Molecular Psychiatry
https://doi.org/10.1038/s41380-020-0742-4

ARTICLE

# Liposome-based targeting of dopamine to the brain: a novel approach for the treatment of Parkinson's disease

Meygal Kahana[1] · Abraham Weizman[2,3,4] · Martin Gabay[1] · Yelena Loboda[1] · Hadar Segal-Gavish[3,5,6] · Avishai Gavish[3,7] · Yael Barhum[5] · Dani Offen[3,4,5] · John Finberg[1] · Nahum Allon[1] · Moshe Gavish[1]

Received: 23 October 2019 / Revised: 8 March 2020 / Accepted: 20 April 2020
© The Author(s), under exclusive licence to Springer Nature Limited 2020

## Abstract

Delivery of drugs into the brain is poor due to the blood brain barrier (BBB). This study describes the development of a novel liposome-based brain-targeting drug delivery system. The liposomes incorporate a diacylglycerol moiety coupled through a linker to a peptide of 5 amino acids selected from amyloid precursor protein (APP), which is recognized by specific transporter(s)/receptor(s) in the BBB. This liposomal system enables the delivery of drugs across the BBB into the brain. The brain-directed liposomal system was used in a mouse model of Parkinson's disease (PD). Intra-peritoneal (IP) administration of liposomes loaded with dopamine (DA) demonstrated a good correlation between liposomal DA dose and the behavioral effects in hemiparkinsonian amphetamine-treated mice, with an optimal DA dose of 60 µg/kg. This is significantly lower dose than commonly used doses of the DA precursor levodopa (in the mg/kg range). IP injection of the APP-targeted liposomes loaded with a DA dose of 800 µg/kg, resulted in a significant increase in striatal DA within 5 min (6.9-fold, $p < 0.05$), in amphetamine-treated mice. The increase in striatal DA content persisted for at least 3 h after administration, which indicates a slow DA release from the delivery system. No elevation in DA content was detected in the heart or the liver. Similar increases in striatal DA were observed also in rats and mini-pigs. The liposomal delivery system enables penetration of compounds through the BBB and may be a candidate for the treatment of PD and other brain diseases.

## Introduction

The symptoms of Parkinson's disease (PD) are related to loss of dopamine (DA)-ergic neurons in the substantia nigra [1]. The available pharmacological treatments are directed at replacement of the DA deficiency, by enhancing brain DA levels (levodopa; MAO-B and COMT inhibitors) or by DA agonists. DA does not cross the blood brain barrier (BBB) due to its polar nature and therefore its administration is not used for PD therapy. The natural DA precursor, levodopa (L-DOPA) is used for the treatment of PD, due to its ability to cross the BBB via a transporter protein (SLC7A5) to a limited extent (0.5–5%); in the brain, L-DOPA is converted to DA [2, 3]. A delivery system that enables penetration of DA into the brain is an unmet need. To this end we developed a brain-targeted liposomal system (BTLS) as carriers for transporting DA via the BBB in a safe, controlled, and effective manner. In the present study, we describe the design and efficacy of an APP-derived peptide to target specific BBB transporter(s)/receptor(s).

The peptide fragment (RERMS) derived from APP that recognizes BBB transporter(s)/receptor(s) [4–7] was bound

Deceased: Nahum Allon.

**Supplementary information** The online version of this article (https://doi.org/10.1038/s41380-020-0742-4) contains supplementary material, which is available to authorized users.

✉ Moshe Gavish
mgavish@technion.ac.il

1 The Ruth and Bruce Rappaport Faculty of Medicine, Technion Institute of Technology, 31096 Haifa, Israel

2 Research Unit at Geha Mental Health Center and the Laboratory of Biological Psychiatry, Felsenstein Medical Research Center, 4910002 Petah Tikva, Israel

