ACS Chemical Neuroscience

Research Article

pubs.acs.org/chemneuro

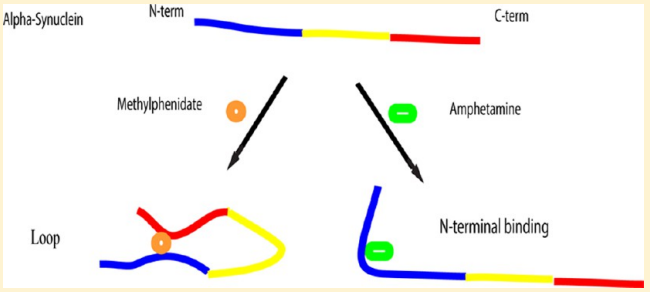
Drugs That Bind to α-Synuclein: Neuroprotective or Neurotoxic?

Joe Kakish, Dongsoo Lee, and Jeremy S. Lee*

Department of Biochemistry, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Canada, S7N 0W0

Supporting Information

ABSTRACT: The misfolding of α-synuclein is a critical event in the death of dopaminergic neurons and the progression of Parkinson's disease. Drugs that bind to α-synuclein and form a loop structure between the N- and C-terminus tend to be neuroprotective, whereas others that cause a more compact structure tend to be neurotoxic. The binding of several natural products and other drugs that are involved in dopamine metabolism were investigated by nanopore analysis and isothermal titration calorimetry. The antinausea drugs, cinnarizine and metoclopramide, do not bind to α-synuclein, whereas amphetamine and the herbicides, paraquat and rotenone, bind tightly and cause α-synuclein to adopt a more compact conformation. The recreational drug, cocaine, binds to α-synuclein, whereas heroin and methadone do not. Metformin, which is prescribed for diabetes and is neuroprotective, binds well without causing α-synuclein to adopt a more compact conformation. Methylphenidate (ritalin) binds to sites in both the N- and C-terminus and causes α-synuclein to adopt a loop conformation. In contrast, amphetamine only binds to the N-terminus. Except for cinnarizine and metoclopramide, there is a good correlation between the mode of binding to α-synuclein and whether a drug is neuroprotective or neurotoxic.



KEYWORDS: *Parkinson's disease, α-synuclein, neurotoxic drugs, neuroprotective drugs, nanopore analysis, protein folding, isothermal titration calorimetry*

A unifying concept for neurodegenerative diseases states that the pathogenesis is caused by the misfolding of proteins, for example, Aβ peptide in Alzheimer's disease, α-synuclein (AS) in Parkinson's disease, and prion proteins in transmissible spongiform encephalopathies (TSEs).[1−3] The misfolded proteins form aggregates and fibrils through β-sheet formation, which are toxic to neurons and are visible upon *postmortem* examination as amyloid plaques in Alzheimer's disease, Lewy bodies in Parkinson's disease, and spongiform morphology in TSEs.[4−7] As the name suggests, TSEs are readily spread through exposure to infected materials, but Alzheimer and Parkinson's diseases can also be induced in naïve animals by cranial injection of extracts from diseased animals.[8−10] In other words, pre-existing fibrils can spread to surrounding cells and cause further cell death, providing an explanation for the progressive nature of these diseases.[11,12] Another unifying concept is that the diseases have a clear genetic component and that, although rare, mutations in the genes themselves encoding the misfolding proteins can lead to early onset and familial forms of the diseases.[13−15] For example, the D23N mutation in Aβ peptide and A30P mutation in AS both cause early onset Alzheimer's or Parkinson's disease, respectively, and both mutant forms of the proteins misfold and aggregate more readily.[16,17] Suprisingly, mutations may also be protective; the recently evolved G127V mutation in the prion protein appears to protect against Kuru (a human TSE) and the Icelandic A673T mutation in Aβ peptide may protect against Alzheimer's disease.[18,19]

The environment may also play a role in neurodegenerative diseases since traumatic brain injury leading to concussion has been shown to increase the incidence of Alzheimer's disease and related dementias with a concomitant decrease in the age of onset.[20] For Parkinson's disease, a link to previous concussions is less persuasive, but there is ample evidence that exposure to certain drugs, whether accidental or deliberate, can lead to an increase in the incidence of the disease.[21,22] For example, exposure to rotenone and paraquat, which are widely used as insecticides and pesticides, causes an increase in the incidence of Parkinson's disease.[23,24] Their mechanism of action involves inhibition of the electron transport chain, an increase in the levels of reactive oxygen species (ROS), and mitophagy.[25−28] There is also evidence that the drugs can increase the rate of fibrillization of AS.[29] Prescription drugs such as cinnarazine (for motion sickness) and metoclopramide (for indigestion) have also been shown to give rise to a Parkinson-like syndrome, although the mechanism of action is not understood.[30,31] Perhaps the best example is provided by the psychotic drug methamphetamine because, in several epidemiological studies, chronic abuse increases the incidence of Parkinson's disease by as much as 3-fold.[32,33] We have recently demonstrated by nanopore analysis that methamphetamine binds to AS and causes it to have a more compact

Received: June 23, 2015
Revised: August 4, 2015
Published: September 17, 2015

ACS Publications © 2015 American Chemical Society

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

conformation.[34] Therefore, it is tempting to propose that methamphetamine binding to AS increases the likelihood of misfolding and, as a direct, consequence increases the incidence of the disease.

In contrast, several natural products have been identified whose use may be neuroprotective and lower the incidence of the disease.[35−40] These include caffeine, nicotine, and curcumin, which have also been shown to bind to AS, but the binding site and conformational changes are different from those induced by methamphetamine.[41] Whereas methamphetamine binds to the N-terminus of AS, drugs such as nicotine have binding sites in both the N- and C-terminus and may cause AS to fold back on itself to form a loop.[41] Previously, we have postulated that loop formation prevents aggregation of AS because the central NAC region can no longer form a linear structure required for participation in β-sheet formation.[41] In the long term, further analysis of the mode of action of these compounds may lead to the development of effective drug therapy for Parkinson's disease.

In this article, the binding to AS of a number of natural products and other drugs has been investigated. The structures are shown in Figure 1. The compounds were chosen on the basis of their (a) structural similarity to known AS binders, e.g., amphetamine; (b) interference in dopamine transport or metabolism, e.g., methylphenidate or cocaine; and (c) being implicated in inducing Parkinson's-like syndrome, e.g., cinnarizine. The goals are 3-fold: First, to provide further confirmation of the correlation between the mode of drug binding and possible neuroprotective or neurotoxic effects. Second, the use or abuse of several of these drugs is widespread. For example, methylphenidate (ritalin, concerta) has been prescribed to as many as 10% of children in parts of the USA, and 2% of young adults admit to its use as a "study" drug to improve concentration.[42] Since methylphenidate exposure in mice has been linked to changes in the substantia nigra, the brain region affected by Parkinson's disease, it is important to understand if it also binds to AS.[43] The third goal is to identify possible lead compounds that might be developed into therapeutics to slow the progression of neuronal death in Parkinson's disease.

