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CREB phosphorylation in nucleus accumbens underlies sustained recovery of sensorimotor gating following repeated D₂-like receptor agonist treatment in rats

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Abstract

Background—Prepulse inhibition (PPI) is a cross-species measure of sensorimotor gating. PPI deficits are observed in humans and rats upon acute treatment with dopamine D₂-like receptor agonists, and in patients with schizophrenia. Repeated treatment with a D₂-like agonist, however, reverses PPI deficits and increases cAMP signaling in the nucleus accumbens (NAc). The present study examined the short and long-term effects on PPI of treatment with quinpirole and ropinirole, dopamine D₂/D₃ receptor agonists, and the molecular mechanism by which they occur.

Methods—PPI was assessed in adult male Sprague-Dawley rats following acute and chronic treatment with quinpirole or ropinirole, and 1, 2, 3, and 4 weeks after termination of repeated ropinirole treatment. Finally, the effect of dominant negative mutant CREB overexpression in the NAc on PPI following chronic quinpirole treatment was assessed.

Results—Acute quinpirole produced dose-dependent PPI deficits, while ropinirole caused consistent PPI reduction at all but the highest dose. Repeated ropinirole treatment significantly increased PPI compared to acute treatment, and increased CREB phosphorylation in NAc neurons.

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Subsequent ropinirole challenge had no effect as long as 28 days later, at which time NAc CREB phosphorylation had normalized. Overexpression of dominant negative mutant CREB prevented PPI recovery induced by chronic quinpirole treatment.

Conclusions—Chronic quinpirole or ropinirole treatment produces sustained PPI recovery; CREB activity in the NAc is required to induce PPI recovery, but not to maintain it. The results suggest that transcriptional regulation by CREB mediates long-lasting changes occurring within NAc circuits to promote recovery of sensorimotor gating.

Keywords

D₂-like receptors; dopamine; quinpirole; ropinirole; CREB; prepulse inhibition

Introduction

Sensorimotor gating, the neural mechanism underlying the integration and processing of sensory information, is disrupted in patients with schizophrenia (1). Defects in sensorimotor gating theoretically reflect sensory flooding and cognitive fragmentation (1,2) and can be detected across species by quantifying prepulse inhibition of the acoustic startle response (PPI). PPI is the reduction in startle response that occurs when the startling stimulus or “pulse” is preceded by a barely audible sensory event or “prepulse” up to 500 msec earlier. Normally, forebrain processing of a prepulse stimulus can inhibit the brain stem startle response to a subsequent pulse stimulus.

Dopamine acts primarily through dopamine D₂-like receptors in the nucleus accumbens (NAc) to reduce PPI in rats (3). For example, systemic administration of the direct and indirect agonists, apomorphine and amphetamine, disrupts PPI in rats (4), as do more selective D₂-like receptor agonists, such as quinpirole (5) and ropinirole (6). The importance of D₂-like receptors in the NAc was confirmed by selective regional infusion of quinpirole, which disrupts PPI (3). In contrast to acute treatment, repeated treatment with indirect dopamine agonists such as cocaine or amphetamine attenuates PPI disruption (7,8) and repeated daily treatment with quinpirole completely reverses acute disruption, which we term PPI recovery (9).

In addition to regulating PPI, acute D₂-like receptor stimulation reduces adenylate cyclase (AC) activity via G_i/G_o protein coupling, which in turn decreases cAMP activity (10,11). Prolonged activation of these receptors *in vitro* produces heterologous sensitization, characterized by enhanced AC responsiveness giving rise to increased cAMP accumulation (12). The molecular mechanisms underlying this effect are not well understood, although agonist-induced enhancement of G_s protein-AC interaction might be crucial (12). Repeated treatment with quinpirole *in vivo* leads to recovery of PPI (9) accompanied by increased phosphorylation of cAMP response element binding protein (CREB) in the NAc (13). However it is unknown whether CREB phosphorylation and its subsequent regulation of target genes are necessary to sustain PPI recovery even in the absence of further agonist administration.