3 Sackler Faculty of Medicine, Tel Aviv University, 6997801 Tel Aviv, Israel

4 Sagol School of Neuroscience, Tel-Aviv University, 6997801 Tel Aviv, Israel

5 Laboratory of Neuroscience, Felsenstein Medical Research Center, 4910002 Petah Tikva, Israel

6 The Child Psychiatry Division, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer 5262000, Israel

7 Goldschleger Eye Institute, Sheba Medical Center, Tel Hashomer 5262000, Israel

Published online: 05 May 2020

to diglycerol succinate by forming an amide bond between the carboxyl end of the succinate and the N terminal of the peptide, yielding: 1,2-dioleoyl-sn-glycero-3-succinate-A-H-RERMS-COOH [7] to target the BBB transporter(s)/receptor(s) [4–7]. Histidine (H) was added to RERMS sequence based on Ninomiya et al. [4] that showed a small, but better, trophic effect of HRERMS compared with RERMS. Alanine (A) served as a spacer for this linkage. We assumed that the APP-targeted liposomes could enable transfer of DA over the BBB, with limited DA exposure of peripheral organs and tissues. Beneficial effects of low doses of DA, administered by APP-targeted liposomes were assessed in a mouse model of PD. In addition, we evaluated the pharmacokinetics of DA accumulation in the striatum, liver and heart, in mice, rats and mini-pigs. The technological approach is similar to that of our liposome-based gene delivery system targeting lung cells [8].

## Materials and methods

### Materials

Cholesterol (Chol), 1,2-dioleyl-sn-glycero-3-phosphocholine (DOPC), and L-α-phosphatidic acid (PA) were purchased from Avanti Polar Lipids, Alabaster (Birmingham, AL), Texas Red from Molecular Probes (Eugene, OR), ammonium sulfate, methanol, sucrose, DA, levodopa (L-DOPA), carbidopa, 6-hydroxydopamine (6-OHDA), apomorphine, amphetamine, HCl, and ascorbic acid from Sigma–Aldrich (St. Louis, MO), chloroform and acetic acid from Bio lab (Jerusalem, Israel), APP from Atlanticpeptides (Lewisburg, PA), perchloric acid from Merck (Darmstadt, Germany), ketamine from Vetoquinol (Paris, France) and xylazine from Phibro (Petah Tikva, Israel).

### APP-targeted liposome preparation

The preparation of the APP-targeted liposome is illustrated in Supplementary Fig. 1. Liposomes with a composition of DOPC/Chol/PA at a molar ratio of 5.5:4.0:0.5, respectively, were prepared and dissolved in chloroform. The APP targeter was added to the mixture at a concentration of 0.5 mol %. The chloroform was removed by rotary evaporation (IKA, RV-10, Staufen im Breisgau, Germany) at 37 °C for 1 h. The dried lipid film was lyophilized overnight using a Virtis Benchtop SLC lyophilizer (SP Industries, Model 4KBTXL-75, Warminster, PA). The next day the dry film was hydrated with ammonium sulfate (0.2 M) and was sonicated (VWR, Model 75 HT, Bridgeport, PA) until all the dry lipid film was dissolved (between 6 to 9 min). The hydrated liposomes were dialyzed (dialysis tubing, high retention seamless cellulose tubing 12400 MWCO, Merck, Darmstadt, Germany) in order to remove the ammonium sulfate against 10% sucrose solution (four solution changes). In the next day, the liposomes were subjected to six freezing (dry ice) and thawing (37 °C water bath) cycles, followed by extrusion through 200-nm-pore and then 100-nm-pore filters (Whatman, polycarbonate and polyester membranes, cyclopore PC circles, Merck, Darmstadt, Germany) using an extrusion device (Northern Lipids Inc., Burnaby, Canada). Liposomes were then lyophilized overnight and kept at 4 °C in sealed vials until use (Supplementary Fig. 1).

In order to establish a standard method for loading DA into the liposomes in a way that can ensure reproducible drug concentration without leakage, we applied the "remote loading" method [9–11]. The active loading of liposomes with DA occurred in response to an ammonium sulfate gradient preloaded in the liposomes (to generate a negatively charged ionic pump). This procedure enabled accelerated DA accumulation (driving force). Efficient loading of DA in the liposomes is dependent on the trans-membrane ammonium sulfate gradient, liposome lipid composition, liposomal zeta potential, and loading capacity. This procedure generated small unilamellar liposomes reproducible by size (90–120 nm), zeta potential (−27 to −33.7 mV), and loading capacity (15 μg DA/mg phospholipids). No observable behavioral changes were detected following 1 h IP administration of unloaded APP-targeted liposomes to naïve mice.

### Formulation of DA loaded APP-targeted liposomes

In order to produce stable biodegradable liposomes that can protect the incorporated DA from peripheral degradation, we used Chol and phosphatidylcholine (egg source) as the main components of the liposomes. The APP-targeted lyophilized liposomes remained intact for at least 1 month when kept at 4 °C (Supplementary Table 1).