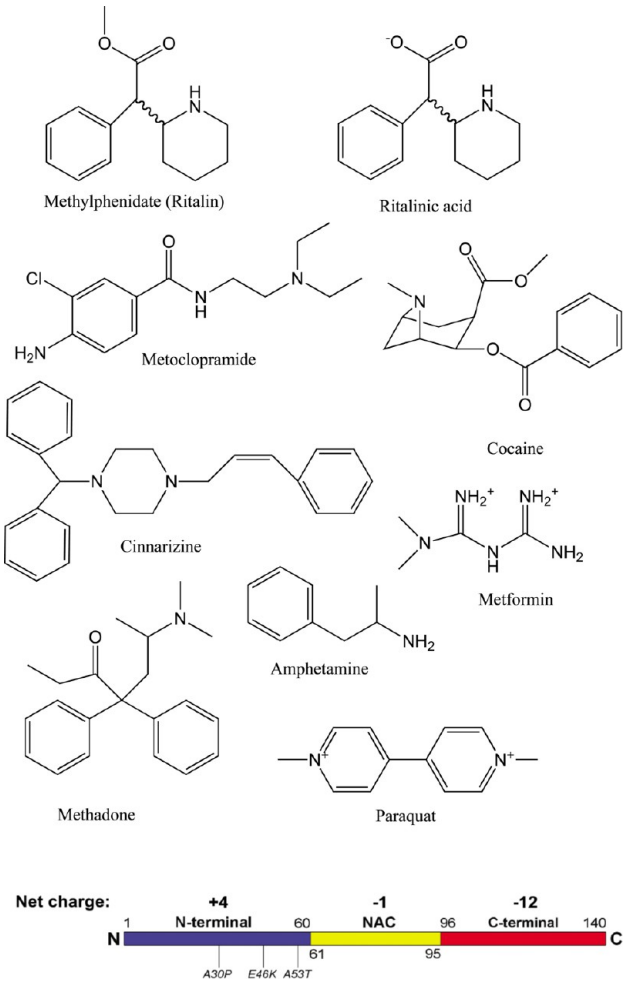


Figure 1. Drug structures and domain structure of AS.

RESULTS AND DISCUSSION

Nanopore analysis generates event profiles in the form of current blockade histograms and event time histograms in which every event represents the interaction of a single AS molecule with the pore.[44,45] The magnitude of the blockade current, I, and the time of the event, T, yield information about the conformation of the AS molecule at the instant when it interacts with the pore.[46] Under standard conditions, AS is unfolded and will translocate through the pore, giving an event with large I and long T.[41] In contrast, if AS folds into a compact conformation or aggregates, then it is more likely to bump into the pore, which yields events with a small I and short T.[41] Previously, we showed by nanopore analysis that a derivative of rasagiline, 1-aminoindan, binds to AS.[47] Event profiles for other derivatives of 1-aminoindan are shown in Figure 2. 1-Hydroxyindan (Figure 2a) gives a major peak at −87 pA due to translocation of AS through the pore and a minor peak at −26 pA due to bumping of AS into the pore. This profile is very similar to that of AS alone, demonstrating that 1-hydroxyindan does not bind to AS.[47] Similarly, it can be concluded from Figure 2b that 1,2,3,4-tetrahydro-1-naphylamine does not bind to AS. For 1-aminonaphthylamine (Figure 2c), there is a small decrease in the proportion of translocation events, suggesting that it binds weakly to AS. In contrast, 4-fluoro-1-aminoindan (Figure 2d) binds tightly because there are large changes in the profile: the translocation peak is shifted to about −80 pA, indicative of a conformational change, and the majority of events are now bumping. At higher ratios of drug to AS (Figure S1), only bumping events were recorded. Taken together, these results demonstrate that the binding site for 1-aminoindan is quite specific and will not tolerate a six-membered ring or a replacement of the amine for a hydroxyl group. On the other hand, simple substitutions on the phenyl ring are allowed.

We next tested a group of drugs for which there is epidemiological evidence for neurotoxicity. Cinnarizine and metoclopramide are prescribed for the treatment of motion sickness and nausea, respectively, and in both cases, there is good evidence that long-term use gives rise to drug-induced Parkinsonism.[30,31] As shown in Figure 3a,b, there are only small changes in the event profiles in the presence of the drugs, so the binding is very weak. It has been suggested that it is a metabolite of cinnarizine that is responsible for its adverse side effects, but, unfortunately, this compound was not available. Upon cessation of drug use, the Parkinsonism eventually disappears, which is in contrast to idiopathic PD that generally progresses.[30,31] Thus, it appears to be reasonable that the target of cinnarizine and metoclopromide is not AS.

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

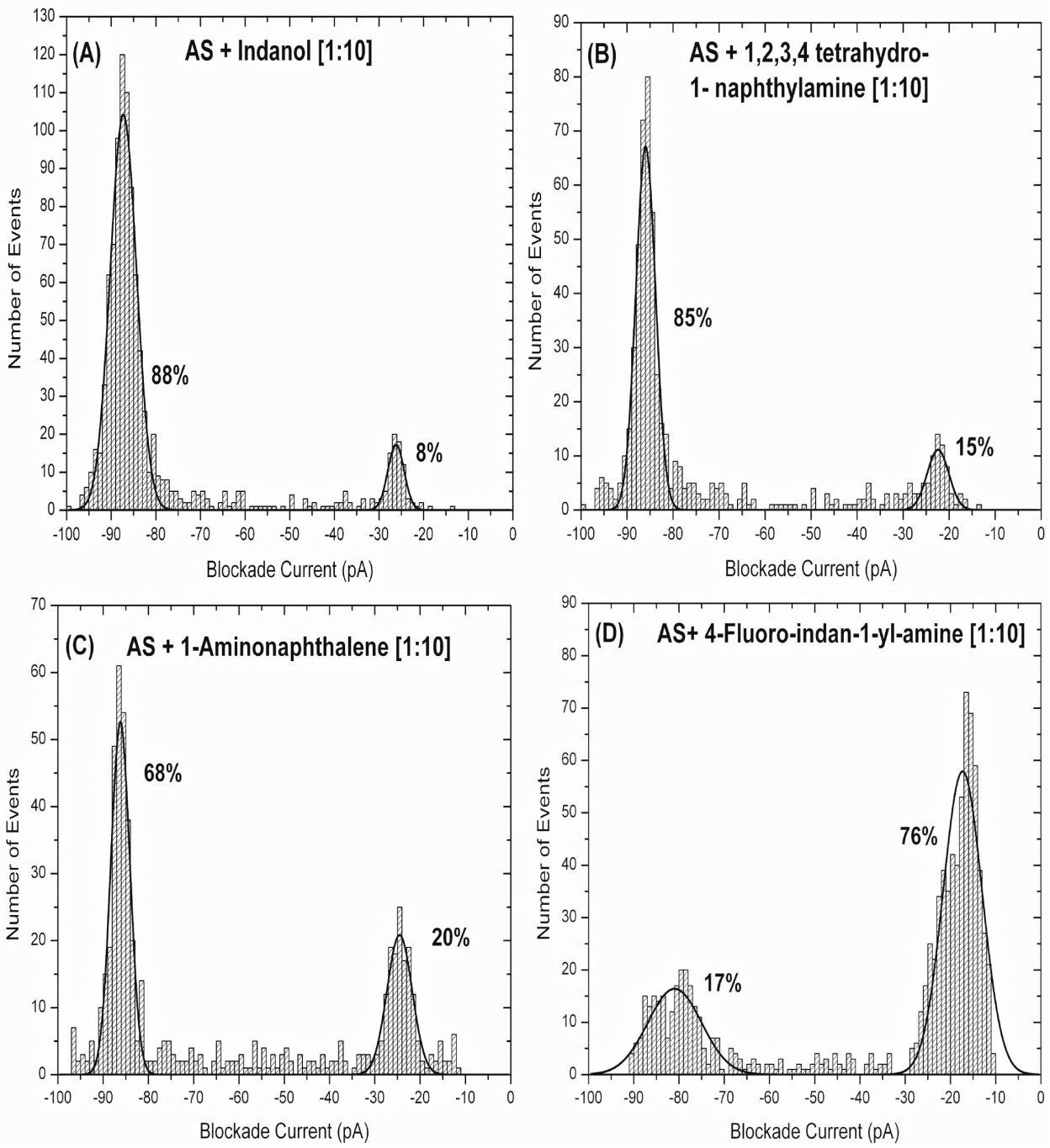


Figure 2. Blockade current histograms for 10 μM drug with 1 μM AS: (A) 1-indanol, (B) 1,2,3,4-tetrahydro-1-naphthylamine, (C) 1-aminonapthalene, and (D) 4-fluoro-1-aminoindan.

