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Hippocampal *NPY* gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP

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Abstract

Recently, hippocampal neuropeptide Y (*NPY*) gene therapy has been shown to effectively suppress both acute and chronic seizures in animal model of epilepsy, thus representing a promising novel antiepileptic treatment strategy, particularly for patients with intractable mesial temporal lobe epilepsy (TLE). However, our previous studies show that recombinant adeno-associated viral (rAAV)-*NPY* treatment in naive rats attenuates long-term potentiation (LTP) and transiently impairs hippocampal learning process, indicating that negative effect on memory function could be a potential side effect of *NPY* gene therapy.

Here we report how rAAV vector-mediated overexpression of *NPY* in the hippocampus affects rapid kindling, and subsequently explore how synaptic plasticity and transmission is affected by kindling and *NPY* overexpression by field recordings in CA1 stratum radiatum of brain slices. In animals injected with rAAV-*NPY*, we show that rapid kindling-induced hippocampal seizures *in vivo* are effectively suppressed as compared to rAAV-empty injected (control) rats. Six to nine weeks later, basal synaptic transmission and short-term synaptic plasticity are unchanged after rapid kindling, while LTP is significantly attenuated *in vitro*. Importantly, transgene *NPY* overexpression has no effect on short-term synaptic plasticity, and does not further compromise LTP in kindled animals. These data suggest that epileptic seizure-induced impairment of memory function in the hippocampus may not be further affected by rAAV-*NPY* treatment, and may be considered less critical for clinical application in epilepsy patients already experiencing memory disturbances.

Keywords

NPY; Gene therapy; Epilepsy; Rapid kindling; Synaptic transmission; Synaptic plasticity; LTP; Hippocampus

Introduction

Neuropeptide Y (*NPY*) is an abundantly expressed peptide in the brain involved in diverse functions such as food intake, anxiety, blood pressure and memory (Pedrazzini et al., 2003; Lin et al., 2004). Strong antiepileptic effects of *NPY* have also been reported in numerous

studies, proposing a critical role of endogenous NPY in seizure regulation by controlling neuronal excitability (Vezzani et al., 1999; DePrato Primeaux et al., 2000). Mice lacking the *NPY* gene are more disposed to seizures (Erickson et al., 1996; Baraban et al., 1997), whereas rats overexpressing NPY show decreased seizure susceptibility and epileptogenesis (Vezzani et al., 2002). At a cellular level, NPY is normally expressed in a subset of GABAergic interneurons and is preferentially released at high frequency neuronal firing (Thureson-Klein et al., 1986; Kits and Mansvelder, 2000). When released, NPY can modulate inhibitory GABA-mediated (Sun et al., 2003) and excitatory glutamate-mediated (Haas et al., 1987) synaptic transmission. In the hippocampus, NPY has profound inhibitory effect on presynaptic glutamate release and can reduce the magnitude of evoked excitatory responses (Colmers et al., 1985), probably by reducing Ca^{2+} -influx in axonal terminals of principal glutamatergic neurons (Colmers et al., 1988). This mechanism probably also underlies the inhibitory effect of NPY on the generation of long-term potentiation (LTP) in hippocampus (Whittaker et al., 1999), which is a form of long-lasting and activity-dependent synaptic plasticity probably underlying learning and memory (Lynch, 2004).

Recently, a novel gene therapy strategy based on the recombinant adeno-associated viral (rAAV) vector carrying the *NPY* gene has been developed to treat epilepsy, in particular intractable temporal lobe epilepsy (TLE) (Noè et al., 2007). Strong seizure-suppressant effects have been demonstrated in both acute and chronic epilepsy models in animals with rAAV-mediated hippocampal NPY overexpression (Richichi et al., 2004; Lin et al., 2006; Noè et al., 2008), and these data have provided the framework of a protocol for *NPY* gene transfer in epilepsy patients, which is currently under evaluation by the FDA (Vezzani, 2007). One main concern of using this treatment in clinical applications is a potential side effect that may exacerbate cognitive function, which is usually already compromised in epilepsy patients. Previously, we have shown that naive rats with rAAV-mediated transgene NPY overexpression in the hippocampus have a transient delay of hippocampal-based learning which is paralleled by an attenuation of LTP in CA1 area (Sørensen et al., 2008b). However, until now it is unknown to what extent LTP is affected by transgene NPY in animals that have already experienced epileptogenesis. Therefore, in the present study we determined alterations in synaptic transmission and plasticity in slices from animals injected with rAAV-NPY and subjected to 40 rapid kindling stimulation-induced seizures, which trigger a process of epileptogenesis and leads to permanent hyperexcitability in the hippocampus (Elmér et al., 1996).

