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Postsynaptic Mechanisms Render *Syn I/II/III* Mice Highly Responsive to Psychostimulants

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Abstract

Background: Synapsins are encoded by *SYN I*, *SYN II*, and *SYN III*, and they regulate neurotransmitter release by maintaining a reserve pool of synaptic vesicles.
Methods: Presynaptic dopamine responses to cocaine were examined by microdialysis, and postsynaptic responses were evaluated to various dopamine receptor agonists in the open field with *SynI/SynII/SynIII* triple knockout mice.
Results: Triple knockout mice showed enhanced spontaneous locomotion in a novel environment and were hyper-responsive to indirect and direct D1 and D2 dopamine agonists. Triple knockout animals appeared sensitized to cocaine upon first open field exposure; sensitization developed across days in wild-type controls. When mutants were preexposed to a novel environment before injection, cocaine-stimulated locomotion was reduced and behavioral sensitization retarded. Baseline dopamine turnover was enhanced in mutants and novel open field exposure increased their striatal dopamine synthesis rates. As KCl-depolarization stimulated comparable dopamine release in both genotypes, their readily releasable pools appeared indistinguishable. Similarly, cocaine-induced hyperlocomotion was indifferent to blockade of newly synthesized dopamine and depletion of releasable dopamine pools. Extracellular dopamine release was similar in wild-type and triple knockout mice preexposed to the open field and given cocaine or placed immediately into the arena following injection. Since motor effects to novelty and psychostimulants depend upon frontocortical-striatal inputs, we inhibited triple knockout medial frontal cortex with GABA agonists. Locomotion was transiently increased in cocaine-injected mutants, while their supersensitive cocaine response to novelty was lost.
Conclusions: These results reveal presynaptic dopamine release is not indicative of agonist-induced triple knockout hyperlocomotion. Instead, their novelty response occurs primarily through postsynaptic mechanisms and network effects.

Keywords: behavior, psychostimulants, dopamine, microdialysis, synapsin

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Significance Statement

Synapsins (*SYNI*, *SYNII*, and *SYNIII*) are a family of genes whose protein products regulate neurotransmitter release by maintaining a reserve pool of synaptic vesicles in presynaptic neurons. We have analyzed effects of deleting all 3 synaptic genes in mice according to their pre- and postsynaptic dopamine responses to cocaine. Surprisingly, we find that presynaptic dopamine secretion to cocaine in triple synapsin knockout mice is largely similar to that of wild-type controls. However, their responses to various dopamine receptor agonists are enhanced in behavioral assays when the mice are exposed to a novel environment. This augmented behavioral response to novelty appears to occur primarily through postsynaptic mechanisms and neural network effects.

Introduction

Synapsins are a family of phosphoproteins associated with synaptic vesicle trafficking (Hilfiker et al., 1999; Bykhovskaia, 2011; Song and Augustine, 2015). In mammals, synapsins are encoded by *SYN I*, *SYN II*, and *SYN III*. Alternative splicing produces 2 isoforms each for SYN I and II (a-b) and 6 isoforms (a-f) for SYN III (Südhof et al., 1989; Porton et al., 1999). Synapsins participate in developmental processes that include synapse, lamellipodial, and neurite formation and axon differentiation (Ferreira et al., 1998; Feng et al., 2002). Importantly, synapsins regulate neurotransmitter release by maintaining a reserve pool of synaptic vesicles through their binding to synaptic vesicles and clustering these vesicles within the reserve pool (Greengard et al., 1993). Neuronal activity is thought to disrupt vesicle clustering through activity-dependent phosphorylation of synapsins, thereby mobilizing vesicles into a readily releasable pool at exocytosis sites. Consistent with this model, deletion of *Syn I* or *II* as well as disruption of all 3 *Syn* genes (triple knockout [TKO]) in mice decreases the clustering of synaptic vesicles at hippocampal synapses and reduces the rate of mobilization of synaptic vesicles from the reserve pool (Li et al., 1995; Gitler et al., 2004).

Beyond presynaptic actions at glutamatergic and GABAergic synapses (Terada et al., 1999; Gitler et al., 2004), synapsins are involved at other stages of synaptic vesicle trafficking. For instance, synapsins have been implicated in synchronizing fusion of both glutamatergic and GABAergic vesicles with the plasma membrane (Hilfiker et al., 1999; Humeau et al., 2001; Song and Augustine, 2015) as well as in inhibiting fusion of vesicles containing dopamine (DA) (Kile et al., 2010) and regulating synaptic vesicle endocytosis (Evergren et al., 2004). With respect to monoamines, voltammetry studies reveal that electrically stimulated DA release is increased in TKO compared with wild-type mice *in vitro* and *in vivo* in anesthetized animals (Kile et al., 2010). Although serotonin levels are increased under both conditions, they are not distinguished between genotypes. However, when cocaine is injected, the electrically stimulated striatal DA release is reduced in TKOs relative to their wild-type controls (Venton et al., 2006). Hence, the DA pool mobilized by cocaine appears to be deficient in anesthetized TKO mice. We have examined both pre- and postsynaptic responses to DA agonists in freely moving TKO animals. We find spontaneous locomotor activities in a novel open field are enhanced in TKO mice, and these mutants are more responsive to DA agonists than wild-type controls. Although synapsin loss has been ascribed primarily to presynaptic mechanisms, our microdialysis and pharmacology studies indicate that postsynaptic responses are important and that neural input from the frontal cortex to striatum mediates the enhanced locomotor responses of TKO mice in a novel environment.

Methods

Subjects

Adult male and female wild-type and TKO mice were obtained from Dr Paul Greengard (Rockefeller University, New York). C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) served as wild-type controls in some studies since the Syn wild-type mice had been extensively backcrossed to this strain. Animals were housed 3 to 5 mice per cage on a 14:10-hour-light/-dark cycle (lights on at 7:00 AM) in a humidity- and temperature-controlled room with chow and water provided *ad libitum*. Behavioral testing occurred between 10:00 AM and 3:00 PM, and microdialysis studies were conducted between 10:00 AM and 5:00 PM. All experiments were conducted with an approved animal protocol from the Duke University Institutional Animal Care and Use Committee. A summary of experiments is provided in Table 1. Assignment to experiments was dependent upon results from the preceding experiment and receipt of the mice. The experimenter was blinded to the genotype of the mice in all experiments but reconstituted and injected the drugs.

Drugs and Injections

Quinpirole, SKF-81297, *d*-amphetamine, cocaine, and α-methyl-*para*-tyrosine methyl ester HCl (Sigma-Aldrich, St. Louis, MO) were dissolved in saline. NSD-1015 (RBI-Sigma, Natick, MA) was administered in distilled water. Apomorphine (Sigma-Aldrich) was solubilized in saline with 0.2% ascorbic acid. Reserpine (Sigma-Aldrich) was predissolved in a drop of citric acid and diluted to volume with sterile water. All drugs were injected at 5 mL/kg (i.p.). To avoid handling-induced seizures, mice were lifted by the tail, allowed to grip the top edge of the home cage wall with its hind paws, and injected. For intracranial microinjections, muscimol and baclofen (Sigma-Aldrich) were dissolved in artificial cerebrospinal fluid (aCSF; CMA Microdialysis, Krista, Sweden). Ketamine (100 mg/mL, Henry Schein, Melville, NY) and xylazine (10 mg/mL, Akom Inc., Decatur, IL) were used in surgery, and pentobarbital (100 mg/kg, Sigma-Aldrich) was for perfusion with phosphate-buffered saline and 4% paraformaldehyde.

Open Field Activity

The open field (42 × 42 × 20 cm; Omnitech Inc., Columbus, OH) was illuminated at 340 lux (Pogorelov et al., 2005). Horizontal and vertical activities were monitored by infrared diodes interfaced to a computer running Fusion software (Omnitech) and expressed as the distance traveled or vertical activity (beam-breaks) over 5-minute intervals or as total distance over 60 or 100 minutes.

Table 1. Cohorts of Mice Used in the Pharmacology and Microdialysis Experiments

Cohort	Genotype	0 wk	1 wk	4 wk	8 wk
1	WT/TKO	Open field (no drugs)	Open field amphetamine	Open field apomorphine quinpirole SKF-81297	Open field reserpine
2	WT/TKO	Open field cocaine	Open field quinpirole SKF-81297	Open field αMPT	
3	WT/TKO	NPE cocaine sensitization			
4	WT/TKO	PE cocaine sensitization			
5	WT/TKO	Surgical cannula implantation	Home cage microdialysis		
6	WT/TKO	Surgical cannula implantation	Open field microdialysis		
7	WT/TKO	Surgical cannula implantation	FC injection baclofen/muscimol open field cocaine		

αMPT, α-methyl-*para*-tyrosine-methyl ester HCl; FC, frontal cortex; NPE, non-preexposure to the open field; PE, preexposure to the open field; TKO, triple knockout; WT, wild type.