Previously, it was shown that methamphetamine binds to AS, and from Figure 3c, it is clear that amphetamine also binds since there is large increase in the proportion of bumping events at −26 pA.[34] As with methamphetamine, the proportion of bumping events increases with drug concentration (Figure S2), but the translocation peak remains at −86 pA. Several epidemiological studies have shown that amphetamine abuse leads to an increase in the incidence of PD by as much as 3-fold.[32,33] Similarly, paraquat and rotenone, which are widely used as herbicides, have long been associated with an increase in the incidence of PD.[28] The event profiles of Figure 3d,e provide good evidence for binding to AS since, as with amphetamine, there is a large increase in the proportion of bumping events, which is concentration-dependent (Figures S3 and S4). Both drugs disrupt the electron transport chain and causes formation of reactive oxygen species. Thus, they lead to oxidative stress and mitochondrial dysfunction, both of which are linked to neuronal cell death in the substantia nigra, the hallmark of PD.[25−27] However, the nanopore results demonstrate that they cause AS to adopt a more compact conformation that may misfold more readily, providing another possible mechanism for their association with PD.

The third group of drugs was selected on the basis of their known interference in dopamine metabolism or transport. It is estimated that at least 15% of American adults have used cocaine at least once, and there is ample evidence that it binds

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

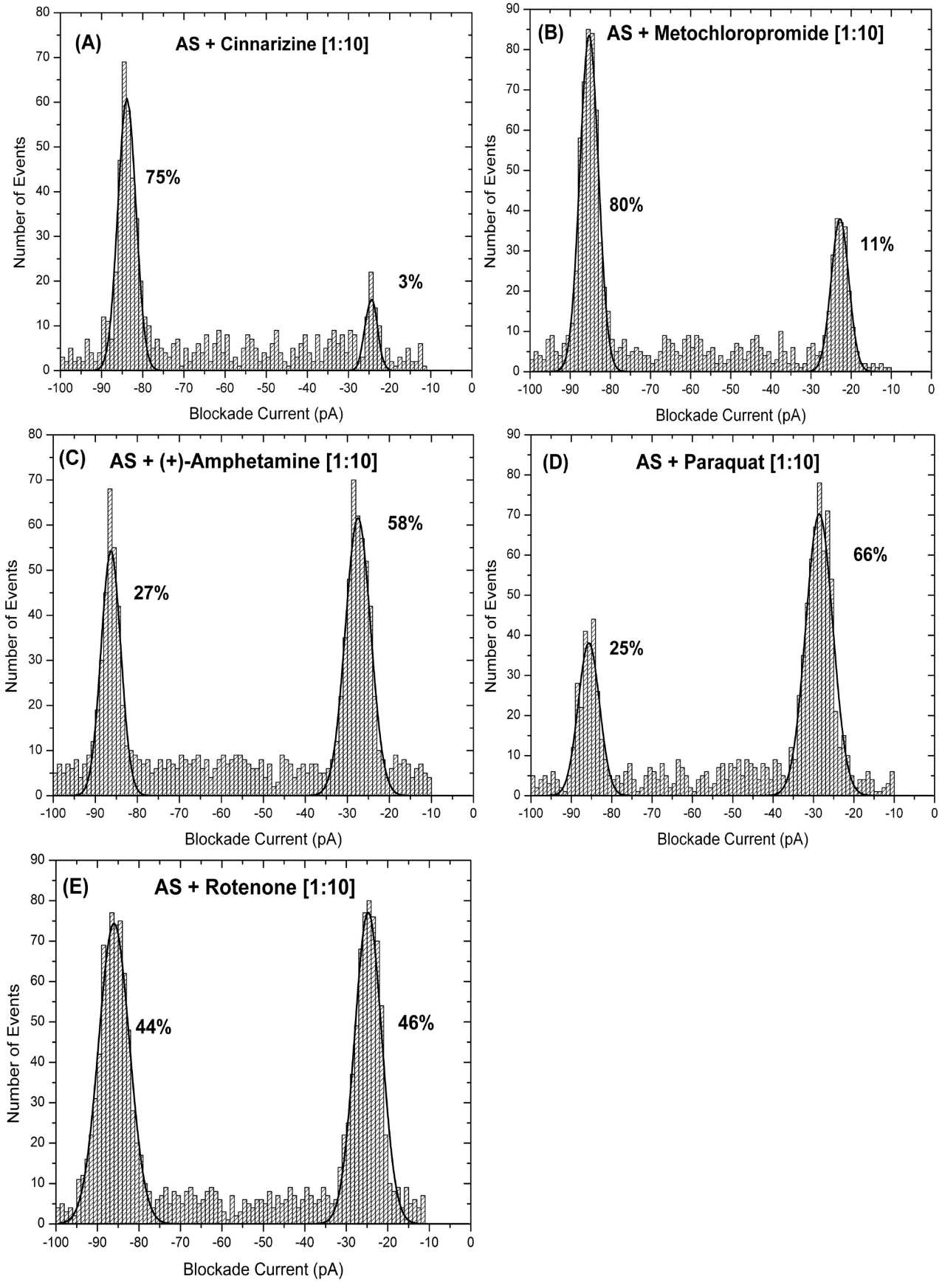


Figure 3. Blockade current histograms for 10 μM drug with 1 μM AS: (A) cinnarizine, (B) metoclopromide, (C) (+)-amphetamine, (D) paraquat, and (E) rotenone.

