The role of subicular VIP-expressing interneurons on seizure dynamics in the intrahippocampal kainic acid model of temporal lobe epilepsy

Short title: VIP-interneurons in temporal lobe epilepsy

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Abstract

The subiculum, a key output region of the hippocampus, is increasingly recognized as playing a crucial role in seizure initiation and spread. The subiculum consists of glutamatergic pyramidal cells, which show alterations in intrinsic excitability in the course of epilepsy, and multiple types of GABAergic interneurons, which exhibit varying characteristics in epilepsy. In this study, we aimed to assess the role of the vasoactive intestinal peptide interneurons (VIP-INs) of the ventral subiculum in the pathophysiology of temporal lobe epilepsy. We observed that an anatomically restricted inhibition of VIP-INs of the ventral subiculum was sufficient to reduce seizures in the intrahippocampal kainic acid model of epilepsy, changing the circadian rhythm of seizures, emphasizing the critical role of this small cell population in modulating TLE. As we expected, permanent unilateral or bilateral silencing of VIP-INs of the ventral subiculum in non-epileptic animals did not induce seizures or epileptiform activity. Interestingly, transient activation of VIP-INs of the ventral subiculum was enough to increase the frequency of seizures in the acute seizure model. Our results offer new perspectives on the crucial involvement of VIP-INs of the ventral subiculum in the pathophysiology of TLE. Given the observed predominant disinhibitory role of the VIP-INs input in subicular microcircuits, modifications of this input could be considered in the development of therapeutic strategies to improve seizure control.

Introduction

Accumulating evidence from both clinical and experimental studies has provided insights into the molecular changes, hypersynchrony, and abnormal anatomical structure of the subiculum that contribute to epileptic seizures (1). Over two decades ago, in brain slices obtained from patients with temporal lobe epilepsy (TLE), a spontaneous rhythmic activity exclusively originating in the subiculum was observed. These synchronous events resembled the interictal discharges recorded in the patients' electroencephalograms (EEG) (2). Analysis of resected hippocampi from individuals with TLE revealed no significant differences in the spontaneous epileptiform activities in the subiculum between sclerotic and non-sclerotic tissues (3). This suggested that modifications in synaptic transmission and intrinsic properties of the subiculum play a crucial role in the development of epilepsy, rather than deafferentation-induced alterations. Consistent with clinical findings indicating relatively preserved integrity of the subiculum in TLE (4), animal models of TLE also demonstrated less noticeable neuron loss in the subiculum compared to the CA1/ CA3 regions (5).

The subiculum consists of glutamatergic pyramidal cells (PCs) that undergo alterations in intrinsic excitability during the course of epilepsy, and various types of GABAergic interneurons (GABAergic-INs) that exhibit diverse characteristics in epilepsy (6–8). Although GABAergic-INs constitute only 10% to 15% of the total neuronal population in the hippocampus (9), their extensive anatomical and physiological diversity enables them to exert significant regulatory influence on cellular and circuit functions (10). The dysfunction of subicular INs in epilepsy can be simplified as an imbalance of excitation and inhibition (E/I). However, these INs form intricate circuit patterns, including feedforward, feedback, and disinhibitory connectivity (11). Consequently, the three major subclasses of subicular INs - parvalbumin-expressing (PV-INs), somatostatin-expressing (SOM-INs), and vasocactive intestinal polypeptide-expressing (VIP-INs) - may play opposing roles in the pathophysiology of TLE. Animal models of TLE have demonstrated selective degeneration of subicular and CA1 PV-INs (12, 13). Moreover, silencing PV-containing basket cells and axo-axonic cells in the subiculum led to recurrent series of interictal spikes and spontaneous seizures in over 60 percent of

51 the animals (14). Loss of SOM-INs in the CA1 region of the hippocampus following pilocarpine-
52 induced seizures has also been reported (15). Additionally, decreased dendritic inhibition of PCs
53 resulting from permanent functional silencing of subicular SOM-INs caused spontaneous seizures in
54 approximately one-third of the animals (16). While the role, changes in the numbers, and properties
55 of subicular PV-INs and SOM-INs in epilepsy are well documented (1), the impact of subicular VIP-INs
56 in epilepsy remains poorly understood.

57 Hippocampal VIP-INs can be classified into two main groups based on their targets (17). The first
58 group consists of VIP-basket cells (VIP-BCs), which co-express cholecystokinin (CCK) and primarily
59 inhibit the somatic region of PCs. The second group comprises interneuron selective interneurons (IS
60 INs), which selectively target other INs. IS INs can be further divided into two subtypes: (a)
61 Interneurons co-expressing calretinin (CR), with cell bodies located in the stratum pyramidale (SP) or
62 near it, projecting to the stratum oriens/alveus border (referred to as VIP-IS O/A INs or IS type III).
63 These interneurons mainly target SOM-expressing oriens lacunosum-moleculare (OLM) interneurons.
64 (b) VIP-INs that project their axons to the stratum radiatum (SR), with cell bodies located either at
65 the SR/ stratum lacunosum-moleculare border (IS type II) or at SR/SP. These VIP-INs target
66 interneurons that mainly innervate proximal dendrites of PCs in stratum radiatum (17–19).

67 In human TLE, the loss of pyramidal neurons has been associated with upregulation of VIP receptors
68 in the hippocampus, while the distribution and pattern of VIP-INs remained unchanged (20). Children
69 with chronic epilepsy have been found to exhibit elevated levels of VIP in their cerebrospinal fluid
70 (21). Moreover, in focal cortical dysplasia (type IIb), primary common cause of medically refractory
71 pediatric epilepsy, there is a notable increase in the ratio of VIP-INs to other interneurons (22). In
72 rodent models, increased firing rates of VIP-INs were observed in cortical slices treated with
73 tubocurarine as spikes and waves developed (23). Interestingly, most VIP-INs are preserved in
74 animal models of epilepsy (24, 25), although the OLM interneurons targeted by VIP-INs are
75 selectively lost (26).

76 While there is growing evidence suggesting the potential significance of VIP-INs in TLE, there is a lack
77 of consensus regarding their role. The upregulation VIP receptors or lack of change in VIP-INs can be
78 interpreted as either causative (22, 24) or compensatory (27) in the context of epileptic
79 excitotoxicity. Additionally, no studies have explored the functional role of VIP-INs specifically in the
80 subiculum. Although VIP-INs are relatively sparse compared to PV-INs and SOM-INs, their unique
81 connectivity patterns suggest they may play a key role in the subicular circuit. However, their precise
82 function in epilepsy remains unclear.
83 To investigate the potential role of VIP-INs of the ventral subiculum in the development and
84 maintenance of TLE, we employed a targeted approach to inhibit neurotransmitter release from
85 these neurons. Our study involved both healthy control mice and a chronic mouse model of epilepsy,
86 with continuous EEG monitoring using telemetric recording. In mice with epilepsy, the silencing of
87 VIP-INs of the ventral subiculum leads to a reduction in the severity of seizures, while silencing them
88 in healthy mice does not induce epileptiform activity. Furthermore, we conducted experiments using
89 chemogenetic activation of these interneurons in a model of acute seizures, which resulted in an
90 increase in epileptiform activity. These findings provide novel insights into the significant
91 involvement of VIP-INs of the ventral subiculum in the pathophysiology of TLE.
92

93 ## Results

94 ### Silencing of VIP-INs of the ventral subiculum in epileptic mice dampens seizures

95 Consistent with the CA1 region (17), we also identified three distinct subgroups of VIP-INs in the
96 ventral subiculum (*n* = 3, VIP:Ai9-tdTomato mice). These subgroups include INs expressing VIP alone
97 (IS type II), INs co-expressing VIP and CCK (VIP-BCs), and INs co-expressing VIP and CR (IS type III; Fig
98 1A). Next, we conducted experiments to examine whether dysfunction of VIP-INs of the ventral
99 subiculum can influence seizure severity and epileptogenesis. For this purpose, we utilized an animal
100 model of TLE induced by intrahippocampal injection of kainic acid (IH-KA, 200 ng). Telemetric EEG