To evaluate spontaneous activity, mice were placed into the open field for 60 minutes. Mice received acute injections of amphetamine, cocaine, apomorphine, SKF-81297, or quinpirole and were placed immediately into the open field. Mice were pretreated with α-methyl-*para*-tyrosine or reserpine at 2 or 24 hours, respectively, before open field testing with cocaine. In experiments where mice were tested repeatedly with different drugs (Table 1), the doses were assigned in an order such that the initial vehicle group received the highest dose, the lower dose group received a higher dose, etc. Cocaine sensitization was conducted with 2 different protocols using separate cohorts. In one experiment, animals were given vehicle or cocaine and placed immediately into the open field. In a second study, mice were preexposed to the open field for 60 minutes, injected with vehicle or cocaine, and returned immediately to the open field for 60 minutes. Injections occurred over 5 consecutive days with a 5-day hiatus, followed by a challenge on day 11 where all groups received the same cocaine dose as on day 1. Locomotor responses to cocaine were analyzed for days 1, 5, and 11.

Apomorphine-Induced Stereotypy and Climbing

Apomorphine-induced behavior was evaluated in wire-mesh cylinders (30 cm high, 12 cm diameter, 1 cm mesh size) on a Plexiglas surface with the top covered. Naïve animals were habituated to the cylinders for 30 minutes. The next day they were given 0, 1, or 3 mg/kg apomorphine and placed immediately into the cylinders. A blinded observer scored behaviors over 5 consecutive 1-minute intervals at 10, 35, and 60 minutes postinjection. Mice were observed for absence (0) or presence (1) of climbing (4 paws on the mesh), oral stereotypies (licking, gnawing), and immobility. The total score (maximum of 15) represented the sum over all intervals for each behavior.

Tissue Levels of DA and Its Metabolites and DA Synthesis Rate

Brain DA and its metabolites were measured as described (Pogorelov et al., 2005). Briefly, within 30 to 40 seconds wild-type or TKO mice were removed individually from their home cages, transported to an adjacent room, and euthanized by cervical dislocation and decapitation. Brains were removed rapidly, placed ventral side-up onto a cold metal block, and the dorsal and ventral striatum and frontal cortex were dissected and frozen in liquid nitrogen. Subsequently, samples were weighed, thawed, and sonicated in an ice-cold 0.1 M HCl-0.1 mM sodium metabisulfate solution with 33 to 100 ng/mL 3,4-dihydroxybenzylamine (Bioanalytical Systems Inc., West Lafayette, IN) as an internal standard. After centrifugation at 10000 × *g* for 10 minutes at 4°C, samples were filtered through 0.22-μm filters (Millipore, Bedford, MA). Ten μL of filtrate was injected onto the high-pressure liquid chromatograph.

For determination of DA synthesis rates, animals were given 100 mg/kg NSD-1015 and placed immediately into the open field. After 40 minutes, mice were euthanized, and the frontal cortex and dorsal and ventral striata were dissected on ice. Samples were homogenized as described above, and L-3,4-dihydroxyphenylalanine contents were determined by high-pressure liquid chromatography.

Microdialysis

Mice were anesthetized with ketamine/xylazine and a CMA-7 guide cannula was implanted into the right striatum (AP: +0.5, L: +1.9, DV: −2.2; relative to bregma). Five days later, a CMA-7 microdialysis probe (Cuprophane, 6 kDa cut-off, 0.24 mm o.d., 2-mm membrane length; CMA Microdialysis) was inserted into the guide. The next day probes were perfused at 1.7 μL/min with aCSF (in mM: 147 NaCl, 2.7 KCl, 1.2 $CaCl_2$, and 0.85 $MgCl_2$) and

equilibrated for 3 to 5 hours before collecting baseline samples. Microdialysis samples were collected at 20-minute intervals into 0.5-mL polypropylene tubes containing a 16-µL mixture of 1 mM oxalic acid, 100 mM acetic acid, and 3 mM l-cysteine. The first 3 samples were collected in the home cage as baseline. In one experiment, mice were injected with 20 mg/kg cocaine in the home cage and samples were collected over 140 minutes. The next day, 60 mM KCl was perfused through the probe. In a separate study, 1 group (non-preexposed) received 20 mg/kg cocaine and was placed immediately into the open field. Another group (preexposed) was placed into the open field, and three 20-minute samples were collected 60 minutes before the cocaine injection, after which 5 additional samples were collected in the open field.

Chromatography

Samples were analyzed by high-pressure liquid chromatography with electrochemical detection using an Alexys monoamine analyzer (Antec, Palm Bay, FL). The apparatus consisted of a DECADE II detector coupled to a VT-03 flow cell (Antec). DA was separated at 50 µL/min on a C18 reverse-phase 1×50 mm column (3-µm particle size, ALF-105; Antec) using a mobile phase (50 mM phosphoric acid, 0.1 mM EDTA, 8 mM KCl, 11% methanol, and 500 mg/mL 1-octane sulfonic acid sodium salt, pH 6.0) with the detector set at +0.3 volts. DA metabolites were separated on a different 1×150 mm column (ALF-115, 3 µm; Antec) with a mobile phase containing 50 mM phosphoric acid, 10 mM citric acid, 0.1 mM EDTA, 8 mM KCl, 10% methanol, and 500 mg/mL 1-octane sulfonic acid sodium salt, pH 3.25, and detected at +0.62 V. Chromatograms were analyzed using Clarity software (DataApex, Prague, Czech Republic), with a signal-to-noise ratio of 3 as the limit of detection.

Frontal Cortex Microinjections

Stainless-steel bilateral guide cannulae (PlasticsOne, Roanoke, VA) were implanted over the medial frontal cortex (AP+1.2, L±0.25, V –0.5; relative to bregma) under ketamine/xylazine anesthesia. Mice were housed in individual cages and acclimated to the test room for 5 days. On day 6, the guides were replaced by hand with double injection cannulae that extended 0.7 mm below the guide. They were connected to FEP tubing (0.1 mm i.d.; Eicom USA, San Diego, CA) and two 2-µL Hamilton syringes (Reno, NV). The mouse was returned to its home cage while 0.3 µL of a muscimol/baclofen cocktail (200 ng each) was infused over 2 minutes. The injectors remained in place for 2 additional minutes before removal. Three minutes later, the animal was administered vehicle or cocaine and placed into the open field for 100 minutes. After experiments, some mice were anesthetized with pentobarbital and perfused with phosphate-buffered saline and 4% paraformaldehyde. Their brains were stained with hematoxylin-eosin to determine cannulae placement. Other animals were anesthetized with pentobarbital, and 0.3 µL toluidine blue was injected through cannulae into the cortex. Their brains were dissected 15 minutes later, frozen on dry ice, and fresh-cut to determine the dye location in the cortex.

Statistical Analyses

The results are presented as means and SEM. All analyses were performed with the Statistical Package for the Social Sciences, version 22.0 (IBM, Armonk, NY). A *t* test was used for genotype comparisons. Two-way ANOVA examined the between subjects effects of genotype and drug doses. Repeated-measures ANOVA (RMANOVA) assessed within-subjects effects of time within the same animals in the open field and microdialysis studies; the between-subjects tests determined genotype or treatment effects. Bonferroni corrected pair-wise tests were used for *posthoc* comparisons. A $P<0.05$ was considered statistically significant.

Results

Novelty Induces Transient Hyperactivity in TKO Mice

In the open field, locomotor activities over the first 15 minutes were higher (*Ps* ≤0.034) in naïve TKO than wild-type mice (Figure 1A). Subsequently, locomotion was similar between genotypes, suggesting the initial activity increase was due to enhanced novelty responses in TKO animals. In contrast, rearing was similar between genotypes (Figure 1B), except at 40 minutes ($P=0.045$), when it was lower in mutants. The augmented locomotion in TKO mice suggests DA neurotransmission may be enhanced initially, but this activity habituates to wild-type levels (see Fornaguera and Schwarting, 1999).

TKO Mice Are Hyper-Responsive to Psychostimulants

To examine the role of DA in TKO hyperlocomotion, acute responses to psychostimulants were assessed. Amphetamine elevates extracellular DA through a variety of mechanisms, and it increased locomotion in both genotypes (Figure 1C). This effect was dose-dependent and more robust in TKO than wild-type mice at 2 mg/kg amphetamine and relative to vehicle (*Ps*<0.001).