### Liposome loading with DA

A solution containing 300 μl of DA (1.5 mg) was added to each of the vials with the dry liposomes (26 mg phospholipids in each vial), vortexed for 1 min and incubated for 1 h at 4 °C. In order to stabilize the system and to protect the DA from oxidation, the liposomes were kept in 50 mM ascorbic acid at pH 5.7 until administered to the animals. The amount of loaded DA inside the liposomes was measured by HPLC (Coulochem II ESA, Stockholm, Sweden) with suitable electrochemical detector. The samples were ultracentrifuged (Thermofisher centrifuge U.M.C RC M150GX, Waltham, MA) at 100,000 × *g* at 4 °C for 1 h. Two phases were obtained in the tube, the lower phase was a transparent liquid that contained the DA that did not penetrate the liposomes and the upper phase was oily and contained liposomes loaded with DA. In order to determine

the degree of incorporation of DA, each phase was treated with 1 ml of 0.2 M perchlorate and mixed by vortex for 30 s followed by 10 min centrifugation at 1000 × *g* at 4 °C. The supernatants were collected for HPLC analysis. The liposomal DA content was measured by HPLC and ranged between 10−15 µg DA per 1.0 mg liposomal phospholipids.

### In vivo animal studies

Animals and source**:** male C57BL/6 mice, aged 8–10 weeks (Central Lab. Animal Inc Harlan, Yokneam, Israel), weighing 26–30 g, male Sprague Dawley rats aged 6 weeks (Central Lab. Animal Inc Harlan, Yokneam, Israel), weighing 160–180 g, and male Sinclair mini-pigs aged 6 weeks (Ben Meir farm, Yokneam, Israel), weighing 2200–3300 g were used in the study. All animals were provided with food and water ad libitum and kept in 12-h light/dark cycles. No animals were excluded from the analyses. No randomization or blinding was used in the experiments, however, the laboratory staff was blind to group allocation.

### Assessment of APP-targeted/non-targeted liposome BBB penetration using Texas Red labeling

IP injection of liposomes labeled with Texas Red with or without APP targeter to naïve mice was used for evaluation of liposomal penetration to the brain. One hour after the injection, mice, under anesthesia, were perfused with saline to remove the liposome residues. Brains were removed and fixed in formaldehyde then were examined in a fluorescence imaging device (Cri Maestro™ device, St. Louis, MO), excitation 595 nm and emission 635 nm (Fig. 1). Three groups were compared: APP-targeted liposomes labeled with Texas Red, non-targeted liposomes labeled with Texas Red and untreated mice.

### Stereotaxic surgery and in vivo validation of the striatal DA deficiency

Hemiparkinsonism was induced in mice by injection of 6-OHDA to the right striatum. The stereotaxic surgery and the rotational behavioral tests in the hemiparkinsonian mice were conducted as described previously [12–14] and are described in Supplementary Information 1. Amphetamine treatment was used for presynaptic DA depletion (Supplementary Information 2).

### Hemiparkinsonian/amphetamine mouse model

No increase in striatal DA content, as assessed by HPLC, was obtained in naïve mice following administration of APP-targeted liposomes loaded with DA. Thus, in an attempt to enhance the uptake of exogenous DA into the striatal dopaminergic neurons, we treated the hemiparkinsonian or naïve mice with repeated amphetamine administration to obtain depletion of vesicular DA. Repeated amphetamine administration (1 µg/kg every 2 weeks for 6 weeks) resulted in striatal DA depletion to a value of a tenth of untreated mice (Supplementary information 2). This observation is consistent with previous reports [15–18]. Hemiparkinsonian mice that participated in the rotational experiment were further tested for the striatal DA content after 2 months. Hemiparkinsonian mice were injected SC with amphetamine (1 mg/kg), beginning 3 weeks after the stereotaxic injection of 6-OHDA and then every 2 weeks, for a period of 6 weeks. Single IP injections of APP-targeted liposomes loaded with DA (40 or 60 µg/kg) were administered for behavioral assessments 2 weeks after the last injection of amphetamine.

### Pharmacokinetic studies

For the pharmacokinetic studies, we used a higher DA dose (mouse: 800 µg/kg) than that in the behavioral study (40 and 60 µg/kg) in order to ascertain detectable DA brain content measured by HPLC. Pharmacokinetic studies of brain DA penetration were conducted in amphetamine-treated mice, naïve rats, and naïve mini-pigs. DA contents in the striatum, heart, and liver were assessed by HPLC at various time points after the administration of APP-targeted liposomes loaded with DA. All tested organs were weighed and mixed with 0.1 M perchlorate. The tissue was homogenized at 4 °C for 30 s. The samples were centrifuged at 4 °C for 10 min at 1000 × *g*. The supernatants were collected and stored at −80 °C until the HPLC assay.

