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1 # Glucagon-like peptide-1 (GLP-1) and exendin-4 transiently enhance
2 GABA$_A$ receptor-mediated synaptic and tonic currents in rat
3 hippocampal CA3 pyramidal neurons

4

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24 **Abstract**

25 GLP-1 is a hormone that stimulates insulin secretion. Receptors for GLP-1 are also found in the brain,
26 including the hippocampus, the centre for memory and learning. Diabetes mellitus is a risk factor for
27 decreased memory functions. We studied effects of GLP-1 and exendin-4, a GLP-1 receptor agonist, on
28 γ-aminobutyric acid (GABA) signaling in hippocampal CA3 pyramidal neurons. GABA is the main
29 inhibitory neurotransmitter and decreases neuronal excitability. GLP-1 (0.01 – 1 nmol/L) transiently
30 enhanced synaptic and tonic currents and the effects were blocked by exendin(9–39). Ten pmol/L GLP-
31 1 increased both the spontaneous inhibitory postsynaptic current (sIPSC) amplitudes and frequency by
32 a factor of 1.8. In 0.1, 1 nmol/L GLP-1 or 10, 50 or 100 nmol/L exendin-4, only the sIPSC frequency
33 increased. The tonic current was enhanced by 0.01 – 1 nmol/L GLP-1 and by 0.5 – 100 nmol/L
34 exendin-4. When action potentials were inhibited by tetrodotoxin (TTX), IPSCs decreased and currents
35 were no longer potentiated by GLP-1 or exendin-4. In contrast, although the tonic current decreased in
36 TTX, it was still enhanced by GLP-1 or exendin-4. The results demonstrate GLP-1 receptor regulation
37 of hippocampal function and are consistent with GLP-1 receptor agonists enhancing $GABA_A$ signaling
38 by pre- and postsynaptic mechanisms.

39

40

41 **Key words**: Diabetes mellitus, cognition, GABA, $GABA_A$ receptor, type 2 diabetes, hippocampus, memory,
42 incretin.

43

44 In recent years compelling evidence has emerged suggesting that diabetes mellitus increases the risk for
45 cognitive impairments in the elderly (1-8). How this comes about is not resolved but interestingly, the
46 brain contains receptors for many metabolic hormones, among those receptors for insulin and the
47 incretins. To-date, with the exception of the hypothalamus, we know relatively little about how
48 metabolic hormones affect neuronal activity and thereby brain function. The hippocampus is central for
49 cognitive functions and is the centre for memory and learning (9; 10). It has prominent expression for
50 receptors activated by metabolic hormones (10). Furthermore, via neurons in the septum, the
51 hippocampus regulates the activity of a number of hypothalamic nuclei (11; 12). Glucagon-like
52 peptide-1 (GLP-1) is a gut hormone that is secreted by intestinal L-cells in response to food intake, and
53 the GLP-1 receptor is expressed in the hippocampus (10; 13). GLP-1 crosses the blood-brain barrier
54 but it is also a neurotransmitter produced by neurons with cell bodies in the brainstem (12-15). The
55 best known effects of GLP-1 are to stimulate insulin and inhibit glucagon secretion in a glucose
56 dependent manner in the pancreatic islets to regulate glucose homeostasis after a meal (13). Although
57 the GLP-1 receptor is expressed in the hippocampus (10; 16; 17) and GLP-1 and its mimetics e.g.
58 exendin-4, liraglutide, might potentially be used to treat cognitive declines related to diabetes mellitus
59 (6; 7), to-date not much is known about the effects of GLP-1 on neuronal signaling and hence how
60 GLP-1 affects cognition and hippocampal regulation of metabolic homeostasis.

61

62 The GLP-1 is a 30 amino acids long peptide and is derived from posttranslational processing of the
63 preproglucagon gene (18). Initially, the peptide GLP-1(1–37) was identified from this processing but
64 later it was shown that there were two shorter peptides, GLP-1(7–37) and GLP-1(7–36) amide, that
65 were the active species *in vivo*. The half-life of the peptides in plasma is very short, only about 1 to 2
66 min (13; 19) due to its degradation by the enzyme dipeptidyl peptidase-4 (DPP-4) (13). In the

67 pancreatic islets, the GLP-1 receptor is internalized following GLP-1 induced activation (20-23) and
68 passes through recycling endosomes before it appears in the plasma membrane again (23). The GLP-1
69 receptor is widely distributed in the brain (10; 16; 24), including in the hippocampal CA3 pyramidal
70 neurons (16; 25), and GLP-1 and other agonists at the GLP-1 receptor have been reported to regulate
71 food intake (26), be neuroprotective (27), anti-inflammatory (28) and modulate synaptic plasticity and
72 memory formation (28-32).

73

74 GABA the major inhibitory neurotransmitter in the central nervous system (CNS) activates synaptic
75 and extrasynaptic $GABA_A$ receptors that mediate synaptic and tonic currents, respectively, regulating
76 activity of neurons and neuronal circuits (33). Metabolic hormones are emerging as modulators of
77 GABA signaling in hippocampal neurons. Already in 1984 Palovcik et al. (34) demonstrated that
78 insulin inhibits pyramidal neurons in hippocampal slices. Later insulin was shown to enhance miniature
79 inhibitory postsynaptic currents in cultured hippocampal neurons (35) and recently we demonstrated
80 that insulin turns-on high-affinity $GABA_A$ receptors that generate tonic currents in hippocampal CA1
81 pyramidal neurons in rat brain slices (36). In the present study we examined the effects of GLP-1 and
82 exendin-4 on $GABA_A$ signaling in hippocampal CA3 pyramidal neurons in rat brain slices. We found
83 that low physiological GLP-1 concentrations (pmol/L–nmol/L) and clinically relevant exendin-4
84 concentrations transiently potentiate synaptic and tonic GABA-activated currents.

85

86 **Research design and methods**

87 **Hippocampal slice preparation**

88 Brain slices were dissected for electrophysiological recordings from 16–22 days old Wistar rats. All
89 animal procedures were conducted in accordance with the local ethical guidelines and approved animal
90 care protocols by the Uppsala djurförsöksetiska nämnd, Uppsala, Sweden (Uppsala Animal Ethical
91 Board). Hippocampal slices were prepared as previously described (37). Briefly, the animal was
92 decapitated, the brain rapidly removed and immersed into the ice-cold artificial cerebrospinal fluid
93 (ACSF) containing (in mmol/L): 124 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, 2.5 Na_2HPO_4
94 and 10 glucose with pH 7.3–7.4 when bubbled with 95% O_2 and 5% CO_2. Sagittal or coronal
95 hippocampal slices 400 μm thick were prepared with a vibratome (Leica VT1200S) in the ice-cold
96 ACSF gassed with 95% O_2 and 5% CO_2. Slices were incubated in the same ACSF at 37 ºC for 1 h and
97 kept at room temperature (20–22 ºC) during experiments.

