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1 **Imaging and manipulating pituitary function in the awake mouse**

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20

21 **Running title:** Pituitary initiative

22

23 **Key words:** In vivo imaging, optogenetic tools, viral infection, endocrine manipulation, hormone
24 rhythms

25

26 Extensive efforts have been made to explore how the activities of multiple brain cells combine to
27 alter physiology through imaging and cell-specific manipulation in different animal models.
28 However, the temporal regulation of peripheral organs by the neuroendocrine factors released by
29 the brain is poorly understood. We have established a suite of adaptable methodologies to
30 interrogate *in vivo* the relationship of hypothalamic regulation with the secretory output of the
31 pituitary gland, which has complex functional networks of multiple cell types intermingled with
32 the vasculature. These allow imaging and optogenetic manipulation of cell activities in the
33 pituitary gland in awake mouse models, in which both neuronal regulatory activity and hormonal
34 output are preserved. This methodology is now readily applicable for longitudinal studies of short-
35 lived events (e.g. calcium signals controlling hormone exocytosis) and slowly-evolving processes
36 such as tissue remodelling in health and disease over a period of days to weeks.

37 **Introduction**

38 In the past decade, there has been an exponential increase in the technical development of novel tools
39 allowing interrogation of the functional interactions of the complex architecture of the mammalian brain
40 in health and disease. These have principally been developed in mouse models, where both organisation
41 and function of the brain largely recapitulates that of higher mammals including humans (1). The
42 availability of a wide-range of genetically-modified mice, combined with novel virus-based approaches
43 to infect specific mouse brain regions, has allowed identification of specific cell-types, manipulation of
44 neuronal circuits with optogenetic techniques and *in vivo* monitoring of cell activity. Combining these
45 with recently developed optical techniques, such as the use of a gradient-index (GRIN) lens for imaging
46 deep brain regions (2), has resulted in rapid mapping of the activity and connectivity of neuronal
47 networks (3). Although the mammalian brain is exceptionally complex, the increasing prevalence of
48 neurological and neuropsychiatric defects has recently inspired large-scale research programmes, such
49 as the NIH Brain Research through Advancing Innovative Neuro-technologies (BRAIN) Initiative (4,
50 5), to meet this challenge.

51 The brain does not simply work as an isolated unit but forms a functional continuum with other
52 physiological processes (6), especially with the endocrine systems that control basic body functions (7,
53 8). These endocrine systems share complex functional features with the brain, such as hierarchal multi-
54 cellular organization (e.g. presence of “hub” cells which control neighbours (9, 10)), adaptive plasticity
55 (11) and long-term memory (9), suggesting that studies of their function would benefit from application
56 of the novel tools and techniques developed for neuroscience. This is exemplified by the pituitary gland,
57 which acts as an intermediate between the brain and periphery, with endocrine and neural lobes (nerve
58 terminals emanating from hypothalamic vasopressin and oxytocin neurons) connected to the brain by
59 the pituitary stalk and surrounded by brain meninges (see Fig. 1). Interest in monitoring the *in vivo*
60 function of this gland has recently been increased by large-scale *ex vivo* imaging, which has revealed
61 3D cell networks that are structurally and functionally organised within the endocrine anterior pituitary
62 (also called the pars distalis); this cell network connectivity is essential for normal gland development
63 (12), coordination of gene expression (13) and pulsatile release of hormones to the periphery (8). To

64 date, *in vivo* studies have been limited by the location of the pituitary on the ventral side of the brain,
65 with extensive microsurgery required to expose the gland through the palate bone in terminally-
66 anaesthetised mice to record and manipulate cell function (14). These surgical procedures preclude both
67 longitudinal studies and functional investigation in awake mice.

68 Here, we describe a toolkit for imaging and manipulating pituitary cells *in vivo* over periods of days to
69 weeks in awake mouse models. We have used these tools to: image the dynamics of pituitary
70 microvascular function and cell signalling (calcium events); locally express exogenous proteins through
71 injection of viral constructs within the parenchyma; and, optogenetically manipulate specific cell
72 networks while monitoring their secretory outputs into the bloodstream. This range of techniques allows
73 analysis of the pituitary gland in awake mammalian models in unparalleled detail, complementing large-
74 scale studies of the brain to further understand neural control of complex physiological systems via
75 endocrine signals.

76 **Materials and Methods**

77 **Animals**

78
79 Tg(Gh1-cre)^{bKnmn} (called GH-Cre) (R Kineman, Jesse Brown Veterans Administration Medical
80 Center, Chicago, USA) (15), ROSA26-*fl/fl*-ChR2-dtTomato and wild-type C57BL/6 mice (6–12 wk
81 old) as indicated in figure legends, were housed in a 12-h light/12-h dark cycle (lights on at 0800 hours
82 and off at 2000 hours) with food and water available *ad libitum*. All animal procedures were approved
83 by the local ethical committee under agreement CEEA-LR-12185 according to EU Directive
84 2010/63/EU. Since this study included only one experimental group of animals, no randomization or
85 blinding were required.

87 **Stereotaxic injections of AAV**

88 Adult GH-Cre mice and wild-type C57BL/6 were anesthetized with Ketamine/Xylazine (0.1/0.02 mg/g),
89 placed in a stereotaxic apparatus, and given bilateral 1 μ L injections of AAV5-CAG-dflox-GCaMP6s-
90 WPRE-SV40 (2.52×10^{13} GC/mL; Penn Vector Core), AAV5-CAG-GCaMP6s-WPRE-SV40 (2.23

91 $\times 10^{13}$ GC/mL; Penn Vector Core), AAV2-CAG-GFP (gift from Margarita Arango, IGF, Montpellier),
92 rAAV5/sspEMBOL-CBA-GFP (8×10^{12} GC/mL; UNC Vector Core), rAAV8/sspEMBOL-CAG-
93 GFP (8×10^{12} GC/mL; UNC Vector Core) or rAAV9/sspEMBOL-CAG-GFP (9.2×10^{12} GC/mL;
94 UNC Vector Core) into the pituitary gland at a rate of 100 nL/min. Coordinates were -2.5mm antero-
95 posterior, ± 0.4 mm lateral to midline, pointed as zero at the superior sagittal sinus. Two dorso-ventral
96 positions were used for injection, 50 μ m and 400 μ m over the sella turca -6.15/5.75 mm for ventral
97 injection and -5.6/5.3 mm for dorsal injection. Experiments were conducted from 4 weeks on after
98 injection.

99

100 **Optical imaging through a GRIN lens in awake head-fixed mice**

101 Adult mice were anesthetized with Ketamine/Xylazine (0.1/0.02 mg/g) and placed in a stereotaxic
102 apparatus to implant a GRIN lens (0.6 mm diameter, 1.5 pitch, 7.5mm length and 150 μ m working
103 distance, GRINTECH Germany) immediately above the pituitary gland. After a large part of skull was
104 exposed, the GRIN lens was placed in 20G1/2 Gauge needle (Ultra-Thin wall, Terumo, USA), with
105 movement restricted by placing a metal rod above it. The needle was inserted at the coordinates -2.5mm
106 antero-posterior, ± 0.4 mm lateral to midline pointed as zero at the superior sagittal sinus
107 -5.5/5.1 mm dorso-ventral. Then, the needle was removed with the metal rod kept in place so that the
108 GRIN lens stayed in place at the dorsal side of the pituitary. Finally, the metal rod was removed. The
109 GRIN lens and a head-plate were fixed with UV-retractable cement. Prior to and starting from two
110 weeks after surgery, mice were habituated to the wheel and the head-plate fixation system under the
111 microscope every two to three days. Four weeks after surgery, mice were placed on the wheel, the head-
112 plate fixed, and fluorescence imaging was performed using a stereomicroscope (Zeiss Discovery V.12,
113 Germany), which was fitted with a fluorescence lamp (Lambda LS, Sutter Instrument company, USA),
114 a shutter (Lambda 10-B Smart Shutter, Sutter Instrument Company) and a CMOS ORCA Flash 4.0
115 camera (C11440 Hamamatsu, Japan), all controlled with MetaMorph 7.8.9 software (Molecular
116 Devices, USA).

117

118 **In vivo imaging in terminally-anesthetized mice**

119 Details of the methods can be found in Lafont et al. (2010) (14). In brief, male, 2- to 4-month-old
120 transgenic GH-Cre mice and GH-ROSA26-*fl/fl*-ChR2-dtTomato mice on a C57Bl6 background were
121 anesthetized by inhalation of isoflurane (1.5% in O₂). After dividing the mandibular symphysis, the
122 mucosa overlying the hard palate was parted by blunt dissection under a stereomicroscope to expose an
123 area of palatal periosteal bone. This was thinned with a felt polisher (drill; World Precision Instruments,
124 USA) and then removed with a hook and forceps. The exposed surface of the pituitary gland, visible
125 through the hole in the bone, was continuously superfused with a physiological solution.

126

127 **In vivo monitoring of blood flow and calcium signals**

128 Mice underwent surgery (see above) to visualize either the ventral (terminally-anesthetized animals) or
129 the dorsal side (awake animals) of the pituitary gland. Using the ventral approach 100µl of
130 tetramethylrhodamine isocyanate 150kDa dextran (Sigma Aldrich, USA) was injected into the jugular
131 vein or in the retro-orbital sinus for GRIN lens approach. Imaging of blood flow was performed at 150
132 to 200 frames/sec using 545nm excitation and 570nm emission filters. When calcium signals were
133 recorded *in vivo*, experiments were performed as described as above four weeks after stereotaxic
134 injection of GCAMP6s-expressing AAV5. Multi-cellular calcium imaging was typically performed at
135 2-4 frames/sec, using 480nm excitation and 520nm emission filters.

136

137 **Optogenetic photostimulation in awake mice**

138 GH Cre x ROSA26-*fl/fl*-ChR2-dtTomato mice were anesthetized with Ketamine/Xylazine (0.1/0.02
139 mg/g) and placed in a stereotaxic apparatus to implant an optical fiber (diameter: 200µm, Doric Lenses,
140 Canada) immediately above the pituitary gland (stereotaxic coordinates described above). The optical
141 fiber was fixed using UV-retractable cement. Two weeks later, an optical fiber was connected to the one
142 previously implanted, and laser stimulation (488nm) was delivered at 10mW and using various patterns
143 (frequency: 1Hz, exposure time: 300ms) while blood samples were collected as described below.