101 transmitters were surgically implanted in 16 VIP-cre mice (8 males and 8 females). The animals were
102 randomly assigned to two groups, with one group receiving AAV-GFP and the other group receiving
103 AAV-TeLC, after a 7-day period (Fig 1B). Prior to vector injection, the severity of status epilepticus
104 (SE) and subsequent spontaneous recurrent seizures were evaluated to establish the baseline
105 epileptic activity for both experimental groups. During SE on Day 1, the median (range) number of
106 seizures observed was 10 (1 to 19) and 8.5 (1 to 14) for the GFP and TeLC groups, respectively (Fig
107 1D). The median (range) cumulative duration of seizures was 314.1 s (10.34 s to 1098 s) for the GFP
108 group and 266.10 s (17 s to 703 s) for the TeLC group (Fig 1G). No significant difference was observed
109 between the groups based on the number or time spent in seizures during SE. Since seizures during
110 SE may not always exhibit postictal depression (Supplementary Fig. S1B), the number of seizures
111 could be underestimated. Therefore, in addition to seizures, we also compared the groups based on
112 the number of epileptiform spikes and spike trains. During SE, no significant differences were
113 observed between the groups regarding the number of epileptiform spikes (Supplementary Fig. S1C-
114 D) or spike trains (Supplementary Fig. S1E-F). Additionally, during the six days of recording after SE
115 and before viral vector injection, the number of spontaneous recurrent seizures (SRSs) was similar
116 between the two experimental groups (Supplementary Fig. S2A).
117 Following a two-week waiting period after viral vector injection, the number and duration of seizures
118 were assessed and compared between the groups, revealing a significant difference (Supplementary
119 Fig. S2B). Taking into account the inter-individual differences in the number of seizures in the IH-KA
120 model, we further evaluated the seizure ratio for each animal by comparing the number of seizures
121 before and after vector injection. The median (range) seizure ratio was 0.55 (-1.00 to 0.86) for the
122 GFP group and -0.33 (-1.00 to 0.42) for the TeLC group (Fig 1E). GFP-injected mice generally exhibited
123 an increasing trend in the number of seizures, whereas the TeLC group showed a decreasing ratio,
124 indicating a significant difference ($p < 0.05$) between the two groups. Upon evaluating circadian
125 changes in the seizure ratio for both groups (Supplementary Fig. S3), a significant reduction was
126 observed during the light phase ($p < 0.05$). However, this reduction was not significant during the

127 dark phase (Fig 1F). The vigilance state of the animals was assessed both before and after vector
128 injection. Interestingly, it was found that the alteration in the circadian rhythm of the seizures was
129 not correlated with changes in the vigilance state. Furthermore, no significant changes in sleep
130 macro and micro architecture were observed that corresponded to these seizure rhythm changes
131 (Supplementary Fig. S4).
132 We next evaluated the duration of seizures in GFP and TeLC-injected mice. Before vector injection,
133 the time spent in seizures was comparable between the experimental groups (Supplementary Fig.
134 S5A). However, following vector injection, a significant difference in seizure duration was observed
135 between the groups ($p < 0.05$, Supplementary Fig. S5B). Additionally, we evaluated the seizure
136 duration ratio for each animal, revealing a median (range) of 0.66 (-1.0 to 0.96) for GFP mice and -
137 0.36 (-1.0 to 0.34) for TeLC mice, indicating a significant difference ($p < 0.05$, Fig 1H). Notably, the
138 reduction in time spent in seizures was more pronounced during the light phase ($p < 0.05$, Fig 1I),
139 aligning with the trend observed for the number of seizures.
140

141 **Silencing VIP-INs of the ventral subiculum in healthy mice does not induce seizures or**
142 **epileptiform activity**

143 In our previous research, we reported that silencing PV-INs (14) and SOM-INs (16) of the ventral
144 subiculum results in the development of spontaneous recurrent seizures (SRSs). However, our
145 current findings demonstrate that silencing VIP-INs of the ventral subiculum can actually alleviate
146 seizures in epileptic animals. Therefore, based on these findings, if we were to silence VIP-INs of the
147 ventral subiculum in healthy mice, it is unlikely to induce any SRSs. To investigate this, we analyzed
148 EEG patterns in healthy male VIP-cre mice unilaterally injected with either AAV-TeLC ($n = 5$) or a
149 control AAV-GFP ($n = 5$) viral vector into the ventral subicular (Fig 2A). Telemetric EEG recordings
150 were conducted continuously for 50 days, and the recorded EEG traces were visually reviewed to
151 identify seizures (see Methods). No seizures were detected in any of the AAV-TeLC- or AAV-GFP-
152 injected animals (Fig 2B). At the injection site, the median (range) percentage of the VIP-INs

153 expressed GFP was 46.67% (41.18% to 90%), while 95.84% (82.35% to 100%) of the GFP-expressing
154 INs also co-expressed VIP (Fig 2C), highlighting the specificity of the experimental manipulation using
155 the TeLC vector.
156 To ensure the detection of subtle focal epileptiform activity, continuous local field potential (LFP)
157 recording was conducted in mice that underwent bilateral injection of AAV-TeLC (n = 4) or AAV-GFP
158 (n = 4) into the ventral subiculum (Fig 2D). The recordings were performed for 21 days, and data
159 were visually reviewed. No seizures were detected in either group. Furthermore, epileptiform spikes
160 and spike trains were assessed using a custom-made MATLAB code for three consecutive days, with 6
161 hours of recording per day (from 18:00 to 24:00, starting from Day 18). We observed a minimal
162 number of epileptiform spikes and spike trains. The median (range) cumulative number of
163 epileptiform spikes was 148 (3 to 1687) for GFP animals and 1500 (24 to 4723) for TeLC animals (Fig
164 2E). No significant difference was found between the groups. Additionally, the median (range)
165 number of spike trains was 0.0 (0.0 to 37) and 13.50 (0.0 to 31) for GFP and TeLC animals,
166 respectively (Fig 2F), and there was no significant difference between the groups.
167

168 **Activating VIP-INs of the ventral subiculum in epileptic mice increases seizures**

169 If silencing VIP-INs of the ventral subiculum reduces seizures in the chronic model of epilepsy, it is
170 expected that activation of these INs would increase seizure activity. To test this hypothesis, we
171 utilized an experimental model of acute focal seizures induced by IH-KA injection (7 ng). AAV-
172 hM3D(Gq) was injected into 15 VIP-Cre mice (10 males, 5 females), and the acute seizure model was
173 induced on day 10 by IH-KA injection (Fig 1A). The animals were randomly divided into two groups,
174 CNO (n = 9) or NaCl (n = 6), and received an i.p. injection of CNO or NaCl, respectively, half an hour
175 before the IH-KA injection. All animals developed seizures within the first hour after IH-KA injection
176 (Fig 1B-C). The median (range) number of seizures in the NaCl group was 2 (1 to 4), whereas CNO
177 group exhibited a higher median value of 5 (2 to 7) seizures (Fig 1D). The CNO-injected mice
178 demonstrated a significant higher number of seizures compared to the NaCl group ($p < 0.05$). The

179 median (range) cumulative duration of seizures for the NaCl group was 76.02 s (33.08 s to 150.50 s),
180 while for the CNO group it was 138.70 s (51.89 s to 225.0 s), indicating a trend towards increased
181 seizure duration in the CNO group compared to control mice (Fig 1E). The findings from the acute
182 seizure model suggest that the enhanced activity of VIP-INs during seizure induction is associated
183 with heightened seizure severity.

184 Discussion

185 Epileptic seizures are a result of excessive electrical discharges (28), caused by an imbalance between
186 inhibition and excitation in the brain (29). The subiculum, which serves as a key output region of the
187 hippocampus, is increasingly recognized for its crucial role in seizure initiation and propagation (1).
188 The concept of dynamic excitation/inhibition balance, modulated by circuit disinhibition, has
189 emerged as an important mechanism for information-processing (19). However, the specific role of a
190 distinct subpopulation of subicular GABAergic cells expressing VIP in epilepsy-related disinhibitory
191 modulation has not been investigated in previous studies. Despite the heterogeneity of subicular VIP-
192 INs and their lower numbers compared to PV-INs and SOM-INs (14, 16), selectively inhibiting VIP-INs
193 of the ventral subiculum within a restricted anatomical region proved sufficient to reduce seizures in
194 the chronic model of epilepsy, underscoring the critical role of this small cell population in
195 modulating TLE. As anticipated, permanent unilateral or bilateral silencing of VIP-INs of the ventral
196 subiculum in non-epileptic animals did not induce seizures or epileptiform activity, whereas transient
197 activation of VIP-INs of the ventral subiculum was found to increase the frequency of seizures in the
198 acute seizure model (Fig 4). Our findings offer new insights into the pivotal role of VIP-INs of the
199 ventral subiculum in regulating the excitability of the subicular network within the context of
200 epilepsy.
201 Hippocampal VIP-INs consist of two main subtypes, VIP-BCs and ISs (IS type II and III). These subtypes
202 have opposing roles in hippocampal circuits, with VIP-BCs directly inhibiting PCs while ISs disinhibit
203 PCs (19). Our findings in both healthy and epileptic animals suggest a more prominent role for ISs. In