Methods and materials

Animals

A total of 34 adult male Sprague Dawley rats (250 g; Charles River, Germany) were used. Animals were housed in individual cages at 22 °C under a 12-hour light/dark cycle with free access to food and water. Experimental procedures were approved by the local Ethical Committee for Experimental Animals, and followed guidelines in accordance of European Community Council Directive for the Care and Use of Laboratory Animals.

Viral vector injection

The rAAV vectors were produced as described elsewhere (During et al., 2003; Richichi et al., 2004). Briefly, a plasmid containing the human prepro-NPY cDNA was subcloned into an expression cassette made of the rat neuron-specific enolase (NSE) promoter, woodchuck post-translational regulatory element (WPRE) and a bovine growth hormone polyA (BGHpA) signal, flanked by AAV2 inverted terminal repeats (pAM/NSE-NPY-WPRE-BGHPA). This cassette was cloned into the backbone of the chimeric AAV vector (mix of AAV serotype 1 and 2 capsid helper plasmid) and purified. An empty control vector carrying no transgene

(pAM/NSE-empty-WPRE-BGHpA) was produced as above. For injection of viral vectors, animals were anesthetized by intra-peritoneal injection of ketamine (80 mg/kg) and xylazine (15 mg/kg) mixture, and placed into a KOPF stereotaxic frame (David Kopf Instruments, Tujunga, CA). Through drill holes made in the skull, viral vector solutions were injected bilaterally at one site in the dorsal (AP −3.3, ML ±1.8, V −2.6) and at two sites in the ventral (AP −4.8, ML ±5.2, V −6.4 and −3.8) hippocampus (in mm). Reference points for all coordinates were bregma, midline and dura, tooth bar at −3.3 mm (Paxinos and Watson, 1996). At each site, 1 μ l vector suspension (with genomic titer of 1×10^{13} particles per ml for rAAV-NPY and rAAV-empty vector) was injected during 5 min (0.2 μ l per min) and the pipette was left in place for additional 3 min to minimize backflow through the injection track when retracting the pipette.

Electrode implantation

Two weeks following viral vector injection, animals were anesthetized as described above. A bipolar stainless-steel stimulating/recording electrode (PlasticsOne, Roanoke, VA) was implanted stereotaxically into the left ventral hippocampus at the following coordinates (in mm): AP −4.8, ML −5.2, V −6.3 and tooth bar at −3.3. This electrode and a reference electrode (inserted into the cheek muscle) were fixed in a pedestal and onto the skull with dental cement. Animals were allowed to recover for one week before undergoing electrical stimulation.

Electrical rapid kindling stimulations

The threshold for inducing focal epileptiform activity of more than 5 s duration was determined in each animal by applying stepwise stimulations (10 μ A steps, 1 s, 100 Hz) at increasing current intensity, starting at 10 μ A. Focal epileptiform activity (afterdischarge, AD) was detected by electroencephalographic (EEG) recording. During rapid kindling stimulation, consisting of 40 suprathreshold stimulation trains (10 s, 1 ms square wave pulses at 50 Hz, 400 μ A intensity) separated by 5 min interval between stimulations, EEG activity was continuously recorded on a MacLab system (ADInstruments, Bella Vista, Australia) for 200 min except during stimulations. Behavioral seizures were scored according to the scale of Racine (1972): stage 0, no behavioral changes; stage 1, facial twitches; stage 2, chewing and head nodding; stage 3, unilateral forelimb clonus; stage 4, rearing, body jerks, bilateral forelimb clonus; stage 5, imbalance. Rats injected with rAAV-NPY or rAAV-empty vector, and used for rapid kindling (RK) were designated as RK-rAAV-NPY ($n=12$) and RK-rAAV-empty ($n=9$) injected animals, respectively.