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

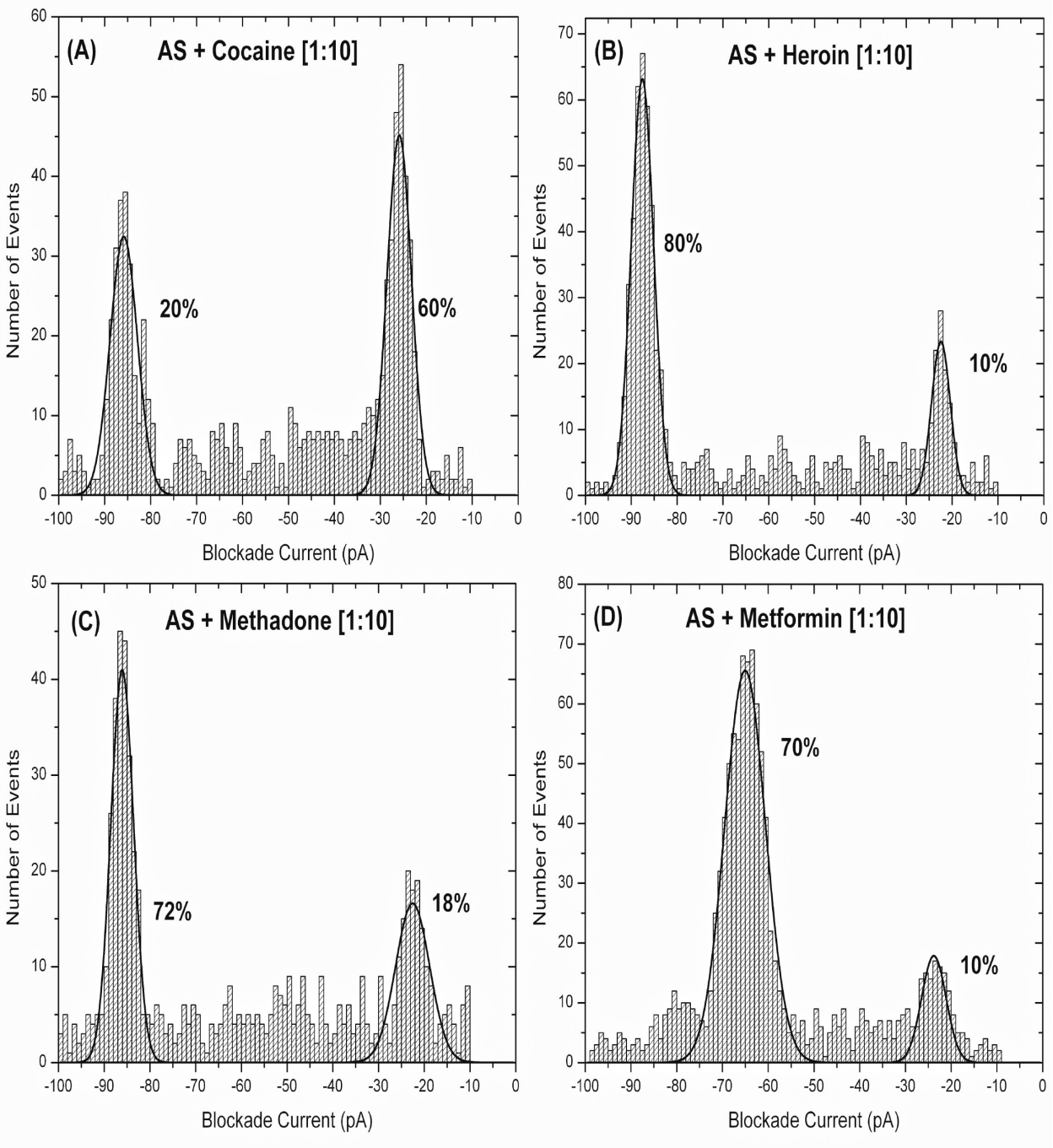


Figure 4. Blockade current histograms for 10 μM drug with 1 μM AS: (A) cocaine, (B) heroin, (C) methadone, and (D) metformin.

to the dopamine transporter, blocking reuptake of dopamine from the synaptic cleft.[48] Although there are changes in brain structure, the evidence for an increase in PD among cocaine users is controversial.[49−51,32] As shown in Figure 4a, it binds well to AS since the major peak in the event profile is a bumping peak at about −26 pA. Again, the proportion of bumping events increases with drug concentration (Figure S5). Although the stucture of cocaine is very different from that of amphetamines, they both cause AS to adopt a more compact conformation. In contrast, other recreational drugs such as heroin and methadone have little effect on the event profile of AS (Figure 4b,c). We also tested metformin, which is one of the most common drugs prescribed for the treatment of diabetes. It is known to reduce oxidative stress in several cell lines and is neuroprotective in an MPTP mouse model of PD.[52] Perhaps suprisingly, since its structure is not related to that of any of the other drugs, it binds well to AS because the major peak is now shifted to −65 pA (Figure 4d); therefore, we would predict that it is neuroprotective. At other drug concentrations, multiple peaks are observed in the events profiles, some of which have different blockade currents (Figure S6). For example, at an AS/metformin ratio of 1:20, a single peak is observed at −52 pA (Figure S6d), suggesting that their may be multiple binding sites.

Finally, methylphenidate was investigated in detail because of its widespread use and abuse and because it shares an aryl-carbon-carbon-amino pharmacophore with the amphetamines (see Figure 1). The major metabolite of methylphenidate is

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

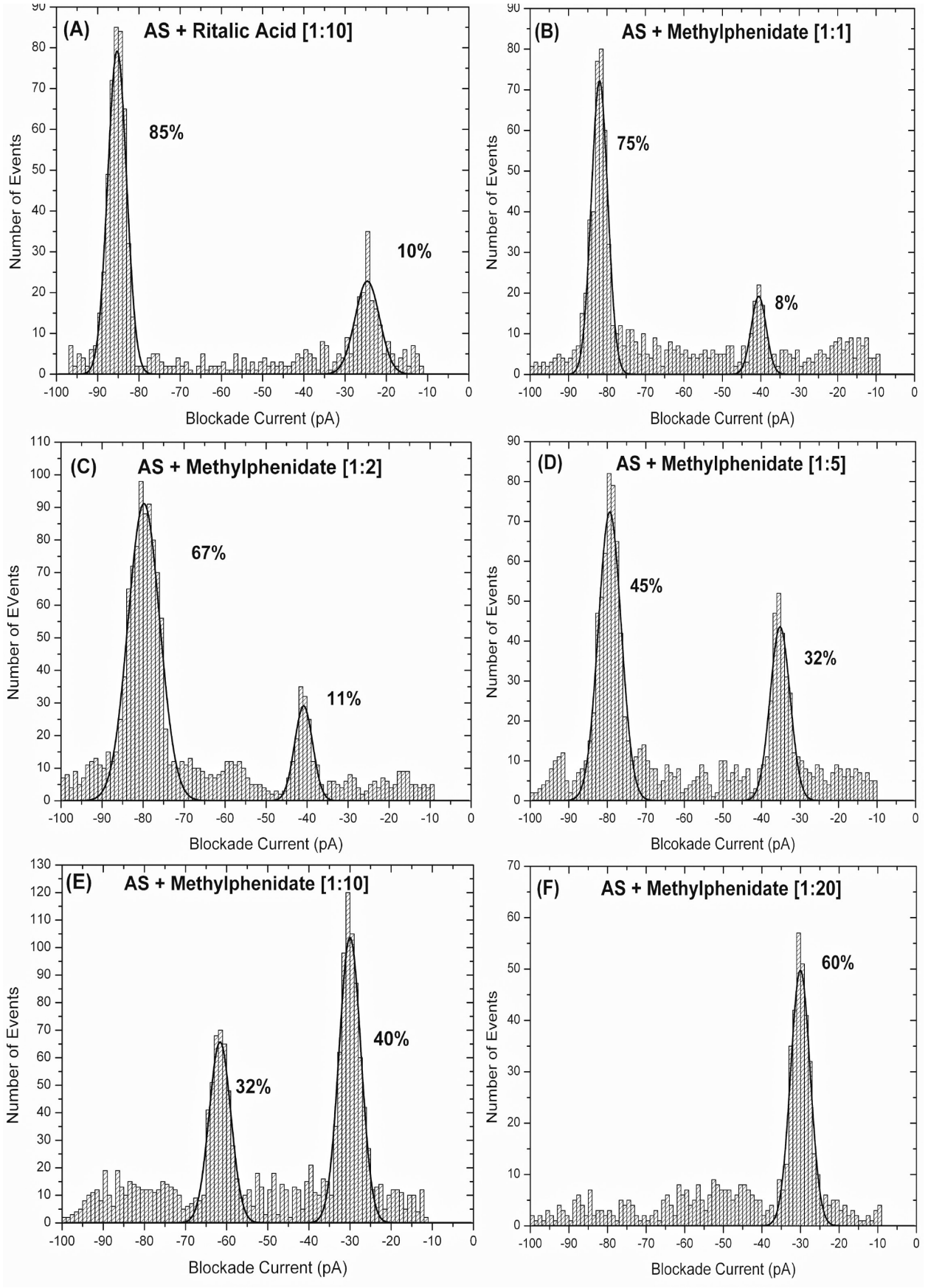


Figure 5. Blockade current histograms for 1 μM AS with (A) 10 μM ritalic acid, (B) 1 μM methylphenidate, (C) 2 μM methylphenidate, (D) 5 μM methylphenidate, (E) 10 μM methylphenidate, and (F) 20 μM methylphenidate.