204 our previous studies, we demonstrated that silencing PV-INs (14) and SOM-INs (16) of the ventral
205 subiculum alone is sufficient of induce seizures. However, this is not the case for VIP-INs of the
206 ventral subiculum, as silencing these neurons in healthy animals did not lead to seizures. This
207 indicates a potential disinhibitory function VIP-INs of the ventral subiculum in the context of epilepsy.
208 Furthermore, silencing of VIP-INs of the ventral subiculum in epileptic animals resulted in a reduction
209 of seizure activity, while activating VIP-INs of the ventral subiculum in an acute model of focal
210 seizures increased the occurrence of seizures. These findings collectively highlight the significant
211 disinhibitory role played by VIP-INs of the ventral subiculum in the context of epilepsy.
212 To the best of our knowledge, our study represents the first attempt to investigate the involvement
213 of VIP-INs of the ventral subiculum in the development of TLE. However, a limited number of studies
214 have examined both VIP-BCs and ISs in hippocampal sector CA1 in various animal models of epilepsy.
215 In the IH-KA model, it has been reported that the number of CCK-expressing INs (of which VIP-BCs
216 constitute approximately 27% (9)) was reduced in the ventral hippocampus following KA injection,
217 although the surviving CCK-BCs did not exhibit significant changes in their intrinsic and synaptic
218 properties or connectivity with CA1-PCs (30). In the pilocarpine model of epilepsy in mice, the overall
219 density of IS type III INs remained relatively preserved (26, 25), but the number of axonal boutons
220 produced by these cells within the CA1 oriens/alveus was significantly lower after SE (26).
221 Furthermore, VIP+/CR- INs, which include both IS type II and VIP-BCs, decreased to 54% compared to
222 non-epileptic animals (25). However, no study has specifically evaluated the vulnerability of IS type II
223 cells in the context of epilepsy. It may be argued, that VIP-BCs are more susceptible to damage than
224 ISs in the IH-KA model of epilepsy. Consequently, the balance of cell types may shift, leading to a
225 greater impact from ISs when VIP-INs are silenced in the chronic model of epilepsy. However, the
226 results of silencing VIP-INs in non-epileptic animals and in the model of acute seizures support the
227 predominant role of ISs, regardless of the number of viable cells.
228 The role of VIP-INs of the ventral subiculum in TLE can be influenced not only by the release of GABA
229 from these INs, but also by endogenous VIP. In the hippocampus, VIP is exclusively expressed by

230 GABAergic-INs (31) and interacts with two receptors, VPAC1 and VPAC2, as well as PAC1 with lower
231 affinity (27). These receptors activate the cAMP/PKA signaling pathway and are positively coupled to
232 $G_{\alpha s}$ (32). Hippocampal VPAC1 receptors can also couple to $G_{i/o}$ proteins (33). VPAC2 receptors are
233 primarily responsible for mediating VIP-induced enhancement of PCs excitability and NMDA receptor
234 currents, which mainly occur post-synaptically and independently of GABAergic transmission (34). In
235 human hippocampi surgically removed from patients with medically intractable TLE, a significant
236 upregulation of VIP receptors on PCs has been observed (20), indicating an increased excitation of
237 these cells by VIP. *In vitro* studies have shown that the selective VPAC1 antagonist PG 97-269
238 contributes to the inhibition of LTP following interictal-like activity in hippocampal slices (35).
239 Additionally, a recent study in a lithium-pilocarpine mouse model demonstrated a compensatory
240 decrease in VPAC1 receptor levels and an increase in VPAC2 receptor levels in hippocampal
241 membranes (36), underscoring the critical role of VIP and its receptors in the pathophysiology of TLE.
242 These reports align with our findings, which demonstrate both the protective effect of reduced VIP-
243 INs activity and the intensified impact of heightened VIP-INs activity on epileptogenic markers in
244 mice.
245 Several limitations need to be considered in relation to this study that. The primary limitation was
246 the high variation in the number of seizures observed among animals, particularly in female mice, in
247 the chronic model of epilepsy. However, this variation was expected (37). To address this issue and
248 ensure the robustness of our findings, we also reported the number (and duration) of seizures as a
249 ratio for each animal, comparing them to the number (and duration) of seizures before injection. As
250 anticipated, individual female mice exhibited a greater degree of variation due to significant changes
251 in hippocampal excitability during the estrous cycle (38, 39). Therefore, it is necessary to conduct
252 additional research that specifically focuses on gender, taking into account the timing of KA injection
253 in accordance with the estrous cycle of female mice.
254 Another limitation of the study concerns the sensitivity of targeting VIP-INs of the ventral subiculum
255 through the injection of AAV-vector expressing TeLC. While our injection of AAV-TeLC was highly

256 specific (over 90%), we were unable to target all VIP-INs in the subiculum, as reflected by a mean
257 sensitivity of approximately 60%. Additionally, we observed variable expression of TeLC in other
258 brain regions, including the ventral CA1, and occasionally in parts of the entorhinal cortex. Future
259 studies utilizing alternative technologies with higher spatial precision may allow for more accurate
260 targeting of the subiculum and its interneurons, including VIP-INs.
261 In our study, we employed an experimental model of acute focal seizures and a chronic model of
262 epilepsy, both induced by IH-KA injection, to assess the role of VIP-INs of the ventral subiculum in the
263 context of epilepsy. It is important to emphasize that conducting similar investigations in diverse
264 animal models of epilepsy can provide additional valuable insights into the subject matter.
265 In conclusion, our study presents compelling evidence for the direct involvement of subicular VIP-INs
266 in the pathogenesis of TLE. This finding represents a significant contribution to the field as it sheds
267 light on the crucial role played by VIP-INs in subicular microcircuits and their potential as targets for
268 therapeutic interventions aimed at enhancing seizure control. Future research employing techniques
269 such as cell type-specific electrophysiology and *in vivo* imaging will further advance our
270 understanding of subicular VIP-INs in the context of epilepsy. Moreover, these investigations will
271 pave the way for the development of gene-targeted and cell type-specific therapies, offering
272 promising avenues for more effective treatment strategies.
273

274 Materials and Methods

275

276 Mice

277 The experiments involving animals were conducted in strict adherence to national regulations and
278 European Community laws. Approval for the animal experiments was obtained from the Committee
279 for Animal Protection of the Austrian Ministry of Science. The VIP-cre transgenic mice (Vip$^{tm1(cre)Zjh}$/J,
280 RRID:IMSR_JAX:010908), which express Cre-recombinase under the VIP promoter, were initially

281 obtained from The Jackson Laboratory through Charles River and were maintained on a C57BL/6N
282 background. C57BL/6N wild-type mice were obtained from Charles River. The mice were housed in
283 ventilated cages, with 3 to 5 mice per group, and had access to food and water *ad libitum.* Standard
284 laboratory conditions were maintained, including a 12-hour light/dark cycle with lights on at 7:00
285 A.M. Male and female heterozygous mice aged 10 to 14 weeks were used for the experiments.
286 For VIP co-localization studies only, VIP-cre homozygous mice were crossed with Ai9-tdTomato mice
287 (B6.Cg-Gt(ROSA)$^{26Sortm9(CAG\text{-}tdTomato)Hze}$/J; RRID:IMSR_JAX:007909) to generate VIP-cre:Ai9 double
288 heterozygous mice.
289

290 **Preparation of the vectors**

291 The AAV2/6 vectors carried either tetanus toxin light chain (TeLC) fused with a GFP tag or GFP alone.
292 The reading frames for TeLC/GFP and GFP were inverted within a FLEX cassette allowing for AAV-
293 mediated transgene expression specifically in VIP-INs at the injection site (40). Pseudotyped AAV2/6
294 vectors were produced and purified as previously described (41). In brief, HEK293 cells were co-
295 transfected with rAAV plasmids pAM-FLEX-TeLC-GFP or pAM-FLEX-GFP, along with pDP1rs helper
296 plasmids, using calcium phosphate co-precipitation. The resulting cleared cell lysates, treated with
297 Benzonase, were purified by HPLC on AVB columns and subsequently subjected to dialysis. The AAV
298 titers were determined by PCR as the number of genomic particles per milliliter (gp/ml), with titers of
299 1×10^{12} gp/ml for pAM-FLEX-TeLC-GFP and 1×10^{13} gp/ml for pAM-FLEX-GFP. To ensure the full
300 activity of TeLC, we evaluated all seizure-related factors after 14 days following the injection,
301 considering that the silencing effects would remain permanent thereafter (40).
302 For experiments involving DREADD (Designer Receptors Exclusively Activated by Designer Drugs)
303 technology, we used AAV6-hSyn-DIO-hM3D(Gq)-mCherry (Addgene 44361), kindly provided by B.
304 Roth and obtained from Addgene (USA). This vector was packaged into AAV2 capsids and injected
305 with a final titer of 9×10^{12} gp/ml, as previously described in detail (42).
306

307 **Surgery**

308 Stereotaxic surgeries were performed following previously described procedures (14). Male and
309 female heterozygous VIP-cre transgenic mice aged 10–14 weeks were administered the analgesic
310 drug meloxicam (5 mg/kg, s.c.) 15 min prior to surgery. Anesthesia was induced using 150 mg/kg of
311 ketamine (50 mg/ml stock solution; Ketasol, Ogris Pharma Vertriebs-GmbH, Wels, Austria) via
312 intraperitoneal injection, followed by maintenance anesthesia using 1–3% sevoflurane (3% for
313 induction, 1–1.5% for maintenance; Sevorane, Abbott, IL, USA) administered through a veterinary
314 anesthesia mask with oxygen (400 ml/min) as the carrier gas. The mice were secured in a stereotaxic
315 frame (David Kopf Instruments, Tujunga, CA, USA), and the skin above the skull was opened.

316 A telemetry EEG transmitter (ETA-F10, Data Sciences International, St. Paul, MN, USA) was placed in
317 a subcutaneous pocket on the left abdominal wall, with the electrode wires carefully pulled through
318 a subcutaneous channel between the pocket and the exposed skull. The pocket was sutured after the
319 procedure. Bilateral holes were drilled for AAV vector injection, insertion of an epidural recording
320 electrode (coordinates from bregma: posterior 2.0 mm, lateral, 1.6 mm), and placement of an
321 epidural reference electrode (coordinates from bregma: posterior 2.0 mm, lateral -1.6 mm). In a
322 subset of animals, local field potential (LFP) was recorded to detect focal epileptiform activity. For
323 these animals, a depth electrode (coordinates from bregma: posterior 1.9 mm, lateral 1.6 mm,
324 ventral 1.9 mm) was inserted above the ipsilateral CA1 instead of the epidural recording electrode.
325 The electrodes were secured to the skull using dental cement (Paladur, Heraeus Kulzer supplied by
326 Henry Schein, Innsbruck, Austria).