144

145 **GH pulse profiling in mice and GH ELISA**

146 A tail-tip blood collection procedure was used to sample blood from C57BL/6 adult mice or transgenic
147 GH-Cre mice; 3µl blood samples were analyzed for GH content by ELISA (16).

148

149 **iDISCO+**

150 Pituitary glands were removed and fixed by overnight immersion in 4% paraformaldehyde. For the
151 immunofluorescence labelling and clearing, an iDISCO+ clearing protocol was used as described in detail
152 elsewhere(17). Primary antibodies were rat anti-Meca32 (1:100, BD Biosciences Cat# 550563,
153 RRID:AB_393754)(18), guinea pig anti-GH (1:2500, NIDDK-NHPP Cat# AFP12121390,
154 RRID:AB_2756840)(19), rabbit anti-GFP (1:250, Molecular Probes Cat# A-6455,
155 RRID:AB_221570)(20) and secondary antibodies were anti-rat Alexa 647 (Jackson ImmunoResearch
156 Labs Cat# 712-606-150, RRID:AB_2340695)(21), anti-guinea pig Alexa 510 (Jackson
157 ImmunoResearch Labs Cat# 706-166-148, RRID:AB_2340461)(22) and anti-rabbit Alexa 488
158 (Molecular Probes Cat# A-21206, RRID:AB_141708)(23) (dilution: 1:2000). After clearing, transparent
159 pituitary glands were mounted in well glass slides (065230, Dominique Dutscher) in DiBenzyl Ether
160 (Sigma Aldrich). Coverslips were sealed with nail varnish.

161

162 **Immunofluorescence staining in fixed pituitary slices**

163 Pituitary glands were collected from terminally-anesthetized mice and fixed by overnight immersion in
164 4% paraformaldehyde at 4°C, serial cuts were done at 40µm-thick tissue sections using a vibratome
165 (Leica, Germany). Combinations of the following antibodies were used: guinea pig anti- GH (NIDDK-
166 NHPP Cat# AFP12121390, RRID:AB_2756840)(19), LH (NIDDK-NHPP Cat# rLHb, also
167 AFP571292393, RRID:AB_2665511)(24), PRL (NIDDK-NHPP Cat# AFP65191,
168 RRID:AB_2756841)(25), TSH (NIDDK-NHPP Cat# AFP9370793, RRID:AB_2756856)(26) or ACTH
169 (NIDDK-NHPP Cat# AFP71111591, RRID:AB_2756855)(27) (dilution: 1:2500), rabbit anti-GFP
170 (1:250, Molecular Probes Cat# A-6455, RRID:AB_221570)(20) and rabbit anti-RFP (1:500, Rockland
171 Cat# 600-401-379, RRID:AB_2209751)(28). Primary antibody incubation was performed in PBS, 0.1%

172 Triton X-100, 2% BSA at 4 °C for 48 h. Sections were then incubated with secondary antibodies for 2h
173 at room temperature. Secondary antibodies were anti-rabbit Alexa 488 (Molecular Probes Cat# A-
174 21206, RRID:AB_141708)(23), anti-guinea pig Alexa 510 (Jackson ImmunoResearch Labs Cat# 706-
175 166-148, RRID:AB_2340461)(22), Anti-Rat Alexa 647 (Jackson ImmunoResearch Labs Cat# 712-606-
176 150, RRID:AB_2340695)(21), Anti-Guinea Pig Alexa 488 (Jackson ImmunoResearch Labs Cat# 706-
177 545-148, RRID:AB_2340472)(29) and anti-rabbit 510 (Jackson ImmunoResearch Labs Cat# 711-166-
178 152, RRID:AB_2313568)(30) (1:2000 in PBS, 0.1% Triton X-100, 2% BSA).

179

180 **Confocal imaging**

181 Fluorescence images of both sliced pituitaries and whole clarified pituitaries were acquired on a Zeiss
182 LSM 780 confocal microscope with 20x, 40x, 63x objectives. Images were analyzed using Imaris
183 (Bitplane, UK).

184

185 **MRI image acquisition from mouse brain**

186 Animals were scanned on a 9.4T Agilent Varian MRI scanner. A volumic RF43 antenna (Rapid
187 Biomedical) was used. For image acquisition, mice were anesthetized with isoflurane and their heads
188 secured with bite and ear bars. Respiration rate and heart rate were monitored. Animals were scanned
189 using a spin echo sequence with the following parameters: Repetition time 500ms, echo time 10ms, 1
190 echo, averaging 16 times, matrix of 256 × 256 pixels in a FOV of 30x30mm, slices thickness 0.5mm.
191 Total imaging time was 34 min.

192

193 **Analysis**

194 Blood flow changes were estimated from red blood cell velocities as previously described (14) and
195 analysed using a two-tailed variance ratio test followed by a Mann–Whitney U test for any differences
196 directly attributable to treatment application. Estimation of decay time ($\tau = 5\text{sec}$) from calcium signals
197 (27 single calcium transients) recorded in vivo was used to generate simulated calcium rises due to trains
198 of calcium spikes firing at frequencies of either 0.4 or 1Hz. Spike frequencies high enough (1Hz) to

199 generate robust plateau rises in cytosolic calcium (Figure supplement 6)(31) then guided selection of
200 appropriate frequencies of laser light pulses during optogenetic experiments.

201 **Results**

202 **Longitudinal optical monitoring of pituitary blood flow in awake mice**

203 Unravelling the intricacies of pituitary function with cellular *in vivo* imaging studies lasting days to
204 weeks requires optical access to the gland whilst maintaining both its integrity and that of surrounding
205 tissue. The location of the pituitary (Fig. 1A-C, sagittal and coronal MRI sections of mouse heads and
206 relative schemas, respectively), suggested that the least invasive strategy would be insertion of a GRIN
207 lens through the cortex towards the dorsal side of the pituitary using a stereotaxic frame in anesthetized
208 animals. To overcome the major challenge of crossing the meninges covering the ventral brain without
209 damaging the nearby pituitary tissue (Fig. 1B), the GRIN lens was inserted into the lumen of a needle
210 which was then retracted once the GRIN lens was located correctly (Fig. 1D). The GRIN lens was then
211 fixed to the cranium with UV-retractable cement and a titanium bar with a central opening for the lens
212 was attached to the skull. After at least 3-4 weeks of mouse habituation to being head-fixed under a
213 stereomicroscope fitted with a x20 objective, with the body and limbs being able to move on a treadmill
214 (Fig. 1E), pituitary blood flow was imaged for 0.5 to 2 hours in animals pre-injected in the retro-orbital
215 sinus with fluorescent 150kDa dextran (Fig. 1F, video 1)(31). These *in vivo* imaging sessions were
216 repeatable every 3-4 days and up to several months after GRIN lens implantation with no alteration in
217 blood flow, assessed by measurements of red blood cell velocities (Fig. 1G). Imaging pituitary blood
218 flow in awake mice using a GRIN lens with a numerical aperture of 0.5 provided image resolution
219 similar to that obtained in terminally-anesthetized animals with ventral surgery and imaged with a long-
220 range (2 cm working distance, N.A. 0.5) objective (Fig. 1H, I) (14). All imaging sessions were
221 performed between one and six months after GRIN lens implantation without noticeable changes of
222 pituitary function, based on preservation of endogenous hormone rhythms (Figure supplement 1)(31).
223 Thus implantation of thin GRIN lenses through two layers of meninges, one at the level of the cortex

224 and the other covering the ventral side of the brain, allowed long-lasting *in vivo* imaging of the dorsal
225 side of the pituitary whilst preserving characteristic features of pituitary function.

226 **Selective viral delivery and fluorescent protein expression in the pituitary parenchyma**

227 Local stereotaxic delivery for expression of specific genes, for example by viral transduction (2), has
228 been an important tool for monitoring the activities of cells in selective brain regions. Whilst this
229 approach has been applied to very large pituitary tumors by trans-auricular injection (32, 33), it has not
230 been described in the pituitary of healthy mice. We developed stereotaxic delivery of viral particles that
231 could easily be combined with *in vivo* imaging using GRIN lenses with minimal pituitary damage. We
232 first inserted vertically the AAV-containing needle via the cortex and then positioned the needle tip to
233 touch the palate bone. After waiting 5 min, the needle was retracted by 50 μ m and 400 μ m to target the
234 ventral and dorsal regions of the pituitary, respectively (Fig. 2A). AAV particles were then injected
235 using a controlled pneumatic pump to transduce cells with an expression cassette encoding the calcium
236 sensor GCAMP6s (34) or GFP under the control of the strong ubiquitous CAG promoter. Virus was
237 routinely injected in both pituitary “wings” (lateral regions are 500-700 μ m thick). Pituitaries were then
238 dissected and fixed 1, 14 and 28 days after viral injection (Fig. 2B-C). Although a small region of tissue
239 damage was apparent one day after AAV injection using a needle with an outer diameter of 210 μ m
240 diameter, this was markedly reduced or absent 2 weeks post-injection and apparently fully repaired after
241 4 weeks. Pituitary tissues were immunostained for fenestrated vessel markers (MECA32), pituitary
242 hormones (e.g. GH) and GCAMP6s in thick pituitary sections (Fig. 2B, top left panels and tissue
243 clarified with the iDISCO+ protocol (Fig. 2C, top right panel) (17). This showed that expression of
244 AAV-CAG-expressed GCAMP6s could be detected 2 weeks post-infection (Fig. 2B-C, middle panels)
245 but was increased and more extensive after 4 weeks (Fig. 2B-C, bottom panels). Consistent with the
246 apparently complete tissue recovery one month after AAV infection (Fig. 2B-C), endogenous (Fig. 2D)
247 and hormonal responses to hypothalamic agonists (Figure supplement 2)(31) were unaltered following
248 stereotaxic injection of AAV.

249 As the pituitary gland contains five endocrine cell types secreting specific hormones (PRL, LH/FSH,
250 GH, ACTH and TSH), we tested the efficiency of viral transduction in each of these by a range of AAV
251 serotypes expressing CAG promoter driven GFP. All pituitary hormonal cell types were transduced with
252 variable efficiency depending of AAV serotype (Fig. 3, Figures supplement 3-5)(31). For all AAV
253 serotypes, expression of GFP could readily be detected by immunostaining from constructs utilizing a
254 CAG promoter but not those with a CMV promoter (data not shown).