Cocaine, which increases extracellular DA levels by blocking both DA uptake and mobilization of the synapsin-dependent reserve pool (Venton et al., 2006), produced effects similar to those of amphetamine. Cocaine dose-dependently stimulated locomotion (Figure 1D), with 20 mg/kg significantly increasing activity, compared with wild-type mice at the same dose ($P=0.001$) and to both genotypes at the other doses (*Ps*≤0.014). Hence, TKO animals appear hyper-responsive to drugs that increase extracellular DA.

Effects of a Mixed D1R/D2R Agonist

The enhanced TKO responsiveness to psychostimulants may be due to presynaptic and/or postsynaptic mechanisms. Parenthetically, D1 receptors (D1Rs) are localized on postsynaptic, whereas D2 receptors (D2Rs) are present on presynaptic DA neurons as autoreceptors and on postsynaptic neurons. The mixed DA receptor agonist, apomorphine, stimulates D1Rs and D2Rs. At all doses examined (0.5–3 mg/kg), apomorphine inhibited open field locomotion in wild-type mice relative to vehicle (*Ps*≤0.003) (Figure 2A). By contrast, only 0.5 mg/kg reduced activity in TKO animals ($P=0.034$). At 0.5 and 1 mg/kg, TKO mice were more active than wild-type controls (*Ps*≤0.010).

As an index of postsynaptic DA receptor activation, climbing behavior and oral stereotypies were evaluated. While apomorphine produced dose-dependent increases in climbing behavior, it did so equally well in both genotypes (Table 2). TKO mice displayed more gnawing relative to wild-type controls at 3 mg/kg apomorphine ($P<0.001$). Since gnawing is mediated primarily through postsynaptic DA receptors, it appears that these receptors may be supersensitive in the mutants. However, from this experiment it is unclear whether D1Rs, D2Rs, or both are supersensitive. In contrast, at the same dose, TKO mice engaged in less licking behavior ($P<0.001$) but were more immobile than

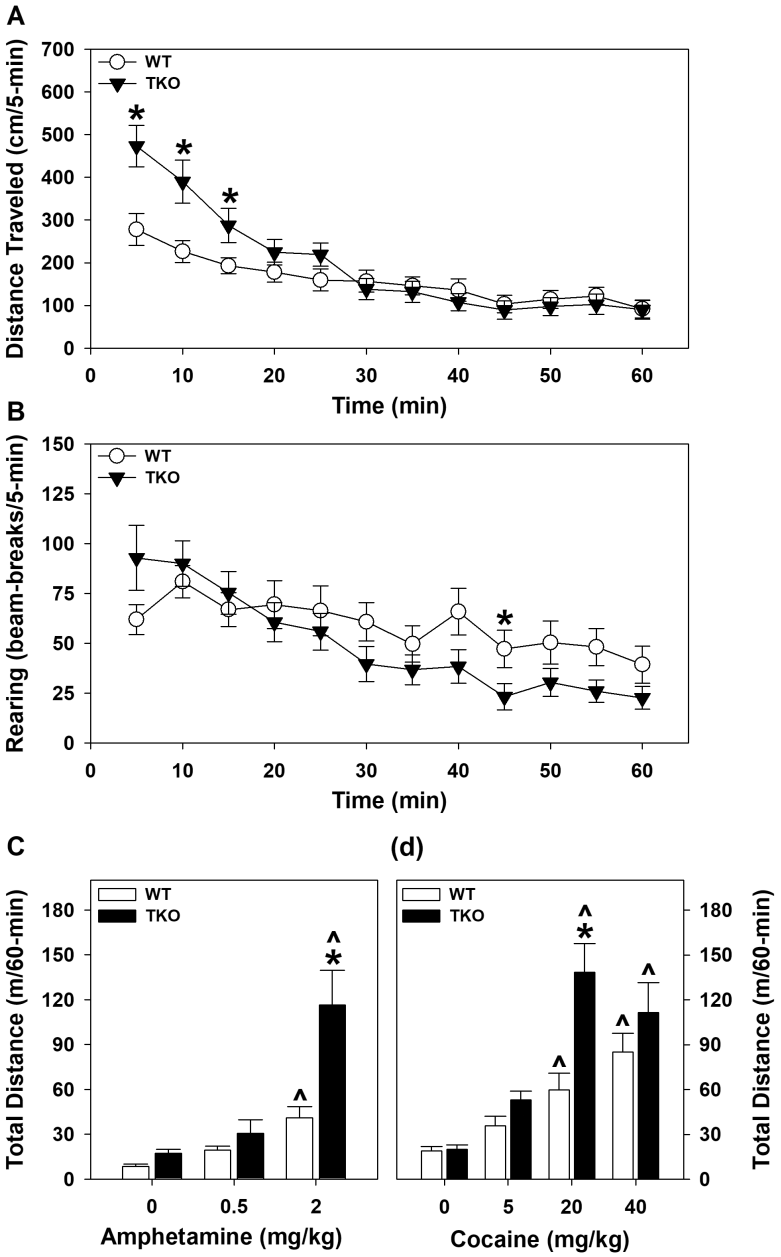

Figure 1. Open field responses of wild-type (WT) and triple knockout (TKO) mice to novelty and indirect dopamine (DA) agonists. (A) Distance traveled in a novel environment by naïve WT and TKO mice. Repeated-measures ANOVA (RMANOVA) for locomotion detected a significant effect of time [$F(11,352)=39.381$, $P<0.001$] and a significant time by genotype interaction [$F(11,352)=7.141$, $P<0.001$]. (B) Rearing activity in the open field by the same mice. RMANOVA for rearing observed a significant effect of time [$F(11,352)=15.787$, $P<0.001$] and a time by genotype interaction [$F(11,352)=3.737$, $P<0.001$], n=16–18 mice/genotype. (C) Effect of D-amphetamine on total distance traveled. ANOVA for locomotion revealed significant effects of genotype [$F(1,40)=12.265$, $P<0.001$] and dose [$F(2,40)=19.740$, $P<0.001$], and a significant genotype by dose interaction [$F(2,40)=5.694$, $P=0.007$]. One-way ANOVA with WT or TKO data indicated a significant effect of dose [$F(2,19)=13.909$, $P<0.001$ and $F(2,21)=13.848$, $P<0.001$, respectively], n=7–8 mice/genotype/dose. (D) Effect of cocaine on total distance traveled. ANOVA for locomotion noted significant effects of genotype [$F(1,58)=9.372$, $P=0.003$] and dose [$F(2,58)=21.448$, $P<0.001$], and a significant genotype by dose interaction [$F(2,58)=5.587$, $P=0.006$]. One-way ANOVA for WT or TKO data indicated a significant effect of dose [$F(2,28)=5.525$, $P=0.020$ and $F(2,30)=17.85$, $P<0.001$, respectively], n=7–14 mice/genotype/dose. *$P<0.05$, vs WT mice; ^$P<0.05$, vs vehicle.

wild-type controls (Ps<0.001). Since this enhanced immobility occurred at the end of the test session, it suggests a decline in DA receptor responsiveness, which likely occurred also in the initial open field study (see Figure 1A).

Both D1Rs and D2Rs Contribute to TKO Responses

More selective DA receptor agonists were used to examine the contributions of D1Rs and D2Rs to locomotion. SKF-81297, a selective D1R agonist, produced a dose-dependent increase in locomotion in both genotypes, with TKO mice responding more robustly ($P<0.001$) than wild-type controls (Figure 2B).