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

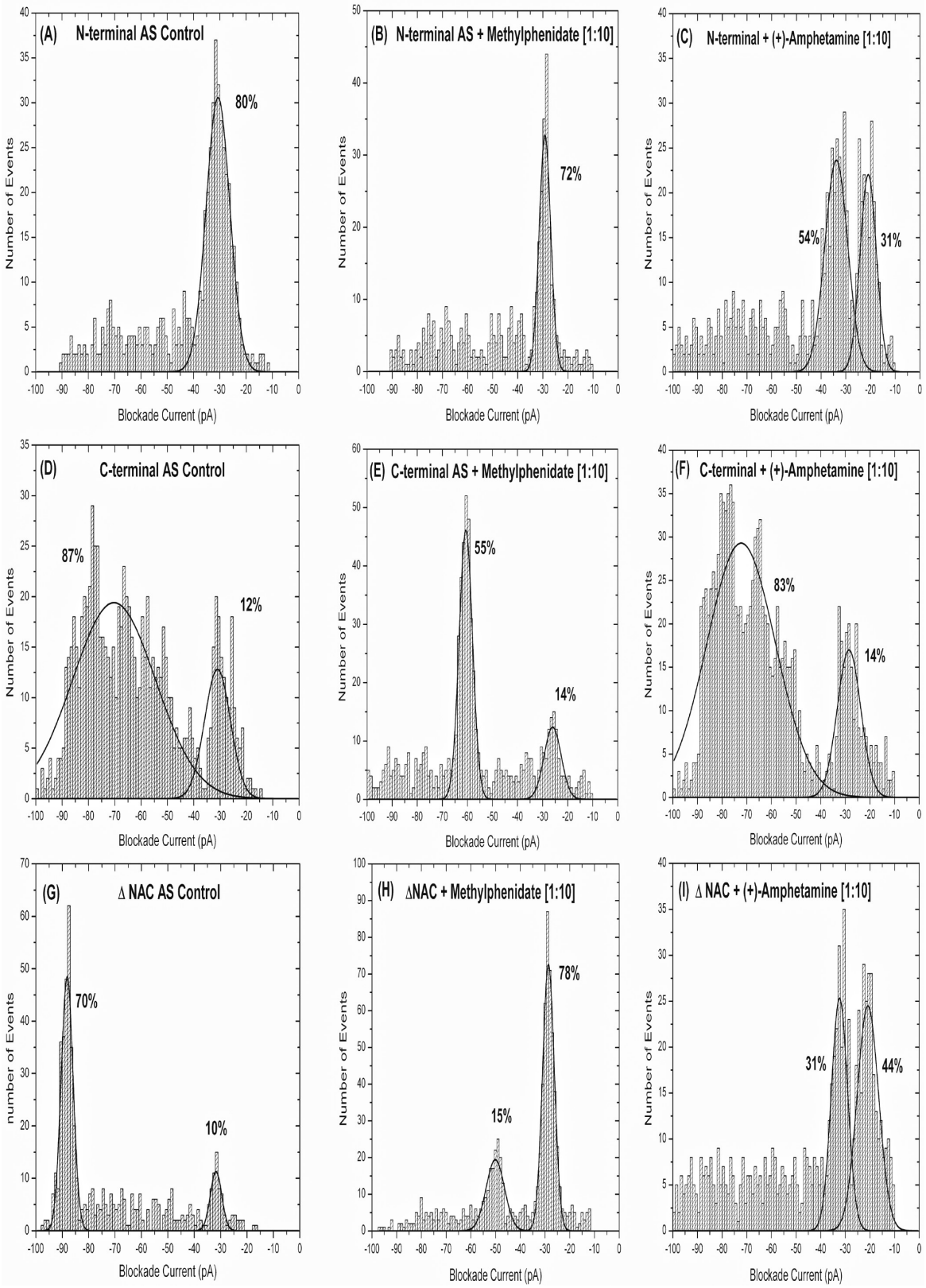


Figure 6. Blockade current histograms for (A) 1 μM N-terminal AS with (B) 10 μM methylphenidate and (C) 10 μM (+)-amphetamine; (D) 1 μM C-terminal AS with (E) 10 μM methylphenidate and (F) 10 μM (+)-amphetamine; and (G) 1 μM ΔNAC AS with (H) 10 μM methylphenidate and (I) 10 μM (+)-amphetamine.

ritalic acid, which was used as a control. As shown in Figure 5a, ritalic acid has little effect on the event profile since the major peak remains at −86 pA. In contrast, on addition of methylphenidate at a ratio of 1:1 (i.e., 1 μM AS to 1 μM drug), the major peak shifts to −81 pA and the minor bumping peak is now found at about −40 pA (Figure 5b). On increasing