255

256 **Pituitary calcium signals in awake mice**

257 Having successfully transduced pituitary cells with constructs expressing GCAMP6s by stereotaxic
258 injection of AAV5-CAG-GCAMP6s, we then explored whether this could be used to monitor
259 multicellular calcium signals in awake mice following AAV injection. GRIN lenses were implanted
260 above the dorsal pituitary at the site where AAVs had previously been injected stereotaxically. One
261 month after GRIN lens implantation, it was possible to monitor a wide range of profiles of pituitary
262 calcium transients in awake mice (Fig. 4A-G, video 2)(31), with evidence of cell-cell coordination (Fig.
263 4B-4E, video 3)(31) similar to that previously reported in *ex vivo* studies on pituitary slice preparations
264 (9, 11, 35). Of note, similar calcium activity was detected *in vivo* using a ventral imaging approach in
265 anaesthetized mice (14) which had been injected with AAV5-CAG-GCAMP6s (Fig. 4H-I), suggesting
266 that the GRIN lens implantation does not affect calcium signaling.

267

268 **Optogenetic manipulation of pituitary hormone pulsatility in awake mice**

269 The ability to implant lenses and optical devices into the pituitary of awake mice also enables control of
270 the secretory activity of pituitary cells. For this, we used a Cre-lox strategy by crossing GH-Cre and
271 R26-*fl-fl*-ChR2-dtTomato mice, resulting in expression of ChR2 specifically in somatotrophs (GH-
272 ChR2; Fig. 5A). To determine which blue laser illumination pattern was efficient at triggering hormone
273 output from somatotrophs, we used the ventral imaging approach in anesthetized mice to stimulate the
274 pituitary cells with a 400 μm diameter fiber optic positioned close to the pituitary surface (Fig. 5B) and

275 measured GH in blood samples collected from the tail (Fig. 5C). The requirement for a 1Hz stimulation
276 for 300ms to elicit a robust output of GH agrees with simulations of the generation of sustained trains
277 of calcium spikes based on from *in vivo* calcium spike kinetics (Figure supplement 6)(31). Application
278 of this pattern of laser light triggered GH pulses in awake GH-ChR2 mice chronically-implanted with
279 an optical fiber which was located above the dorsal side of the pituitary (Fig. 5 D-E).

280

281 **Discussion**

282 By adapting approaches using stereotaxic to access the ventral side of the brain, we have successfully
283 applied a wide range of tools and techniques for imaging and manipulating specific cell activities in the
284 pituitary gland of awake mice. These technical developments now allow the study of the function of this
285 gland and its intimate relationship with the brain in health and disease at a level hitherto not achievable
286 in awake animal models. Analysis of dynamic pituitary function over periods of days to months in
287 animals with intact interactions between multiple organs will provide important insight into a range of
288 conditions with dysregulated physiological function which may occur at different level within an axis.
289 For example, it is unclear to what extent altered pituitary, hypothalamic or ovarian function contributes
290 to the dysregulated LH secretion which is a hallmark of the polycystic ovarian syndrome, the most
291 common endocrine pathology in the reproductive age female (prevalence 7-15% of pre-menopausal
292 women (36).

293 Live imaging with multi-cellular resolution in awake GRIN lens implanted mice is well suited to real-
294 time studies of cell signals, as illustrated here with calcium signals that are essential for hormone
295 exocytosis (8), and can be used to monitor cell-cell communication within the variety of intermingled
296 cell networks wiring the gland (12). As multi-cellular signal events can be directly combined with
297 frequent blood microsamples and high-sensitive hormone ELISA (16), on-line monitoring of 'stimulus-
298 secretion' coupling (37, 38) is now achievable at the organ (pituitary) level in awake animals, avoiding
299 the well-described blunting of hypothalamic inputs by anesthetics (14). In addition, these studies will
300 be augmented by combining laser light-control of cell functions with monitoring cell activity within the

301 same field of view of the GRIN lens, which is now possible given the efficiency of optogenetic tools for
302 the control of pituitary cell networks.

303 On-line monitoring and manipulation of *in vivo* stimulus-secretion coupling is now readily applicable
304 to answer long-standing questions concerning pituitary gland integration of both brain and peripheral
305 signals for the generation of pulsatile hormonal output. For example, it is now clear that dynamic pulses
306 of corticotroph ACTH output is generated by both a combination of both hypothalamic (CRF and
307 vasopressin) inputs and negative cortisol feedback (39). Future use of miniature imaging systems in
308 GRIN lens-implanted animals (3) would allow monitoring and manipulation of corticotroph cell activity
309 regulating the stress axis, with simultaneous modification of environmental conditions in freely-moving
310 mouse models and study of behavioral effects. To date, such interrogation of the role of pituitary
311 corticotrophs in the stress axis has been restricted to simpler animal models, such as larval zebrafish
312 (40), which lack delivery of hypophysiotropic input via a portal blood system and thus may differ in
313 important aspects to humans (8). An ability to manipulate pituitary cell output via optogenetic
314 stimulation and/or inhibition will also allow dissection of the role of specific patterns of pituitary
315 hormone output, for example the sexually dimorphic GH-dependent regulation liver gene expression
316 (41). Male and female GH secretion patterns can now be optogenetically triggered irrespective of sex
317 animal.

318 A remarkable feature of this suite of tools is their capacity to allow long-term pituitary imaging and
319 manipulation in awake animals. With the restriction of studying adult animals, both short-lived cell
320 events (as discussed above) and slowly-evolving remodelling of the tissue, such as angiogenesis and
321 expansion/shrinkage of a cell population can now be examined over weeks to months in individual
322 animals, which act as their own controls (42). This will notably be relevant for visualizing and studying
323 on-line potential repopulation of the pituitary with stem cells/progenitors (43-45) (e.g. fluorescent cells
324 locally injected in immune-suppressed mice), which have the potential to restore cell populations in the
325 hypoplastic pituitary. It will also be possible to explore the function of either sick or healthy tissue zones
326 within one pituitary by local injection of, for example, tumor cells or a virus encoding CRISPR-driven
327 gene mutation in Cas9-expressing mice (46, 47).

328 In summary, the ability to image at multiple time scales and manipulate the pituitary gland enables the
329 interrogation of pituitary gland function in awake mammalian models and study of how it delivers
330 highly-ordered hormone pulses essential for controlling body functions such as reproduction, growth,
331 stress and metabolism. Since endocrine cells can be photo-painted *in situ* (10), longitudinal *in vivo*
332 studies would give access to the history of cells (48) and how they interact with neighbours in their
333 native environment (9, 49). Single-cell multiomics which include transcriptomics, epigenomics and
334 proteomics (50) would then be applicable to individual pituitary cells which have been monitored for
335 days to months in awake mouse models. Together with these newly-developed single-cell level
336 techniques, application of our cellular *in vivo* imaging and manipulation toolkit to longitudinal studies
337 of awake animal models will provide a unique ability to explore the origin and development of pituitary
338 hormone defects.