98

99 **Electrophysiological recording and analysis**

100 All patch-clamp recordings were performed at room temperature (20–22 ºC). Drugs were in general
101 purchased from Sigma-Aldrich (Germany) or Anaspec (GLP-1, exendin-4 and exendin(9–39); USA).
102 Bicuculline methiodide from Santa Cruz Biotechnology (USA) or Sigma-Aldrich (Germany) was used.
103 The pipette solution contained (mmol/L): 140 CsCl, 1 $CaCl_2$, 3 EGTA, 0.5 KCl, 1 $MgCl_2$, 2 ATP-Mg,
104 0.3 GTP-Na, 5 QX-314 bromide, 10 TES, pH of 7.25 with CsOH. In some experiments an inhibitor of
105 the $GABA_B$ receptor, CGP52432 (5 μmol/L), was used but it did not change the results. The recording
106 pipettes were made from borosilicate glass capillaries (Harvard Apparatus; UK) with DMZ-Universal
107 Puller (Zeitz Instruments; Germany) and had resistance of 2 to 4 MΩ when filled with the pipette
108 solution. The holding potential (V_h) was set to –60 mV and used in all experiments. ACSF containing
109 kynurenic acid (3 mmol/L) and other drugs, was continuously perfused (3 mL/min) through the

110 recording chamber during experiments. Patch-clamp recordings were done using Axopatch 200B
111 amplifier (Molecular Devices; USA), filtered at 2 kHz, sampled at 10 kHz by analogue-to-digital
112 converter, Digidata 1322A (Molecular Devices; USA), and stored in a computer. The recordings were
113 analyzed with pClamp 10 (Molecular Devices; USA) and MiniAnalysis 6 (Synaptosoft, Inc.; USA)
114 software. The amplitude of the tonic current was defined as the difference between the baseline current
115 levels before and after the drug application (38) and the frequency of the spontaneous inhibitory
116 postsynaptic currents (sIPSCs) immediately before first drug application was defined as control. The
117 maximal drug effect on the sIPSC frequency was calculated and normalized to its control value in the
118 same cell. The average value of the baseline current during the transient change in the current value
119 during GLP-1 application was fitted with a double exponential function (Eq 1): $y = y_0 + A_1 * exp(-t/\tau_{rise})$
120 $- A_2 * exp(-t/\tau_{decay})$, where y_0, $A_{1,2}$ are arbitrary constants; and the $\tau_{rise/decay}$ are time constants for the rise
121 and the decay phase of the transient current, respectively.
122

123 **Total RNA isolation and reverse transcription PCR (RT-PCR)**

124 Total RNA was isolated from rat hippocampal slices by using GenElute Mammalian Total RNA
125 Miniprep kit (Sigma-Aldrich) and quantified with Nanodrop (Nanodrop Technologies, Inc). Rat
126 hippocampal total RNA (100 ng) was reverse transcribed into cDNA in a 20 µl reaction mixture using
127 Superscript III reverse transcriptase (Invitrogen). Negative control was performed by omitting reverse
128 transcriptase in the reaction in order to confirm no genomic DNA contamination in the isolated RNA.
129 Human hippocampal cDNA was purchased from USBiological (USA). PCRs were done in a 10 µl
130 reaction mixture containing 4 µl cDNA (4 ng), 1x PCR reaction buffer, 3 mmol/L $MgCl_2$, 0.3 mmol/L
131 dNTP, 1x ROX reference dye, 0.7 U JumpStart Taq DNA polymerase (Sigma-Aldrich), 0.5x SYBR
132 Green I (Invitrogen) and 0.4 µmol/L each of forward and reverse primers. The primer pairs were
133 synthesized by Sigma-Aldrich: rat *Glp1r* (forward: GGCATTGTCAAGTATCTCTAC, reverse:

134 GATGAAGACAAGGAAGTTGAC, amplicon size: 123 bp), human *GLP1R* (forward:
135 ACATCAAATGCAGACTTGCC, reverse: TCACAAAGGCAAAGATGACC, amplicon size: 81 bp).
136 Amplification was performed in 384-well optical plates using the ABI PRISM 7900HT Sequence
137 Detection System (Applied Biosystems) with an initial denaturation of 5 min at 95°C, followed by 45
138 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. A melting curve was determined at the end of
139 cycling to ensure the amplification of a single PCR product. 5 µl of each individual PCR reaction was
140 then electrophorized on a 2% agarose gel stained with SYBR Gold (Invitrogen, Carlsbad, CA).
141

142 **Statistical analysis**

143 Statistical analysis was carried out using SigmaPlot 11 (Systat Software; USA), MiniAnalysis 6
144 (Synaptosoft, Inc.; USA) or GraphPad Prism 6 (GraphPad Software; USA) software. Results are
145 presented as mean ± standard error of the mean (SEM). Paired Student's t-test was used for data-sets
146 normally distributed. The Tukey method was used to detect the outliers. Statistical analysis was
147 performed after excluding the outliers. Non-parametric Mann-Whitney test was used for data-sets that
148 were not normally distributed. One way ANOVA Bonferroni *post-hoc* test was used for multiple
149 comparisons with normally distributed data.
150
151
152

153 **Results**

154 The $GABA_A$ mediated whole-cell currents were recorded from rat hippocampal CA3 pyramidal
155 neurons bathed in ACSF in the presence of kynurenic acid. At the end of every experiment bicuculline
156 (100 μmol/L), a $GABA_A$ receptor antagonist, was applied to block GABA-evoked currents. The
157 spontaneous inhibitory postsynaptic currents (sIPSCs) were abolished by 100 μmol/L bicuculline (Fig.
158 1) and the holding current decreased, revealing the prominent tonic GABA-activated current normally
159 present in the hippocampal CA3 pyramidal neurons (24.7 ± 1.5 pA, n = 19; Fig. 1). We then proceeded
160 and examined the effects of GLP-1 on these $GABA_A$ receptor-mediated currents.

161

162 **GLP-1 transiently modulates GABA-activated synaptic and tonic currents in CA3 pyramidal**
163 **neurons.**

164 We examined if GLP-1 concentrations ranging from 10 pmol/L to 10 nmol/L affected the GABA-
165 evoked currents and representative results are shown for three cells in Fig. 2A, B and C. The GLP-1
166 receptor mRNA is expressed in both human and rat hippocampus (Fig. 2D). GLP-1 in a concentration
167 dependent manner transiently enhanced the synaptic and tonic GABA-activated currents in the neurons.
168 The 10 pmol/L GLP-1 concentration was most effective and transiently increased the most frequent
169 sIPSC amplitude by a factor of 1.8 (Fig. 3A) and the average sIPSC frequency was similarly increased
170 (Fig. 3B) by a factor of 1.8 (n = 7) as compared to control. Higher GLP-1 concentrations (100 pmol/L,
171 1 nmol/L, 10 nmol/L; Fig. 3A) did not increase the most frequent sIPSC amplitude. However, in 100
172 pmol/L and 1 nmol/L GLP-1, the average sIPSC frequency still increased by a factor of 1.6 (n = 6) and
173 1.8 (n = 5), respectively, as compared to control (Fig. 3B). The tonic current in the CA3 pyramidal
174 neurons increased when exposed to 10 pmol/L to 1 nmol/L concentrations of GLP-1 but was similar to
175 control in 10 nmol/L GLP-1 (Fig. 3C) resulting in a bell shaped-like concentration-response
176 relationship. When several GLP-1 concentrations were sequentially applied to a neuron then each new

177 application led to a transient increase of the tonic current which subsequently relaxed to the initial
178 current level. Thus, the tonic current amplitude changed transiently in a GLP-1
179 concentration-dependent manner. Just applying the extracellular solution did not induce the transient
180 increase in the tonic current amplitude. The amplitude was not related to whether the response was
181 obtained after a single exposure to GLP-1 (open circles, Fig. 3C) or if the neuron had been previously
182 exposed to another concentration of GLP-1 (filled triangles, Fig. 3C).

183

184 The time course of the tonic current during the first minutes of the GLP-1 application was U-shaped
185 (Fig. 2A–C, 4A, B) and could be fitted with a double exponential function: Eq 1, $y = y_0 + A_1 * exp(-$
186 $t/\tau_{rise}) - A_2 * exp(-t/\tau_{decay})$ where y_0 is the initial baseline current value, $A_{1,2}$ are arbitrary constants but
187 τ_{rise} and τ_{decay} are the time constants for the rise and the decay phase of the transient current,
188 respectively. At all GLP-1 concentrations the current increased with a characteristic time constant of
189 about 2 min (τ_{rise}) and after additional 2 min (τ_{decay}; Fig. 4C), the current had returned to approximately
190 the initial current level. Both the sIPSCs and the tonic currents in the presence of GLP-1 were blocked
191 by 100 μmol/L bicuculline (Fig. 2A–C).