Electrophysiology

For electrophysiology, rats used for rapid kindling (RK-rAAV-NPY and RK-rAAV-empty injected animals) and 13 time-matched control rats not exposed to rapid kindling (8 rats injected with rAAV-empty vector and with no electrode implantation, and 5 naive rats) were briefly sedated with isoflurane before decapitation. Their brains were quickly placed into ice-cold sucrose solution (in mM; 195 sucrose, 2.5 KCl, 0.5 CaCl₂, 7.0 MgCl₂, 28 NaHCO₃, 1.25 NaH₂PO₄, 7.0 glucose, 1.0 ascorbate and 3.0 pyruvate; adjusted to pH 7.4; osmolarity 300 mOsm; oxygenated with 95% O₂/5% CO₂) and 350 μ m transverse slices were prepared from the right hippocampus (i.e. contra-lateral to the stimulation/EEG electrode) using a vibratome (Vibratome 3000, Ted Pella, Inc., Redding, CA) containing the same solution. Slices were maintained for >1 h in artificial cerebrospinal fluid (aCSF) (mM; 119 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 26 NaHCO₃, 1.0 NaH₂PO₄, and 11 glucose; pH 7.4; 296 mOsm; oxygenated, room temperature) before being transferred to the recording chamber, which was constantly perfused (2 ml per min) with oxygenated aCSF. In stratum radiatum of CA1, a bipolar stimulation electrode and a pipette filled with aCSF (tip resistance of 0.8–1.2 M Ω) were placed, separated by approximately 500 μ m. Field excitatory postsynaptic potentials (fEPSPs)

at increasing stimulation intensities were recorded and used for input-output analysis by plotting the presynaptic fiber volley (PSFV; mV) against the slope (mV/ms) of the corresponding fEPSP. Slices generating fEPSPs with PSFV/EPSP ratio of more than 1:3, and/or those with maximal fEPSP amplitudes of less than 1 mV were excluded from the analysis. Stable submaximal baseline fEPSPs (30–40% of maximal fEPSP) were continuously monitored for 5–10 min (at 0.05 Hz) before paired-pulse stimulations were delivered at interstimulus intervals (ISI) of 25, 50, 100 and 200 ms. For LTP, a 15 min fEPSP baseline was recorded (at 0.067 Hz and with 15% acceptable variability) before high-frequency stimulation (HFS, 100 Hz, 1 s) was applied. Field EPSPs were recorded for another 60 min, and analyzed by measuring the initial slope (1 ms), normalized to average baseline values and plotted against time (average of four fEPSPs per min). Data were acquired at 10 kHz and filtered at 2.9 kHz using HEKA amplifier and software (EPC 10, PATCHMASTER, HEKA Elektronik, Lambrecht, Germany) and off-line analysis was performed using FITMASTER (HEKA Elektronik) and Igor Pro (Wavemetrics, Lake Oswego, OR) software.

Immunohistochemistry

All brain slices were processed for visualization of NPY immunoreactivity. Following recording, slices were post-fixed overnight in 4% paraformaldehyde (4 °C), rinsed in KPBS, quenched (3% H₂O₂, 10% MeOH in KPBS) and incubated in a 1:5000 dilution of rabbit antiserum to rat NPY (Sigma-Aldrich, Sweden) in 5% normal goat serum in KPBS for four days at 4 °C. Finally slices were incubated in biotinylated secondary antibody (BA1000; 1:200; Vector Laboratories, Burlingame, CA). The reaction was amplified (Vectastain ABC KIT, Vector Laboratories) and visualized by 3–3'-diaminobenzidine (DAB). For improved image illustrations, some microtome slices (30 µm) were prepared from the slices (350 µm) cut on the Vibratome. These thin slices were processed as above, but incubated in primary antibody in a 1:1000 dilution for 24 h. All images were digitally acquired and no damage caused by either virus or surgery was observed in any slices.