339

340

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360

361 **References**

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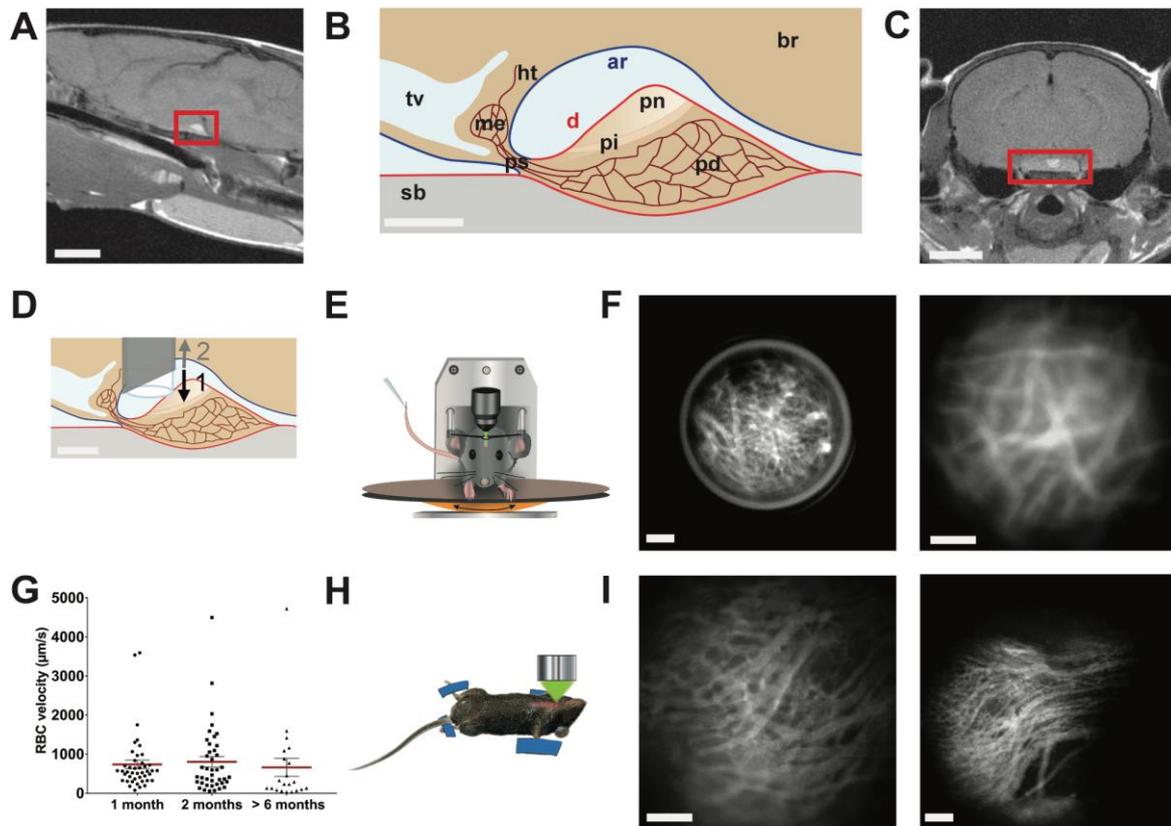
- 363 1. Buzsaki G, Logothetis N, Singer W 2013 Scaling brain size, keeping timing: evolutionary
 364 preservation of brain rhythms. *Neuron* 80:751-764
- 365 2. Deisseroth K, Schnitzer MJ 2013 Engineering approaches to illuminating brain structure and
 366 dynamics. *Neuron* 80:568-577
- 367 3. Li Y, Mathis A, Grewe BF, Osterhout JA, Ahanonu B, Schnitzer MJ, Murthy VN, Dulac C 2017
 368 Neuronal Representation of Social Information in the Medial Amygdala of Awake Behaving
 369 Mice. *Cell* 171:1176-1190 e1117
- 370 4. Ecker JR, Geschwind DH, Kriegstein AR, Ngai J, Osten P, Polioudakis D, Regev A, Sestan N,
 371 Wickersham IR, Zeng H 2017 The BRAIN Initiative Cell Census Consortium: Lessons Learned
 372 toward Generating a Comprehensive Brain Cell Atlas. *Neuron* 96:542-557
- 373 5. Jorgenson LA, Newsome WT, Anderson DJ, Bargmann CI, Brown EN, Deisseroth K, Donoghue
 374 JP, Hudson KL, Ling GS, MacLeish PR, Marder E, Normann RA, Sanes JR, Schnitzer MJ,
 375 Sejnowski TJ, Tank DW, Tsien RY, Ugurbil K, Wingfield JC 2015 The BRAIN Initiative:
 376 developing technology to catalyse neuroscience discovery. *Philosophical transactions of the*
 377 *Royal Society of London Series B, Biological sciences* 370
- 378 6. Sudhof TC 2017 Molecular Neuroscience in the 21st Century: A Personal Perspective. *Neuron*
 379 96:536-541
- 380 7. Herbison AE 2016 Control of puberty onset and fertility by gonadotropin-releasing hormone
 381 neurons. *Nature reviews Endocrinology* 12:452-466
- 382 8. Le Tissier P, Campos P, Lafont C, Romano N, Hodson DJ, Mollard P 2017 An updated view of
 383 hypothalamic-vascular-pituitary unit function and plasticity. *Nature reviews Endocrinology*
 384 13:257-267
- 385 9. Hodson DJ, Schaeffer M, Romano N, Fontanaud P, Lafont C, Birkenstock J, Molino F, Christian
 386 H, Lockey J, Carmignac D, Fernandez-Fuente M, Le Tissier P, Mollard P 2012 Existence of
 387 long-lasting experience-dependent plasticity in endocrine cell networks. *Nature*
 388 *communications* 3:605
- 389 10. Johnston NR, Mitchell RK, Haythorne E, Pessoa MP, Semplici F, Ferrer J, Piemonti L, Marchetti
 390 P, Bugliani M, Bosco D, Berishvili E, Duncanson P, Watkinson M, Broichhagen J, Trauner D,
 391 Rutter GA, Hodson DJ 2016 Beta Cell Hubs Dictate Pancreatic Islet Responses to Glucose. *Cell*
 392 *Metab* 24:389-401
- 393 11. Sanchez-Cardenas C, Fontanaud P, He Z, Lafont C, Meunier AC, Schaeffer M, Carmignac D,
 394 Molino F, Coutry N, Bonnefont X, Gouty-Colomer LA, Gavois E, Hodson DJ, Le Tissier P,
 395 Robinson IC, Mollard P 2010 Pituitary growth hormone network responses are sexually
 396 dimorphic and regulated by gonadal steroids in adulthood. *Proceedings of the National*
 397 *Academy of Sciences of the United States of America* 107:21878-21883
- 398 12. Budry L, Lafont C, El Yandouzi T, Chauvet N, Conejero G, Drouin J, Mollard P 2011 Related
 399 pituitary cell lineages develop into interdigitated 3D cell networks. *Proceedings of the*
 400 *National Academy of Sciences of the United States of America* 108:12515-12520
- 401 13. Featherstone K, Hey K, Momiji H, McNamara AV, Patist AL, Woodburn J, Spiller DG, Christian
 402 HC, McNeilly AS, Mullins JJ, Finkenstadt BF, Rand DA, White MR, Davis JR 2016 Spatially
 403 coordinated dynamic gene transcription in living pituitary tissue. *Elife* 5:e08494
- 404 14. Lafont C, Desarmenien MG, Cassou M, Molino F, Lecoq J, Hodson D, Lacampagne A,
 405 Mennessier G, El Yandouzi T, Carmignac D, Fontanaud P, Christian H, Coutry N, Fernandez-
 406 Fuente M, Charpak S, Le Tissier P, Robinson IC, Mollard P 2010 Cellular in vivo imaging
 407 reveals coordinated regulation of pituitary microcirculation and GH cell network function.
 408 *Proceedings of the National Academy of Sciences of the United States of America* 107:4465-
 409 4470

- 410 15. Luque RM, Amargo G, Ishii S, Lobe C, Franks R, Kiyokawa H, Kineman RD 2007 Reporter
411 expression, induced by a growth hormone promoter-driven Cre recombinase (rGHP-Cre)
412 transgene, questions the developmental relationship between somatotropes and lactotropes
413 in the adult mouse pituitary gland. *Endocrinology* 148:1946-1953
- 414 16. Steyn FJ, Huang L, Ngo ST, Leong JW, Tan HY, Xie TY, Parlow AF, Veldhuis JD, Waters MJ,
415 Chen C 2011 Development of a method for the determination of pulsatile growth hormone
416 secretion in mice. *Endocrinology* 152:3165-3171
- 417 17. Renier N, Adams EL, Kirst C, Wu Z, Azevedo R, Kohl J, Autry AE, Kadiri L, Umadevi Venkataraju
418 K, Zhou Y, Wang VX, Tang CY, Olsen O, Dulac C, Osten P, Tessier-Lavigne M 2016 Mapping of
419 Brain Activity by Automated Volume Analysis of Immediate Early Genes. *Cell* 165:1789-1802
- 420 18. RRID:AB_393754, https://scicrunch.org/resolver/AB_393754
- 421 19. RRID:AB_2756840, https://scicrunch.org/resolver/AB_2756840
- 422 20. RRID:AB_221570, https://scicrunch.org/resolver/AB_221570
- 423 21. RRID:AB_2340695, https://scicrunch.org/resolver/AB_2340695
- 424 22. RRID:AB_2340461, https://scicrunch.org/resolver/AB_2340461
- 425 23. RRID:AB_141708, https://scicrunch.org/resolver/AB_141708
- 426 24. RRID:AB_2665511, https://scicrunch.org/resolver/AB_2665511
- 427 25. RRID:AB_2756841, https://scicrunch.org/resolver/AB_2756841
- 428 26. RRID:AB_2756856, https://scicrunch.org/resolver/AB_2756856
- 429 27. RRID:AB_2756855, https://scicrunch.org/resolver/AB_2756855
- 430 28. RRID:AB_2209751, https://scicrunch.org/resolver/AB_2209751
- 431 29. RRID:AB_2340472, https://scicrunch.org/resolver/AB_2340472
- 432 30. RRID:AB_2313568, https://scicrunch.org/resolver/AB_2313568
- 433 31. Materials to be uploaded to Dryad.
- 434 32. Riley DJ, Nikitin AY, Lee WH 1996 Adenovirus-mediated retinoblastoma gene therapy
435 suppresses spontaneous pituitary melanotroph tumors in Rb+/- mice. *Nat Med* 2:1316-1321
- 436 33. Walls GV, Lemos MC, Javid M, Bazan-Peregrino M, Jeyabalan J, Reed AA, Harding B, Tyler DJ,
437 Stuckey DJ, Piret S, Christie PT, Ansorge O, Clarke K, Seymour L, Thakker RV 2012 MEN1 gene
438 replacement therapy reduces proliferation rates in a mouse model of pituitary adenomas.
439 *Cancer Res* 72:5060-5068
- 440 34. Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger
441 MB, Jayaraman V, Looger LL, Svoboda K, Kim DS 2013 Ultrasensitive fluorescent proteins for
442 imaging neuronal activity. *Nature* 499:295-300
- 443 35. Bonnefont X, Lacampagne A, Sanchez-Hormigo A, Fino E, Creff A, Mathieu MN, Smallwood S,
444 Carmignac D, Fontanaud P, Travo P, Alonso G, Courtois-Coutry N, Pincus SM, Robinson IC,
445 Mollard P 2005 Revealing the large-scale network organization of growth hormone-secreting
446 cells. *Proceedings of the National Academy of Sciences of the United States of America*
447 102:16880-16885
- 448 36. Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R, Karaderi T, Barber TM,
449 McCarthy MI, Franks S, Lindgren CM, Welt CK, Diamanti-Kandarakis E, Panidis D, Goodarzi
450 MO, Azziz R, Zhang Y, James RG, Olivier M, Kissebah AH, Reproductive Medicine N, Stener-
451 Victorin E, Legro RS, Dunaif A 2015 Genome-wide association of polycystic ovary syndrome
452 implicates alterations in gonadotropin secretion in European ancestry populations. *Nature*
453 *communications* 6:7502
- 454 37. Neher E, Marty A 1982 Discrete changes of cell membrane capacitance observed under
455 conditions of enhanced secretion in bovine adrenal chromaffin cells. *Proceedings of the*
456 *National Academy of Sciences of the United States of America* 79:6712-6716
- 457 38. Thomas P, Surprenant A, Almers W 1990 Cytosolic Ca²⁺, exocytosis, and endocytosis in single
458 melanotrophs of the rat pituitary. *Neuron* 5:723-733
- 459 39. Walker JJ, Spiga F, Waite E, Zhao Z, Kershaw Y, Terry JR, Lightman SL 2012 The origin of
460 glucocorticoid hormone oscillations. *PLoS biology* 10:e1001341