327 Subsequently, 1 µL of AAV-TeLC-GFP or AAV-GFP was injected unilaterally into the transition zone of
328 the left ventral subiculum and sector CA1 at a rate of 0.1 µl/min (coordinates from bregma: posterior
329 3.6 mm, lateral 3.2 mm, ventral 3.6 mm). The cannula remained in place for 10 minutes before slowly
330 being retracted. In mice that underwent depth electrode recording, bilateral injections of AAV-TeLC-
331 GFP or AAV-GFP were performed with the same coordinates as the unilateral injection (posterior 3.6
332 mm, lateral ± 3.2 mm, ventral 3.6 mm) to enhance the silencing effect of subicular VIP-INs in the

333 subiculum. The AAV-TeLC-GFP or AAV-GFP injections were performed on the same days in mice from
334 the same litters. Following surgery, the mice were housed individually.
335 For specific and reversible chemogenetic activation of VIP-INs in the subiculum, rAAV6-hSyn-DIO-
336 hM3D(Gq) (0.5 µL) was injected at a rate of 0.1 µL/min into the left ventral subiculum of male and
337 female heterozygous VIP-cre mice. The injections followed the same coordinates and protocol as the
338 AAV-TeLC injections.
339

340 **Induction of the chronic model of epilepsy by IH-KA injection**

341 To induce the chronic model of epilepsy, intrahippocampal injections of kainic acid (KA, Ascent
342 Scientific, North Somerset, UK) were performed during transmitter implantation surgery in male and
343 female heterozygous VIP-cre mice. KA (200 ng in 140 nL phosphate-buffered saline [PBS], pH 7.4) was
344 injected at a speed of 140 nL/min using a 1 µL Hamilton syringe connected to a precision syringe
345 pump (Nexus 3000 by Chemyx, Science Products, Hofheim, Germany). The injection cannula, a
346 polyimide-coated silica capillary tubing with a 150 µm outer diameter (TSP075150, Polymicro
347 Technologies, Phoenix, AZ, USA), was attached to the syringe using fine-bore polythene tubing
348 (800/100/100; Smiths Medical International, Kent, UK). Following the injection, the cannula
349 remained in place for 3 minutes before being slowly retracted. EEG recording was initiated
350 immediately as the mice were transferred back to their cages. All mice in the study experienced
351 acute seizures after the KA injection. The EEG activity returned to baseline after approximately 24
352 hours, indicating the end of status epilepticus (SE). As an exclusion criterion, mice that did not
353 develop SRSs before vector injection (3 mice) were excluded from the rest of study.
354

355 **Experimental model of acute focal seizures induced by injection of IH-KA**

356 To establish an experimental model of acute focal seizures, male and female heterozygous VIP-cre
357 mice were anesthetized briefly with 3% sevoflurane, ten days after the injection of AAV-hM3Dq
358 vector. Subsequently, they received an intrahippocampal injection of KA (7 ng in 500 nL phosphate-

359 buffered saline [PBS], pH 7.4) at a speed of 500 nL/min using a 1 µL Hamilton syringe connected to a
360 precision syringe pump. The injection cannula, a polyimide-coated silica capillary tubing with a 150
361 µm outer diameter, was attached to the syringe via fine-bore polythene tubing. After the injection,
362 the cannula was left in place for 1 minute before being slowly retracted, and the mice were promptly
363 transferred back to their cages for EEG recording.
364 Under these experimental conditions, KA induced acute seizures in 100% of the mice and the EEG
365 activity returned to baseline after 1-2 hours.
366

367 **Transient activation of VIP-INs using the DREADD system**

368 Seven days following the injection of AAV-hM3Dq, male and female heterozygous VIP-cre mice
369 underwent the insertion of an EEG-transmitter, allowing for continuous telemetric EEG recording
370 until the end of the study. Three days later (day 10 after vector injection), the selective hM3Dq
371 agonist clozapine-N-oxide (CNO, Tocris Bioscience, Abingdon, UK) was intraperitoneally injected at a
372 dose of 5 mg/kg. CNO was dissolved in sterile 0.9% NaCl at a concentration of 1.0 mg/ml. The control
373 group of mice that received the AAV-hM3Dq vector injection were injected with saline instead of
374 CNO.
375 Thirty minutes after CNO administration, acute focal-onset seizures were induced by
376 intrahippocampal injection of KA. The effects of activating VIP-INs on the number and duration of
377 seizures were assessed through telemetric EEG recordings.
378

379 **EEG and LFP recordings**

380 Continuous EEG and LFP recordings were conducted using a telemetry system and Ponemah analysis
381 software version 6.0.x. (Data Sciences International, St. Paul, MN, USA). The recordings were
382 obtained at a sampling rate of 1000 Hz and saved onto external hard disk drives without any
383 predetermined filter cutoff. To complement the analysis of behavior, a subset of animals was
384 subjected to continuous video recordings. These recordings were captured using Axis 221 network

385 cameras (Axis Communications, Lund, Sweden) along with infrared illumination (Conrad Electronics,
386 Leonding, Austria).
387

388 **Analysis of epileptiform activity**

389 For the analysis of epileptiform activity, two independent observers visually examined EEG and LFP
390 traces using Ponemah analysis software version 6.0.x. (Data Sciences International, St. Paul, MN,
391 USA). Seizures were identified as periods of uninterrupted activity with a minimum duration of 10
392 seconds and an amplitude at least twice that of the baseline, accompanied by postictal depression
393 (EEG signal below baseline amplitude). In our cohort database, this signal corresponded to seizure
394 Racine scale 3-5 (14), which was determined based on simultaneous video recordings.
395 To differentiate epileptiform spikes from normal baseline spikes or those caused by electrical or
396 mechanical artifacts, specific features of each spike were analyzed, including amplitude, duration,
397 frequency, and inter-spike intervals. An offline spike detection algorithm was implemented using a
398 custom-made program developed in MATLAB 2020b (Mathworks, Natick, MA, USA). This program
399 automatically detected spikes and spike trains based on threshold crossings. Initially, movement
400 artifacts were automatically removed, and then a blind reviewer adjusted the spike detection
401 threshold to account for variations in individual signal amplitude, ranging from 4 to 6 standard
402 deviations. Epileptiform interictal spikes were defined as short (10 to 80 ms) high-amplitude
403 discharges above the threshold, with a minimum peak distance of 150 ms. Detected spikes were
404 visually confirmed by the reviewers.
405 Spike trains were defined as sequences of three or more spikes occurring with a frequency greater
406 than 1 Hz and lasting for at least 1 second (43). Since postictal depression is not always visible during
407 SE, in addition to comparing the groups based on the number of seizures, the severity of SE was also
408 assessed by analyzing the number of epileptiform spikes and spike trains (44).
409

410 **Wake/Sleep annotation**

411 We employed our previously published algorithm to automatically differentiate between sleep and
412 wakefulness in single-channel EEG recordings by utilizing high-frequency oscillations (> 200 Hz). This
413 approach achieved an accuracy of over 90 percent (45). Subsequently, we employed the SCOPRISM
414 algorithm (46) to differentiate between rapid eye movement sleep (REMS) and non-rapid eye
415 movement sleep (NREMS). The original SCOPRISM algorithm suggests dividing theta by delta and
416 employing a threshold of 0.75, classifying values above the threshold as REMS and those below as
417 NREMS. However, in our cohort database, where the vigilance state is manually determined based on
418 EEG analysis from the visual, somatosensory, and motor cortex, as well as the EMG signal, we
419 observed that a threshold of 0.8 produced more accurate results. Consequently, we decided to utilize
420 this threshold for the remainder of the study.
421 For epileptic animals, we compared the vigilance state not only after vector injection (during a 24-
422 hour period 14 days after vector injection), but also before vector injection to establish a baseline for
423 the groups' vigilance patterns. To assess the general sleep architecture, we derived the proportion of
424 vigilance states for non-overlapping two-hour observation episodes throughout the 24-hour period,
425 as well as the distribution of bout lengths for each vigilance state, from the scoring vectors.
426

427 **Double- and triple-labeling by immunofluorescence**

428 At the end of the experiment, which typically occurred 4-8 weeks after the initial vector injection, the
429 mice were deeply anesthetized and euthanized with thiopental (150 mg/kg). Perfusion with 4%
430 paraformaldehyde was then performed to fix the brains. Horizontal sections with a thickness of 40
431 µm were prepared for the immunofluorescence study.
432 Monoclonal rat anti-GFP (1:2000; catalog #04404-84, Nacalai Tesque, obtained through GERBU
433 Biotechnik; RRID:AB_10013361) and rabbit anti-VIP (1:500; catalog #20077, Immunostar, obtained
434 through ACRIS, RRID: AB_572270) were used as primary antibodies. The antibodies had been
435 previously characterized by the supplier or validated in previous experiments (16). For the double
436 labeling of GFP and VIP, the horizontal sections were incubated with 10% normal goat or horse