192

193 **The GLP-1 receptor antagonist exendin(9–39) inhibits GLP-1 modulation of the GABA-activated**
194 **currents.**

195 We examined, if the effects of GLP-1 on the GABA-activated synaptic and tonic currents could be
196 prevented by a GLP-1 receptor antagonist. Exendin(9–39) (Ex9-39), is a competitive inhibitor of GLP-
197 1 at the GLP-1 receptor (23). Since GLP-1 once bound to the GLP-1 receptor starts intracellular
198 cascades leading to activation of various proteins, it is essential to apply the inhibitor first and only
199 then, co-apply GLP-1 together with the inhibitor in order to prevent GLP-1 effects on neuronal function
200 such as modulation of the GABA signaling. In our experiments we, therefore, applied Ex9-39 first and

201 then co-applied GLP-1 together with Ex9-39 to the brain slices. When Ex9-39 was applied to the
202 hippocampal slices it inhibited the effects of 10 pmol/L GLP-1 on the GABA-activated currents but
203 had no effects when applied alone (Fig. 5). The amplitude and frequency of the synaptic currents were
204 now similar to control currents (Fig. 5A–C) and there was no increase in the amplitude of the tonic
205 current (Fig. 5A, D).
206
207 **GLP-1 enhances the tonic but not the synaptic currents in the presence of tetrodotoxin (TTX).**
208 In order to examine if the GLP-1 effects on the currents were due to pre- or postsynaptic mechanisms
209 or both, we studied the influence of GLP-1 on the currents in the presence of the voltage-gated sodium
210 channel blocker TTX (1µmol/L). TTX inhibits action potential-dependent GABA release. The
211 amplitudes of the synaptic currents were similar in TTX and TTX plus 10 pmol/L GLP-1 (Fig. 6A, B).
212 The results further show that the frequency of the IPSCs decreased when the slices were exposed to
213 TTX from 16.8 ± 1.2 to 2.3 ± 0.4 Hz (non-parametric Mann-Whitney test, $P = 0.008$, $n = 5$),
214 respectively, and remained at a similar level in the presences of 10 pmol/L GLP-1 plus TTX (Fig. 6A,
215 C). The results are consistent with that GLP-1 potentiates the release of GABA from presynaptic
216 terminals. The tonic current also decreased in TTX as compared to control but in contrast to the
217 synaptic currents, the effect of GLP-1 on the tonic current was maintained (Fig. 6A, D). The tonic
218 current transiently increased from 11.1 ± 2 pA to 22.7 ± 2.3 pA ($n = 6$) when the slices were exposed to
219 GLP-1 (10 pmol/L) in the presence of TTX (Fig. 6A, D). These results demonstrate that GLP-1
220 signaling modulates high-affinity, extrasynaptic $GABA_A$ receptors in the plasma membrane of CA3
221 pyramidal neurons that generate the tonic current.
222
223 **Exendin-4 transiently modulates GABA-activated synaptic and tonic currents in CA3 pyramidal**
224 **neurons.** Exendin-4 is an agonist at the GLP-1 receptors. We, therefore, examined if exendin-4 had

225 similar effects as GLP-1 on the synaptic and tonic $GABA_A$ receptor-mediated currents in the
226 hippocampal CA3 pyramidal neurons. Representative results for 10, 50 and 100 nmol/L exendin-4 are
227 shown for one cell in Fig. 7A. In a concentration dependent manner, exendin-4 transiently enhanced
228 both the synaptic and tonic GABA-activated currents in the neurons. Exendin-4 did not increase the
229 most frequent sIPSC amplitude (Fig. 7B) whereas the average sIPSC frequency (Fig.7C) was enhanced
230 by a factor of 1.4 (n = 6), 1.5 (n = 6) and 1.4 (n = 6) by 10, 50 and 100 nmol/L exendin-4, respectively,
231 but did not change in 0.5 nmol/L exendin-4. All exendin-4 concentrations tested (0.5, 10, 50 and 100
232 nmol/L) transiently enhanced the tonic current in the CA3 pyramidal neurons (Fig. 8A). The time
233 course of the tonic current enhancement by exendin-4 was U-shaped. It was similar to what was
234 recorded in GLP-1 (Fig. 7A, 8B, C) and could be fitted by Eq 1. ($y = y_0 + A_1*exp(-t/\tau_{rise}) - A_2*exp(-$
235 $t/\tau_{decay}))$. Similar to tonic currents evoked by GLP-1, for all exendin-4 concentrations, the currents
236 increased with a characteristic time constant of about 2 min (τ_{rise}; Fig. 8D) and after additional 2 min
237 (τ_{decay}; Fig. 8D), the current had returned to approximately the initial current level. Both the sIPSCs and
238 the tonic currents in the presence of exendin-4 were inhibited by 100 µmol/L bicuculline (Fig. 7A).
239
240 **Exendin-4 enhances the tonic current but not the synaptic currents in the presence of TTX.**
241 Similar to the GLP-1, effects of exendin-4 on the $GABA_A$ receptor-activated currents might be related
242 to either pre- or postsynaptic mechanisms or both. We, therefore, examined the effects of exendin-4 on
243 the currents in the presence of TTX (1 µmol/L) that inhibits action-potential dependent transmitter
244 release (Fig. 9A–D). The slices were first exposed to TTX to block presynaptic GABA release and then
245 exposed to 10 nmol/L exendin-4 (Fig. 9A). The amplitudes of the synaptic currents were similar in
246 TTX alone or TTX plus exendin-4 (Fig. 9B) but the frequency of the IPCSs decreased to 2.2 ± 0.4 Hz
247 when the slices were exposed to TTX and remained at a similar level in the presence of 10 nmol/L
248 exendin-4 plus TTX (n = 4, Fig. 9C). At the same time, exendin-4 enhanced the tonic current in the

249 presence of TTX (Fig. 9D). The results are consistent with that exendin-4 enhances GABA release
250 from presynaptic terminals and modulates tonic currents in the postsynaptic neurons and are similar to
251 the results obtained with GLP-1.
252
253

254 **Discussion**

255 The metabolic hormone GLP-1 and its mimetic exendin-4 both enhanced GABA signaling in rat
256 hippocampal CA3 pyramidal neurons. The CA3 pyramidal neurons are regulated by a number of
257 inhibitory interneurons and form an important part of the hippocampal neuronal circuit involved in
258 memory formation (9). The synaptic and tonic GABA-activated currents in the CA3 pyramidal neurons
259 were transiently enhanced by GLP-1 at physiologically relevant concentrations and by exendin-4 at
260 concentrations relevant when treating type 2 diabetes. The effects of GLP-1 and exendin-4 on the
261 GABA signaling can be attributed to both presynaptic and postsynaptic mechanisms. The results are
262 consistent with GLP-1 modulating cognitive process in a hippocampal dependent manner.
263
264 There appears to be a number of ways GLP-1 and exendin-4 can enhance GABA signaling in the CA3
265 pyramidal neurons. The most frequent sIPSC amplitude was approximately doubled and the tonic
266 current increased by 60% in 10 pmol/L GLP-1. These increases potentially can be attributed to a
267 number of processes such as increased release of GABA from the presynaptic terminal, increased
268 number of $GABA_A$ receptors in the postsynaptic membrane, increased spillover of GABA from the
269 synaptic cleft or insertion of new or modified $GABA_A$ receptors with higher affinity in the postsynaptic
270 membrane (33; 35; 36; 39). TTX inhibits voltage-gated sodium channels. In solutions containing TTX
271 action potential generation is inhibited resulting in decreased transmitter release from presynaptic
272 terminals. We used TTX to differentiate between presynaptic and postsynaptic effects of GLP-1 and
273 exendin-4 on the GABA signaling. In the presence of TTX, GLP-1 neither affected the amplitudes nor
274 the frequency of the synaptic currents and similarly, exendin-4 in the presence of TTX no longer
275 modulated the frequency of the IPSCs. The enhancing effect of GLP-1 or exendin-4 on the sIPSC
276 frequency in the absence of TTX is, therefore, related to increased release of GABA from presynaptic
277 terminals. The effects on the tonic current were more complex. In the presence of TTX, the tonic