- 461 40. De Marco RJ, Thiemann T, Groneberg AH, Herget U, Ryu S 2016 Optogenetically enhanced
462 pituitary corticotroph cell activity post-stress onset causes rapid organizing effects on
463 behaviour. *Nature communications* 7:12620
- 464 41. Waxman DJ, O'Connor C 2006 Growth hormone regulation of sex-dependent liver gene
465 expression. *Mol Endocrinol* 20:2613-2629
- 466 42. Pilz GA, Bottes S, Betizeau M, Jorg DJ, Carta S, Simons BD, Helmchen F, Jessberger S 2018 Live
467 imaging of neurogenesis in the adult mouse hippocampus. *Science* 359:658-662
- 468 43. Andoniadou CL, Matsushima D, Mousavy Gharavy SN, Signore M, Mackintosh AI, Schaeffer
469 M, Gaston-Massuet C, Mollard P, Jacques TS, Le Tissier P, Dattani MT, Pevny LH, Martinez-
470 Barbera JP 2013 Sox2(+) stem/progenitor cells in the adult mouse pituitary support organ
471 homeostasis and have tumor-inducing potential. *Cell Stem Cell* 13:433-445
- 472 44. Perez Millan MI, Brinkmeier ML, Mortensen AH, Camper SA 2016 PROP1 triggers epithelial-
473 mesenchymal transition-like process in pituitary stem cells. *Elife* 5
- 474 45. Rizzoti K, Akiyama H, Lovell-Badge R 2013 Mobilized adult pituitary stem cells contribute to
475 endocrine regeneration in response to physiological demand. *Cell Stem Cell* 13:419-432
- 476 46. Swiech L, Heidenreich M, Banerjee A, Habib N, Li Y, Trombetta J, Sur M, Zhang F 2015 In vivo
477 interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nat Biotechnol*
478 33:102-106
- 479 47. VanDusen NJ, Guo Y, Gu W, Pu WT 2017 CASAAB: A CRISPR-Based Platform for Rapid
480 Dissection of Gene Function In Vivo. *Curr Protoc Mol Biol* 120:31 11 31-31 11 14
- 481 48. Singh SP, Janjuha S, Hartmann T, Kayisoglu O, Konantz J, Birke S, Murawala P, Alfar EA,
482 Murata K, Eugster A, Tsuji N, Morrissey ER, Brand M, Ninov N 2017 Different developmental
483 histories of beta-cells generate functional and proliferative heterogeneity during islet
484 growth. *Nature communications* 8:664
- 485 49. van der Meulen T, Mawla AM, DiGruccio MR, Adams MW, Nies V, Dolleman S, Liu S,
486 Ackermann AM, Caceres E, Hunter AE, Kaestner KH, Donaldson CJ, Huisman MO 2017 Virgin
487 Beta Cells Persist throughout Life at a Neogenic Niche within Pancreatic Islets. *Cell Metab*
488 25:911-926 e916
- 489 50. Macaulay IC, Ponting CP, Voet T 2017 Single-Cell Multiomics: Multiple Measurements from
490 Single Cells. *Trends Genet* 33:155-168

491

492

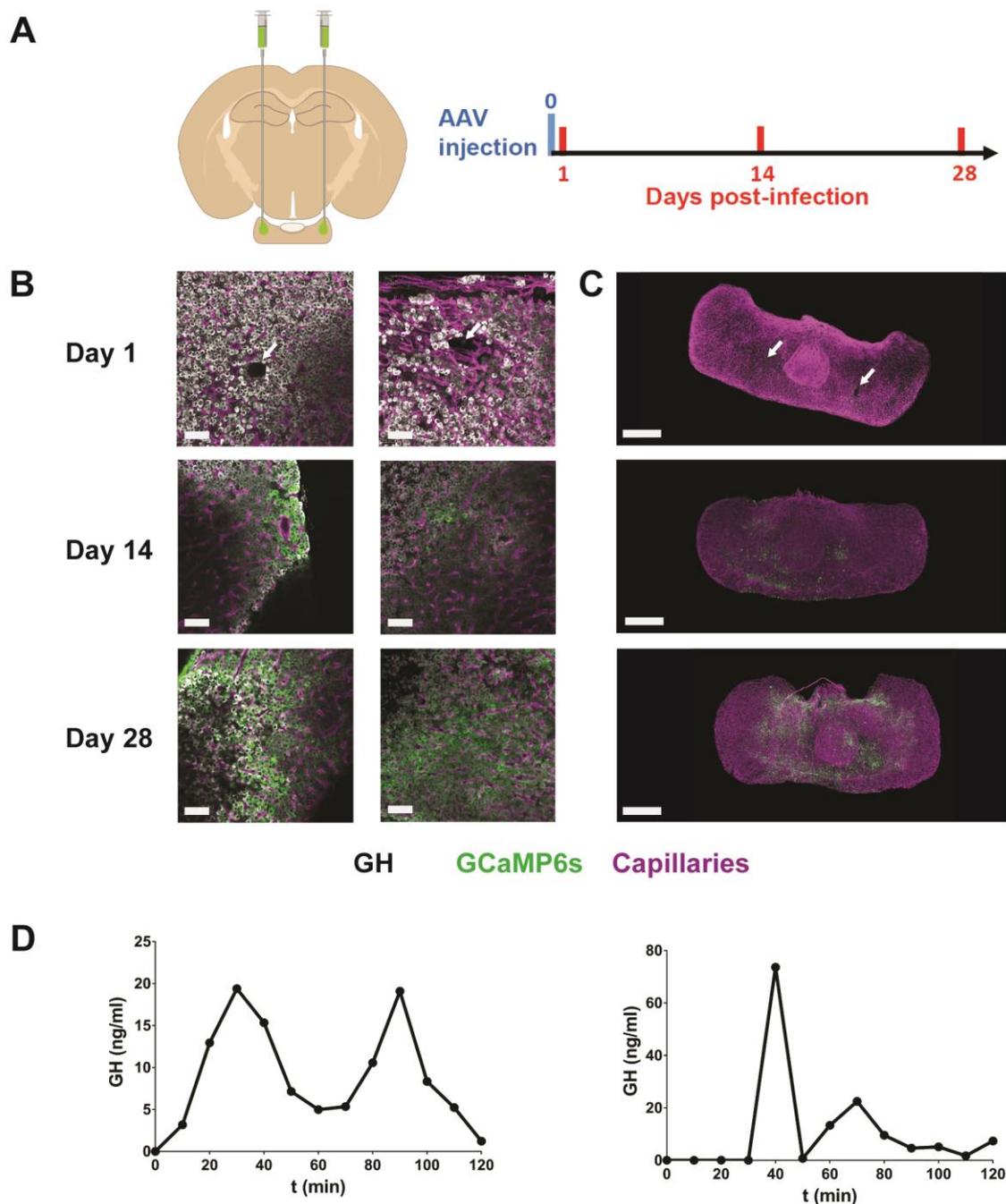


493

494 **Figure 1.** In vivo imaging of pituitary blood flow in the awake mouse. (A) Sagittal MRI view of the
 495 mid-brain from a female mouse. Red rectangle indicates pituitary location below the ventral side of the
 496 brain. Scale bar, 3mm. (B) Drawing of a sagittal view of the hypothalamus-pituitary system. Ht,
 497 hypothalamus; tv, third ventricle; me, median eminence; ps, pituitary stalk; d, dura mater (in red); ar,
 498 arachnoid mater (in blue); pn, pars nervosa; pi, pars intermedia; pd, pars distalis; sb, sphenoidal bone.
 499 Scale bar, 300µm. (C) Coronal MRI view of the brain of a female mouse. Red rectangle indicates
 500 pituitary location. Scale bar, 3mm. (D) Schema showing the GRIN lens implantation in the arachnoid
 501 matter region above the dorsal side of the pituitary. Downward (1) and upward (2) arrows indicate the
 502 sequential needle movements when the GRIN lens is positioned above the pituitary. Scale bar, 300µm.
 503 (E) Head-fixed in vivo imaging of an awake mouse implanted with a GRIN lens which provides an
 504 optical relay between the microscope and pituitary gland. (F) Head-fixed in vivo imaging of pituitary
 505 capillaries at low (left panel) and high magnification (right panel). Scale bar, 100µm; representative
 506 image of n = 5 female mice. (G) Example of longitudinal monitoring of red blood cell velocities in the
 507 same pituitary field viewed from one to six months after GRIN lens implantation; n = 21 to 43 vessels

508 analyzed per animal, n= 4 female mice. **(H)** Schematic arrangement of the ventral in vivo imaging
509 approach in terminally-anesthetized mice (14). **(I)** Ventral in vivo imaging of pituitary capillaries at the
510 level of the pituitary parenchyma (left panel) and entrance (right panel) of different male mice. Scale
511 bar, 100 μ m. See also Figure supplement 1(31).