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

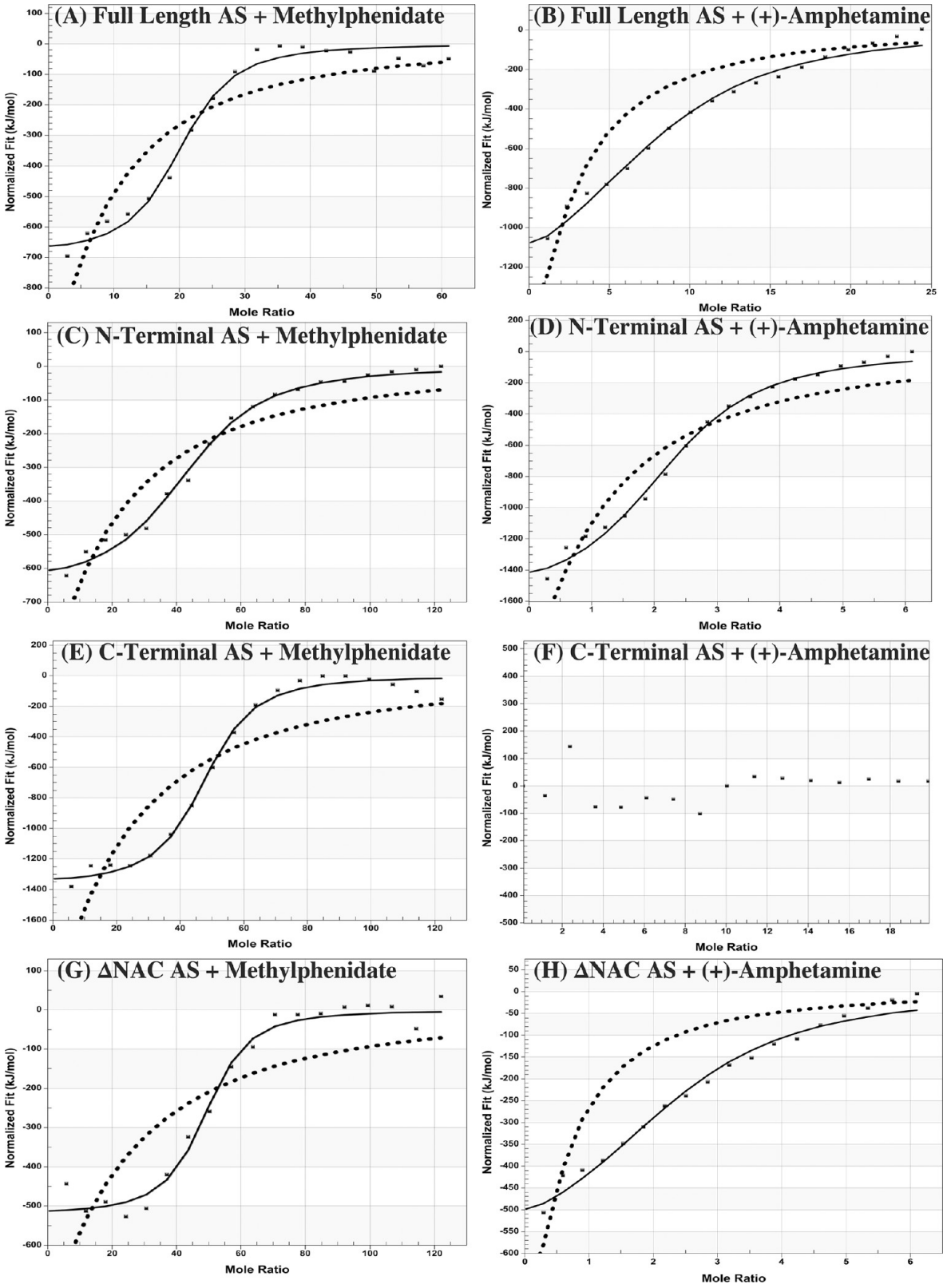


Figure 7. Isothermal titration calorimetry. (A) AS with methylphenidate, (B) AS with (+)-amphetamine, (C) N-terminal AS with methylphenidate, (D) N-terminal AS with (+)-amphetamine, (E) C-terminal AS with methylphenidate, (F) C-terminal AS with (+)-amphetamine, (G) ΔNAC AS with methylphenidate, and (H) ΔNAC AS with (+)-amphetamine. The solid lines are with n = 1, and the dashed lines are for n = 2.

the concentration of drug to 1:2, 1:5, 1:10, and 1:20 (Figure5c–f), the bumping peak increases in intensity to about 60% of the total events and shifts to −30 pA. Concurrently, an intermediate peak at about −60 pA can be seen at a 1:10 ratio. Thus, not only does methylphenidate bind tightly to AS but also the presence of several peaks at different blockade currents suggests that multiple conformations can be adopted. The adoption of multiple conformations has been

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

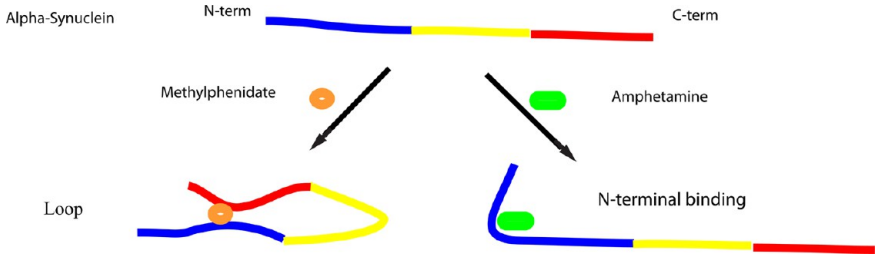


Figure 8. Drugs that cause loop formation may be neuroprotective, whereas those that bind only to the N-terminus may be neurotoxic.

observed previously with drugs such as (±)-nicotine, which can bind to both the N- and C-terminus of AS.[41]

The potential binding site(s) of methylphenidate and amphetamine were investigated in more detail by nanopore analysis and isothermal titration calorimetry (ITC) with peptide fragments of AS. The goal was to highlight differences between the two drugs because numerous studies have indicated that amphetamines increase the incidence of PD, whereas for methylphenidate, no such link has been demonstrated. Figure 6 shows the event profiles, and it is clear that there is good evidence for binding of methylphenidate to ΔNAC and the C-terminus because there are large changes (Figure 6e,h). For the N-terminal fragment, the result is less clear because the profiles are similar except for a reduction in the width of the bumping peak and a small shift from −30 to −28 pA in the presence of drug (Figure 6a,b). The blockade time for these events was also measured, but the change was not significant (0.20 ms without drug and 0.23 ms with drug; data not shown). When the major peak in the event profile is a bumping peak, binding of a drug may not lead to large changes. Therefore, further evidence for binding was obtained from ITC, as shown in Figure 7. The binding constants (average of three experiments with an error of ±10%) were estimated to be 1.8×10^5 M^{-1} for full-length AS, 2.1×10^5 M^{-1} for ΔNAC AS, 5.4×10^4 M^{-1} for N-terminal AS, and 1.7×10^5 M^{-1} for C-terminal AS. In all cases, there was a good fit when n (the number of sites) was constrained to be unity but not for $n = 2$ (Figure 7). Thus, methylphenidate can simultaneously bind both N- and C-terminus but not the NAC region, and since $n = 1$, it must be constraining the AS in a loop conformation.

In contrast, amphetamine binds to the N-terminus of AS because the event profile (Figure 6c) shows a major peak at about −35 pA that is not present in the control (Figure 6a). However, there is little evidence for binding to the C-terminus (compare Figures 6, panels d and f). A preference for an N-terminal binding site was confirmed by ITC (Figure 7) since the binding constants were estimated to be 6.8×10^5 M^{-1} for full-length AS, 3.2×10^5 M^{-1} for ΔNAC AS, and 7.1×10^5 M^{-1} for N-terminal AS; there was no significant change in enthalpy in the presence of the C-terminus.

It is known that C-terminal cleavage of AS increases the rate of aggregation and exacerbates the neurodegeneration and propagation of PD in mouse models.[53,54] The N-terminus and the NAC region become mostly α-helical upon binding membranes, suggesting that in the unbound, mostly disordered form of AS there is a significant interaction between the N- and C-terminal regions that prevents NAC-mediated aggregation.[55,56] Thus, it is perhaps not surprising to find several drugs that can bind to both the N- and C-terminus, enhancing this interaction and inhibiting the misfolding of AS (Figure 8). Such drugs may be neuroprotective. In contrast, drugs that bind only to the N-terminus, such as amphetamines, may prevent the interaction between the N- and C-terminus and thus enhance AS misfolding. Such drugs may be neurotoxic.

METHODS

Nanopore analysis and isothermal titration calorimetry were performed as described previously.[47] The peptides were purchased from rPeptide (Bogart, GA), and the drugs, from Sigma-Aldrich, Ltd. (Oakville, Ontario).