Quinpirole, a D2R/D3R agonist, produced a dose-dependent decrease in locomotion in both genotypes (Figure 2C). However, only 0.5 mg/kg quinpirole reduced activity in TKO mice ($P=0.010$), whereas all doses dramatically depressed locomotion in wild-type controls (Ps<0.001). Collectively, these data show that mutants are more responsive to D1R agonists and

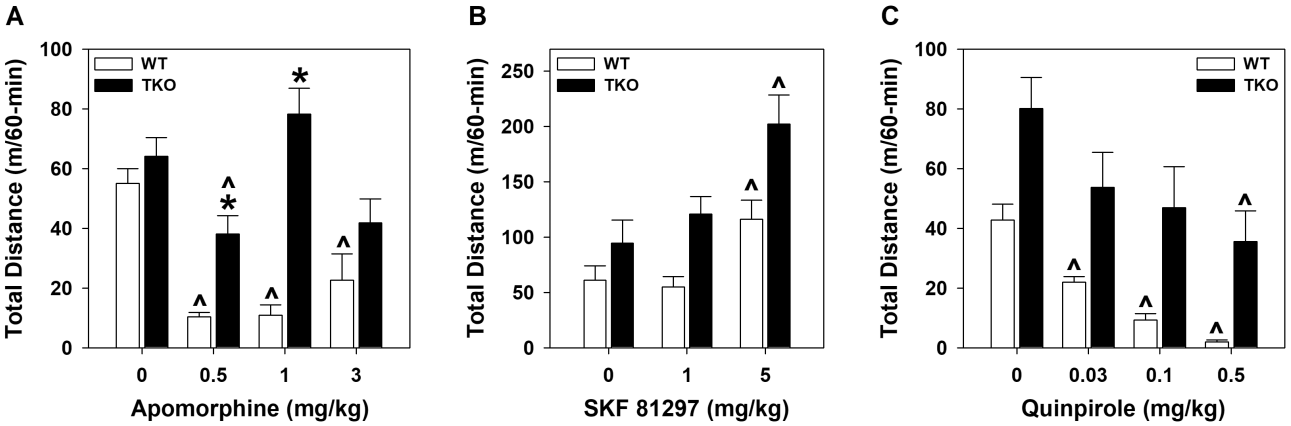

Figure 2. Effects of direct dopamine (DA) receptor agonists on open field activities of wild-type (WT) and triple knockout (TKO) mice. (A) Apomorphine effects on locomotor activity. ANOVA demonstrated significant effects of genotype [$F(1,80) = 38.015$, $P < 0.001$] and dose [$F(3,80) = 12.017$, $P < 0.001$] and a significant genotype by dose interaction [$F(3,80) = 6.004$, $P < 0.001$], n = 9–11 mice/genotype/dose. (B) Effects of SKF81297 on locomotion. ANOVA found the effects of genotype [$F(1,50) = 16.302$, $P < 0.001$] and dose [$F(2,50) = 10.342$, $P < 0.001$] to be significant. One-way ANOVA on WT and TKO data indicated a significant effect of dose [$F(2,24) = 5.901$, $P = 0.008$ and $F(2,26) = 5.505$, $P = 0.010$, respectively], n = 7–13 mice/genotype/dose. (C) Effects of quinpirole on locomotion. ANOVA discerned the effects of genotype [$F(1,72) = 24.772$, $P < 0.001$] and dose [$F(3,72) = 8.421$, $P < 0.001$] to be significant. One-way ANOVA run on separately with the WT or TKO data indicated a significant effect of dose [$F(3,31) = 23.054$, $P < 0.001$ and $F(3,31) = 3.385$, $P = 0.027$, respectively], n = 7–15 mice/genotype/dose. *$P < 0.05$, vs WT mice; ^$P < 0.05$, vs vehicle.

Table 2. Apomorphine-Induced Stereotypies in Climbing Cylinders

Apomorphine[a]	0 mg/kg		1 mg/kg		3 mg/kg	
Genotype	WT	TKO	WT	TKO	WT	TKO
Climbing[b]	2.30 ± 0.94	3.10 ± 1.05	4.4 ± 1.75	7.0 ± 1.98	8.20 ± 1.63	7.30 ± 1.30
Gnawing[c]	0.60 ± 0.19	0.50 ± 0.18	0.3 ± 0.21	1.2 ± 0.80	0.09 ± 0.09	6.20 ± 1.20[f,g]
Licking[d]	0.00 ± 0.00	0.00 ± 0.00	0.6 ± 0.42	0.0 ± 0.00	1.81 ± 0.72[g]	0.27 ± 0.19[f]
Immobility[e]	0.05 ± 0.05	4.93 ± 1.17	4.6 ± 1.50	7.1 ± 0.43	1.40 ± 0.50	4.00 ± 0.55
n	17	16	10	10	11	11

Abbreviations: TKO, triple knockout; WT, wild type.
[a]The results are presented as means and SEMs for the total stereotypy scores over the 60-minute observation period.
[b]ANOVA for climbing found only a significant effect of dose [$F(2,69) = 7.3$, $P < 0.001$].
[c]ANOVA for gnawing observed significant effects of genotype [$F(1,69) = 27.2$, $P < 0.001$] and dose [$F(2,69) = 14.2$, $P < 0.001$] and for the genotype by dose interaction [$F(2,69) = 19.2$, $P < 0.001$].
[d]ANOVA for licking noted significant effects of genotype [$F(1,69) = 8.0$, $P = 0.006$] and dose [$F(2,69) = 6.5$, $P = 0.003$] and for the genotype by dose interaction [$F(2,69) = 3.5$, $P = 0.036$].
[e]ANOVA for immobility reported significant effects of genotype [$F(1,69) = 22.34$, $P < 0.001$] and dose [$F(2,69) = 8.856$, $P < 0.001$].
[f]$P < 0.05$, vs wild-type mice.
[g]$P < 0.05$, vs 0 and 1 mg/kg apomorphine within genotype.

are subsensitive to presynaptic D2R stimulation (see Jeziorski and White, 1989).

Habituation to Novelty Abrogates Sensitized Responses to Cocaine in TKO Mice

Because acute responses to indirect and direct D1R agonists were more robust while D2R appeared less sensitive in TKO than in wild-type mice, we asked whether repeated administration of cocaine would further augment their activities. Parenthetically, repeated exposure to psychostimulants leads to a progressive enhancement in responsiveness or behavioral sensitization resulting in increased DA release, activation of D1Rs, and subsensitivity of D2 autoreceptors on DA cell bodies (Wolf, 1998). Wild-type and TKO mice were administered cocaine and placed immediately into the open field for 5 consecutive days; saline was given as a control. A cocaine challenge was provided on day 11 to all groups.

Cocaine stimulated locomotion in both genotypes on days 1 and 5 relative to their saline controls (*Ps* <0.001); however, cocaine responses were more robust in TKO than wild-type mice on both days (*Ps* ≤ 0.003) (Figure 3A). Wild-type animals given cocaine repeatedly showed progressively increased locomotion on days 5 and 11 compared with day 1 (*Ps* ≤ 0.024). Although TKO mice administered cocaine repeatedly had higher activities on day 5 than day 1 ($P = 0.015$), sensitization was not evident at challenge. On day 11, locomotor activities were higher in wild-type mice that received repeated cocaine injections across days compared with wild-type controls given cocaine only at challenge ($P = 0.012$), further supporting behavioral sensitization for this group. By contrast, at cocaine challenge locomotion in TKO mice that received saline repeatedly did not differ significantly from mutants or wild-type animals given cocaine throughout the experiment (Figure 3A). Together, these results show that cocaine sensitization is evident in wild-type mice across acquisition days and at cocaine challenge, while expression of sensitization is blunted in TKO animals.

Since cocaine responses at challenge in TKO mice that received saline repeatedly did not reach quite the same levels as mutants