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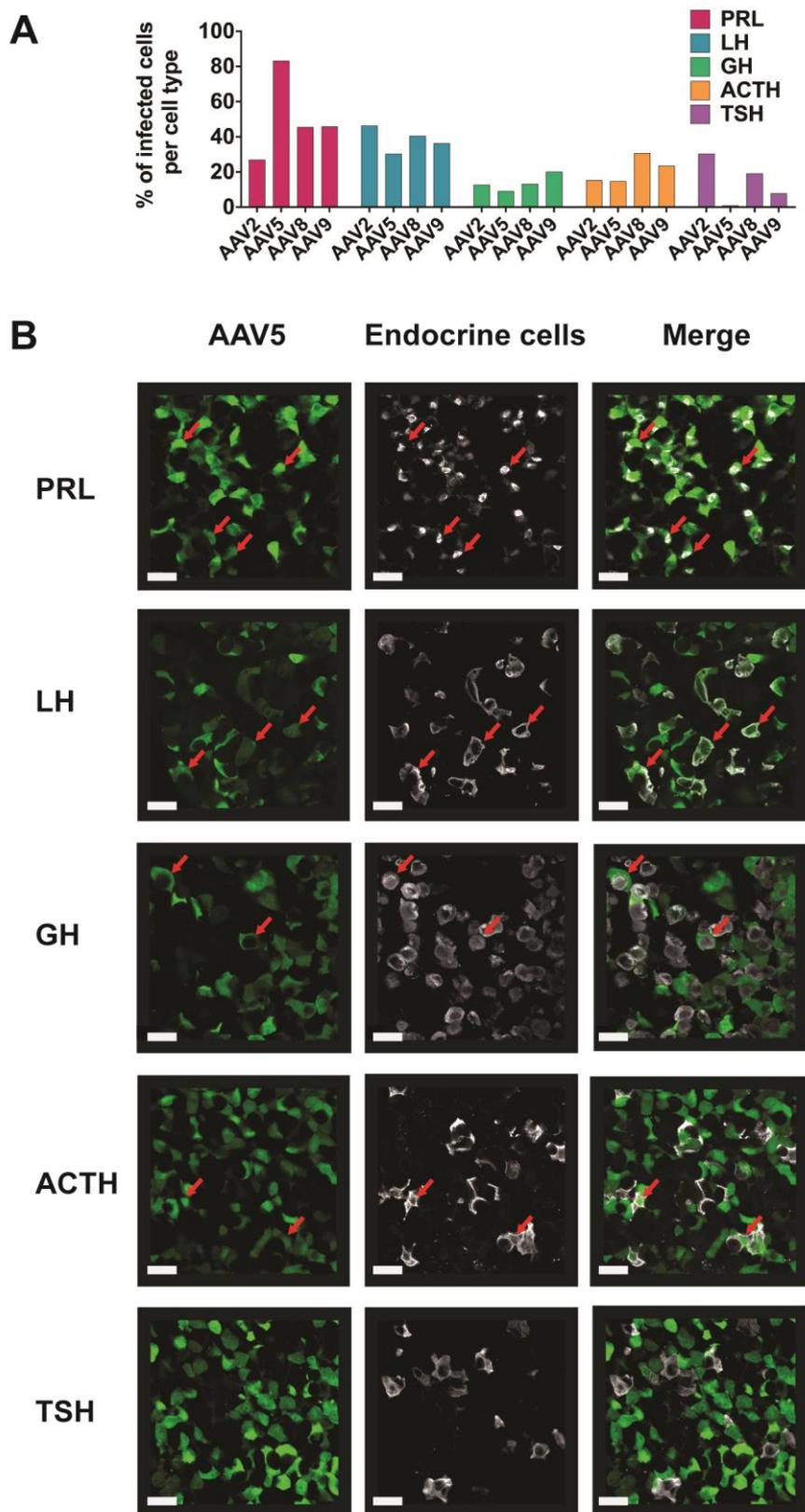


513

514 **Figure 2.** AAV injection into the pituitary. (A) Following bi-lateral AAV injection in anesthetized mice,
 515 pituitaries were dissected from terminally anesthetized animals from 1 to 28 days after GCAMP6s-
 516 expressing AAV5 injection, fixed and subjected to immunostaining and imaging. (B) and (C) pituitary
 517 sections and whole gland (iDISCO+ protocol), respectively. Immunostaining for GH (cells pseudo-
 518 coloured in white), GCAMP6s (green) and MECA32 (a marker of fenestrated capillaries, magenta);
 519 representative images of n = 2-4 male mice per condition. White arrows indicate presumed needle tissue

520 damage. Scale bars, 50 μ m (left panels) and 300 μ m (right panels), respectively. **(D)** Endogenous GH
521 pulses prior to and one month after AAV5 injection in the same animal. 3 μ l blood samples were
522 collected every 5 min at the tail-tip and GH content was then measured using a high-sensitive Elisa
523 assay. See also Figure supplement 2(31).

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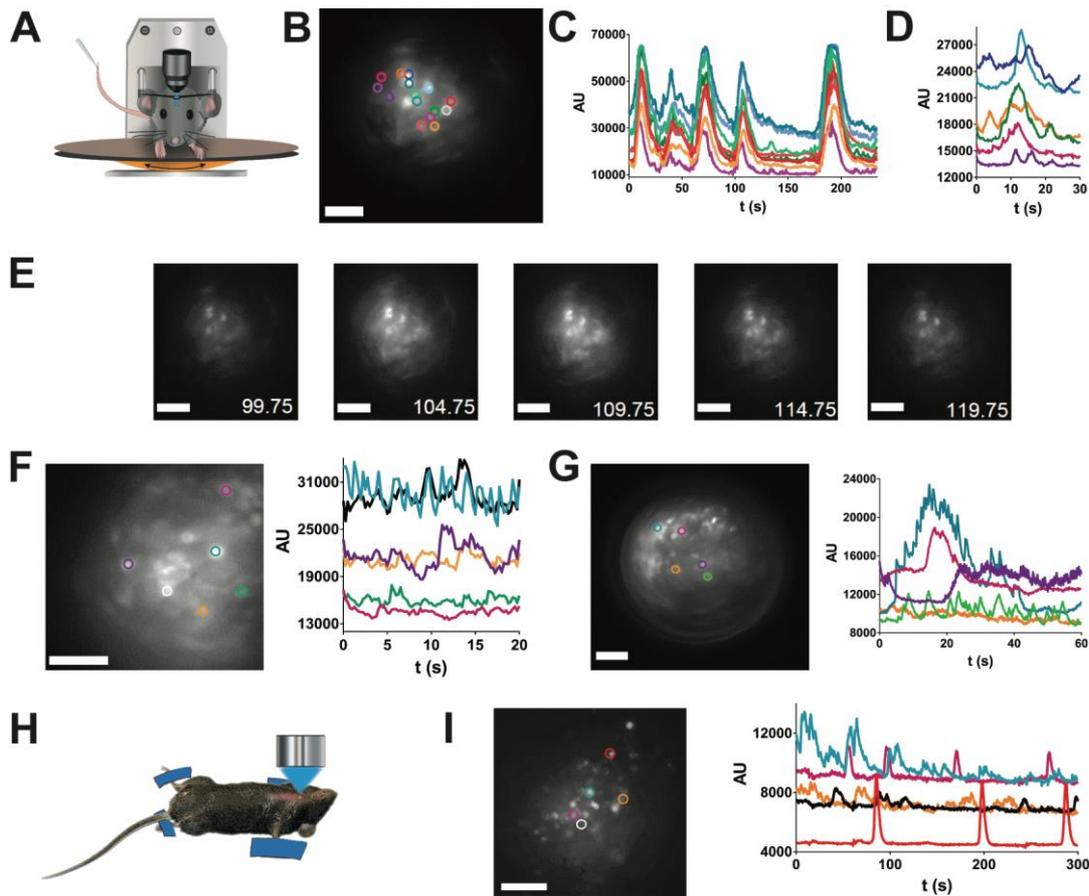


525

526 **Figure 3.** Percentage of infected cells per pituitary cell type. (A) Infection efficiency by different AAV
 527 serotypes (2, 5, 8 and 9) of endocrine cell types (6 tissue sections/pituitary, n = 3 female mice). The

528 percentage of infected cells was counted under microscopic observation within each field of infected
529 cells. **(B)** Examples of co-labelling of endocrine pituitary cells infected by AAV5-CAG-GFP particles
530 (fixed pituitary sections followed by dual immunostaining against hormones and GFP). Scale bars,
531 20µm. See also Figures supplement 3-5(31).

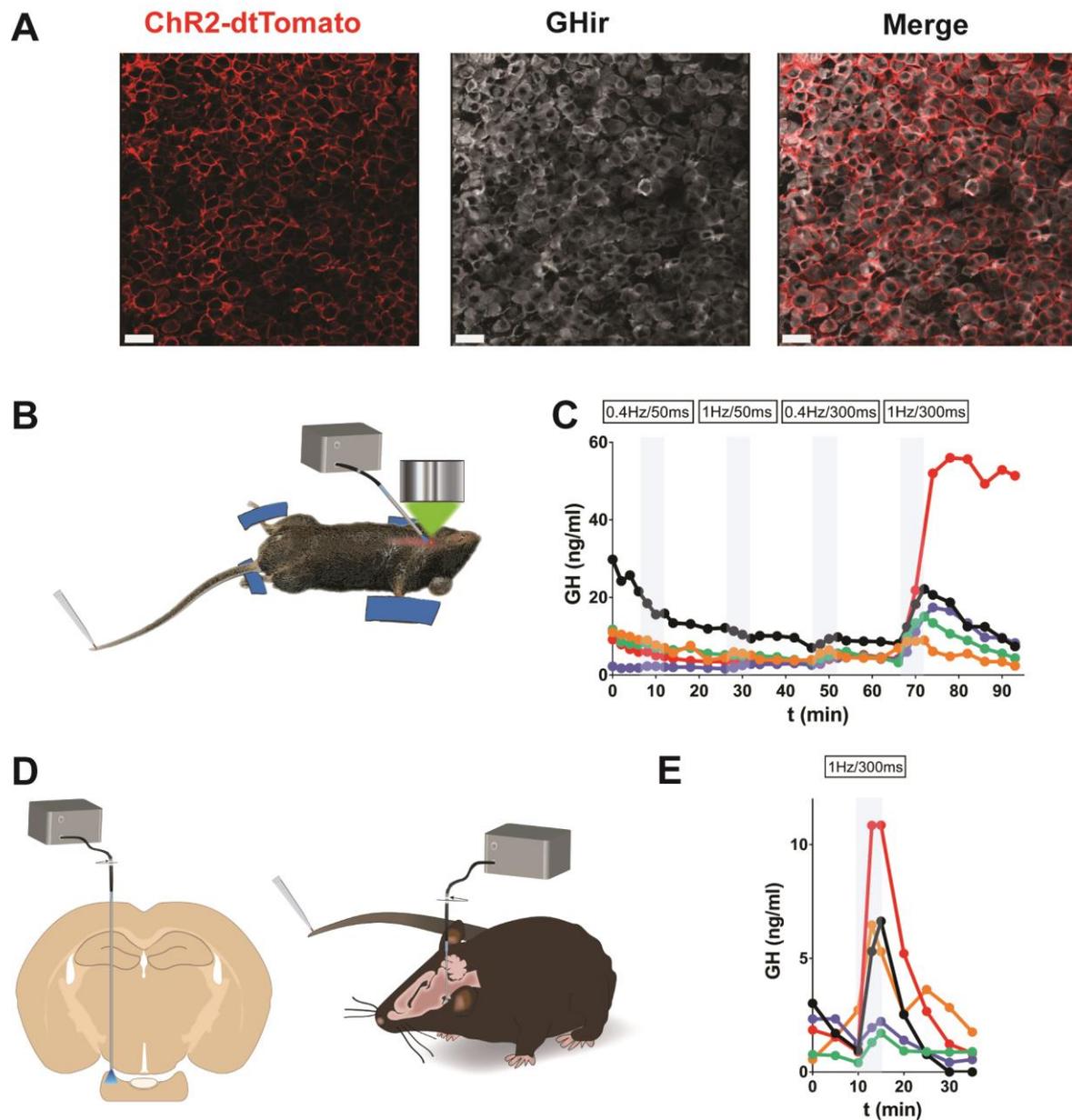
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534 **Figure 4.** *In vivo* calcium imaging in pituitary cells in the awake mouse. (A) Schematic arrangement of
 535 calcium imaging in head-fixed animals injected with AAV5-CAG-GCAMP6s particles into the
 536 pituitary. (B) Field of GCAMP6s cells viewed from the dorsal pituitary side with the selection of cells
 537 as ROIs shown in colored circles in A. Scale bar, 40 μ m; representative image of n=3 female mice. (C)
 538 Coordinated calcium spikes recorded in cells shown in B. (D) Calcium spikes recorded at 10 frames/sec
 539 in cells shown in B. (E) Mosaic of GCAMP6s images (bottom right, recording time in sec) show a
 540 coordinated increase in calcium spike firing. Scale bar, 50 μ m (F) and (G). Two examples of calcium
 541 recordings in other female animals injected with AAV5-CAG-GCAMP6s particles. Scale bar, 100 μ m.
 542 (H) and (I) Calcium signals in pituitary cells (I) imaged from the ventral side in a terminally-anesthetized
 543 animal (H); representative image and trace of n = 2 male mice.