278 current was reduced about 50%. This is in accordance with at least a part of the tonic current amplitude
279 being related to the magnitude of spillover of GABA from synapses (33; 39). However, the remaining
280 tonic current recorded in TTX was still sensitive to GLP-1 and approximately doubled in amplitude
281 when the neurons were exposed to 10 pmol/L GLP-1 and similar results were obtained with exendin-4.
282 These results are consistent with GLP-1 and exendin-4 modulating GABA signaling in the
283 hippocampal CA3 neuron in a postsynaptic manner. The $GABA_A$ receptors generating the tonic current
284 and modulated by GLP-1 receptor-signaling in the postsynaptic neuron are high-affinity, extrasynaptic
285 $GABA_A$ receptors that are activated by the very low, ambient GABA concentrations present around the
286 neurons. Where the GABA originates from is not clear but mechanisms involving non-vesicular release
287 (40-42) such as reversal of GABA transporters or release of GABA from astrocytes have been
288 proposed. That pre- and postsynaptic mechanisms can regulate tonic $GABA_A$ receptor-mediated
289 currents in hippocampal neurons is in accordance with previous reports (33; 35; 36; 39). Interestingly,
290 we have previously reported that another metabolic hormone, insulin, can modulate high-affinity
291 $GABA_A$ receptors in hippocampal CA1 pyramidal neurons (36). Axons from CA3 pyramidal neurons,
292 the Schaffer collaterals, project to the CA1 pyramidal neurons where they synapse. Both the CA3 and
293 the CA1 neurons have critical roles in hippocampal-dependent memory and learning processes (43).
294

295 Metabolic hormones are emerging as important regulators of hippocampal function (28; 44-47). GLP-1
296 has previously been shown to decrease glutamate-generated currents in cultured hippocampal neurons
297 (48), decrease hippocampal theta wave duration in rats (30), and there is an impairment of synaptic
298 plasticity and memory formation in GLP-1 receptor knock-out mice (49). The hippocampus is well
299 known for its role in memory encoding but less focus has been on its function in governing body
300 physiology (10; 11). The hippocampus contains receptors for molecules regulating many physiological
301 processes including receptors for the metabolic hormones (10). Furthermore, via neurons in the septum,

302 the hippocampus maps in a topographical manner onto the hypothalamus and generally results in
303 inhibition of hypothalamic activity (10; 11). In hippocampal CA1 neurons, insulin enhances IPSCs (35)
304 and insulin also turns-on high-affinity $GABA_A$ receptors generating tonic currents in these neurons (36)
305 which normally express very small or no tonic currents (33). GLP-1 is made in the brain stem and by
306 L-cells in the intestine and crosses the blood-brain barrier (13). In plasma the half-life of GLP-1 is 1–2
307 min and the maximal concentration after a meal is less than 40 pmol/L (13). Interestingly, in our
308 experiments, 10 pmol/L GLP-1 and exendin-4 effectively enhanced the GABA-activated current
309 response with a similar time constant of activation (2 min), an apparent synchrony with the life-time of
310 GLP-1 in plasma. Recently, Roed et al. (23), determined the half-maximal concentration for activation
311 (EC_{50}) of the GLP-1 receptor expressed in HEK cells to be 9.8 ± 1.0 pmol/L. They further determined
312 the EC_{50} of GLP-1 for inducing GLP-1 receptors internalization to be 12 ± 5 nmol/L and showed that
313 maximal internalization level occurred with super-saturating concentrations (1 μmol/L) within 15–20
314 min. In the CA3 neurons in the presence of an inhibitor of the GLP-1 receptors, Ex9-39, no effects of
315 GLP-1 on the synaptic or tonic GABA-activated currents were recorded. The activation and decay
316 phase time constants of 2 min for the tonic current were concentration independent, similar for the two
317 agonists and much faster than the reported rate for GLP-1 receptors internalization. The time constant
318 of the tonic currents rising phase reflects the activation of GLP-1 receptors by the agonists, GLP-1 or
319 exendin-4, transduction of the signal inside the neuron and as a result, increased current through the
320 extrasynaptic $GABA_A$ receptors in the postsynaptic plasma membrane. What the decay time constant of
321 the tonic current represents is unclear. However, as the effect of both GLP-1 and exendin-4 on the
322 synaptic currents was transient, it is possible that the decay of the responses is related to desensitization
323 of the GLP-1 receptors (50).
324

325 Midlife type 2 diabetes increases the likelihood for cognitive decline and brain atrophy (1; 2). A
326 number of studies published to-date indicate that diabetes mellitus and poor glycemic control are
327 significant risk factors for decreased memory functions later in life (1-8). GLP-1 receptors agonists e.g.
328 exendin-4, liraglutide, are reported to be neuroprotective and possible rescue cognition in models of
329 Alzheimer's, Parkinson's and Huntington's disease (7; 51). Cognitive decline appears to be a
330 significant complication associated with diabetes mellitus and it is, therefore, of major interest to
331 characterize the effects of molecules activating the GLP-1 receptor in hippocampal brain tissue as these
332 compounds may potentially reverse or slow-down the decline. Our results show that exendin-4
333 application effectively mimics the GLP-1 effects on the $GABA_A$ signaling in the CA3 pyramidal
334 neurons but the modulation varies somewhat between the two GLP-1 receptor agonists e.g. the sIPSC
335 amplitudes were not enhanced by any of exendin-4 concentrations we used in this study whereas the
336 sIPSC amplitudes were enhanced by 10 pM GLP-1.
337
338 In conclusion, the results demonstrate that both GLP-1 and exendin-4 effectively potentiate
339 GABAergic signaling in hippocampal CA3 pyramidal neurons. Fig. 9E shows a cartoon with pre- and
340 postsynaptic neuronal terminals, the synapse and identifies the location of the different receptors. It
341 also shows where the GABA-evoked currents that are modified by the GLP-1 receptor agonists are
342 generated. At physiological GLP-1 concentrations both synaptic and tonic GABA-activated currents
343 were transiently enhanced and the effects were blocked by exendin(9–39). The results demonstrate that
344 GLP-1 and exendin-4 modulate GABA signaling in hippocampal neurons in both pre- and postsynaptic
345 manner. The results imply that in order to combat diabetic associated cognitive dysfunction with GLP-
346 1, or medicines that mimic GLP-1 actions like e.g. exendin-4, a good understanding of GLP-1 receptor
347 agonist effects on hippocampal neuronal functions is desirable. Furthermore, hippocampal-dependent
348 learning and memory mechanisms may potentially contribute directly to control of energy homeostasis

349 by regulating hypothalamic function. In order to elucidate the interplay between cognitive function and
350 diabetes more studies of regulation of hippocampal function by metabolic hormones are required.

351

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361

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480
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482

483 **Figure legends**

484 **Figure 1 – Inherent GABA-activated synaptic and tonic currents in hippocampal CA3 pyramidal**
485 **neuron in rat brain slices**. The currents are inhibited by the $GABA_A$ receptor antagonist bicuculline
486 (100 µmol/L) application. Horizontal bar above the recording denotes the period of inhibitor
487 application. Right panel represents Gaussian fits to the all-points histograms of 30-s segments taken in
488 the middle of control period and after bicuculline application. Peaks of Gaussians are denoted by
489 horizontal dash lines: lower dash line indicates baseline current level in control condition before
490 bicuculline application; upper dash line shows "zero" current level after adding the inhibitor.
491 Difference between marked Gaussian peaks represents the amplitude of the GABA-activated tonic
492 current.

493

494 **Figure 2 –GLP-1 potentiates the spontaneous inhibitory postsynaptic currents (sIPSCs) and the**
495 **GABA-evoked tonic current in hippocampal CA3 pyramidal neurons**. GLP-1 induced increase in
496 frequency and amplitude of the sIPSCs (*A–C*) and enhanced the tonic current manifested by a
497 downward shift of the baseline current, the level indicated by the lowest dash line in *A–C*. The baseline
498 current level before GLP-1 application i.e. control, is indicated by the middle dash line; the dash line on
499 top represents "zero" current level after bicuculline application where all $GABA_A$ receptors have been
500 inhibited. Note, that starting GLP-1 concentration in *A* is 10 pmol/L and 0.1 nmol/L in *B*, sloped lines
501 in *A* indicate a break in the recording. *C*: An example of $GABA_A$ receptor-mediated current inhibited
502 by applying bicuculline at the moment of maximal enhancement of the tonic current. *D*: GLP-1
503 receptor mRNA is expressed in human and rat hippocampus. Horizontal bars above the current
504 recordings show the periods of drug applications.