### Statistical analysis

Statistical analyses were carried out with the GraphPad Prism software (Version 6.01). Unpaired *t*-test, one- and two-way analysis of variance (ANOVA) were conducted as appropriate. The sample sizes of the groups were determined according to reasonable and convenient estimation, as well as expected results following preliminary observations. The variance was similar between the groups that were statistically compared. All results are expressed as mean ± SEM. $p < 0.05$ was considered statistically significant.

## Results

### Liposome preparation

The liposomal preparation is illustrated in Supplementary Fig. 1. The initial zeta potential of the APP-targeted liposomes was −4 mV. However, this condition resulted in

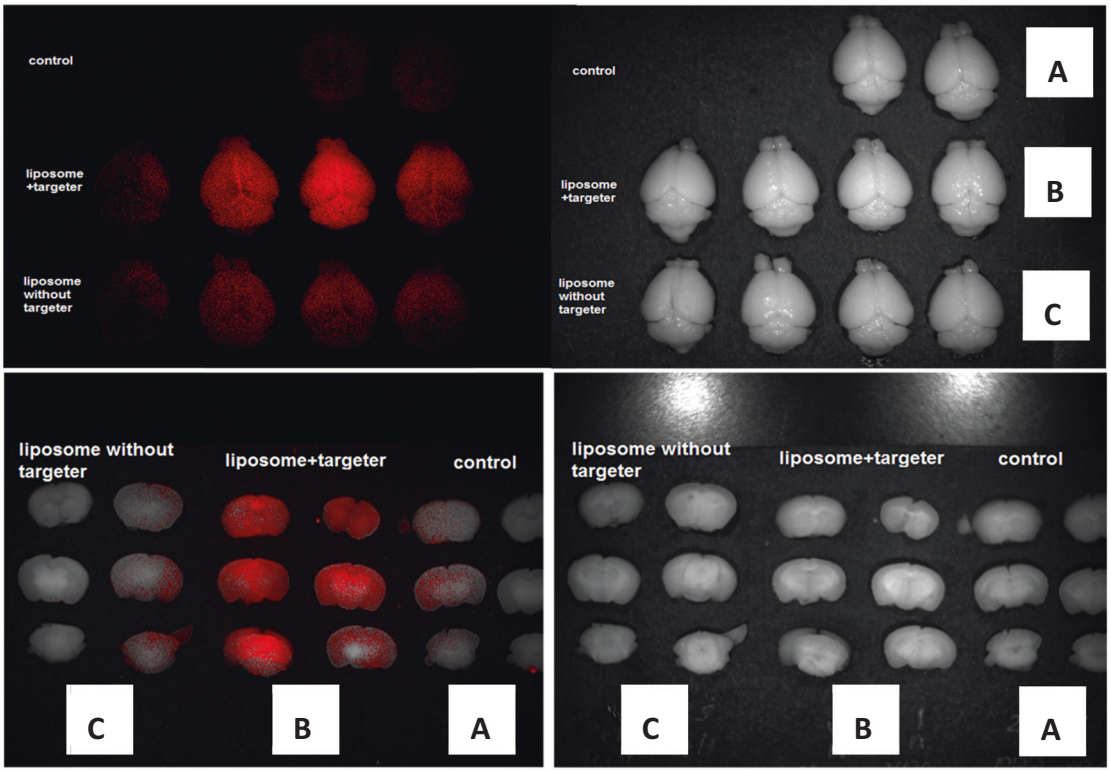


**Fig. 1 Whole brain (upper panels) and coronal brain sections (lower panels) of mice.** Right panels without fluorescent illumination and left panels following exposure to fluorescent illumination. APP-targeted or non-targeted liposomes labeled with Texas Red were administrated to mice by IP injection. One hour after the injection, mice were perfused with saline to remove the liposomal residues from the blood. Brains were removed and fixed in formaldehyde. The brains were examined in the Cri Maestro™ device, excitation 595 nm and emission 635 nm. A—untreated mice (control), B—mice treated with APP-targeted liposomes labeled with Texas Red, C—mice treated with non-targeted liposomes labeled with Texas Red.

instability and aggregation of the liposomes. Thus, we added 5% PA to the lipid mixture. This addition led to a decrease in the zeta potential magnitude to −33.7 mV and the liposomal size was stable around 100 nm. The addition of PA improved the stability and the homogeneity of the liposomes. In order to increase the shelf-life of the liposomes, we lyophilized them before the final DA loading that was conducted just before administration to the animals.