Statistical analysis

The level of significance was $p < 0.05$ and data are presented as mean $\pm$ SEM. Kindling data were analyzed using two-tailed Student's *t*-test. Input-output and paired-pulse facilitation (PPF; calculated as the mean of five consecutive fEPSPs) data were analyzed by ANOVA followed by Bonferroni-Dunn post-hoc test. For LTP analysis, repeated-measures ANOVA and Mann-Whitney test was used. Differences in rate of LTP induction between groups were evaluated by χ^2 -test followed by Fisher's exact test. LTP was determined in individual slices and recognized as an increase by >15% from baseline values, calculated 20–24 min after HFS. Investigators conducting behavioral grading of seizures, EEG analysis, and electrophysiological recordings were blind to group identities of experimental animals and pre-treatment conditions. For electrophysiology, animals were randomly selected for experiments on a day-to-day basis. Since no differences in input-output, PPF and LTP were detected in slices prepared from naive rats and non-stimulated rats injected with rAAV-empty vector, these animals were pooled in one group and are together referred as control slices.

Results

Endogenous and transgene NPY expression

Expression of endogenous and transgene NPY was determined in all slices by immunohistochemistry. Consistent with previous observations (Richichi et al., 2004; Lin et al., 2006; Noè et al., 2008; Sørensen et al., 2008b,a), injection of rAAV-NPY vector into the hippocampus gave rise to long-lasting and strong expression of transgene NPY throughout the hippocampus (Figs. 1A, B, C). RK-rAAV-NPY treated animals had a uniform expression pattern of transgene NPY immunoreactivity as revealed in hippocampal slices used for

electrophysiology 7–9 weeks post viral vector injection. Transgene NPY was observed within the cell layers of CA1 and CA3, including stratum pyramidale, moleculare, radiatum and oriens, as well in the granule cell layer, molecular layer and hilus of the dentate gyrus (Figs. 1A, B, C) in agreement with previous results (for details see Richichi et al., 2004 and Sørensen et al., 2008a). Transgene NPY was predominantly confined to neuronal cell bodies and fibers throughout the hippocampus. In slices from RK-rAAV-empty treated animals, scattered NPY immunoreactivity was observed throughout hippocampus, particularly within the hilus of the dentate gyrus, most likely representing NPY-positive interneurons (Figs. 1D, E, F). In slices from naive control animals, we found similar expression pattern and intensity of NPY immunoreactivity (Figs. 1G, H, I) as described for RK-rAAV-empty treated slices (see above), suggesting that rapid kindling, at least 4–6 weeks post kindling, did not alter endogenous NPY expression.

Transgene NPY provides anticonvulsive effects during rapid kindling

Applying stepwise stimulations at increasing intensities until reaching focal epileptiform activity of more than 5 s duration did not reveal any difference in seizure threshold between RK-rAAV-NPY ($n=12$) and RK-rAAV-empty ($n=9$) treated animals (Fig. 2A). Similarly, during the 40 supra-threshold stimulations with 5 min intervals during 3 h and 20 min, the number of stimuli needed to reach seizure stage 1–5 was similar for both groups (Fig. 2B). However, the mean AD duration recorded using stimuli at threshold intensity strength was significantly shorter ($p<0.01$, t -test) in RK-rAAV-NPY (25.1 ± 6.0 s) as compared to RK-rAAV-empty (72.3 ± 12.8 s) treated animals (Fig. 2C). Also, during the rapid kindling procedure, the mean AD duration at seizure stages 1–4 was significantly shorter in RK-rAAV-NPY as compared to RK-rAAV-empty treated animals (stage 1: 29.4 ± 1.5 s vs. 62.3 ± 3.8 s, $p<0.001$; stage 2: 39.5 ± 3.1 s vs. 91.7 ± 9.6 s, $p<0.001$; stage 3: 37.3 ± 8.3 s vs. 84.9 ± 10.8 s, $p<0.01$; stage 4: 37.7 ± 3.0 s vs. 53.2 ± 5.4 s, $p<0.05$; Fig. 2C). Finally, the average duration of total ADs in each animal during kindling was reduced by ~50% in RK-rAAV-NPY (23 ± 2 min) as compared to RK-rAAV-empty (44 ± 6 min) treated animals ($p<0.001$, t -test).

Basal synaptic transmission and short-term synaptic plasticity

Four to six weeks after animals were subjected to rapid kindling, vibratome slices from the contralateral hippocampus (site with no stimulation/EEG electrode) were prepared. Input-output, paired-pulse and HFS-induced fEPSPs in CA1 were analyzed to determine possible alterations in synaptic transmission and plasticity. Serving as a control, recordings in slices from time-matched animals not subjected to rapid kindling were studied.