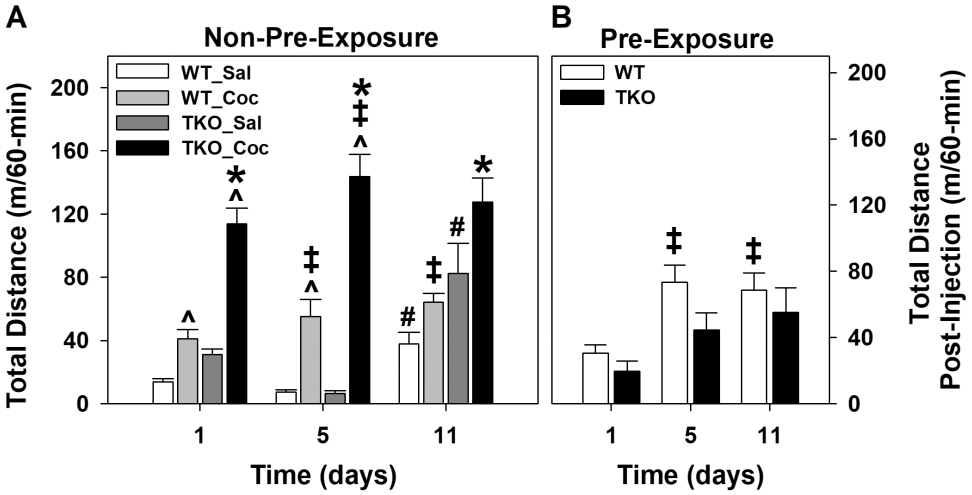

Figure 3. Locomotor sensitization to cocaine in wild-type (WT) and triple knockout (TKO) mice. (A) WT and TKO mice were injected with saline (Sal) or cocaine (Coc) and placed immediately into the open field for 5 consecutive days. On day 11, all mice received a 15-mg/kg Coc challenge. This panel displays locomotion for days 1, 5, and 11. An omnibus repeated-measures ANOVA (RMANOVA) revealed a significant within-subject effect of time [$F(2,66)=15.370$, $P<0.001$]; the time by dose [$F(2,66)=13.740$, $P<0.001$] and time by dose by genotype [$F(2,66)=5.179$, $P=0.008$] interactions were significant. The between-subject effects of genotype [$F(1,33)=45.273$, $P<0.001$] and dose [$F(1,33)=74.673$, $P<0.001$] and the genotype by dose interaction [$F(1,33)=14.791$, $P<0.001$] were also significant. RMANOVA within WT mice indicated a significant effect of time [$F(2,34)=18.761$, $P<0.001$] and dose [$F(1,17)=21.664$, $P<0.001$], and the time by dose interaction [$F(2,34)=4.319$, $P=0.021$]. RMANOVA within TKO mice indicated significant effects of time [$F(2,32)=5.877$, $P=0.007$] and dose [$F(1,16)=51.053$, $P<0.001$], and the time by dose interaction [$F(2,32)=9.771$, $P<0.001$], n = 8–10 mice/genotype/treatment. (B) In a second sensitization study, mice were preexposed to the open field for 60 minutes and then administered Sal or Coc for 5 consecutive days, followed by a cocaine challenge on day 11. This panel displays locomotion for days 1, 5, and 11. An omnibus RMANOVA revealed a significant effect of time [$F(2,36)=15.290$, $P<0.001$]. Separate RMANOVA within genotype revealed significant effects of time for WT [$F(2,18)=11.336$, $P<0.001$] and TKO mice [$F(2,18)=5.479$, $P=0.014$], n = 10 mice/genotype/treatment. *$P<0.05$, WT_Coc vs TKO_Coc group within day; ^$P<0.05$, compared with vehicle within days (1 and 5) and genotype; ‡$P<0.05$, Coc groups vs day 1 Coc within genotypes; #$P<0.05$, vehicle day 1 vs day 11 Coc within genotype.

given cocaine throughout, it is possible that habituation to the open field before cocaine challenge may have mitigated the drug response in the saline-treated mice. To examine this possibility, mice were preexposed to the open field for 60 minutes prior to cocaine injection each day. Locomotor activities in wild-type and TKO mice were similar on days 1 and 5 and at cocaine challenge (day 11) (Figure 3B). When the data were examined within genotype, locomotion in wild-type animals was increased on day 5 and at cocaine challenge (day 11) relative to day 1 ($Ps \leq 0.019$). By comparison, while in TKO mice locomotion increased across days, the changes were not significant. Hence, sensitization was less evident in TKO animals. Thus, novelty appears to play a critical role in determining the enhanced cocaine responses in TKO mice.

Cocaine-Induced DA Release Is Reduced in TKO Mice in a Familiar Environment

To better understand the role of DA in these behavioral studies, striatal extracellular levels of DA were monitored in freely moving mice in their home cages. Baseline DA levels were higher in TKO than in wild-type mice [wild type: 4.72±1.20 pg, TKO: 9.51±1.47 pg; n = 15–17 mice/genotype; $t(1,30)=-2.47$, $P=0.019$], suggesting a basis for their transient novelty-induced hyperlocomotion in the initial open field study (see Figure 1A). To determine whether cocaine could alter DA levels in this familiar environment, mice were injected with saline or 20 mg/kg cocaine. Because baseline DA levels were different between genotypes, the data were normalized as percent change from 100 at time 0. No genotype differences in DA levels were observed following saline injection (Figure 4A). Cocaine stimulated striatal DA release in both genotypes; however, normalized levels were lower in TKO than wild-type mice at 20 to 60 and 140 minutes postinjection ($Ps \leq 0.017$). Parenthetically, in a voltammetry study, DA responses to cocaine were also decreased in TKO mice (Venton et al., 2006). To determine whether the reduced cocaine response in TKO mice was due to compromised vesicular release, 60 mM KCl was infused into the microdialysis probe. DA levels were elevated to similar extents in both genotypes (Figure 4B). Thus, the releasable pool of DA *in vivo* appears similar in both genotypes, even though cocaine responses in TKO mice are blunted in the familiar environment.

No Genotype Differences in Cocaine-Induced DA Release in a Novel Environment

Since locomotor responses to cocaine were robust in TKO animals in a novel environment (see Figures 1D and 3A), we expected DA levels would be increased by novelty and decreased with open field preexposure. In the preexposure condition prior to cocaine injection, no significant genotype differences were observed in DA responses to the novel environment (Figure 4C). Following cocaine injection, elevations in extracellular DA were not different between genotypes in either the preexposure or non-preexposure paradigms (Figure 4D). Hence, extracellular DA responses to cocaine are undifferentiated between genotypes and are insensitive to environmental novelty.

Because cocaine was more potent in stimulating locomotion in TKO mice in the non-preexposure than the preexposure conditions (Figure 3), it was surprising that DA levels were not higher in non-preexposed cocaine-treated mice. However, baseline locomotion in the preexposure condition was higher in TKO than wild-type animals ($P=0.046$) (Figure 4E). Following cocaine injection, locomotor activities were similar under the preexposure and non-preexposure conditions for wild-type animals. By comparison, locomotion in non-preexposed TKO mice was higher than that in the non-preexposed wild-type and preexposed TKO groups ($Ps \leq 0.035$). Together, these findings reveal that DA release is not indicative of genotypic differences in open field locomotion.