As shown in Supplementary Table 2, the zeta potential of the APP-targeted liposomes with or without DA payload and the non-targeted liposomes were between −33.7 to −27.0 mV. The size of the liposomes was around 100 nm that fits the criteria of adequate penetration through the BBB [19, 20]. As shown in Supplementary Table 1, the lyophilized APP-targeted liposomes kept at 4 °C show similar capacity to contain DA at both baseline and after 1 month.

## Penetration of Texas red labeled APP-targeted liposomes into mouse brain, behavioral and pharmacokinetic in mice

Validation of APP-targeted liposomal penetration into the brain was determined by Texas Red labeling of the APP-targeted or non-targeted liposomes. Three groups were included in this ex vivo experiment: untreated mice (control) (A), APP-targeted liposomes (liposomes + APP targeter) (B), and non-targeted liposomes (liposomes without APP targeter) (C).

As shown in Fig. 1 APP-targeted liposomes labeled with Texas Red penetrated the brain and are distributed throughout the brain. The fluorescent dye inside the brain was detected in the mice treated with the APP-targeted liposomes, but only to a very small extent in those administered with the non-targeted liposomes. In order to demonstrate an anti-parkinsonian effect of the BTLS, the hemiparkinsonian mouse model was used. To this end we tested the efficacy of APP-targeted liposomes loaded with DA for the determination of the threshold and optimal dose needed to induce contra-lateral turns in hemiparkinsonian amphetamine-treated mice. Mice turning to the contra-lateral side indicated penetration of DA to the brain and a corresponding agonistic effect at the DA-depleted striatum. The behavioral effectiveness of IP injection of DA, 40 and 60 μg/kg, loaded inside the BTLS was compared with ten-fold dose of free DA (600 μg/kg mouse). Preference to the contralateral side (left) was observed in hemiparkinsonian

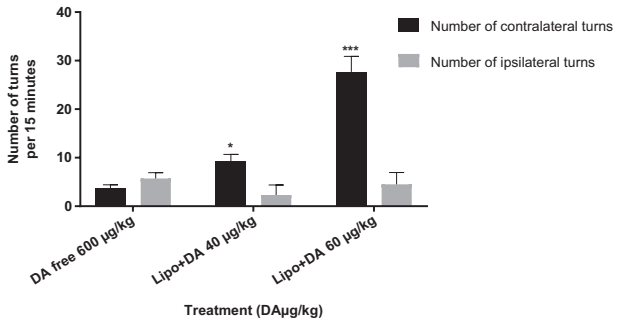


**Fig. 2 The impact of administration of free DA (600 µg/kg) and APP-targeted liposomes loaded with DA (40 and 60 µg/kg) on turns of hemiparkinsonian amphetamine-treated mice (mean ± SEM) for 15 min.** $n = 6$ in each group, two-way ANOVA: F (1,30) = 51.49, $p < 0.0001$: for DA 40 µg/kg versus free DA 600 µg/kg, $p = 0.014$; for DA 60 µg/kg versus free DA 600 µg/kg, $p < 0.0001$. ***$p <$ 0.0001 and *$p < 0.05$ versus corresponding ipsilateral turns. DA dopamine, Lipo APP-targeted liposomes.

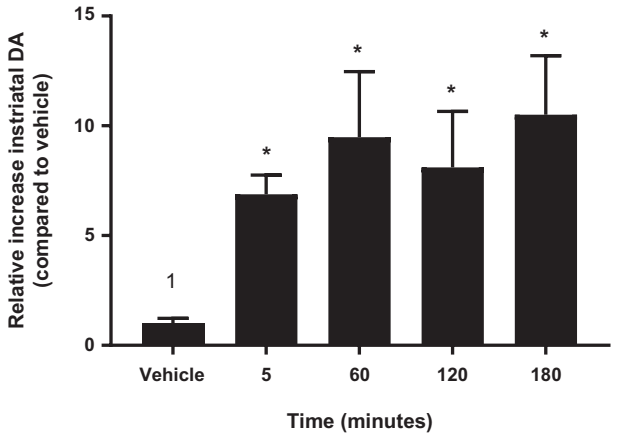


**Fig. 3 The effect of IP administration of APP-targeted liposomes loaded with DA on striatal DA content (picomoles of DA/striatum) in amphetamine-treated mice, expressed by relative increases.** The relative increases in striatal DA content following IP injection of APP-targeted liposome loaded with 800 µg/kg DA over 180 min compared with 5 min after vehicle administration (50 mM ascorbic acid). $n = 3$ in each group, except $n = 4$ in the 5 min liposomal treatment group: *$p <$ 0.05 versus vehicle.

amphetamine-treated mice following injection of APP-targeted liposomes loaded with DA (40 µg/kg and 60 µg/kg). However, the effect of 40 µg/kg was smaller than that achieved with 60 µg/kg. In contrast, administration of free DA (600 µg/kg mouse) was ineffective (Fig. 2).

