiple comparison test; Ipsi: H1b/c$^{+/+}$;H1a$^{+/+}$ = 1.35 ± 0.07, H1b/c$^{+/+}$;H1a$^{+/-}$ = 1.48 ± 0.08,
558 H1b/c$^{+/-}$;H1a$^{+/-}$ = 1.45 ± 0.17, H1b/c$^{+/-}$;H1a$^{-/-}$ = 1.38 ± 0.06, ANOVA, F(3,44) = 0.272, p =
559 0.8456; Newman-Keuls multiple comparison test. Number of animals n: same as (A).
560

561 **Figure 7. mGluR5-H1a binding is required for establishing normal ODI and sustaining**
562 **ODI with longer duration MD.**
563 Data shown as mean ± S.E.M. Response magnitude is of the order x10^{-4}. N, number of animals:
564 FRWT Ctrl,5-6dMD = 8. FRKI Ctrl,5-6dMD = 6. Statistics: paired two-tailed Student's t-test.
565 #p=0.0650 *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
566 (A) Left: Average ODI measurement from control FRWT and FRWT with 5-6 days MD (5-
567 6dMD). FRWT Ctrl = 0.32 ± 0.02, FRWT 5-6dMD = 0.15 ± 0.03, paired t-test p = 0.0009, t
568 = 5.549, dF = 7. Right: Example signals for each eye stimulation for 5-6dMD and Ctrl
569 groups. Scale Bar – 1mm.
570 (B) Contralateral and ipsilateral raw response magnitude with 5-6dMD measured in FRWT mice.
571 FRWT Contra: Ctrl = 2.38 ± 0.30, 5-6dMD = 1.86 ± 0.17, paired t-test p = 0.0650, t = 2.186,
572 dF = 7; FRWT Ipsi: Ctrl = 1.20 ± 0.16, 5-6dMD = 1.32 ± 0.12, paired t-test p = 0.1664, t =
573 1.544, dF = 7.

(C) Left: Average ODI data measure in FRKI with 5-6dMD. FRKI Ctrl = 0.21 ± 0.03, FRKI 5-6dMD = -0.12 ± 0.07, paired t-test p = 0.0061, t = 4.550, dF = 5. Right: Example signals for each eye stimulation for corresponding 5-6dMD and Ctrl groups. Scale bar: 1 mm.

(D) Contralateral and ipsilateral raw response magnitude with 5-6dMD in FRKI mice. FRKI Contra: Ctrl = 2.04 ± 0.19, 5-6dMD = 1.34 ± 0.24, paired t-test p = 0.0275, t = 3.078, dF = 5; FRKI Ipsi: Ctrl = 1.29 ± 0.18, 5-6dMD = 1.62 ± 0.23, paired t-test p = 0.1422, t = 1.741, dF = 5.