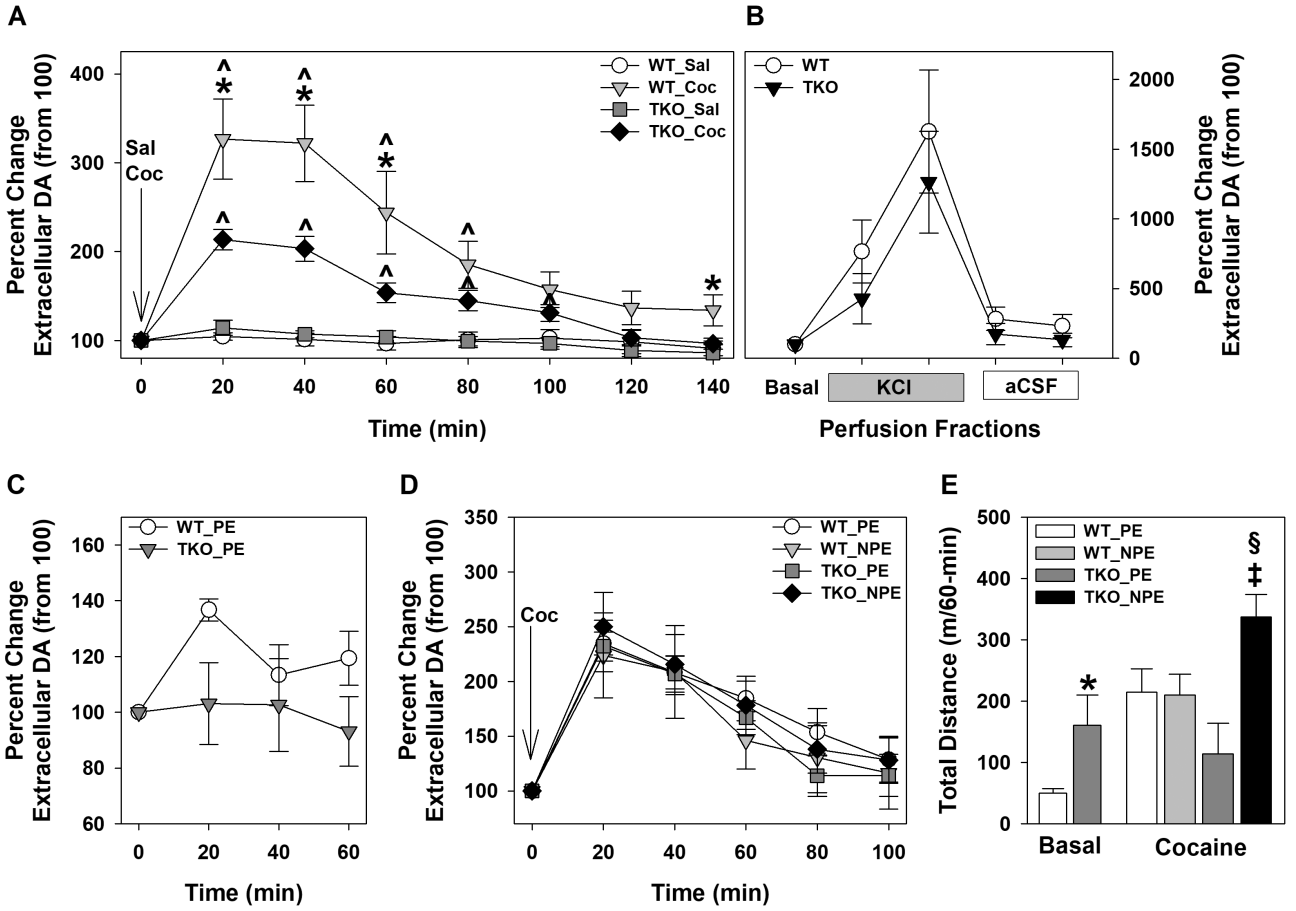

Figure 4. Microdialysis of dopamine (DA) in the home cage and open field. (A) Wild-type (WT) and triple knockout (TKO) mice were injected (arrow) with saline (Sal) or cocaine (Coc) in the home cage. Repeated-measures ANOVA (RMANOVA) detected significant main effects of time [$F(7,196)=25.283$, $P<0.001$] and treatment [$F(1,28)=20.430$, $P<0.001$] as well as the time by treatment [$F(7,196)=18.693$, $P<0.001$] and time by treatment by genotype [$F(7,196)=3.072$, $P=0.004$] interactions, n = 5–6 mice/genotype for Sal and n = 9–12 mice/genotype for Coc. *$P<0.05$, vs WT; ^$P<0.05$, vs vehicle within genotype. (B) Local perfusion into the striatum with 60 mM KCl through the microdialysis probe. Three 20-minute samples with artificial cerebrospinal fluid (aCSF) were collected and are presented as the mean baseline (BASAL). Subsequently, 2 KCl samples were collected at 20-minute intervals (KCl) and then conditions were returned to aCSF for two 20-minute intervals (aCSF), n = 7–8 mice/genotype. (C–E) DA levels were examined into 2 separate procedures. In first, mice were preexposed (PE) to the open field for 60 minutes, administered cocaine (Coc), and returned immediately to the open field for 100 minutes. In a second procedure (non-preexposure or NPE), mice were injected with Coc and placed immediately into the open field. (C) PE procedure at 0 to 60 minutes depicting percent change from baseline DA levels. No significant effects of time or genotype were discerned. (D) Comparison of percent change from baseline DA levels for cocaine postinjected mice (arrow) in the PE and NPE procedures. Note, to compare both experiments, the PE group at 60 minutes was assigned "0 min" in this panel. RMANOVA reported a significant effect of time [$F(5,115)=41.852$, $P<0.001$], but no other main effects or interactions were observed. (E) Locomotor activity in open field in the same mice as in panels C and D. In PE condition, a paired *t* test revealed higher overall pre-injection (basal) activity in TKO mice [$t(1,12)=-2.224$, $P=0.046$]. For post-Coc activities, an ANOVA indicated significant effects of the exposure condition [$F(1,23)=7.208$, $P=0.013$] and the exposure condition by genotype interaction [$F(1,23)=7.927$, $P=0.010$], n = 6–8 mice/genotype. *$P<0.05$, WT-PE vs TKO_PE at baseline; ‡$P<0.05$, WT_NPE vs TKO-NPE after Coc; §$P<0.05$, TKO_PE vs TKO-NPE after Coc.

DA Synthesis Is Increased in Striata of TKO Mice *ex Vivo*

Since basal levels of extracellular DA were higher in mutant than wild-type mice, we examined whether their tissue DA levels were altered. High-pressure liquid chromatography with electrochemical detection analyses revealed that tissue levels of DA were similar between genotypes (Table 3). However, the (3,4-hihydroxyphenylacetic acid + homovanillic acid)/DA turnover ratio was augmented in dorsal striata of TKO mice [wild type: 0.122±0.005, TKO: 0.146±0.007; n = 13 mice/genotype; $t(1,24)=-2.55$, $P=0.017$].

Because turnover tissue levels of DA were enhanced in mutants, *ex vivo* synthesis rates were monitored with NSD-1015 to block conversion of L-3,4-dihydroxyphenylalanine to DA. Mice were pretreated with this compound, exposed to the open field, and then euthanized. Synthesis rates of L-3,4-dihydroxyphenylalanine were increased in the dorsal striata of TKO compared with wild-type animals [$t(1,10)=-3.46$, $P=0.006$] (Table 4). Hence, exposure to the open field increases striatal DA synthesis and turnover, which may compensate for possible depletion of the releasable DA pool during the novelty-enhanced locomotion in TKO mice.

Locomotion Is Indifferent to Blockade of Newly Synthesized DA and Depletion of Releasable DA Pools

To examine the functional consequences of altered DA synthesis and turnover, we determined whether disruption of the vesicular or newly synthesized pool of DA would exert a greater effect on open field activity in TKO than wild-type mice. α-Methyl-*para*-tyrosine blocks the rate-limiting synthesis of DA at tyrosine hydroxylase and thereby depletes the "releasable" pool of DA (Glowinski, 1973).

Table 3. Brain Tissue Levels of DA and Metabolites in WT and TKO Mice

	DA			DOPAC			HVA		
	FC	DS	VS	FC	DS	VS	FC	DS	VS
WT	0.03 ±0.004	18.17 ±0.727	8.31 ±0.541	0.02 ±0.009	0.94 ±0.053	0.92 ±0.038	0.02 ±0.001	1.31 ±0.114	0.65 ±0.041
TKO	0.04 ±0.007	18.12 ±1.004	7.95 ±0.387	0.01 ±0.002	1.02 ±0.071	0.90 ±0.051	0.04[a] ±0.005	1.61 ±0.119	0.75 ±0.042

Abbreviations: FC, frontal cortex; DA, dopamine; DS, dorsal striatum; TKO, triple knockout; VS, ventral striatum; WT, wild type.
The results are presented as means and SEMs for tissue levels (ng/mg wet-tissue weight); n = 13 mice/genotype.
[a]$P < 0.05$, vs WT mice (2-tailed *t* test).

Table 4. Brain Tissue Levels of l-DOPA in WT and TKO Mice After the Open Field

	FC	DS	VS
WT	0.15 ±0.094	1.02 ±0.050	1.03 ±0.045
TKO	0.16 ±0.103	1.50[a] ±0.132	1.08 ±0.059

Abbreviations: FC, frontal cortex; DS, dorsal striatum; TKO, triple knockout; VS, ventral striatum; WT, wild type.
The results are presented as means and SEMs for tissue levels (ng/mg wet-tissue weight); n = 6 mice/genotype.
[a]$P < 0.05$, vs WT mice (2-tailed *t* test).

By comparison, reserpine blocks uptake of monoamines from the cytosol into secretory vesicles and prevents exocytosis (Varoqui and Erickson, 1997). We found that while cocaine-stimulated locomotion was decreased by reserpine in a dose-dependent manner ($Ps \leq 0.050$), it was not altered by α-methyl-*para*-tyrosine in either genotype relative to their respective vehicle control (Figure 5A). Notably, locomotor activities of TKO mice were always higher than wild-type controls in response to vehicle, α-methyl-*para*-tyrosine, and 1 mg/kg reserpine ($Ps \leq 0.042$), except with 5 mg/kg reserpine, where there was virtually no cocaine-induced locomotion. When expressed as a percent change from 100, motor activities in both genotypes were similarly affected by reserpine and were not altered by α-methyl-*para*-tyrosine relative to vehicle (Figure 5B). Thus, TKO mice are not more sensitive to reserpine or α-methyl-*para*-tyrosine than wild-type mice.

Frontal Cortex Modulates Cocaine-Stimulated Hyperlocomotion in TKO Mice

While loss of synapsins is well-known to affect presynaptic physiology (Gitler et al., 2004; Venton et al., 2006; Kile et al., 2010), our findings indicate that DA-mediated postsynaptic responses are aberrant in TKO mice. Because motor activity is mediated by the indirect and direct pathways that converge on the frontal cortex (Kaji, 2001), it is possible that input from the frontal cortex to striatum is responsible for the increased cocaine responses of TKO mice. To test this hypothesis, we temporarily inhibited the medial frontal cortex with $GABA_A$ (muscimol) and $GABA_B$ (baclofen) agonists. Naïve TKO mice were microinjected with vehicle or muscimol/baclofen into frontal cortex, administered 20 mg/kg cocaine 5 minutes later, and placed immediately into the open field. While cocaine briefly increased locomotion in the muscimol/baclofen group, activity was lower than in saline-microinjected mice at 20 to 55 minutes and 70 minutes ($Ps \leq 0.043$) (Figure 5C). Hence, disruption of the frontocortical-striatal circuit abrogated the cocaine stimulatory response in TKO mice in a fashion similar to preexposure to the open field. This result suggests that neural input from the frontal cortex to striatum mediates the enhanced locomotor responses of TKO mice in a novel environment.