505

506 **Figure 3 –GLP-1 modulates the synaptic (sIPSCs) and the tonic GABA-activated current**
507 **characteristics.** *A*: Cumulative probability histograms of sIPSC amplitudes for different GLP-1
508 concentrations increased significantly in amplitudes only at 10 pmol/L GLP-1. Solid and dash lines
509 indicate cumulative probability histograms of sIPSC amplitudes before and after GLP-1 application,
510 respectively. Paired Student's t-test: *P < 0.05, n = 7 for 10 pmol/L; not significant for other GLP-1
511 concentrations, n = 6 for 100 pmol/L, 1 and 10 nmol/L of GLP-1. *B*: sIPSC frequencies increased upon
512 application of 0.01, 0.1 and 1 nmol/L of GLP-1 but not 10 nmol/L. Horizontal dash line represents
513 normalized sIPSC frequency in control for every GLP-1 concentration. Data from each group is
514 presented as a scatter dot plot (open circles) with a mean and a box and whiskers plot with median
515 values plotted by Tukey method to detect the outliers (filled circles above or below the box and
516 whiskers plot). Statistical analysis was performed after excluding the outliers. Non-parametric Mann-
517 Whitney test, 0.01 nmol/L, ***P < 0.001, n = 7; 0.1 nmol/L, **P < 0.01, n = 6; 1 nmol/L, **P < 0.01, n
518 = 5; 10 nmol/L, not significant, n = 4. *C*: Tonic currents in individual neurons at different GLP-1
519 concentrations. Values from experiments with sequential application of different GLP-1 concentrations
520 (filled triangles) are overlaid with values from experiments with application of a single GLP-1
521 concentration (open circles). Data from each group is presented as a scatter dot plot (open circles
522 and/or filled triangles) with mean ± SEM and a box and whiskers plot with median values plotted by
523 Tukey method. No outliers were detected. One way ANOVA Bonferroni *post-hoc* test, multiple
524 comparisons versus control group (0 nmol/L GLP-1, n = 19), ***P < 0.001, 0.01 nmol/L, n = 9; 0.1
525 nmol/L, n = 4; 1 nmol/L, n = 10; 10 nmol/L, n = 4.
526

527 **Figure 4 – The kinetics of the transient tonic current induced by GLP-1**. *A*: A representative
528 example of the transient current evoked by 10 pmol/L GLP-1 application. *B*: A fit to the transient
529 current by Eq 1: $y = y_0 + A_1*exp(-t/\tau_{rise}) - A_2*exp(-t/\tau_{decay})$. *C*: Values of time constants τ_{rise} and τ_{decay}

530 at different GLP-1 concentrations. One way ANOVA on ranks; significant difference was detected for
531 neither of pairs compared (P = 0.214); 0.01 nmol/L, n = 5; 0.1 nmol/L, n = 4; 1 nmol/L, n = 6; 10
532 nmol/L, n = 4.
533
534 **Figure 5 – Exendin(9–39) inhibits GLP-1 modulation of the GABA-activated currents**. *A*: A
535 representative recording showing the effects of GLP-1 and exendin(9–39) (Ex9-39) on the currents. *B*:
536 Cumulative probability histograms of sIPSC amplitudes showed no significant difference in
537 amplitudes. One way ANOVA on ranks, P = 0.336, n = 5. *C*: Difference in frequencies of sIPSCs
538 among control, before and after GLP-1 application in the presence of Ex9-39 was not detected. One
539 way ANOVA, P = 0.919, n = 5. *D*: Difference in tonic current amplitude among control, before and
540 after GLP-1 application in the presence of Ex9-39 was not detected. Data from each group is presented
541 as a scatter dot plot with mean ± SEM and a box and whiskers plot with median values plotted by
542 Tukey method. No outliers were detected. One way ANOVA, P = 0.686, n = 5. The GLP-1 and Ex9-39
543 concentrations used were 10 pmol/L and 100 nmol/L, respectively. Horizontal bars above the current
544 recordings show the periods of drug applications.
545
546 **Figure 6 – GLP-1 enhances the tonic but not the miniature inhibitory postsynaptic currents**
547 **(mIPSCs) in the presence of tetrodotoxin (TTX).** *A*: A representative recording showing the effects
548 of GLP-1 and TTX on the currents. *B*: Cumulative probability histograms of mIPSC amplitudes
549 showed no significant difference in amplitudes before and after GLP-1 application in the presence of
550 TTX. Student's t-test, P = 0.421, n = 5. *C*: Difference in frequencies of mIPSCs before and after GLP-1
551 application in the presence of TTX was not detected. Student's t-test, P = 0.726, n = 5. *D*: Comparison
552 of the tonic current amplitudes before and after GLP-1 application in the absence (–) and presence (+)
553 of TTX. Student's t-test, ***P < 0.001, **P < 0.01; –TTX: control, n = 19, GLP-1, n = 9; +TTX:

554 control, n = 6, GLP-1, n = 6. The GLP-1 and TTX concentrations used were 10 pmol/L and 1 µmol/L,
555 respectively. Horizontal bars above the current recordings show the periods of drug applications.
556
557 **Figure 7 –Exendin-4 (Ex-4) modulates the inhibitory postsynaptic currents in the CA3 pyramidal**
558 **neurons.** *A*: Changes in baseline current level and sIPSCs induced by different Ex-4 concentrations. *B*:
559 Cumulative probability histograms of sIPSC amplitudes for different Ex-4 concentrations showed no
560 significant increase in amplitudes for any of the Ex-4 concentrations tested (500 pmol/L, n = 4; 10, 50,
561 100 nmol/L, n = 6). Solid and dash lines indicate cumulative probability histograms of sIPSC
562 amplitudes before and after Ex-4 application, respectively. *C*: sIPSC frequencies significantly increased
563 upon application of 10, 50 and 100 but not 0.5 nmol/L of Ex-4. Horizontal dash line represents
564 normalized sIPSC frequency in control for every Ex-4 concentration. Data from each group is
565 presented as a scatter dot plot (open circles) with a mean and a box and whiskers plot with a median
566 value plotted by Tukey method. No outliers were detected. Non-parametric Mann-Whitney test, 10, 50,
567 100 nmol/L, $**P < 0.01$, n = 6; 0.5 nmol/L, not significant, n = 4. Horizontal bars above the current
568 recordings show the periods of drug applications.
569
570
571 **Figure 8 –Exendin-4 (Ex-4) enhances the tonic current in CA3 pyramidal neurons**. *A*: Tonic
572 current amplitudes in individual neurons at different Ex-4 concentrations. Data from each group is
573 presented as scatter dot plot (open circles) with mean ± SEM and box and whiskers plot with median
574 values plotted by Tukey method. No outliers were detected. Paired comparisons versus control group (0
575 nmol/L Ex-4, n = 12), $***P < 0.001$, 0.5 nmol/L, n = 5; 10 nmol/L, n = 6 (Student's t-test); $**P < 0.01$,
576 50 nmol/L, n = 10; 100 nmol/L, n = 5 (non-parametric Mann-Whitney test). *B*: An example of the
577 transient current evoked with 10 nmol/L Ex-4 application. *C*: A fit to the transient current by a double