Evoking fEPSPs using stimulations with stepwise increasing intensities, thereby establishing an input-output relationship between the amount of activated afferent axons (estimated by amplitude of PSFV) and the corresponding magnitude of postsynaptic response (estimated by steepness of initial slope of the fEPSP), revealed that basal synaptic transmission was unaltered between RK-rAAV-NPY ($n=25$ slices, 9 animals), RK-rAAV-empty ($n=23$ slices, 11 animals) and control ($n=22$ slices, 8 animals) slices (Fig. 3A). Paired stimulations at different ISIs induced fEPSPs with pronounced PPF (Fig. 3B). No differences in PPF were detected at ISIs of 25, 50 and 100 ms between the groups (RK-rAAV-NPY, $n=20$ slices, 7 animals; RK-rAAV-empty, $n=21$ slices, 9 animals; control, $n=22$ slices, 8 animals). However, at ISI of 200 ms, PPF was significantly higher in RK-rAAV-NPY treated slices ($138\pm3\%$) as compared to control slices ($126\pm4\%$) ($p<0.05$, ANOVA followed by Bonferroni-Dunn post hoc test), but similar to RK-rAAV-empty treated slices. No differences were detected between RK-rAAV-empty and control slices (Fig. 3B).

Long-term potentiation

In control slices, stable HFS-induced LTP was recorded in Schaffer collateral-CA1 synapses, with a $60\pm4\%$ increase of fEPSP initial slope (calculated 20–24 min post HFS) as compared to baseline values ($n=10$ slices, 8 animals), lasting for at least 60 min (Fig. 3C). At a similar time point, the fEPSP initial slope in RK-rAAV-empty treated slices ($n=11$ slices, 9 animals) was only increased by $43\pm3\%$, which was significantly lower ($p<0.05$, repeated measures ANOVA; $p<0.001$, Mann-Whitney test) as compared to control slices. Likewise, in RK-rAAV-NPY treated slices, the magnitude of LTP ($39\pm3\%$, $n=11$ slices, 7 animals) was significantly lower ($p<0.05$, repeated measures ANOVA; $p<0.001$, Mann-Whitney test) as compared to control slices, but similar to RK-rAAV-empty treated slices (Fig. 3C). At the same time, the induction rate of LTP (i.e. at least 15% increase from baseline values) was similar between groups (73% in RK-rAAV-NPY, 68% in RK-rAAV-empty, 71% in control slices; χ^2 -test followed by Fisher's exact test).

Discussion

The present study demonstrates that hippocampal rAAV vector-mediated NPY overexpression significantly reduces seizure duration during rapid kindling stimulations. We also demonstrate for the first time that LTP in the hippocampus is attenuated after rapid kindling, and that transgene NPY has no further detrimental effect on LTP. This is in contrast with our previous studies in naive animals, where LTP was compromised by transgene NPY.

Transgene NPY expression and seizure suppression

In slices transduced with rAAV-NPY, immunohistochemistry revealed increased NPY levels mainly restricted to neurons and fibers throughout the hippocampus. This pattern of transgene NPY expression is in line with previous observations showing that injection of rAAV-NPY vector causes long-lasting and widespread expression of transgene NPY in the hippocampus (Richichi et al., 2004; Noè et al., 2008). At the same time, no alterations are observed in the expression of NPY Y2 receptor subtype (Noè et al., 2008), which appears to be centrally involved in mediating the seizure-suppression effect of transgene NPY (Lin et al., 2006). In rats with hippocampal overexpression of transgene NPY, acute intrahippocampal and intraventricular kainic acid-induced seizures were significantly attenuated revealed by delayed onset and time spent in EEG seizures (Richichi et al., 2004), and after electrical-induced status epilepticus, the progression and frequency of subsequent spontaneous seizures were decreased in a rat model of chronic epilepsy (Noè et al., 2008), indicating that transgene NPY has strong anticonvulsive and antiepileptic effects. In addition, in the rapid kindling model of epilepsy, Richichi et al. (2004) observed a significant delay in kindling acquisition at stage 3–5 and a significant increase in AD threshold, suggesting that transgene NPY may also effectively suppress epileptogenesis. In the present study, the threshold for inducing focal epileptiform activity and kindling development (at any seizure stage) was similar between RK-rAAV-NPY and RK-rAAV-empty treated animals. However, the duration of ADs was dramatically decreased by transgene NPY at threshold stimulation intensity and during seizure stage 1–4 (by almost 50%). Since the experimental conditions in our study were different from Richichi et al. (2004) (i.e. time gap between viral vector injection and rapid kindling was 3 weeks and 8 weeks, respectively), it appears that the seizure parameters affected by transgene NPY could depend on the experimental conditions used. For example, Lin et al. (2006) observed unaltered onset to first motor seizures induced by kainic acid in rAAV-NPY treated mice, while the time spent in seizures was significantly reduced in rAAV-NPY treated animals as compared to controls.