Discussion

SYN polymorphisms have been linked to neuropsychiatric conditions as disparate as schizophrenia (Chen et al., 2004; Lachman et al., 2005), autism (Fassio et al., 2011; Corradi et al., 2014), and epilepsy (Garcia et al., 2004; Fassio et al., 2011). This broad association stems from the ubiquitous presynaptic expression of synapsins throughout brain so that perturbation of *SYN* genes yields an excitatory/inhibitory imbalance in synaptic networks (Gitler et al., 2004; Farisello et al., 2013; Lignani et al., 2013). Here, we report that locomotor responses of TKO mice are supersensitive to direct and indirect DA agonists due to modulation by environmental novelty.

Deletion of single *Syn* genes produces open field hyperactivity in naïve mice (Corradi et al., 2008; Dyck et al., 2009; Porton et al., 2010). Similarly, we found TKO mice were hyperactive relative to wild-type controls, but only over the first 15 minutes in the open field. This transient novelty response suggests that the stress of open field exposure may lead to a short-term increase in DA release. Indeed, novelty can produce a transient enhancement in striatal DA release measured by voltammetry (Rebec et al., 1997). Psychostimulant-induced open field hyperlocomotion depends on stimulation of postsynaptic DA receptors in the basal ganglia through increased DA release (Conti et al., 1997). Since TKO mice were much more responsive to psychostimulants than wild-type mice, we reasoned their responses may reflect alterations in pre- and/or postsynaptic mechanisms.

The functions of D1Rs and D2Rs can be assessed by administration of direct DA agonists, which produce well-characterized behavioral endpoints. Although D2Rs reside both on presynaptic DA neurons and postsynaptic cells, D2R stimulation usually inhibits locomotor activity through presynaptic D2 autoreceptors (Martin and Bendesky, 1984). Low doses of apomorphine decrease locomotion, while higher doses increase activity through actions on postsynaptic DA receptors (Kelly et al., 1975). In our study, apomorphine depressed locomotion in wild-type mice across doses. In contrast, TKO motor activities were affected only at 0.5 mg/kg apomorphine, suggesting that presynaptic D2Rs in TKO mice are subsensitive. This effect was confirmed with quinpirole, a D2/D3R agonist. This drug depressed TKO motor activity only at the highest dose, whereas all doses suppressed wild-type responses, supporting a role for D2 autoreceptor activity (see Jackson et al., 1989). By contrast, responses to a selective D1 agonist (SKF 81297) were augmented in TKO mice, revealing an enhanced stimulation of postsynaptic D1Rs. Combined stimulation of postsynaptic D1R/

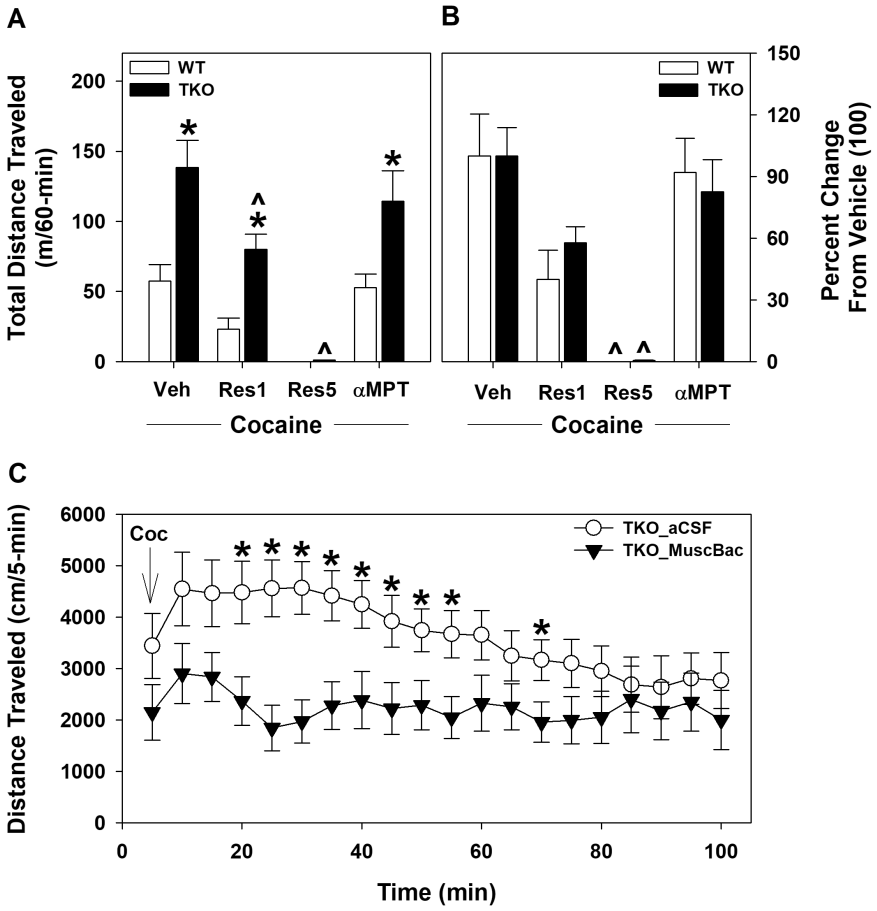

Figure 5. Effects of monoamine depletion and microinjection of a muscimol/baclofen mixture into the medial frontal cortex on cocaine-induced locomotor activity. (A) Cocaine (20 mg/kg) was administered to all mice pretreated with vehicle (Veh: n = 13–14 mice/genotype), 1 or 5 mg/kg reserpine (Res1, Res5: n = 4–6 mice/genotype), or 250 mg/kg α-methyl-*para*-tyrosine (αMPT: n = 8–9 mice/genotype). In the reserpine experiment, ANOVA determined the effects of genotype [$F(1,42) = 9.280$, $P = 0.004$] and dose [$F(2,42) = 15.279$, $P < 0.001$] were significant. In the αMPT experiment, there were significant effects only of genotype [$F(1,40) = 16.627$, $P < 0.001$]. (B) Percent change in total distance traveled relative to that for vehicle. In the reserpine experiment, there was a significant effect of dose [$F(2,42) = 14.258$, $P < 0.001$]; no significant effects were observed in the αMPT experiment. *$P < 0.05$, vs wild type (WT); ^$P < 0.05$, vs vehicle. (C) Naïve triple knockout (TKO) mice were microinjected into the frontal cortex with artificial cerebrospinal fluid (aCSF) or a muscimol/baclofen (MuscBac) mixture, and 5 minutes later they were given 20 mg/kg cocaine (arrow) and were placed immediately into the open field. Repeated-measures ANOVA (RMANOVA) revealed the main effects of time [$F(19,361) = 2.837$, $P < 0.001$] and treatment [$F(1,19) = 6.385$, $P = 0.021$] and the time by treatment interaction [$F(19,361) = 1.858$, $P = 0.016$] to be significant, n = 10–11 mice/group; *$P < 0.05$, vs the aCSF group.

D2Rs produces both climbing and stereotypies in mice (Geter-Douglass et al., 1997). In our study, apomorphine stimulated climbing to similar extents in both genotypes; however, it produced more gnawing in TKO mice, suggestive of postsynaptic DA receptor supersensitivity.

Cocaine can serve as an indirect DA receptor agonist. During repeated cocaine administration, locomotion in TKO mice was significantly higher than in wild-type controls. By contrast, at challenge cocaine responses in vehicle-treated mutants were similar to sensitized wild-type mice. This result suggests that long-term habituation to the open field may attenuate some effects of novelty stress and mitigate cocaine responses. We examined this possibility by preexposing mice to the open field before administering cocaine in a sensitization paradigm. One hour of open field preexposure rendered the TKO cocaine-stimulated activity indistinguishable from that of wild-type controls, both across days and in response to cocaine challenge on day 11. Because novelty stress augments DA activities, we examined whether presynaptic DA function was altered in TKO mice during novelty exposure.