544



545

546 **Figure 5.** Optogenetic stimulation of GH pulses *in vivo*. (A) Co-labelling of dtTomato and GH in the
 547 pituitary from a GH-Cre mouse injected with Cre-activated AAV5-GAG-ChR2-dtTomato particles. (B)
 548 Laser light illumination of the ventral pituitary side in terminally-anesthetized mice subjected to tail-tip
 549 blood sampling (3 μ l every 3 min). (C) In experimental conditions as in B, trains of blue laser light pulses
 550 (300 msec pulses at 1Hz) were able to trigger GH pulses (n = 5 male mice). (D) Laser light illumination
 551 of the pituitary in the awake mouse in which tail-tip blood sampling was carried out. (E) GH pulses
 552 triggered by a train of laser light pulses (300 msec pulses at 1Hz) in GH-Cre mice injected with Cre-
 553 selective AAV5-GAG-ChR2-dtTomato particles (n = 5 male mice). See also Figure supplement 6(31).

554

555 **Supplementary information**

556

557 **Supplementary information**

558 Imaging and manipulating pituitary function in the awake mouse.

559

560 Hoa O, Lafont C, Fontanaud P, Guillou A, Kemkem Y, Kineman RD, Luque RM, Fiordelasio Coll T,

561 Le Tissier P, Mollard P

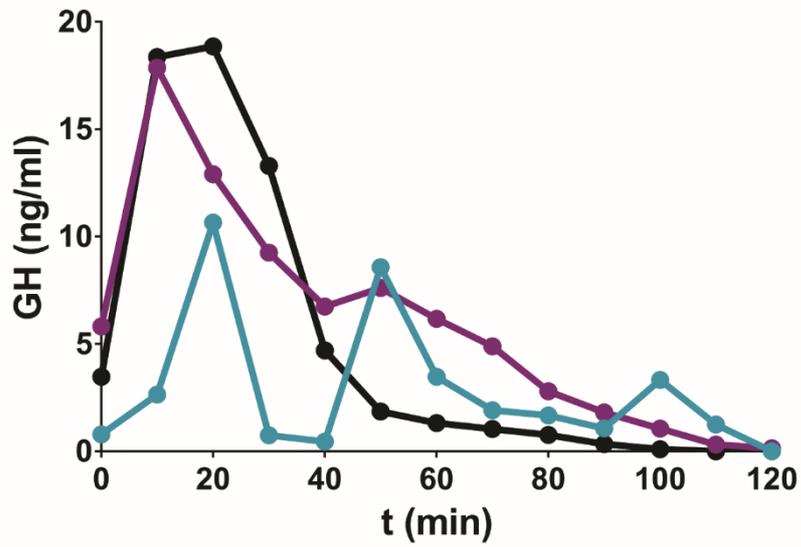
562 Endocrinology. 2019 Jul 22. pii: en.2019-00297. doi:10.1210/en.2019-00297.

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570 **Figure supplement 1.** Endogenous GH pulses in 3 male mice implanted with a GRIN-lens. Related to

571 Figure 1. In awake animals, 3 μ l blood aliquots were tail-tip collected every 10min.

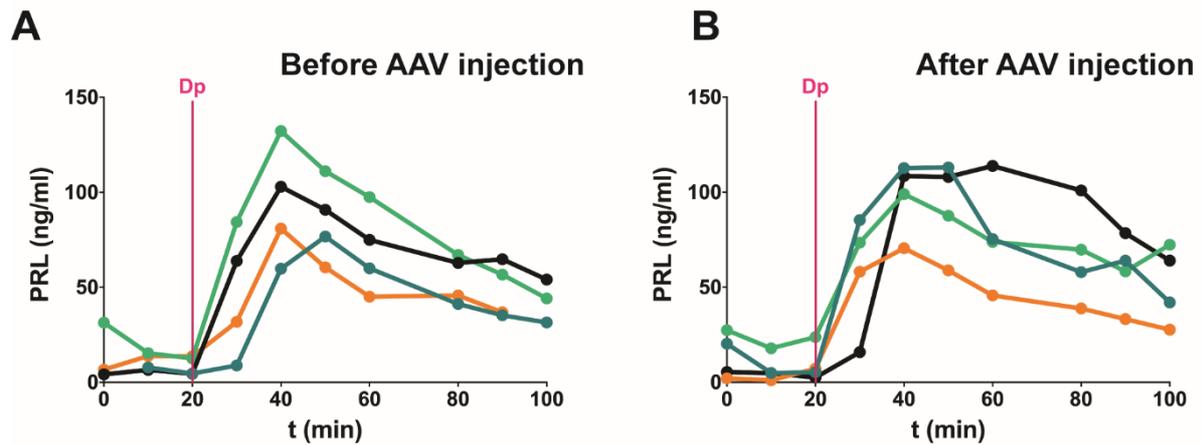
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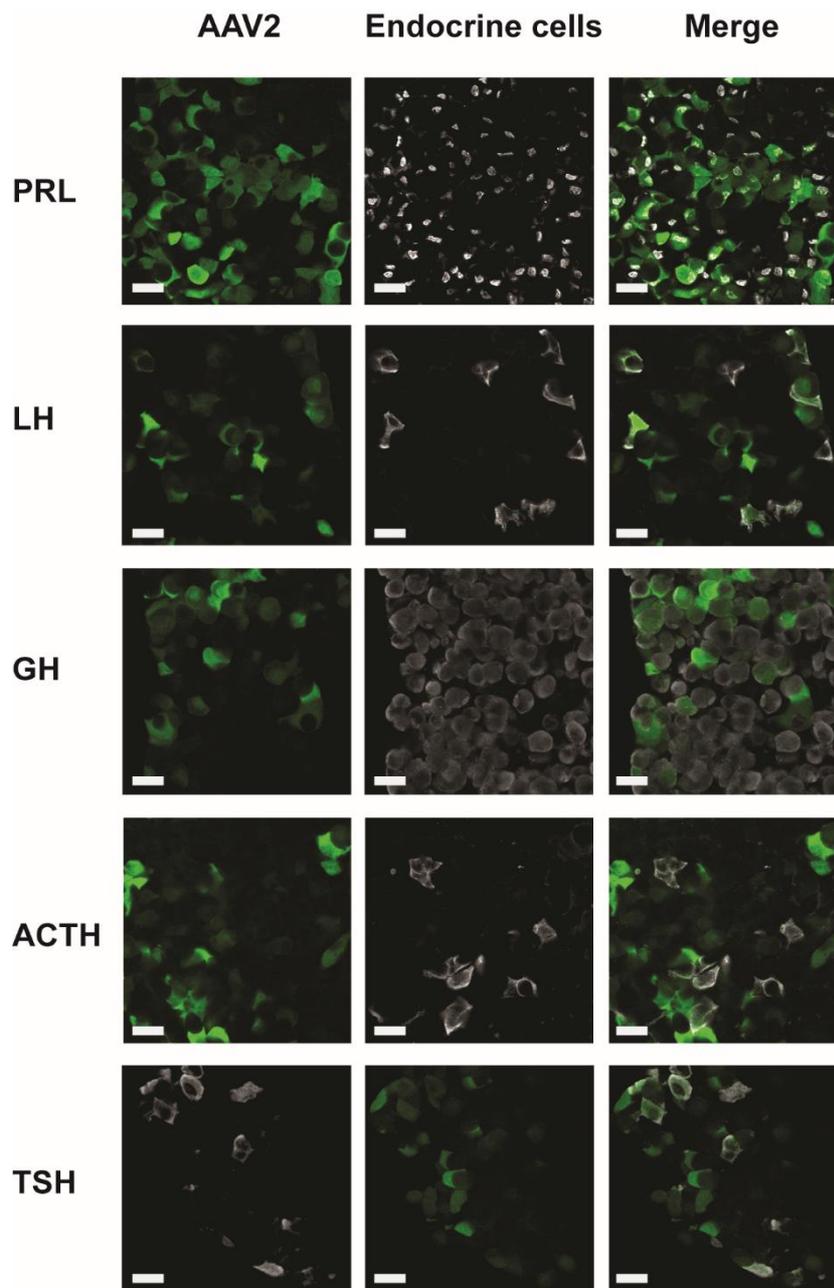
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577

578 **Figure supplement 2.** Prolactin secretion response in response to the D2 receptor antagonist
 579 domperidone (Dp) in mice. Related to Figure 2. In awake animals prior to (A) or one month after
 580 pituitary infection (B) with AAV5-CAG-GFP virus particles (about 80% of lactotrophs were infected),
 581 domperidone (20 mg/kg (Abcam Biochemicals)) was injected i.p. and tail-tip blood samples were then
 582 processed using a high-sensitive mPRL Elisa; n = 4 female mice.