437 serum (catalog #26050088, Gibco, through Thermo Fischer) in Tris-HCl-buffered saline (TBS; 50 mm),
438 pH 7.2, containing 0.4% Triton X-100 (TBS-Triton) for 90 minutes. Subsequently, the sections were
439 incubated with the respective primary antisera for 16 hours at room temperature.
440 After washing with TBS-Triton, a secondary reaction was performed by simultaneously incubating the
441 sections with a donkey antibody directly conjugated to Alexa Fluor 488 (1:500; catalog #A-21208,
442 Molecular Probes) for GFP and a biotin-coupled goat anti-rabbit antibody (1:500; catalog #A-16027,
443 Invitrogen) for VIP at room temperature for 120 minutes. The sections were then washed with TBS
444 and incubated in streptavidin (1:100; catalog #SA 5649, Vektor) for 100 minutes. Following another
445 wash with TBS, the sections were mounted on slides in gelatin and covered with mounting medium
446 (86% glycerol and 2.5% DABCO in PBS).
447 To characterize the three subgroups of subicular VIP-INs, we utilized VIP:Ai9-tdTomato mice. These
448 mice were deeply anesthetized with thiopental (150 mg/kg) and perfused to prepare 40-µm-thick
449 coronal sections for immunofluorescence analysis. We already reported a high level of colocalization
450 between tdTomato and VIP in VIP:Ai9-tdTomato mice, indicating a sensitivity and specificity
451 exceeding 90% (47). For the immunofluorescence labeling of VIP (tdTomato), Calretinin (CR), and
452 Cholecystokinin (CCK), the free-floating coronal sections were incubated with 10% normal goat
453 serum (catalog #5425, Cell Signaling) in Tris-HCl-buffered saline, pH 7.2, containing 0.4% Triton X-100
454 (TBS-Triton) for 90 minutes at room temperature. Subsequently, the sections were incubated with
455 the respective primary antisera for 20 h at 4°C. The primary antibodies used were rabbit anti-CR
456 antibody (1:1000; catalog 7769/3H, SWant) and guinea pig anti-CCK antibody (1:1000; catalog #438-
457 004; Synaptic Systems).,
458 After washing with TBS-Triton, a secondary reaction was performed by simultaneously incubating the
459 sections with a rabbit antibody directly conjugated to Alexa Fluor 488 (1:500; catalog #4412S, Cell
460 Signaling) for CR and with a guinea pig antibody directly conjugated to Alexa Fluor 647 (1:500;
461 catalog #ab150187, Abcam) for CCK at room temperature for 120 minutes. The sections were then

462 washed with TBS and mounted on slides with gelatin. Finally, the slides were covered with mounting
463 medium composed of 86% glycerol and 2.5% DABCO in PBS.
464 To determine the number of neurons co-expressing TeLC/GFP and VIP at the AAV vector injection
465 site, microphotographs of 40-µm-thick horizontal double immunofluorescence-labeled sections (four
466 sections per mouse, ten mice) were captured at 20× primary magnification using a fluorescence
467 microscope (Imager.M1, Carl Zeiss). The acquired images were imported into NIH ImageJ 1.51d
468 (National Institutes of Health, Bethesda, MD, USA), and the photographs of the individual channels
469 were displayed side by side for analysis. The specific area of interest for evaluation was defined by
470 the region containing GFP-positive neurons, which were directly affected by the vector injection. The
471 numbers of single-labeled (TeLC/GFP or VIP) and double-labeled (co-expressing TeLV/GFP and VIP)
472 cells were manually determined by visual inspection of the images.
473

474 **Statistical Analyses**

475 Statistical analyses were performed using GraphPad Prism statistical software (version 9.1.1;
476 GraphPad Software). Due to the significant variation in seizure numbers observed in the IH-KA model
477 (37), the ratio of seizures for each animal was calculated in addition to reporting the raw number and
478 duration of seizures. The ratio was determined by comparing the number (or duration) of seizures
479 after vector injection to the number (or duration) of seizures before injection using the formula
480 (seizures after - seizures before) divided by (seizures after + seizures before).
481 The differences between the two groups were analyzed using the non-parametric Mann–Whitney U
482 test. To compare seizures, epileptiform spikes, spike trains, and sleep-related factors before and after
483 vector injection over a specific time period, a two-way repeated-measures ANOVA was conducted,
484 followed by Šídák's multiple-comparison post hoc test. A p-value of less than 0.05 was considered
485 statistically significant. Graphs were generated using GraphPad Prism 9.1.1 and MATLAB 2020b.

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Data availability

The EEG recordings and all algorithms implemented in this work can be made available upon request by contacting the first author (Dr. Sadegh Rahimi, Medical University of Innsbruck, Austria, sadegh.rahimi@i-med.ac.at).

Author contributions

S.R. designed the experiments, conducted surgeries, performed EEG recordings and analyses, and wrote the manuscript.

P.S. contributed to the analysis, and revised the draft manuscript.

P.M. contributed to the depth electrode recordings.

A.S. analyzed the interictal spikes and the circadian change in seizures, and contributed to immunohistochemistry assessment.

A.R.-P. contributed to immunohistochemistry assessment, and revised the draft manuscript.

R.T. provided vectors for circuit manipulation.

M.D. conceived and designed the experiments and revised the manuscript.

Competing interests

The authors declare no competing interests.

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Figure legends

Fig 1. Silencing VIP-INs of the ventral subiculum in epileptic mice alleviates seizures.

(A) Representative example of triple labeling in brain slices from the ventral subiculum of VIP:Ai9-tdTomato mice (n = 3).

(B) Study protocol and injection coordinates for intrahippocampal KA (IH-KA, 200 ng), AAV-TeLC-GFP (n=8), and AAV-GFP (n=8). Seizure frequency and duration were evaluated on Day one (SE), the six days following KA injection (Day 2 to Day 7), as well as on the six days commencing two weeks after vector injection (Day 22 to Day 27).

(C) Representative seizure recorded 3 days after IH-KA injection. Seizures are typically preceded by a series of epileptiform spikes and were identified as continuous activity lasting at least 10 seconds with an amplitude at least twice that of the baseline, accompanied by postictal depression.

(D) During SE, the median (range) number of seizures was 10 (1 to 19) for the GFP group (n=8) and 8.5 (1 to 14) for the TeLC group (n=8), with no significant difference between the groups (two-tailed Mann-Whitney test; p = 0.625).

(E) The median (range) seizure ratio was 0.55 (-1.00 to 0.86) for the GFP group (n=8) and -0.33 (-1.00 to 0.42) for the TeLC group (n=8), with a significant difference according to the two-tailed Mann-Whitney test (p = 0.022).

(F) The reduction in the number of seizures was more prominent during the light phase (p = 0.044, post-hoc Šídák test) compared to the dark phase (p = 0.369, post-hoc Šídák test) according to two-way ANOVA test.

(G) During SE, the median (range) cumulative duration of seizures was 314.1 s (10.34 s to 1098 s) for the GFP group (n=8) and 266.10 s (17 s to 703 s) for the TeLC group (n=8), with no significant difference between the groups according to the two-tailed Mann-Whitney test (p = 0.645).

(H) The median (range) seizure duration ratio was 0.66 (-1.0 to 0.96) for GFP mice (n=8) and -0.36 (-1.0 to 0.34) for TeLC mice (n=8), with a significant difference according to the two-tailed Mann-Whitney test (p = 0.027).

(I) The decrease in the cumulative duration of seizures was more pronounced during the light phase (p = 0.042, post-hoc Šídák test) compared to the dark phase (p = 0.323, post-hoc Šídák test) according to the two-way ANOVA test.

Male individuals are represented by half-filled shapes in both groups.

Mouse coronal slices in (B) are adapted from the Allen Mouse Brain Atlas, available online: http://atlas.brain-map.org/atlas. The walking mouse (right) is sourced from https://scidraw.io.

Figure 2. Silencing of VIP-INs of the ventral subiculum does not induce seizures in healthy mice.

(A) Study protocol and injection coordinates for intrahippocampal AAV-TeLC-GFP (*n*=5) and AAV-GFP (*n*=5) injections in non-epileptic mice, followed by long-term telemetric EEG recordings.

(B) Representative 1-minute EEG-trace obtained 14 days after AAV-TeLC injection. No seizures were observed during the 50-day recording period.

(C) Rate of GFP expression in cells. At the injection site, the median (range) percentage of VIP-INs expressed GFP (101 cells out of 169 cells) was 46.67% (41.18% to 90%), while 95.84% (82.35% to 100%) of the GFP-expressing interneurons also co-expressed VIP (101 cells out of 108 cells; *n*=4 mice from the GFP group, *n*=4 mice from the TeLC group).

(D) Study protocol and injection coordinates for bilateral intrahippocampal AAV-TeLC-GFP (*n*=4) and AAV-GFP (*n*=4) injections in non-epileptic mice, followed by long-term LFP recording from dorsal CA1. No seizures were observed during the 21-day recording period.

(E) Median (range) cumulative number of epileptiform spikes (for the entire 24-hour assessment) was 148 (3 to 1687) for the GFP group (*n*=4) and 1500 (24 to 4723) for the TeLC group (*n*=4). There was no significant difference between the groups according to the two-tailed Mann-Whitney test (p=0.343).