578 exponential function; $y = y_0 + A_1 * exp(-t/\tau_{rise}) - A_2 * exp(-t/\tau_{decay})$, where y_0, $A_{1,2}$ are arbitrary constants;
579 $\tau_{rise/decay}$ are time constants for the rise or the decay phase of the transient current, respectively. *D*:
580 Values of time constants τ_{rise} and τ_{decay} at different Ex-4 concentrations. One way ANOVA; significant
581 difference was not detected for any pairs compared (P = 0.971); 0.5 nmol/L, n = 4; 10 nmol/L, n = 6;
582 50 nmol/L, n = 5; 100 nmol/L, n = 5.
583
584 **Figure 9 – Exendin-4 enhances the tonic but not the miniature inhibitory postsynaptic currents**
585 **(mIPSCs) in the presence of tetrodotoxin.** *A*: A representative current recording from a CA3
586 pyramidal neuron, exendin-4 (Ex-4) was 10 nmol/L and TTX 1 µmol/L**.** Horizontal bars above the
587 current show the periods of drug applications *B*: Cumulative probability histograms of mIPSC
588 amplitudes showed no significant difference in amplitudes before and after Ex-4 application in the
589 presence of TTX. Student's t-test, P = 0.432, n = 4. *C*: Difference in frequencies of mIPSCs before and
590 after Ex-4 application in the presence of TTX was not detected. Student's t-test, P = 0.872, n = 4. *D*:
591 Tonic current amplitudes before and after 10 nmol/L Ex-4 application in the presence of TTX. Data
592 from each group is presented as a scatter dot plot (open circles) with a mean ± SEM and a box and
593 whiskers plot with a median value plotted by Tukey method. No outliers were detected. Student's t-test,
594 *P < 0.05, n = 4. *E*: A cartoon illustrating GABA signaling in hippocampal neurons identifying: the
595 pre- and postsynaptic neuron, the neurotransmitter GABA, synaptic and extrasynaptic $GABA_A$
596 receptors ($GABA_AR$) and the GLP-1 receptor (GLP-1R). The phasic currents (inhibitory postsynaptic
597 currents, IPSCs) are mediated by synaptic $GABA_A$ receptors and the tonic current is mediated by
598 extrasynaptic $GABA_A$ receptors.
599

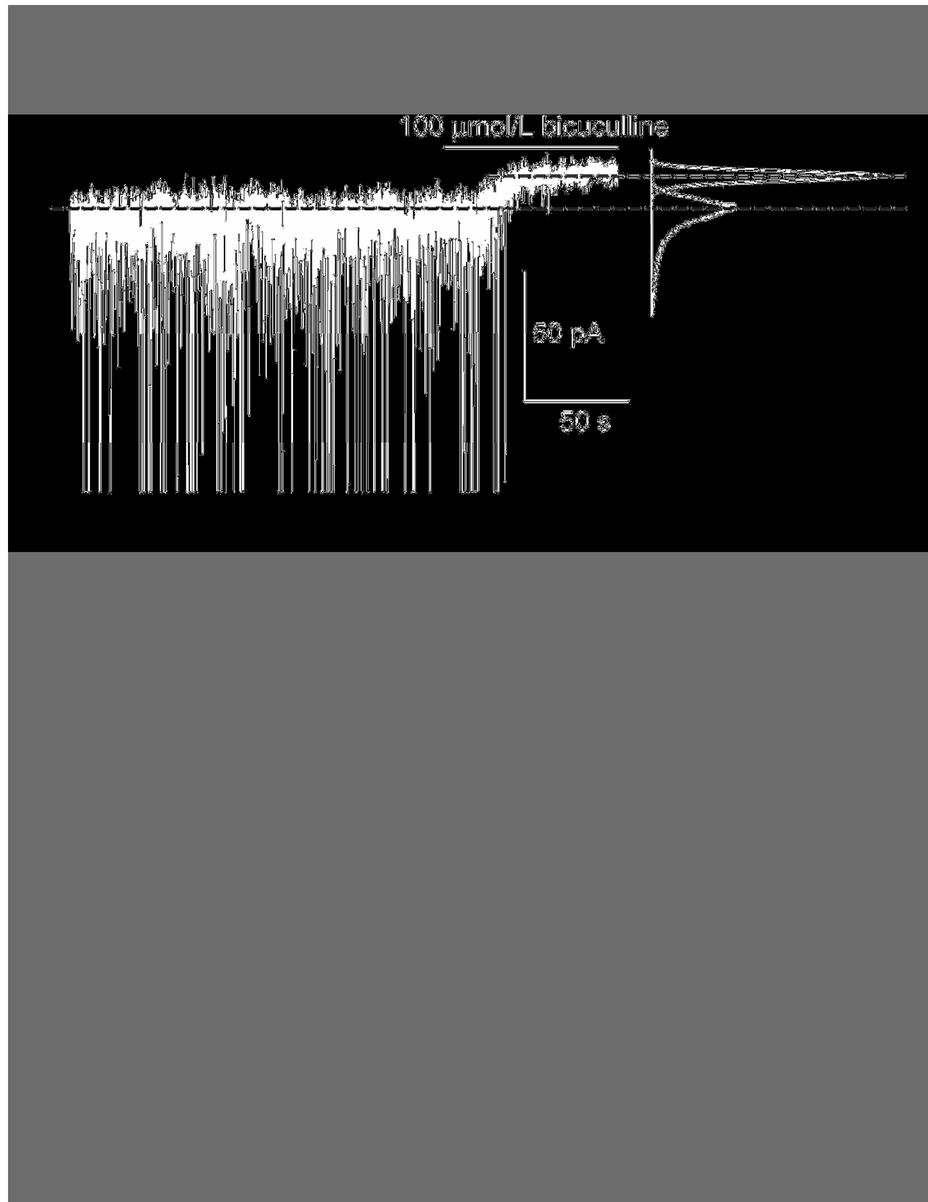

Figure 1
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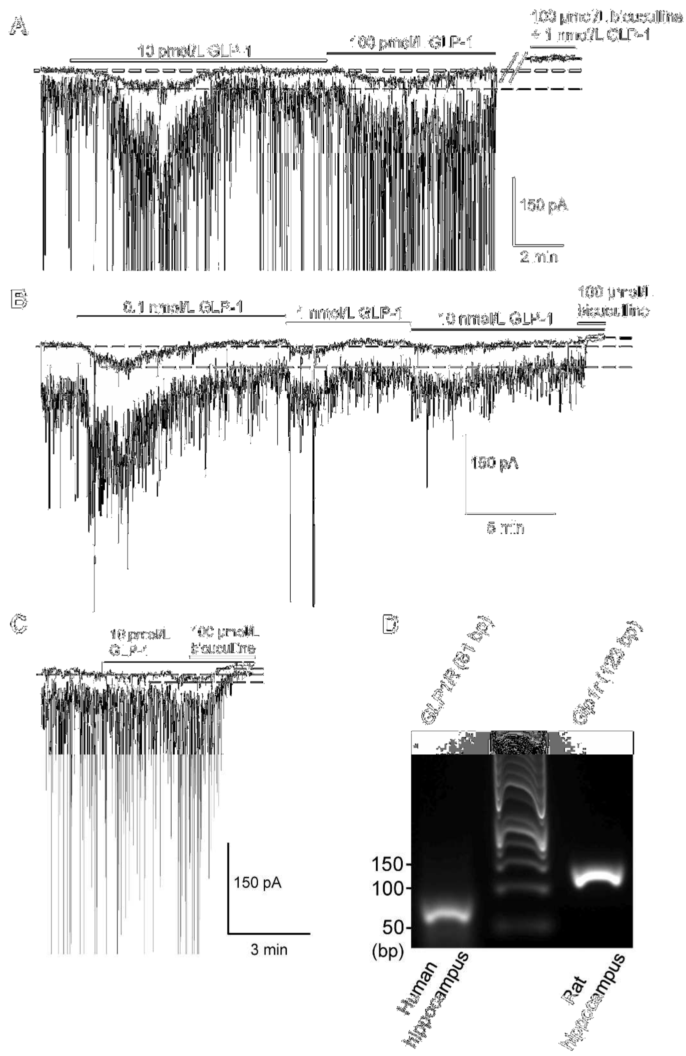