In order to validate the contribution of amphetamine treatment in depleting striatal DA content and enhancement of liposomal DA uptake to the striatum, we administered 800 µg/kg DA loaded in APP-targeted liposomes to amphetamine-treated mice. A significant increase in striatal DA was detected within 5 min following IP injection of APP-targeted liposomes loaded with DA (800 µg/kg) in naïve mice (Fig. 3). The increase in DA content in the striatum persisted for up to 180 min. No such increase was

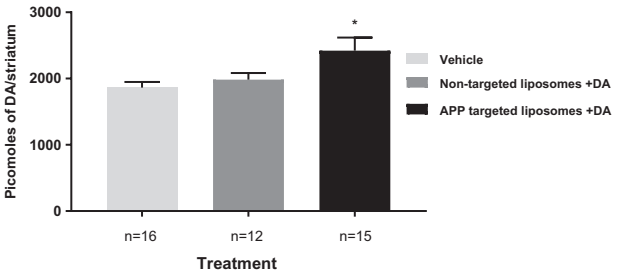


**Fig. 4 Changes in striatal DA content 30 min after IP injection of APP-targeted liposomes loaded with DA (400 µg/kg) to naïve rats compared with non-targeted liposomes loaded with DA (400 µg/kg) and vehicle (50 mM ascorbic acid).** One-way ANOVA: F (2, 40) = 4.62, *$p < 0.05$ (APP-targeted liposomes versus both non-targeted liposomes and vehicle).

detected in the mice treated with the vehicle. DA was undetectable in the liver or heart.

In order to examine the effect of DA-liposomal treatment in other animal species, we tested the pharmacokinetics of APP-targeted liposomes loaded with DA in another rodent model (rats) and mini-pigs [21], both without amphetamine pretreatment, as an additional mammalian model.

### Rats

A significant increase (+23%, $p < 0.05$) was observed in rat striatal DA 30 min following IP administration of BTLS loaded with DA (400 µg/kg) compared with non-targeted liposomes loaded with DA (400 µg/kg) and vehicle (Fig. 4). DA was undetectable in the liver or heart.

### Mini-pigs

IV administration of BTLS loaded with DA (360 µg/kg) to naïve mini-pigs resulted in a significant mild increase in the striatal DA content after 30 min (+17%, $t = 1.76$, df = 14, $p < 0.05$) (Fig. 5). DA was undetectable in the liver or heart.

## Discussion

We created small, stable unilamellar liposomes constructed from 1,2-dioleyl-sn-glycero-3-phosphocholine (DOPC), Chol and L-α-phosphatidic acid (PA) in a molar ratio of 5.5:4.0:0.5; the unilamellar membrane of these liposomes incorporated a five-peptide sequence of the APP recognized by transporter(s)/receptor(s) engaged in mediating transport across the BBB into the brain. IP administered of DA-loaded BTLS led to significantly increased striatal DA concentrations in mice, rats, and mini-pigs. Importantly, behavioral study supports incorporation of the liposome-delivered DA into functional striatal circuits; specifically, DA-loaded BTLS led to dose-dependent increase in the

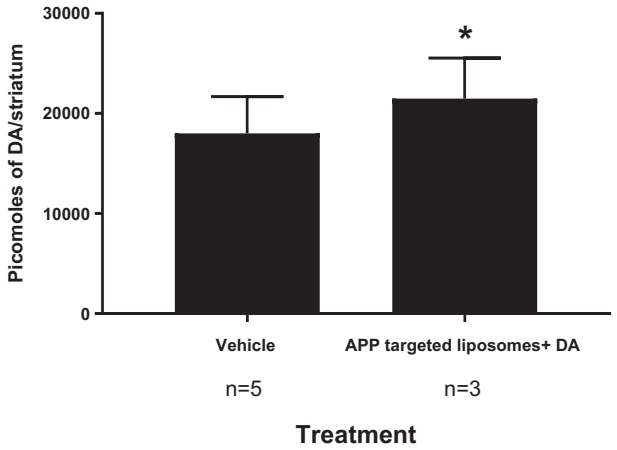


**Fig. 5 The impact of APP-targeted liposomes loaded with DA compared with vehicle on striatal DA content in mini-pigs.** Changes in striatal DA content following IV injection of APP-targeted liposome loaded with DA (360 µg/kg) to naïve mini-pigs after 30 min compared with vehicle (saline). One-tailed unpaired *t* test: $*p < 0.05$.