Few changes in tissue DA levels were observed in TKO mice in a familiar environment (home cage); the only significant differences were elevated homovanillic acid in frontal cortex and increased metabolites/DA contents in striatum. The latter metric suggests elevated release and metabolism (turnover) of DA in TKO mice. This possibility was supported by increased extracellular levels of striatal DA in TKO mice in the home cage. DA levels measured by microdialysis depend on neural activities of DA neurons (Arbuthnott et al., 1990). Thus, enhanced basal DA levels in TKO mice should reflect increased incidences of DA transients (Wightman et al., 2007). Kile and colleagues (2010) reported that TKO neurons release more DA that wild-type neurons in response to brief striatal electrical stimulation. It has been proposed that loss of synapsins enhances the mobility of reserve pool vesicles, thereby increasing their redistribution to the readily releasable vesicle pool (Brenes et al., 2015). If this occurs in DA neurons, it should yield greater DA release. Alternatively, increased basal DA levels in TKO animals may reflect enhanced DA population activity or augmented excitability of DA neurons, resulting in higher bursting activity (Chiappalone et al., 2009). While the first possibility has not been studied, the latter mechanism is unlikely because it should produce large increases in extracellular DA following blockade of DA transporters (Floresco et al., 2003). However, cocaine treatment of TKO mice yielded the opposite effect.

Since D2 autoreceptors appear subsensitive in TKO mice while basal DA release is augmented with Km values for DA uptake similar between genotypes (Kile et al., 2010), cocaine may be expected to release greater amounts of DA in TKO mice. However, we found cocaine-stimulated DA release was lower in TKO than wild-type striatum in the home cage. This result concurs with reports that cocaine has a lower potency for releasing DA from striatal TKO slices (Kile et al., 2010) or from anesthetized TKO mice (Venton et al., 2006). In contrast, DA release evoked by high K^+ was similar between genotypes. Hence, the reservoir pool of DA may be reduced in TKO animals (as for GABAergic and glutamatergic synaptic vesicles; Terada et al., 1999; Gitler et al., 2004, 2008), so that this pool is unable to supply sufficient DA to the ready-releasable pool (see Pieribone et al., 1995) to maintain DA release during cocaine exposure.

The striking differences in cocaine-induced locomotion of TKO mice in the non-preexposure and preexposure conditions indicate that environmental novelty is an important factor in their cocaine response. The stress associated with preexposure may cause a rapid release and depletion of the ready-releasable DA store, thereby rendering cocaine less efficacious. Surprisingly, in the preexposure condition, novelty-induced DA release in untreated TKO mice was similar to wild-type controls. Additionally, cocaine-stimulated locomotion in preexposed TKO mice was blunted relative to non-preexposed TKO mice, despite both groups showing similar levels of DA release. Of note, a limitation of microdialysis is that it reflects integral neuronal activity over minutes; hence, this time resolution may miss transient spikes in DA release in response to novelty. Nevertheless, the increased DA synthesis rates during novelty exposure may provide additional DA for release, thereby masking a potential decline in extracellular DA levels caused by depletion of the ready-releasable pool.

Further dissection of presynaptic mechanisms via pharmacological interrogation of reserpine-sensitive or the ready-releasable (i.e., α-methyl-*para*-tyrosine) pools of neurotransmitters revealed that the sensitivities of these pools to disruption was similar between genotypes. Thus, presynaptic pools of DA do not appear to contribute to the differences in cocaine-stimulated locomotion in TKO compared with wild-type mice. Together, changes in the presynaptic DA system cannot explain the hyper-responsiveness of TKO mice to cocaine or the blunting of cocaine-evoked locomotion in the preexposure condition. Postsynaptic mechanisms were examined next. Although supersensitive postsynaptic DA receptors can contribute to increased TKO cocaine responses, this cannot account for the abrupt blunting of the cocaine response by preexposure to the open field. Alterations in DA receptor sensitivity that produce changes in behavior usually occur over prolonged periods of time (Direnfeld et al., 1978; Jeziorski and White, 1989; Hu and White, 1992) rather than within 60 minutes. Another postsynaptic mechanism relates to the regulation of DA receptor function by afferent systems in the basal ganglia.

The basal ganglia contain parallel D1R- and D2R-containing circuits linking the thalamus and cerebral cortex. In these circuits, dopaminergic responses are modulated through glutamatergic and GABAergic neurotransmission, both of which are abnormal in synapsin-deficient mice (Terada et al., 1999; Gitler et al., 2004). The frontal cortex provides massive glutamatergic inputs both to the striatum (Divac et al., 1977) and the ventral tegmentum (Carr and Sesack, 2000), where DA nerve terminals and cell bodies reside, respectively. These inputs have been implicated in different behavioral outcomes. First, striatal microinjections of ionotropic (Thanos et al., 1992) or metabotropic (Kim and Vezina, 1997) glutamate receptor agonists potentiate locomotor activity. Second, disruption of this input by lesions of the frontal cortex, which decrease levels of glutamate in the ventral striatum, block expression of cocaine-induced behavioral sensitization (Pierce et al., 1998). Third, strengthening of the glutamatergic input to the ventral striatum contributes to relapse to cocaine-seeking (Luis et al., 2017). Finally, changes in glutamate receptor function have been proposed as a common mechanism for the effects of both psychostimulants and stress on mesolimbic DA neurons (Fitzgerald et al., 1996), with the stress of novelty-strengthening excitatory synapses on nucleus accumbens neurons (Rothwell et al., 2011).

A prominent phenotype of TKO mice is overt seizures evoked by sensory stimulation (Gitler et al., 2004). Although we did not observe overt seizures, handling can induce electroencephalographic seizures without visible effects in *Syn* mutants (Etholm et al., 2011). Notably, in animal models of generalized epilepsy, cortical electro-encephalographic seizures are associated with increased glutamate efflux in striatum (Kovács et al., 2003; Crick et al., 2014), and electrical kindling of the prefrontal cortex produces sensitized locomotor responses to cocaine (Schenk and Snow, 1994). Hence, in the non-preexposure condition, the novelty-stimulating effects may be due to abnormal neural input from the frontal cortex to the striatum. To test this hypothesis, we inhibited the prefrontal cortex with GABA agonists. This treatment caused cocaine-stimulated locomotion in TKO mice to be lower than the saline-microinjected mutants. The effect is reminiscent of the reduced stimulatory effect of cocaine in TKO mice after open field preexposure. Such an effect may underlie the heightened cocaine responses of non-preexposed TKO mice where the initial hyperlocomotion in TKO mice can result from increased excitatory glutamatergic input from frontal cortex. Following dissipation of the novelty stress-induced cortical activation, responses of postsynaptic striatal neurons to DA may decrease due to the decline in cortical glutamatergic input. This decrement in excitatory tone could be promoted by the high rate of synaptic depression at excitatory synapses in TKO mice (Gitler et al., 2004). Thus, the protracted stress of novelty in TKO mice may attenuate glutamatergic excitation of striatal neurons, which may decrease psychostimulant responses (see Pulvirenti et al., 1989; Tzschentke and Schmidt, 1998) and reduce activity in the open field and climbing tests.

Synapsins control many presynaptic responses (Greengard et al., 1993; Li et al., 1995; Ferreira et al., 1998; Gitler et al., 2004; Kile et al., 2010; Song and Augustine, 2015). These effects have been ascribed to functional consequences. For instance, Syn I and Syn II KO mice engage in increased exploratory activity and are deficient in social behavior; both these mutants and Syn III KO animals are deficient in novel object recognition memory and fear conditioning (Corradi et al., 2008; Dyck et al., 2009; Porton et al., 2010; Greco et al., 2013). Impaired spatial memory is observed in Syn II KO (Corradi et al., 2008) and TKO mice (Gitler et al., 2004). In humans polymorphisms in *SYN* genes are associated with schizophrenia (Porton et al., 2004; Chen et al., 2004; Lachman et al., 2005), bipolar disorder (Vawter et al., 2002), autism (Fassio et al., 2011; Corradi et al., 2014), and epilepsy (Garcia et al., 2004; Fassio et al., 2011). We found that deletion of *Syn I/II/III* renders mice more responsive to psychostimulants. While synapsin dysfunction has been ascribed to presynaptic disturbances, we find that DA receptor responses are aberrant at both presynaptic and postsynaptic sites. Hence, synapsins regulate neurotransmission not only through direct effects on neurotransmitter release, but also secondarily through widespread network effects that exert pervasive roles on behavior. In this way, synapsin dysfunction may contribute to diverse neuropsychiatric conditions.

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Statement of Interest

Drs Pogorelov, Kao, and Augustine have no conflicts of interest to declare in relation to this manuscript. Dr Wetsel has no conflict of interest to declare in relation to this manuscript; however, he does receive royalties from the National Institutes of Health for making antisera to the protein kinase C isoforms and has research funds from Rugen Holdings (Cayman) for preclinical studies related to obsessive-compulsive disorder and autism.

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