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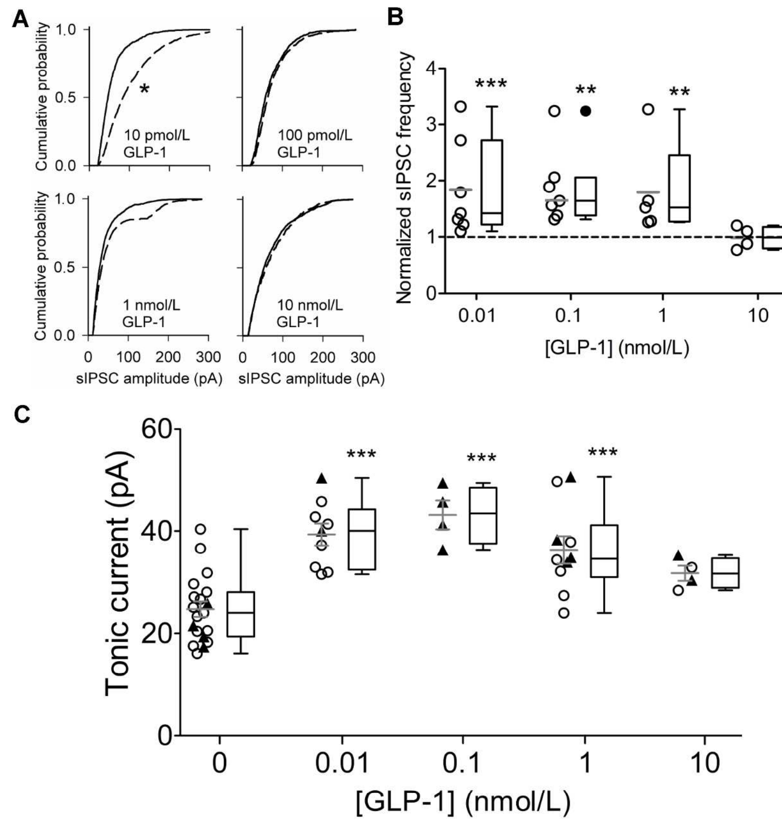

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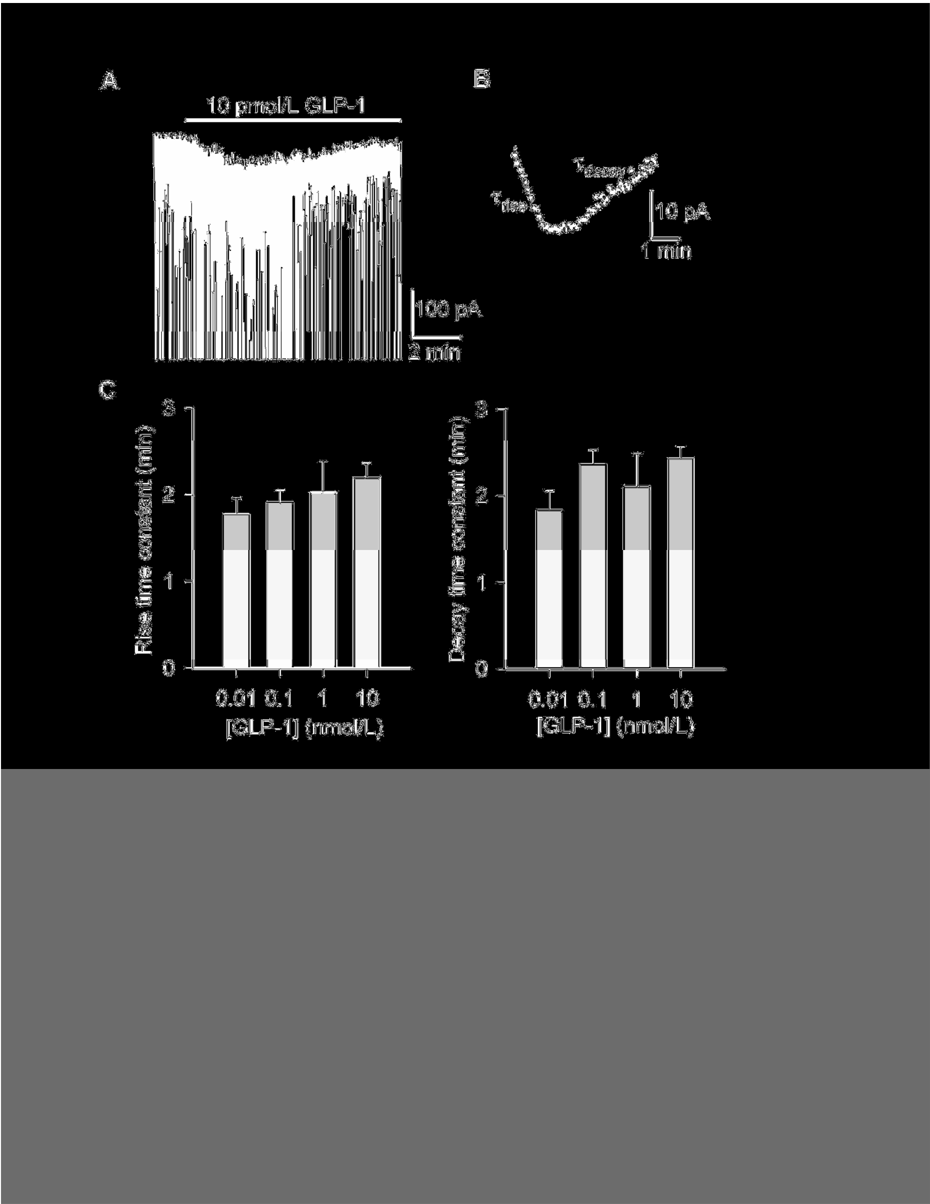

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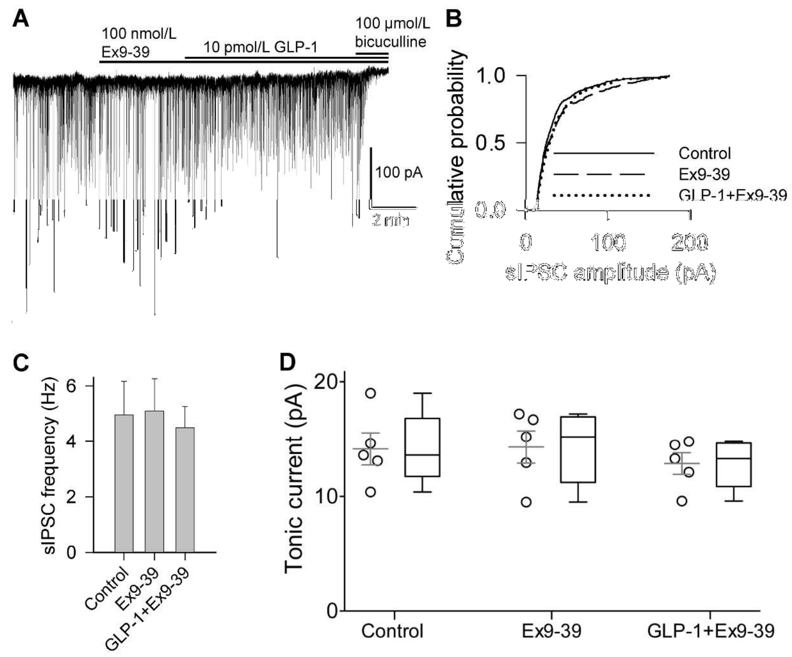