number of contralateral turns in a 15-min period made by hemiparkinsonian mice. Moreover, in the hemiparkinsonian mice, whose striatal content of DA was further depleted by prior treatment with amphetamine, the striatal content of DA was significantly increased as early as 5 min after IP administration of the DA-loaded BTLS. The data clearly show that the BTLS is a viable strategy for drug delivery of polar molecules into the brain.

Moreover, it appears that our novel BTLS can be utilized for transporting compounds through the BBB in a simple, controlled, and effective manner and without accumulation in peripheral organs, indicating minimal risk and good tolerability. Achievement of DA delivery to the striatum may serve as a potential treatment for PD and other DA-deficiency states.

It seems that the novel BTLS is capable of transporting compounds over the BBB into the brain, storing and releasing them for hours, while bypassing peripheral metabolism. The BTLS unique structure allows minimal DA doses for the treatment of PD, as well as a rapid penetration into the brain, so that free DA and DA metabolite levels as assessed by HPLC are undetectable in the periphery. Notably, other similar liposomal delivery systems are also avoid of peripheral DA metabolism [22–24]. However, direct evidence for the presence of a BBB cell-surface recognition molecule for the targeter RERMS is still lacking [4].

It should be noted that alternative approaches exist for treating neurodegenerative disorders. Namely, rather than delivering agents into the brain, extracorporeal apheresis therapy targeting relevant lipids, stress, and inflammation can be used to halt the progression of the neurodegenerative process [25].

In conclusion, the current study demonstrated the ability of a novel BTLS to deliver DA into the brain without accumulation in peripheral organs (liver and heart). In addition, due to the persistent and stable release of DA into the brain, the risk for the emergence of severe neuropsychiatric complications such as dyskinetic movements or psychosis could be minimal. The novel drug delivery system is designed for transporting compounds in a minimal effective dosage through the BBB for the treatment of brain diseases. Further preclinical and clinical studies are needed before implementation of the system in clinical practice. Future studies will explore incorporation of site-selective honing strategies (e.g., lectins, polysaccharides, and peptide fragments) into the small, stable unilamellar liposomal membrane in order to achieve delivery of candidate therapeutic agents to specific disease-relevant anatomic regions of interest.

**Acknowledgements** This work was partially supported by the KAMIN project (Technion in collaboration with Tel Aviv University) of the Israel Innovation Authority [IIA] in the Ministry of Economy and Industry. The grant was given to MG and AW.

## Compliance with ethical standards

**Conflict of interest** None of the authors report any conflict of interest. They would like to note, however, that the present technology directed at liposome-based targeting of dopamine to the brain was described and patented by some of them.

**Ethical approval** The study was approved by the Tel Aviv University and the Technion Institute of Technology Animal Care Committees and maintenance according to the guidelines of these committees

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

1. Sveinbjornsdottir S. The clinical symptoms of Parkinson's disease. J Neurochem. 2016;139:318–24.
2. Nissbrandt H, Sundström E, Jonsson G, Hjorth S, Carlsson A. Synthesis and release of dopamine in R Brain: comparison between substantia Nigra pars compacta, pars reticulata, and striatum. J Neurochem. 1989;52:1170–82.
3. Haddad F, Sawalha M, Khawaja Y, Najjar A, Karaman R. Dopamine and levodopa prodrugs for the treatment of Parkinson's Disease. Molecules. 2017;23:40.
4. Ninomiya H, Roch JM, Sundsmo MP, Otero DA, Saitoh T. Amino acid sequence RERMS represents the active domain of amyloid beta/A4 protein precursor that promotes fibroblast growth. J Cell Biol. 1993;121:879–86.
5. Mileusnic R, Lancashire C, Clark J, Rose SP. Protection against Abeta-induced memory loss by tripeptide D-Arg-L-Glu-L-Arg. Behav Pharm. 2007;18:231–8.
6. Pawlik M, Otero DA, Park M, Fischer WH, Levy E, Saitoh T. Proteins that bind to the RERMS region of beta amyloid precursor protein. Biochem Biophys Res Commun. 2007;355:907–12.
7. Jin LW, Ninomiya H, Roch JM, Schubert D, Masliah E, Otero DA, et al. Peptides containing the RERMS sequence of amyloid