Nanopore Analysis. Briefly, a lipid membrane was painted over a small hole in a Teflon cup separating two chambers. One milliliter of nanopore buffer (1 M KCl and 10 mM Hepes/KOH (pH 7.8) was added to the cis and trans sides, and the membrane was thinned to a capacitance of <65 pF by repeated brush strokes. Aliquots of 5 μL of 1 μg/mL of α-hemolysin were added to the cis chamber until stable pore formation was observed by a jump in the open pore current to 100 pA under an applied voltage of −100 mV. AS (1 μM final concentration) was added to the cis side, and about 1000 events were recorded to ensure that a major peak was observed at the standard blockade current of −86 pA. The drugs were dissolved in methanol and added to the cis side to a maximum final concentration of 1% methanol. Events were then recorded until the membrane broke or the pore closed. Experiments were performed in duplicate or triplicate. Event profiles of blockade currents were fitted to a Gaussian distribution with Clampfit (Axon Instruments) and Origin 7. The error is estimated to be ±1 pA.

Isothermal Titration Calorimetry. ITC was performed with samples of 50 μM drug in the syringe and 5 μM AS in the thermal chamber, in a Nano-ITC calorimeter (TA Instruments, New Castle, PA, USA). The nanopore buffer, 1 M KCl and 10 mM Hepes/KOH (pH 7.8), was used throughout at 20 °C with a stir rate of 250 rpm. After degassing, 21 aliquots of 2.5 μL were added at 250 s intervals. A control was performed without drug, and the signal was subtracted from the results with drug. The experiments were performed in triplicate, and each data point was averaged with an estimated error of ±10%. NanoAnalyze (TA Instruments) was used to fit the data, with values of n (number of AS molecules per drug molecule) set at one or two.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00172.

Blockade current histograms for 4-fluoro-1-aminoindan, (+)-amphetamine, paraquat, rotenone, cocaine, and metformin at AS/drug ratios of 1:1, 1:2, 1:5, and 1:20 (Figures S1−S6) (PDF).

AUTHOR INFORMATION

Corresponding Author

*Telephone: 1-306-966-4371. E-mail: jeremy.lee@usask.ca.

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

Author Contributions

J.K. and D.L. performed the experiments under the supervision of J.S.L.

Funding

Funding was provided by an NSERC discovery grant.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank the Protein Characterization and Crystallization Facility (PCCF) of the College of Medicine, University of Saskatchewan and Dr. Michal T. Boniecki for help with ITC.

■ REFERENCES

(1) Lansbury, P. T., and Lashuel, H. A. (2006) A century-old debate on protein aggregation and neurodegeneration enters the clinic. *Nature 443*, 774.

(2) Chiti, F., and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem. 75*, 333−66.

(3) Schnabel, J. (2010) The dark side of proteins. *Nature 464*, 828−829.

(4) Mucke, L. (2009) Neuroscience: Alzheimer's disease. *Nature 461*, 895−897.

(5) Samii, A., Nutt, J. G., and Ransom, B. R. (2004) Parkinson's disease. *Lancet 363*, 1783−93.

(6) Davie, C. A. (2008) A review of Parkinson's disease. *Br. Med. Bull. 86*, 109−127.

(7) Cobb, N. J., and Surewicz, W. K. (2009) Prion Diseases and Their Biochemical Mechanisms. *Biochemistry 48*, 2574−2585.

(8) Prusiner, S. B. (2012) A unifying role for prions in neurodegenerative diseases. *Science 336*, 1511−1513.

(9) Kraus, A., Groveman, B. R., and Caughey, B. (2013) Prions and the potential transmissability of protein misfolding diseases. *Annu. Rev. Microbiol. 67*, 543−564.

(10) Luk, K. C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J. Q., and Lee, V. M.-Y. (2012) Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science 338*, 949−953.

(11) Jucker, M., and Walker, L. C. (2013) Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature 501*, 45−51.

(12) Hardy, J. (2009) The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *J. Neurochem. 110*, 1129−1134.

(13) Klein, C., and Westenberger, A. (2012) Genetics of Parkinson's Disease. *Cold Spring Harbor Perspect. Med. 2*, a008888.

(14) Spinney, L. (2014) The forgetting gene. *Nature 510*, 26−28.

(15) Mastrianni, J. A. (2010) The genetics of prion diseases. *Genet. Med. 12*, 187−195.

(16) Grabowski, T. J., Cho, H. S., Vonsattel, J. P., Rebeck, G. W., and Greenberg, S. M. (2001) Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. *Ann. Neurol. 49*, 697−705.

(17) Li, J., Uversky, V. N., and Fink, A. L. (2001) Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human alpha-synuclein. *Biochemistry 40*, 11604−11613.

(18) Mead, S., Whitfield, J., Poulter, M., Shah, P., Uphill, J., Campbell, T., Al-Dujaily, H., Hummerich, H., Beck, J., Mein, C. A., Verzilli, C., Whittaker, J., Alpers, M. P., and Collinge, J. (2009) A novel protective prion protein variant that colocalizes with Kuru exposure. *N. Engl. J. Med. 361*, 2056−2065.

(19) Jonsson, T., Atwal, J. K., Steinberg, S., Snaedal, J., Jonsson, P. V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., Hoyte, K., Gustafson, A., Liu, Y., Lu, Y., Bhangale, T., Graham, R. R., Huttenlocher, J., Bjornsdottir, G., Andreassen, O. A., Jonsson, E. G., Palotie, A., Behrens, T. W., Magnusson, O. T., Kong, A., Thorsteinsdottir, U., Watts, R. J., and Stefansson, K. (2012) A mutation in APP protects against Alzheimer's dusease and age-related cognitive decline. *Nature 488*, 96−99.

(20) Guskiewicz, K. M., Marshall, S. W., Bailes, J., McCrea, M., Cantu, R. C., Randolph, C., and Jordan, B. D. (2005) Association between recurrent concussion and late-life cognitive impairment in retired football players. *Neurosurgery 57*, 719−726.

(21) Rugbjerg, K., Ritz, B., Korbo, L., Martinussen, N., and Olsen, J. H. (2008) Risk of Parkinson's disease after hospital contact for head injury: population based case-control study. *BMJ 337*, a2494.

(22) Kamel, F. (2013) Paths from pesticides to Parkinson's. *Science 341*, 722−723.

(23) Wang, A., Costello, S., Cockburn, M., Zhang, X., Bronstein, J., and Ritz, B. (2011) Parkinson's risk from ambient exposure to pesticides. *Eur. J. Epidemiol. 26*, 547−555.

(24) Tanner, C. M., Kamel, F., Ross, G. W., Hoppin, J. A., Goldman, S. M., Korell, M., Marras, G. S., Bhudhikanok, M., Kasten, A. R., Chade, K., Comyns, M. B., Richards, C., Meng, C., Priestley, B., Fernandez, H. H., Cambi, F., Umbach, D. M., Blair, A., Sandler, D. P., and Langston, J. W. (2011) Rotenone, paraquat, and Parkinson's disease. *Environ. Health Perspect. 119*, 866−872.

(25) Sherer, T. B., Richardson, J. R., Testa, C. M., Seo, B. B., Panov, A. V., Yagi, T., Matsuno-Yagi, A., Miller, G. W., and Greenamyre, J. T. (2007) Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease. *J. Neurochem. 100*, 1469−1479.

(26) McCormack, A. L., Thiruchelvam, M., Manning-Bog, A. B., Thiffault, C., Langston, J. W., Cory-Slechta, D. A., and Di Monte, D. A. (2002) Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. *Neurobiol. Dis. 10*, 119−127.

(27) Kuter, K., Nowak, P., Golembiowska, K., and Ossowska, K. (2010) Increased reactive oxygen species production in the brain after repeated low-dose pesticide paraquat exposure in rats. A comparison with peripheral tissues. *Neurochem. Res. 35*, 1121−1130.