(F) Median (range) number of spike trains was 0.0 (0.0 to 37) for the GFP group (*n*=4) and 13.50 (0.0 to 31) for the TeLC group (*n*=4), with no significant difference between the groups according to the two-tailed Mann-Whitney test ($p > 0.99$).

Mouse coronal slices in (A) and (D) are adapted from the Allen Mouse Brain Atlas, available online: http://atlas.brain-map.org/atlas.

Figure 3. Activating VIP-INs of the ventral subiculum in model of acute seizures results in increased seizure frequency.

(A) Study protocol and injection coordinates for AAV-hM3D(Gq) (n=15) and intrahippocampal KA (IH-KA, 7 ng) injections. The animals were blindly divided into two groups, NaCl (n=6) and CNO (n=9), receiving intraperitoneal (i.p.) injection of NaCl and CNO at Day 10, respectively. The i.p. injection was administered 30 minutes prior to the IH-KA injection.

(B) Number of seizures for each individual animal in the first 60 minutes after IH-KA injection (10-minute bin).

(C) After IH-KA injection, animals in the NaCl (n=6) and CNO (n=9) groups developed seizures. A significant difference was found between the groups (Two-way ANOVA; F = 5.353, p = 0.037).

(D) Median (range) number of seizures in the NaCl group was 2 (1 to 4), while it was 5 (2 to 7) for the CNO group. A significant difference was reported according to the two-tailed Mann-Whitney test (p = 0.034).

(E) Median (range) cumulative duration of seizures for the NaCl group was 76.02 s (33.08 s to 150.50 s), and for the CNO group it was 138.70 s (51.89 s to 225.0 s). There were no significant differences between the groups, despite a strong trend (p = 0.087, according to the two-tailed Mann-Whitney test).

Male individuals are represented by half-filled shapes in both groups.

Mouse coronal slices in (B) are adapted from the Allen Mouse Brain Atlas, available online: http://atlas.brain-map.org/atlas.

Figure 4. How manipulating subicular VIP-INs can alter seizure occurrence.

(A) In the balanced state (left), VIP basket cells (VIP-BC) directly innervate principal cells (PCs), while interneuron-specific type II and III VIP-interneurons (IS 2/3) primarily target other interneurons, resulting in a mixture of inhibition and disinhibition of PCs. However, when both groups of VIP-interneurons were silenced (right), we observed a predominant role of IS type II and III interneurons, leading to the lack of seizure development.

(B) In the epileptic condition, silencing VIP-INs may have an opposite effect (left). Silencing VIP basket cells can exacerbate seizures, while silencing IS type II and III VIP-INs indirectly increases the overall inhibition of principal cells. Our experimental results support a prominent role of IS type II and III VIP-INs over VIP basket cells in the development of epilepsy. Activation of VIP-INs (right) can also have an opposite effect. Activation of VIP basket cells can decrease seizure occurrence, but activating IS type II and III VIP-INs can enhance seizure development. However, the outcome of the experiment highlights the greater impact of IS type II and III compared to VIP basket cells in the development of seizures.

Supplementary Figure S1. No difference was observed in severity of SE between groups.

(A) The study protocol and the coordinates and for intrahippocampal injection of KA (IH-KA) (*n*=16). The animals were blindly divided into two groups, GFP (*n*=8) and TeLC (*n*=8), receiving AAV-GFP or AAV-TeLC after 7 days, respectively.

(B) Representative example of status epilepticus (SE) in the first 24 hours after intrahippocampal KA injection. Postictal depression is not always visible during SE.

(C) During SE, the median (range) hourly number of epileptiform spikes was 3149 (1786 to 4071) for the GFP group (n=8) and 3767 (206.8 to 4633) for the TeLC group (*n*=8). No significant difference was found between the groups according to a two-way ANOVA test ($F = 0.252$, $p = 0.623$).

(D) The median (range) number of epileptiform spikes in the GFP group (*n*=8) was 54511 (32651 to 153106), and for the TeLC group (n=8) it was 68674 (33018 to 153892). There was no significant difference between the groups according to a two-tailed Mann-Whitney test ($p = 0.878$).

(E) During SE, the median (range) hourly number of spike trains was 283 (110.4 to 401.6) for the GFP group (N=8) and 324.2 (6.62 to 471.90) for the TeLC group (*n*=8). No significant difference was found between the groups according to a two-way ANOVA test ($F = 0.058$, $p = 0.813$).

(F) The median (range) number of spike trains was 3519 (1993 to 18226) for the GFP group (*n*=8) and 6439 (1996 to 15306) for the TeLC group (*n*=8). There was no significant difference between the groups according to a two-tailed Mann-Whitney test ($p = 0.645$).

Male individuals are represented by half-filled shapes in both groups.

Mouse coronal slices in (A) are adapted from the Allen Mouse Brain Atlas, available online: http://atlas.brain-map.org/atlas.

Supplementary Figure S2. Daily number of seizures before and after vector injection.

(A) The number of seizures during Day 2 to Day 7 (before vector injection). Both groups developed spontaneous seizures. A two-way ANOVA test revealed a significant difference between days ($F = 4.879$, $p = 0.005$), but not between groups ($F = 1.829$, $p = 0.198$, $n = 8$ for both GFP and TeLC groups).

(B) The number of seizures during Day 22 to Day 27 (after vector injection). Both groups had a stable daily number of seizures ($F = 1.540$, $p = 0.188$, no significant difference between days, according to a two-way ANOVA test). However, the TeLC group had a significantly lower number of seizures compared to the GFP group ($F = 6.296$, $p = 0.0250$, $n = 8$ for both groups).

Male individuals are represented by half-filled shapes in both groups.

Supplementary Figure S3. Circadian variation in seizure ratio among individual animals.

(A) The seizure ratio was calculated for each animal by comparing the number of seizures after vector injection (Day 22 to Day 27) to the number of seizures before vector injection (Day 2 to Day 7).

(B) Each cycle represents a single animal from either the GFP (n = 8) or TeLC group (n = 8), with each rectangle indicating the seizure ratio during one hour of the 24-hour day.

Supplementary Figure S4. Silencing of VIP-INs of the ventral subiculum in epileptic mice causes no changes in sleep pattern.

(A) Before vector injection (three days after intrahippocampal KA injection [IH-KA]), both groups exhibited altered patterns in the three vigilance states due to epilepsy, resulting in the disappearance of the typical nocturnal pattern of wakefulness.

(B) Two weeks after vector injection (Day 22), both groups showed a restoration of the nocturnal pattern of wakefulness and a recovery in sleep architecture.

(C) During the light phase, before vector injection, there were no significant differences in the percentage of wakefulness, non-rapid eye movement sleep (NREMS), and rapid eye movement sleep (REMS) between the GFP (n=8) and TeLC (n=7, since signal from one mouse was not fit to our sleep scoring algorithm) groups. Also after vector injection, there were no significant differences observed in the percentage of wakefulness, NREMS, and REMS between the groups. Data were analyzed by fitting a mixed model.

(D) During the dark phase, before and after vector injection, there were no significant differences in the percentage of wakefulness, NREMS, and REMS between the GFP (n=8) and TeLC (n=7, since signal from one mouse was not fit to our sleep scoring algorithm) groups. Data were analyzed by fitting a mixed model.

(E) During the light phase, before vector injection, there were no significant differences in the bout length of wakefulness, NREMS, and REMS between the GFP (n=8) and TeLC (n=7, since signal from one mouse was not fit to our sleep scoring algorithm) groups. Also after vector injection, there were no significant differences observed in bout length of wakefulness, NREMS, and REMS between the groups. Data were analyzed by fitting a mixed model.

(F) During the dark phase, before vector injection, there were no significant differences in the bout length of wakefulness, NREMS, and REMS between the GFP (n=8) and TeLC (n=7) groups. After vector injection, there were no significant differences observed in the bout

length of wakefulness and NREMS between the two groups, but a significant difference was observed in the bout length of REMS (p = 0.02, Šídák post hoc test). However, analyzed by fitting a mixed model, also for REMS, no difference was observed between groups (F = 2.77, p = 0.12).