beta/A4 protein precursor bind cell surface and promote neurite extension. J Neurosci. 1994;14:5461–70.
8. Allon N, Saxena A, Chambers C, Doctor BP. A new liposome-based gene delivery system targeting lung epithelial cells using endothelin antagonist. J Control Release. 2012;160:217–24.
9. Haran G, Cohen R, Bar LK, Barenholz Y. Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochim Biophys Acta. 1993;1151:201–15.
10. Zucker D, Marcus D, Barenholz Y, Goldblum A. Liposome drugs' loading efficiency: a working model based on loading conditions and drug's physicochemical properties. J Control Release. 2009;139:73–80.
11. Zhigaltsev IV, Kaplun AP, Kucheryanu VG, Kryzhanovsky GN, Kolomeichuk SN, Shvets VI, et al. Liposomes containing dopamine entrapped in response to transmembrane ammonium sulfate gradient as carrier system for dopamine delivery into the brain of parkinsonian mice. J Liposome Res. 2001;11:55–71.
12. Lev N, Barhum Y, Ben-Zur T, Melamed E, Steiner I, Offen D. Knocking out DJ-1 attenuates astrocytes neuroprotection against 6-hydroxydopamine toxicity. J Mol Neurosci. 2013;50:542–50.
13. Stott SRW, Barker RA. Time course of dopamine neuron loss and glial response in the 6-OHDA striatal mouse model of Parkinson's disease. Eur J Neurosci. 2014;39:1042–56.
14. Bagga V, Dunnett SB, Fricker RA. The 6-OHDA mouse model of Parkinson's disease—terminal striatal lesions provide a superior measure of neuronal loss and replacement than median forebrain bundle lesions. Behav Brain Res. 2015;288:107–17.
15. Stephans SE, Yamamoto BK. Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. Synapse. 1994;17:203–9.
16. Wagner GC, Ricaurte GA, Seiden LS, Schuster CR, Miller RJ, Westley J. Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. Brain Res. 1980;181:151–60.
17. Ricaurte GA, Schuster CR, Seiden LS. Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. Brain Res. 1980;193:153–63.
18. Ricaurte GA, Seiden LS, Schuster CR. Further evidence that amphetamines produce long-lasting dopamine neurochemical deficits by destroying dopamine nerve fibers. Brain Res. 1984;303:359–64.
19. Laouini A, Jaafar-Maalej C, Sfar S, Charcosset C, Fessi H. Liposome preparation using a hollow fiber membrane contactor-application to spironolactone encapsulation. Int J Pharm. 2011;415:53–61.
20. Yingchoncharoen P, Kalinowski DS, Richardson DR. Lipid-based drug delivery systems in cancer therapy: what is available and what is yet to come. Pharm Rev. 2016;68:701–87.
21. Christensen AB, Sørensen JCH, Ettrup KS, Orlowski D, Bjarkam CR. Pirouetting pigs: a large non-primate animal model based on unilateral 6-hydroxydopamine lesioning of the nigrostriatal pathway. Brain Res Bull. 2018;139:167–73.
22. Johnsen KB, Burkhart A, Melander F, Kempen PJ, Vejlebo JB, Siupka P, et al. Targeting transferrin receptors at the blood-brain barrier improves the uptake of immunoliposomes and subsequent cargo transport into the brain parenchyma. Sci Rep. 2017;7:10396.
23. Qua M, Lin Q, He S, Wanga L, Fua Y, Zhanga Z, et al. A brain targeting functionalized liposomes of the dopamine derivative N-3, 4-bis(pivaloyloxy)-dopamine for treatment of Parkinson's disease. J Control Release. 2018;277:173–82.
24. Vieira DB, Gamarra LF. Getting into the brain: liposome-based strategies for effective drug delivery across the blood-brain barrier. Int J Nanomed. 2016;11:5381–414.
25. Bornstein SR, Voit-Bak K, Rosenthal P, Tselmin S, Julius U, Schatz U, et al. Extracorporeal apheresis therapy for Alzheimer disease-targeting lipids, stress, and inflammation. Mol Psychiatry. 2019. https://doi.org/10.1038/s41380-019-0542-x.