(28) Dinis-Oliveira, R. J., Remiao, F., Carmo, H., Duarte, J. A., Navarro, A. S., Bastos, M. L., and Carvalho, F. (2006) Paraquat exposure as an etiological factor of Parkinson's disease. *Neurotoxicology 27*, 1110−1122.

(29) Uversky, V. N., Li, J., Bower, K., and Fink, A. L. (2002) Synergistic effects of pesticides and metals on the fibrillation of alpha-synuclein:implications for Parkinson's disease. *NeuroToxicology 23*, 527−536.

(30) Marti Masso, J. F., Obeso, J. A., Carrera, N., and Martinez-lage, J. M. (1987) Aggravation of Parkinson's disease by cinnarizine. *J. Neurol., Neurosurg. Psychiatry 50*, 804−805.

(31) Indo, T., and Ando, K. (1982) Metoclopramide-induced parkinsonism. Clinical characteristics of ten cases. *Arch. Neurol. 39*, 494−496.

(32) Curtin, K., Fleckenstein, A. E., Robison, R. J., Crookston, M. J., Smith, K. R., and Hanson, G. R. (2015) Methamphetamine/amphetamine abuse and risk of Parkinson's disease in Utah: a population-based assessment. *Drug Alcohol Depend. 146*, 30−38.

(33) Callaghan, R. C., Cunningham, J. K., Sykes, J., and Kish, S. J. (2012) Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs. *Drug Alcohol Depend. 120*, 35−40.

(34) Tavassoly, O., and Lee, J. S. (2012) Methamphetamine binds to α-synuclein and causes a conformational change which can be detected by nanopore analysis. *FEBS Lett. 586*, 3222−3228.

(35) Postuma, R. B., Lang, A. E., Munhoz, R. P., Charland, K., Pelletier, A., Moscovich, M., Filla, L., Zanatta, D., Romenets, S. R., Altman, R., and Chuang, R. (2012) Caffeine for treatment of Parkinson disease: A randomized controlled trial. *Neurology 79*, 651−658.

(36) Prediger, R. D. (2010) Effects of caffeine in Parkinson's disease: from neuroprotection to the management of motor and non-motor symptoms. *J. Alzheimers Dis. 20*, 205−220.

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940

(37) Ross, G. W., and Petrovitch, H. (2001) Current evidence for neuroprotective effects of nicotine and caffeine against Parkinson's disease. *Drugs Aging 18*, 797−806.
(38) Quik, M., Perez, X. A., and Bordia, T. (2012) Nicotine as a potential neuroprotective agent for Parkinson's disease. *Mov. Disord. 27*, 947−957.
(39) Quik, M. (2004) Smoking, nicotine and Parkinson's disease. *Trends Neurosci. 27*, 561−568.
(40) Mythri, R. B., and Bharath, M. M. (2012) Curcumin: a potential neuroprotective agent in Parkinson's disease. *Curr. Pharm. Des. 18*, 91−99.
(41) Tavassoly, O., Kakish, J., Nokhrin, S., Dmitriev, O., and Lee, J. S. (2014) The use of nanopore analysis for discovering drugs which bind to α-synuclein for treatment of Parkinson's disease. *Eur. J. Med. Chem. 88*, 42−52.
(42) Visser, S. N., Danielson, M. L., Bitsko, R. H., Holbrook, J. R., Kogan, M. D., Ghandour, R. M., Perou, R., and Blumberg, S. J. (2014) Trends in the Parent-Report of Health Care Provider-Diagnosis and Medication Treatment for ADHD disorder: United States, 2003−2011. *J. Am. Acad. Child Adolesc. Psychiatry 53*, 34−46.
(43) Sadasivan, S., Pond, B. B., Pani, A. K., Qu, C., Jiao, Y., and Smeyne, R. J. (2012) Methylphenidate Exposure Induces Dopamine Neuron Loss and Activation of Microglia in the Basal Ganglia of Mice. *PLoS One 7* (3), e33693.
(44) Stefureac, R., Long, Y. T., Kraatz, H. B., Howard, P., and Lee, J. S. (2006) Transport of alpha-helical peptides through alpha-hemolysin and aerolysin pores. *Biochemistry 45*, 9172−9179.
(45) Kasianowicz, J. J., Robertson, J. W. F., Chan, E. R., Reiner, J. E., and Stanford, V. M. (2008) Nanoscopic porous sensors. *Annu. Rev. Anal. Chem. 1*, 737−766.
(46) Madampage, C., Tavassoly, O., Christensen, C., Kumari, M., and Lee, J. S. (2012) Nanopore analysis: An emerging technique for studying the folding and misfolding of proteins. *Prion 6*, 116−123.
(47) Kakish, J., Tavassoly, O., and Lee, J. S. (2015) Rasagiline, a suicide inhibitor of MAO-B, binds to alpha-synuclein. *ACS Chem. Neurosci. 6*, 347−355.
(48) (2010) *Research report series: Cocaine*, NIH Publication No. 10-4166, NIDA, Bethesda, MD. https://teens.drugabuse.gov/sites/default/files/cocainerrs.pdf.
(49) Lloyd, S. A., Faherty, C. J., and Smeyne, R. J. (2006) Adult and in utero exposure to cocaine alters sensitivity to the parkonsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neuroscience 137*, 905−913.
(50) Mash, D. C., Ouyang, Q., Pablo, J., Basile, M., Izenwasser, S., Lieberman, A., and Perrin, R. J. (2003) Cocaine abusers have an over expression of alpha-synuclein in dopamine neurons. *J. Neurosci. 23*, 2564−2571.
(51) Dhopesh, V. P., Yagnik, P. M., and Weddington, W. W. (1997) Can cocaine cause parkinism? *Am. J. Addict. 6*, 177−179.
(52) Patil, S. P., Jain, P. D., Ghumatkar, P. J., Tambe, R., and Sathaye, S. (2014) Neuroprotective effect of metformin in MPTP-induced Parkinson's disease in mice. *Neuroscience 277*, 747−754.
(53) Mishizen-Eberz, A. J., Guttmann, R. P., Giasson, B. I., Day, G. A., Hodara, R., Ischiropoulos, H., Lee, V. M., Trojanowski, J. Q., and Lynch, D. R. (2003) Distinct cleavage patterns of normal and pathologic forms of alpha-synuclein by calpain I in vitro. *J. Neurochem. 86*, 836−847.
(54) Games, D., Valera, E., Spencer, B., Rockenstein, E., Mante, M., Adame, A., Patrick, C., Ubhi, K., Nuber, S., Sacayon, P., Zago, W., Seubert, P., Barbour, R., Schenk, D., and Masliah, E. (2014) Reducing C-terminal-truncated alpha-synuclein by immunotherapy attenuates neurodegeneration and propagation in Parkinson's disease-like models. *J. Neurosci. 34*, 9441−9454.
(55) Periquet, M., Fulga, T., Myllykangas, L., Schlossmacher, M. G., and Feany, M. B. (2007) Aggregated α-synuclein mediates dopaminergic neurotoxicity In Vivo. *J. Neurosci. 27*, 3338−3346.
(56) Allison, J. R., Rivers, R. C., Christodoulou, J. C., Vendruscolo, M., and Dobson, C. M. (2014) A relationship between the transient structure in the monomeric state and the aggregation propensities of α-synuclein and β-synuclein. *Biochemistry 53*, 7170−7183.

DOI: 10.1021/acschemneuro.5b00172
ACS Chem. Neurosci. 2015, 6, 1930−1940