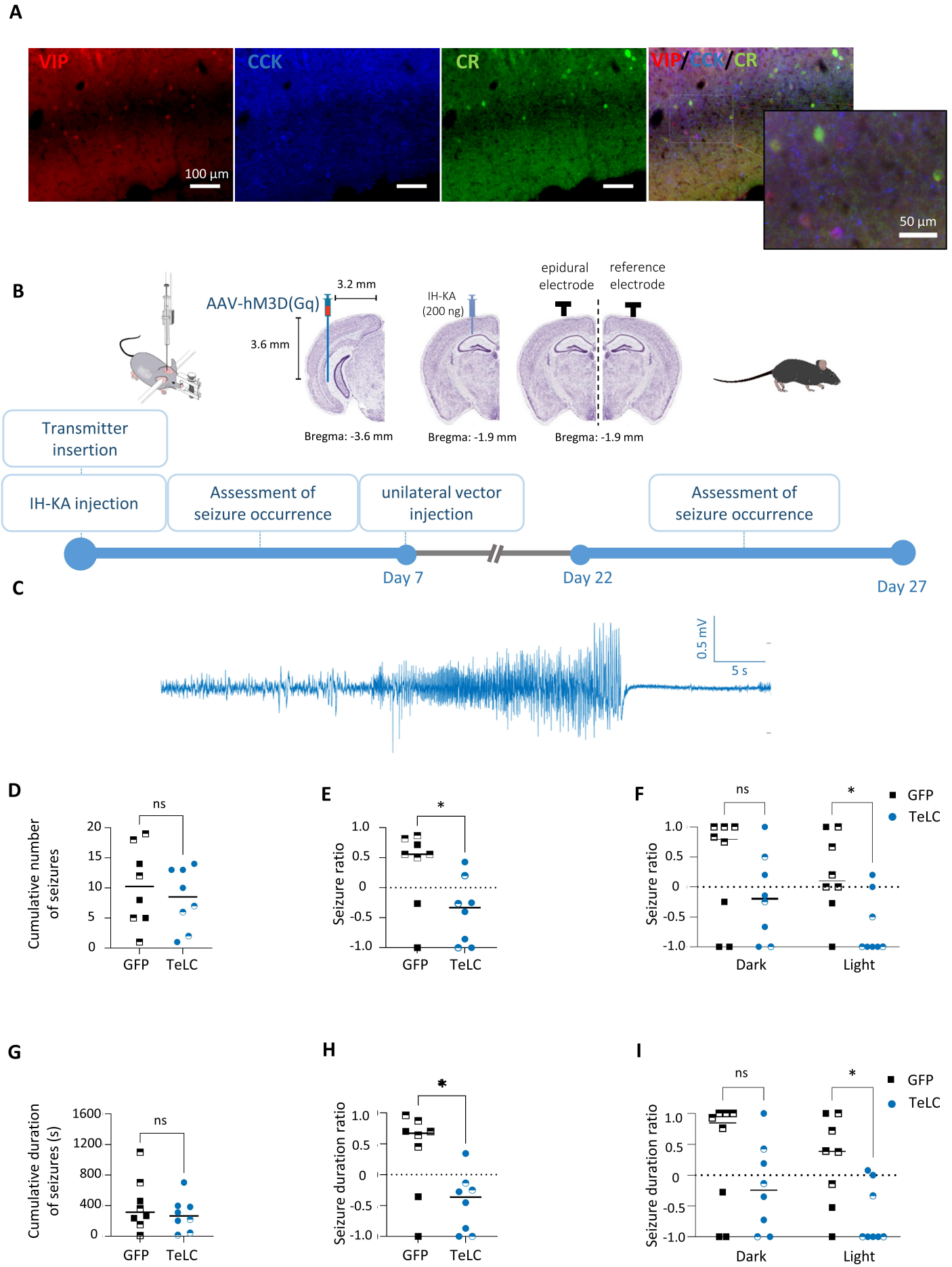
Male individuals are represented by half-filled shapes in both groups.

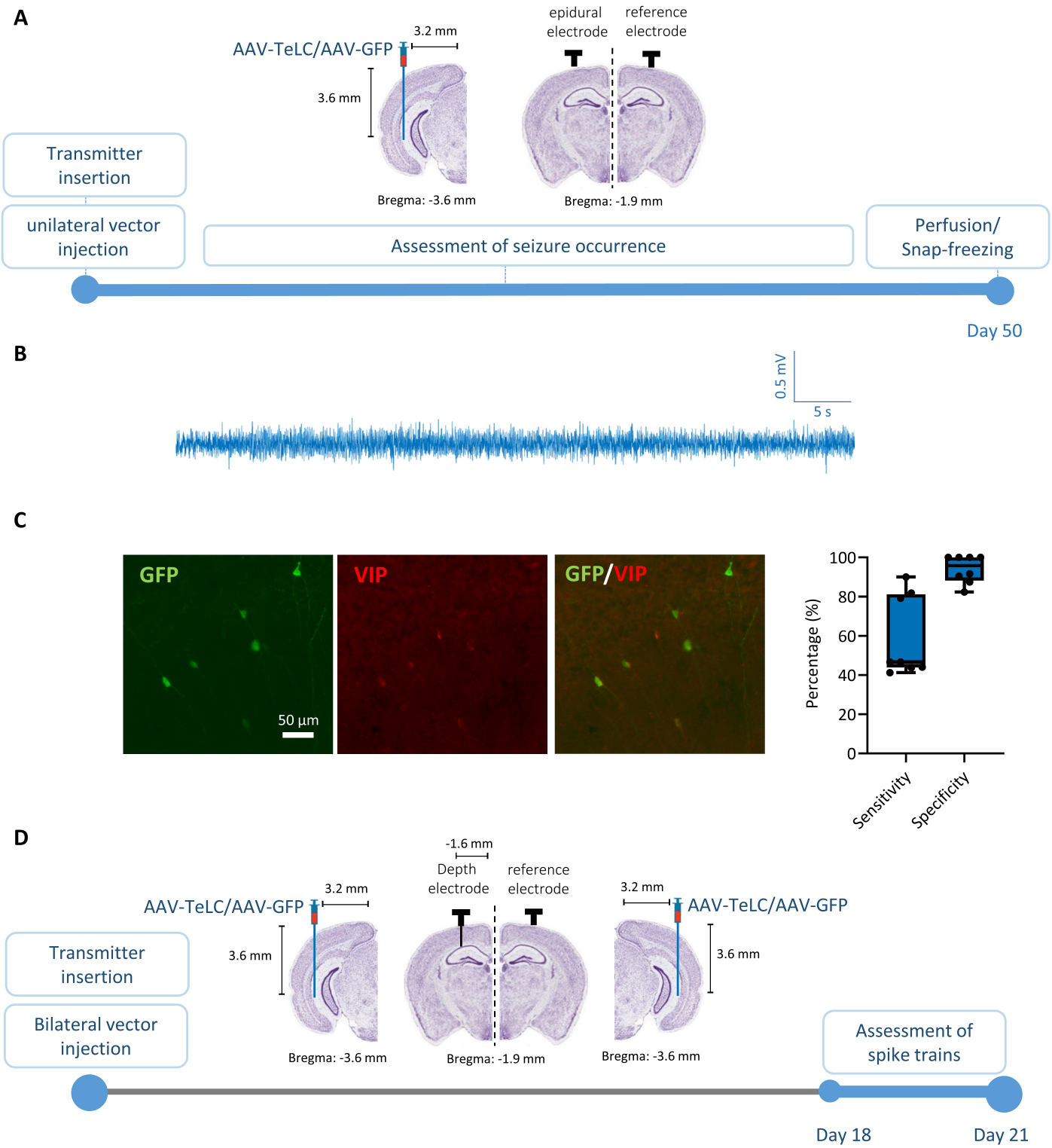
Supplementary Figure S5. Daily duration of seizures before and after vector injection.

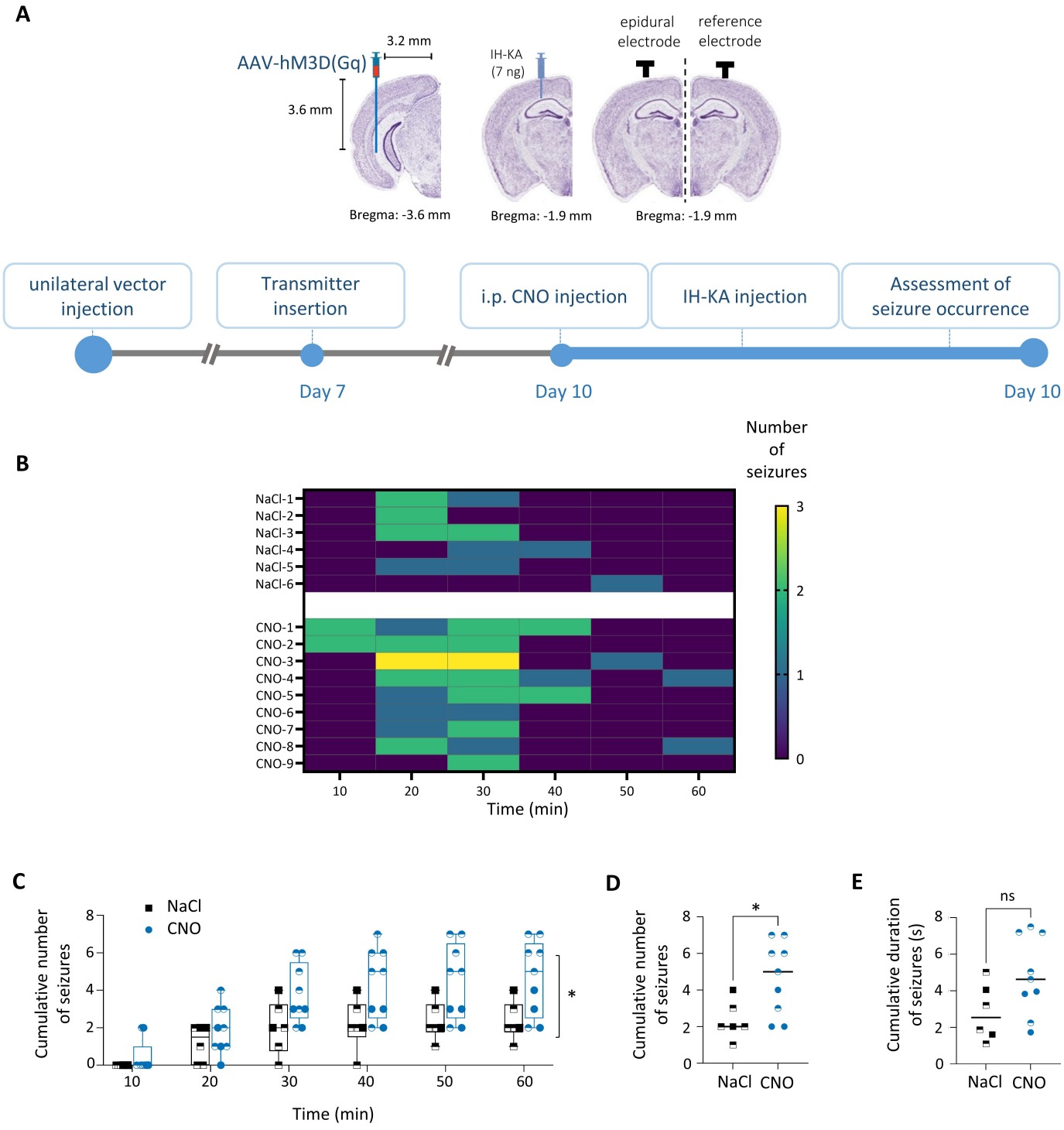
(A) The daily time spent in seizures during Day 2 to Day 7 (before vector injection). Both groups developed spontaneous seizures. There was a significant difference in the time spent in seizures between days (F = 4.561, p = 0.005), but no significant difference between the GFP and TeLC groups (F = 1.695, p = 0.687, n = 8 for both groups).