Influence on synaptic transmission and short-term synaptic plasticity

In vivo recordings reveal that stimulation-induced EPSPs in CA1 of kindled animals can be enhanced as long as up till 28 days after the last kindling stimulation (Leung and Shen, 1991), but remain unchanged when assessed by *in vitro* recordings in brain slices. *In vitro*, basal synaptic transmission of kindled animals remains unaltered both shortly after the last kindling stimulation (<24 h) (Leung and Wu, 2003) and at later time points (>3 weeks) (Zhao and Leung, 1991; Leung et al., 1994). Similarly, our input-output analysis did not reveal any changes in basal synaptic transmission neither in slices from RK-rAAV-empty injected animals, nor in those injected by rAAV-NPY vector. PPF was also unchanged between the groups, except at ISI of 200 ms, where the rate of facilitation was slightly, but significantly, higher in RK-rAAV-NPY as compared to control slices. Thus, it appears that short-term synaptic plasticity is mostly unaffected by rapid kindling and transgene NPY. In other models of epilepsy, e.g., after traditional electrical kindling (Zhao and Leung, 1991; Leung and Wu, 2003) and during the latent period following status epilepticus (El-Hassar et al., 2007), an increase in PPF has been observed *in vitro*. Interestingly, even after less severe seizures during conventional kindling, such as after partial kindling, i.e., when animals do not experience any generalized seizures, PPF was persistently increased 6–8 weeks after the last stimulation (Leung et al., 1994). Moreover, partial kindling induced similar synaptic changes in the contralateral to the stimulation hippocampal CA1 site (Leung et al., 1994). Taken together, these data indicate that these two models of epilepsy differ in how excitatory synaptic transmission and short-term synaptic plasticity are affected, and therefore may differ in mechanistic aspects of epileptogenesis and development of increased excitability in the hippocampus. The fact that transgene NPY had no effect on PPF indicates that probably very limited amount of transgene NPY is released during low frequency stimulations. This is in line with our previous studies, where we showed that transgene NPY is released predominantly during HFS (Sørensen et al., 2008b,a).

Attenuation of long-term potentiation

The ability to express LTP is markedly reduced in the surgically resected human hippocampal specimens from TLE patients (Beck et al., 2000) and often these patients have complains for impaired memory function (Helmstaedter et al., 2003; Elger et al., 2004), supporting the idea that LTP may be a synaptic correlate of memory (Lynch, 2004). Similarly, in animals, isolated CA1 slice preparation exposed to repeated seizure-like activity can totally loose the ability to generate LTP (Hu et al., 2005), and several reports have demonstrated that both electrical and chemical kindling can severely attenuate LTP *in vitro* (Leung and Wu, 2003; Schubert et al., 2005) and induce spatial memory deficits (Leung and Shen, 1991; Leung et al., 1994; Mortazavi et al., 2005). In the present study, we now also show for the first time that *rapid kindling* significantly reduces LTP in the hippocampal formation. In addition, our data show, also for the first time, that in slices from RK-rAAV-NPY injected animals, LTP is impaired to a similar magnitude as slices from RK-rAAV-empty injected animals, both compared to control slices from non-kindled animals. This is an important finding bearing in mind that hippocampal rAAV-NPY gene therapy is being considered for clinical application in epilepsy.