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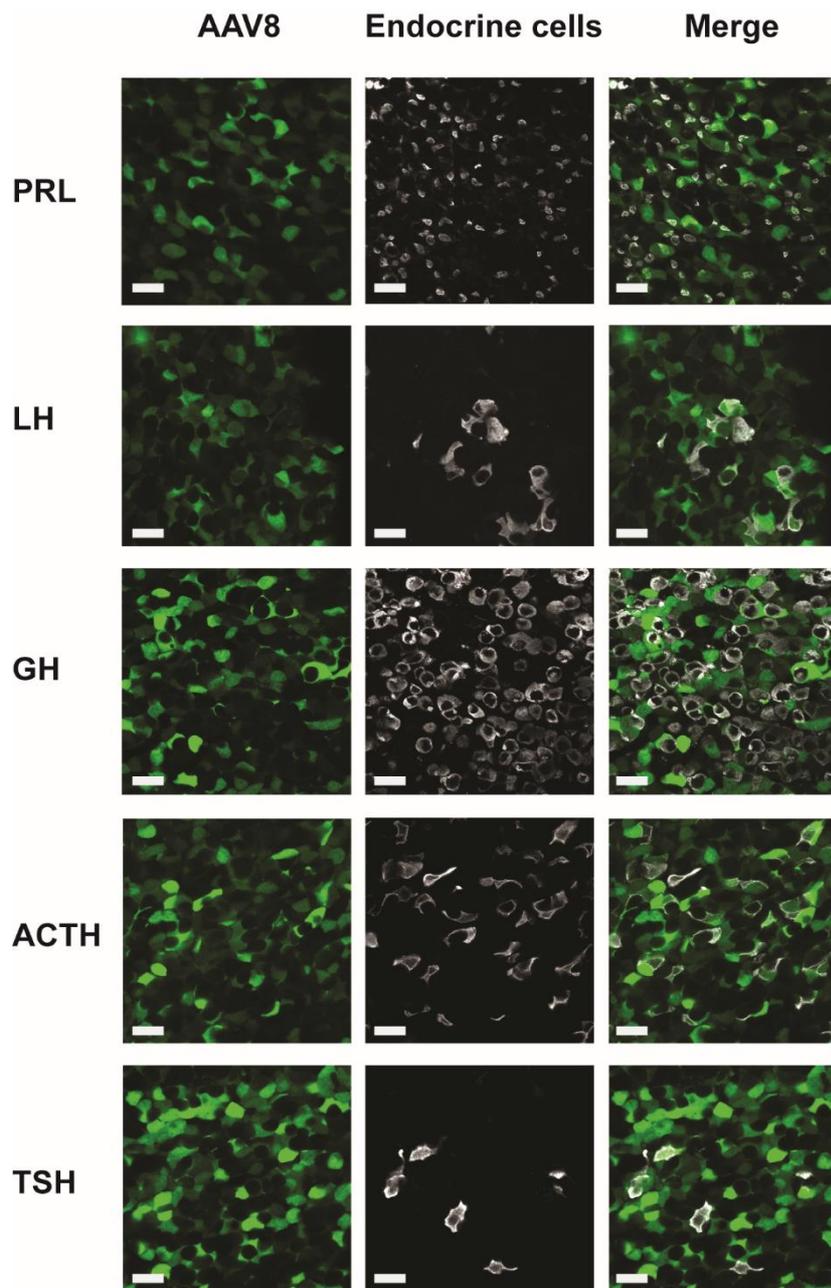
584

585 **Figure supplement 3.** Representative examples of co-labelling of endocrine pituitary cells infected by

586 AAV2-CAG-GFP particles (fixed pituitary sections) in 3 tissue sections /pituitary of 3 female mice.

587 Related to Figure 3. Scale bars, 20 μ m.

588

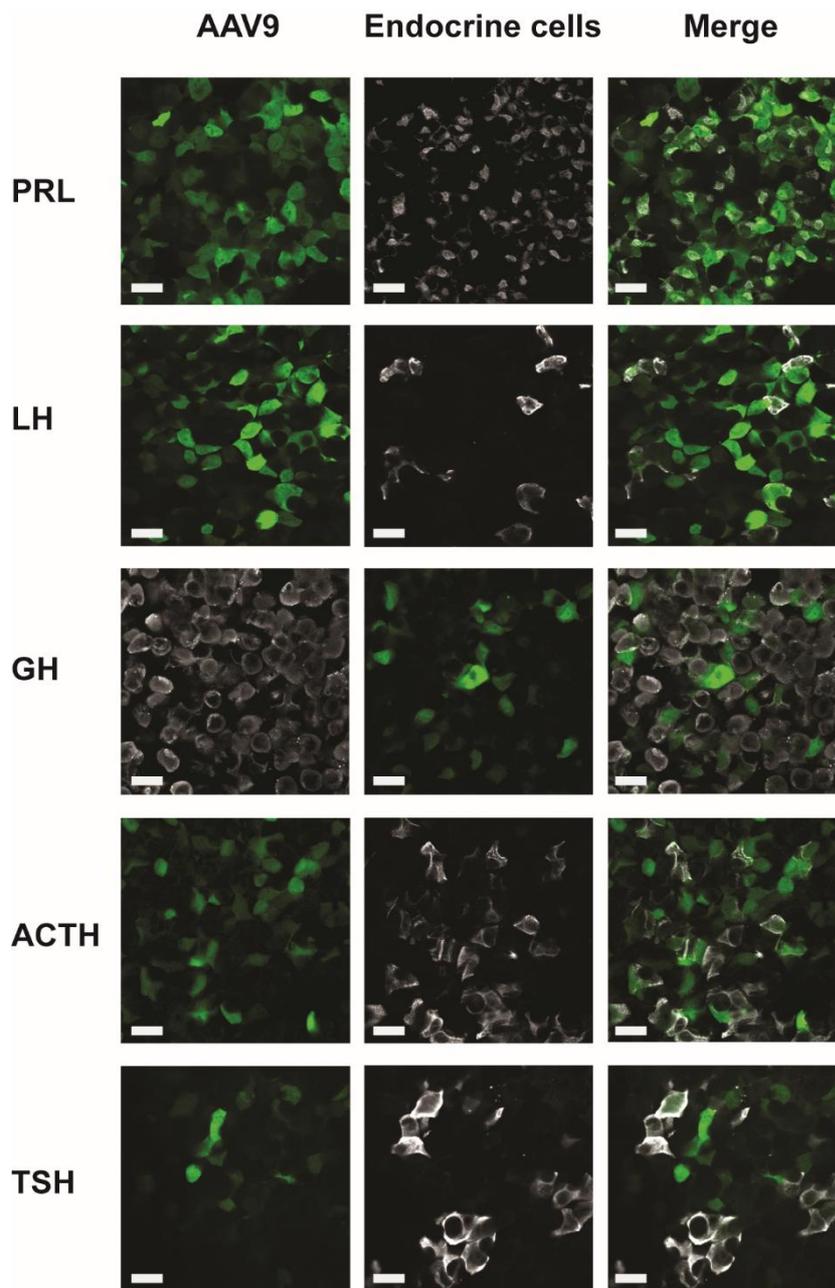


589

590 **Figure supplement 4.** Representative examples of co-labelling of endocrine pituitary cells infected by
591 AAV8-CAG-GFP particles (fixed pituitary sections) in 3 tissue sections /pituitary of 3 female mice.

592 Related to Figure 3. Scale bars, 20 μ m.

593



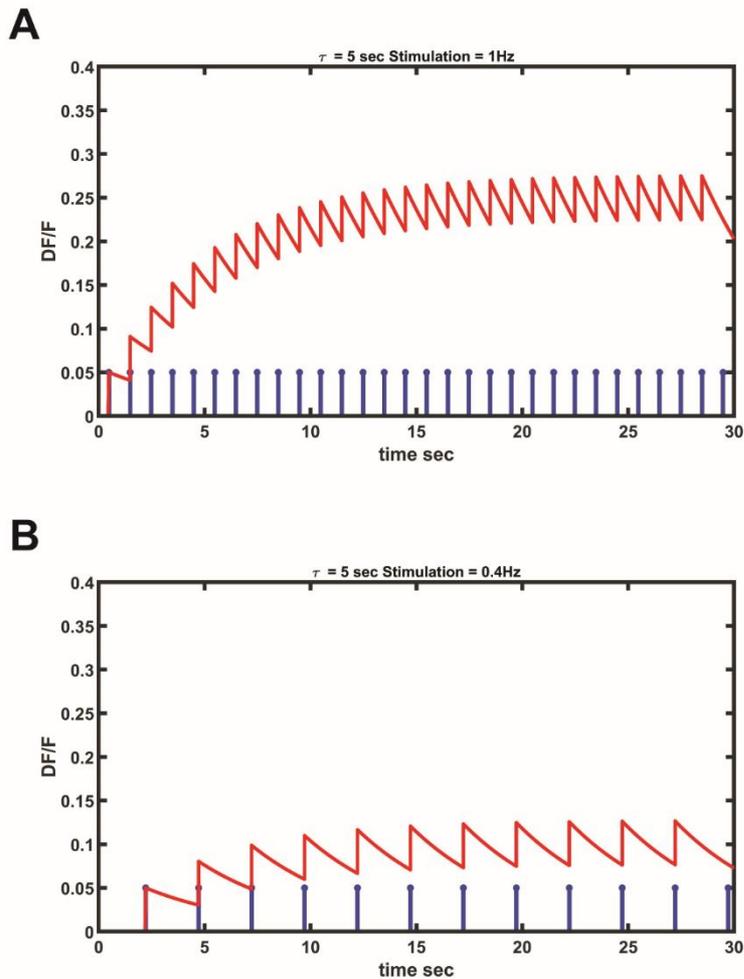
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595 **Figure supplement 5.** Representative examples of co-labelling of endocrine pituitary cells infected by

596 AAV9-CAG-GFP particles (fixed pituitary sections) in 3 tissue sections /pituitary of 3 female mice.

597 Related to Figure 3. Scale bars, 20 μ m.

598



599

600 **Figure supplement 6.** Representation of simulated trains of *in vivo* calcium spikes from pituitary cells.
601 Based on simulated calcium spikes with a 5sec decay time (see Materials and Methods for details),
602 stimulation at frequencies of 1Hz (A), but not 0.4 Hz (B) was efficient at eliciting a robust calcium
603 plateau rise. Related to Figure 5.