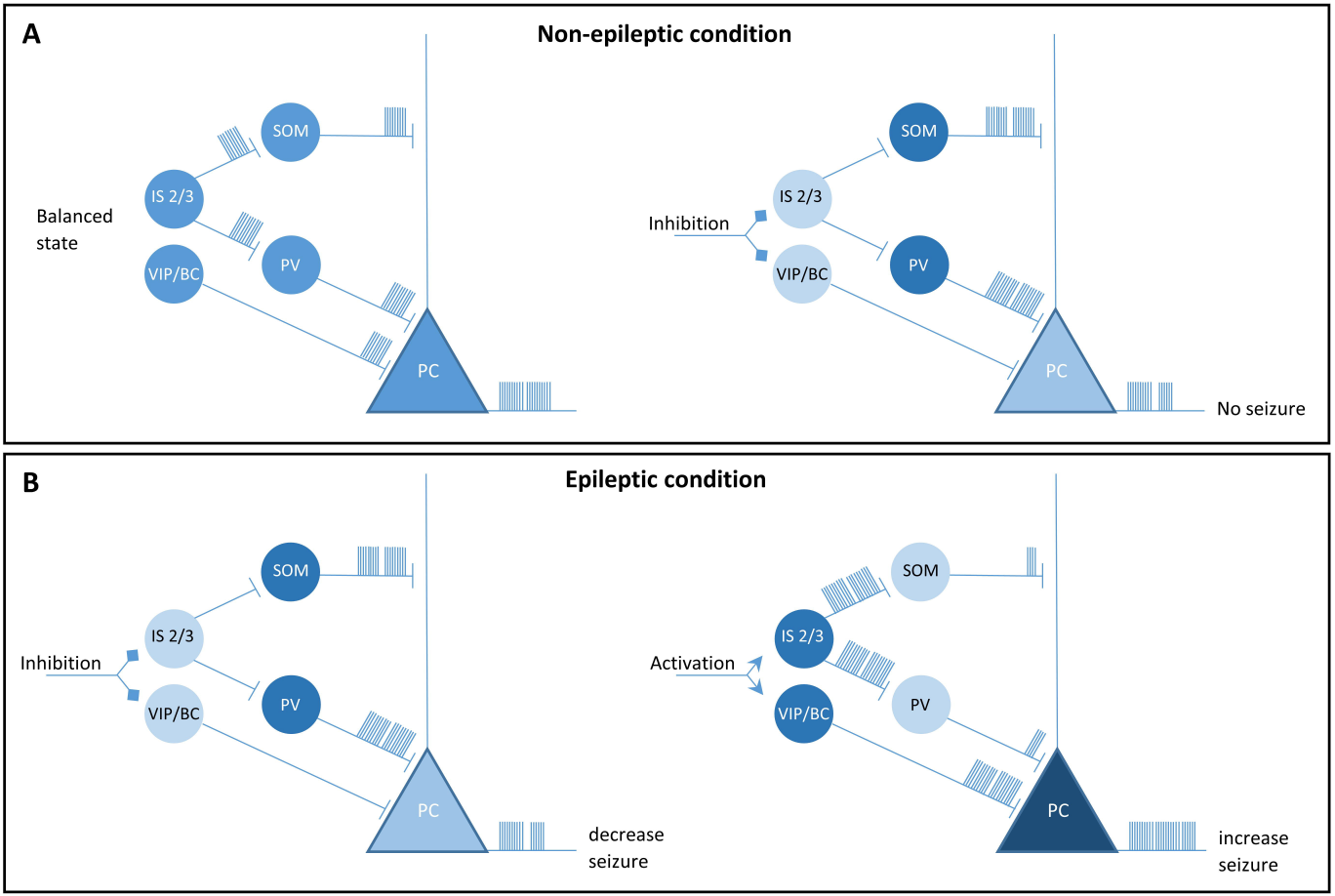
(B) The daily time spent in seizures during Day 22 to Day 27 (after vector injection). Both groups maintained stable values for the time spent in seizures (F = 1.286, p = 0.287, no significant difference between days, according to two-way ANOVA test); however, the mice in the TeLC group spent significantly less time seizures compared to the GFP group (F = 10.350, p = 0.006, n = 8 for both groups).

Male individuals are represented by half-filled shapes in both groups.


A
VIP
CCK
CR
VIP/CCK/CR
100 µm
50 µm
B
AAV-hM3D(Gq)
3.2 mm
3.6 mm
IH-KA
(200 ng)
epidural
electrode
reference
electrode
Bregma: -3.6 mm
Bregma: -1.9 mm
Bregma: -1.9 mm
Transmitter
insertion
IH-KA injection
Assessment of
seizure occurrence
unilateral vector
injection
Assessment of
seizure occurrence
Day 7
Day 22
Day 27
C
0.5 mV
5 s
D
Cumulative number
of seizures
ns
GFP
TeLC
E
Seizure ratio
*
GFP
TeLC
F
Seizure ratio
ns
*
GFP
TeLC
Dark
Light
G
Cumulative duration
of seizures (s)
ns
GFP
TeLC
H
Seizure duration ratio
*
GFP
TeLC
I
Seizure duration ratio
ns
*
GFP
TeLC
Dark
Light


A
AAV-TeLC/AAV-GFP
3.2 mm
3.6 mm
epidural electrode
reference electrode
Bregma: -3.6 mm
Bregma: -1.9 mm
Transmitter insertion
unilateral vector injection
Assessment of seizure occurrence
Perfusion/ Snap-freezing
Day 50
B
0.5 mV
5 s
C
GFP
VIP
GFP/VIP
50 µm
Percentage (%)
100
80
60
40
20
0
Sensitivity
Specificity
D
-1.6 mm
Depth electrode
reference electrode
AAV-TeLC/AAV-GFP
3.2 mm
3.6 mm
Bregma: -3.6 mm
Bregma: -1.9 mm
Transmitter insertion
Bilateral vector injection
Assessment of spike trains
Day 18
Day 21
E
Cumulative number of epileptiform spikes
ns
5000
4000
3000
2000
1000
0
GFP
TeLC
F
Cumulative number of spike trains
ns
5000
4000
3000
2000
1000
0
GFP
TeLC


A
AAV-hM3D(Gq)
3.2 mm
3.6 mm
IH-KA
(7 ng)
epidural
electrode
reference
electrode
Bregma: -3.6 mm
Bregma: -1.9 mm
Bregma: -1.9 mm
unilateral vector injection
Transmitter insertion
i.p. CNO injection
IH-KA injection
Assessment of seizure occurrence
Day 7
Day 10
Day 10
B
Number of seizures
NaCl-1
NaCl-2
NaCl-3
NaCl-4
NaCl-5
NaCl-6
CNO-1
CNO-2
CNO-3
CNO-4
CNO-5
CNO-6
CNO-7
CNO-8
CNO-9
Time (min)
C
NaCl
CNO
Cumulative number of seizures
Time (min)
D
Cumulative number of seizures
NaCl
CNO
E
Cumulative duration of seizures (s)
ns
NaCl
CNO


A
Non-epileptic condition
Balanced state
IS 2/3
SOM
VIP/BC
PV
PC
Inhibition
IS 2/3
SOM
VIP/BC
PV
PC
No seizure
B
Epileptic condition
Inhibition
IS 2/3
SOM
VIP/BC
PV
PC
decrease seizure
Activation
IS 2/3
SOM
VIP/BC
PV
PC
increase seizure