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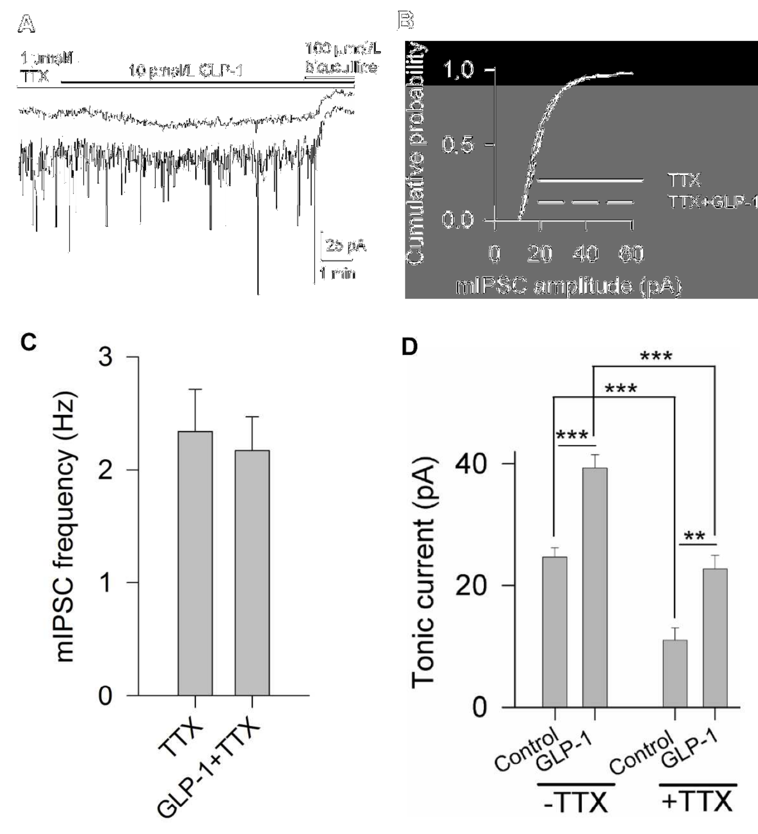

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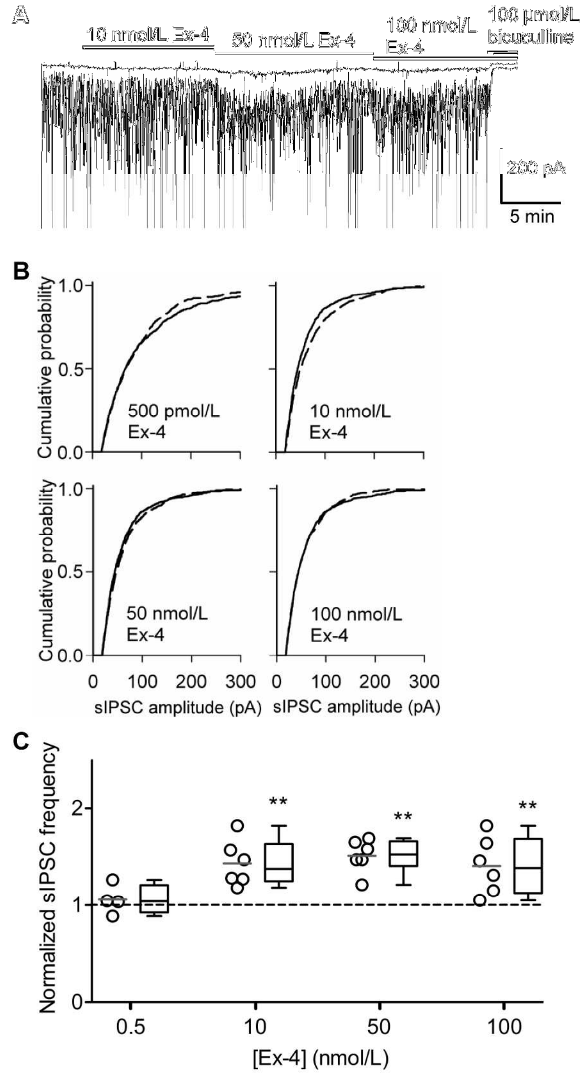

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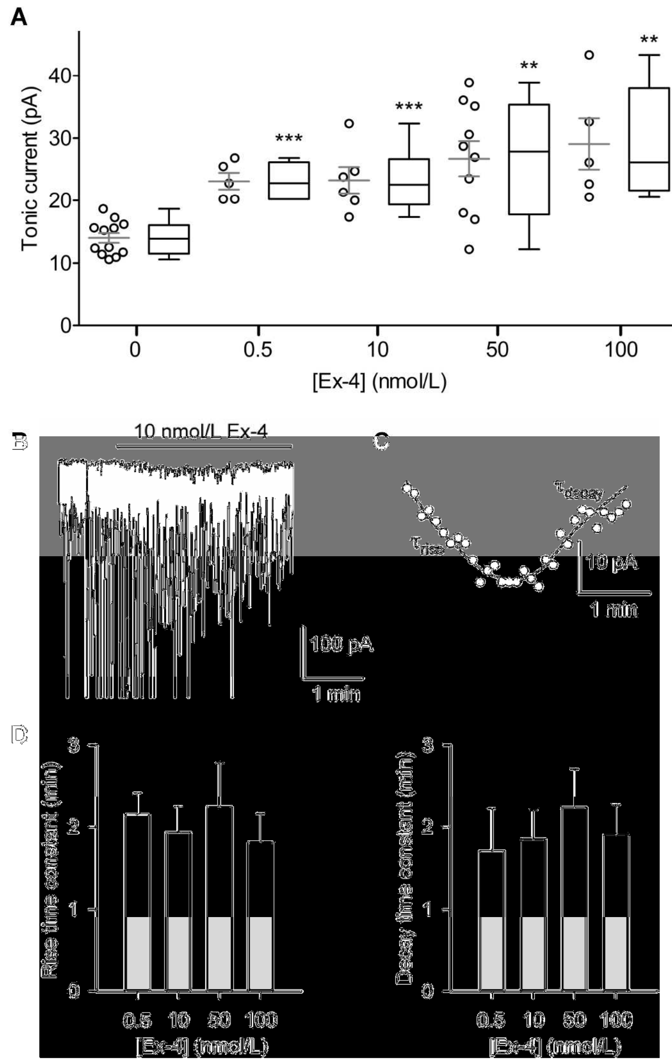

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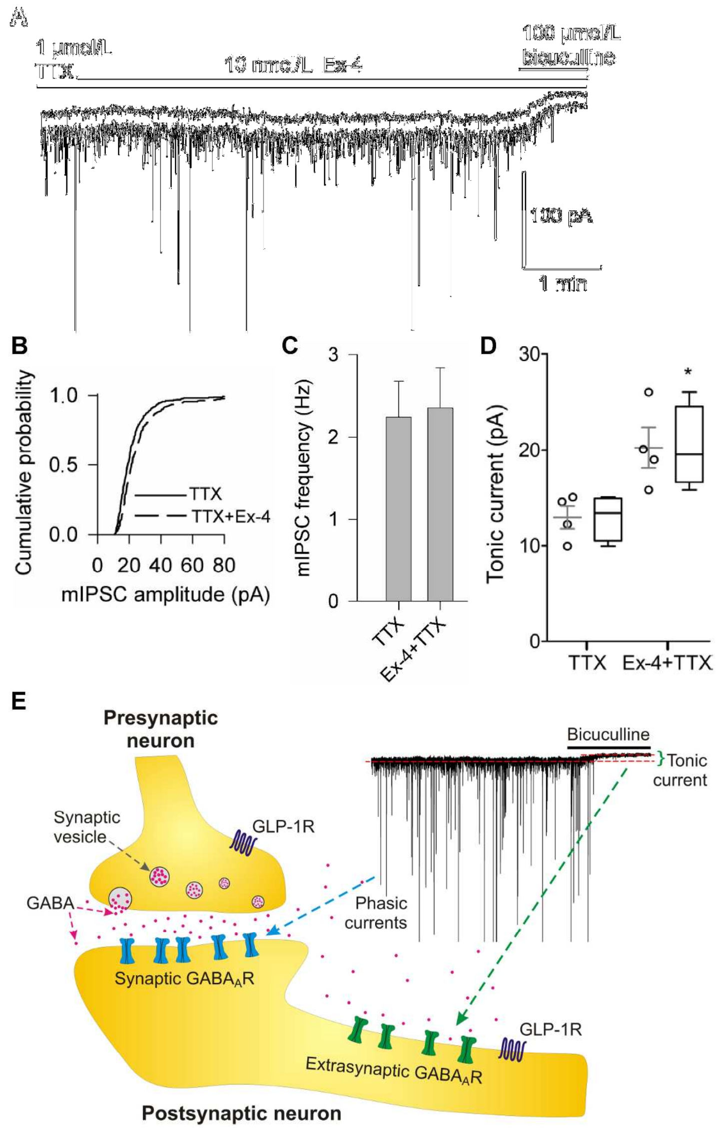

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