Concluding remarks

In contrast to our previous findings, where LTP was strongly attenuated in CA1 of hippocampal slices in naive rats (Sørensen et al., 2008b), our present data suggest that rAAV-NPY therapy in the epileptic brain does not seem to exacerbate the degree of memory deficit already existing in epileptic patients. The detailed mechanisms of why transgene NPY limit seizure duration without affecting LTP need to be further investigated. Nevertheless, it is clear that there may be multiple different mechanisms for how NPY interferes with these two processes in the normal versus epileptic hippocampus. For example, apart from inhibiting glutamate release

from excitatory synapses in the hippocampal formation (Haas et al., 1987; Colmers et al., 1988), NPY has an effect on excitatory and inhibitory transmission onto a subpopulation of inhibitory interneurons in the dentate gyrus, which appear to be highly vulnerable to epileptic insults (our unpublished data).

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Fig. 1.

Expression of endogenous and transgene NPY in the hippocampus. (A, B, C) Representative slices from a RK-rAAV-NPY treated animal showing high levels of transgene NPY immunoreactivity throughout the whole hippocampus with particular dense transgene NPY expression in hilus of the dentate gyrus and in mossy fiber terminals in stratum oriens of CA3. (D, E, F) NPY immunoreactivity in a slice from a RK-rAAV-empty treated animal showing scattered NPY positive cells and fibers throughout the hippocampus (indicated by arrows in hilus of dentate gyrus), most likely resembling NPY containing interneurons. (G, H, I) Endogenous immunoreactivity of NPY is confined to interneurons clearly identified in the hilus of the dentate gyrus (shown by arrows), but is detectable throughout hippocampus. Boxes in left images are magnified and shown on right. Dashed lines show the borders of the principal cell layer. Scale bar in A is 1 mm and applies for D and G. Scale in B is 100 μ m and applies for C, E, F, H, and I.

Fig. 2. Transgene NPY decreases seizure duration in rapid kindling. (A) Mean afterdischarge (AD) threshold (μ A) inducing focal epileptiform activity of more than 5 s duration. (B) Mean number of suprathreshold stimuli needed to reach seizure stage 1–5 during 40 rapid kindling stimulations. (C) Mean AD duration at threshold (th) stimuli intensity, and during seizure stage 1–5 at suprathreshold stimulation intensity. In all figures, black bars represent RK-rAAV-NPY ($n=12$) and white bars represent RK-rAAV-empty ($n=9$) treated animals, respectively. Values are mean $\pm$ SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, t -test.

Fig. 3.

Alterations in synaptic transmission and plasticity in CA1 after rapid kindling in rAAV-NPY-treated and control animals. (A) The amplitude (mV) of the presynaptic fiber volley (PSFV) is plotted against the initial slope (mV/ms) of the corresponding fEPSP for RK-rAAV-NPY ($n=25$ slices, 9 animals), RK-rAAV-empty ($n=23$ slices, 11 animals) and control ($n=22$ slices, 8 animals) animals ($p>0.05$, ANOVA with Bonferroni-Dunn test). Each trace is labeled as shown in C. (B) Mean evoked fEPSP showing pronounced PPF at different ISIs (at 25, 50, 100 and 200 ms) in RK-rAAV-NPY ($n=20$ slices, 7 animals), RK-rAAV-empty ($n=21$ slices, 9 animals) and control ($n=22$ slices, 8 animals) animals. * $p<0.05$, ANOVA followed by Bonferroni-Dunn test. (C) HFS-induced LTP of fEPSPs expressed as initial slope normalized to average baseline values and plotted against time (average of four fEPSPs per min) for RK-rAAV-NPY ($n=11$ slices, 7 animals), RK-rAAV-empty ($n=11$ slices, 9 animals) and control ($n=10$ slices, 7 animals) animals. HFS-induced LTP is significantly attenuated in RK-rAAV-NPY and RK-rAAV-empty treated slices as compared to control slices, revealed by repeated measures ANOVA ($p<0.05$) and Mann-Whitney test ($p<0.001$). Representative fEPSPs (average of four traces) acquired during baseline and 20 min post HFS are shown for each group. Data in A, B and C are shown as mean $\pm$ SEM.