Ropinirole is a selective D₂/D₃ dopamine agonist used in the treatment of Parkinson's disease and restless leg syndrome (14). Both quinpirole and ropinirole have greater potency at the D₃- than the D₂-receptor, however, quinpirole is seven-fold more potent than ropinirole at the D₃-receptor (15,16). Acute administration of ropinirole derived from crushed formulated tablets is known to partially reduce PPI in rats (6) and in humans (17).

The present study assessed the effects of pharmaceutical-grade ropinirole compared to quinpirole on PPI and striatal CREB phosphorylation after acute or repeated treatment, and

examined the sustained effect of repeated ropinirole treatment. The involvement of CREB activation in the process of PPI recovery was examined directly using adeno-associated viral-mediated gene transfer to overexpress CREB or dominant-negative mutant CREB (mCREB) in the NAc during chronic quinpirole treatment.

Methods and Materials

Animals and drug treatment

For all of the following experiments animals were provided with food and water *ad libitum* while housed in a climate-controlled facility with reverse light/dark cycles (lights off at 0900 h, on at 1900 h). Rats were allowed to acclimate to the laboratory for seven days prior to handling and habituation to the behavioral testing apparatus. All experiments were approved by the Tufts-New England Medical Center and the University of Arizona Institutional Animal Care and Use Committees and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Male Sprague-Dawley rats (Charles River Laboratories; Kingston, RI) weighing 250–300 g were habituated to handling and subcutaneous (sc) saline injection, and placed into a Startle Monitor behavior testing chamber (Kinder Scientific, Poway, CA) with 70 dB ambient noise for 5 min daily on each of two days prior to baseline PPI testing. Baseline PPI was assessed as described below starting 10 min after 0.9% sterile saline vehicle injection (1.0 ml/kg, sc) on two consecutive days to ensure a reliable mean value. Treatment groups were normalized according to the mean acoustic startle response observed during baseline testing.

Prepulse inhibition testing

All PPI testing was conducted during the dark phase (1000 – 1400 h). Each animal was exposed to 70 dB ambient noise for 5 min followed by a test session, which consisted of 4 pulse trials (120 dB, 40 ms pulses), followed by a randomized presentation of 10 pulse and 30 prepulse trials (10 each of 73 dB, 76 dB, and 82 dB, 20 ms prepulses, followed 100 ms later by a pulse), and ending with 4 pulse trials. The inter-trial interval was an average of 15 sec (range: 8–22 sec). Percent PPI was calculated using the equation, $100 - [(\text{mean prepulse response} / \text{mean pulse response}) \times 100]$. Data were compared using repeated measures ANOVA in each treatment group, followed by a Fisher's Least Squares Difference post hoc test.

Experiment I

Several days after normalization, rats received either quinpirole HCl (0.0, 0.05, 0.1, or 0.3 mg/kg, sc; Sigma-Aldrich, St. Louis, MO) or ropinirole HCl (0.0, 0.05, 0.1, 0.5, or 1.0 mg/kg, sc; generously provided by GlaxoSmithKline, Research Triangle Park, NC) in 0.9% sterile saline followed by PPI testing 10 min later. Ten days later, during which time the animals were handled daily but did not receive injections, quinpirole-treated animals received a dose of ropinirole or vice versa, followed by PPI testing 10 min later.

Experiment II

Following habituation and normalization of groups, rats received repeated treatment once daily for 28 consecutive days with the same dose of ropinirole HCl (0.0, 0.05, 0.1, or 0.5 mg/kg sc.) in 0.9% sterile saline vehicle. PPI was assessed on the first and last day of drug administration as described above.

Immunohistochemistry—On the 29th day, rats were challenged with the same dose of ropinirole they received previously. Twenty minutes after the challenge injection, sodium

pentobarbital (100 mg/kg, ip) was administered, rats were perfused and brains were removed and processed for immunohistochemistry to label CREB phosphorylated at serine 133, as previously described (13). The number of immunolabeled profiles was quantified in the NAc and caudatoputamen using NIH ImageJ following background correction, and application of a set labeling and size threshold across all sections. See Supplement: Methods and Materials for details.

Experiment III

Following habituation and normalization of groups, rats received repeated drug treatment once daily for 28 consecutive days with ropinirole HCl (0.0 or 0.1 mg/kg sc) in 0.9% sterile saline vehicle. Startle amplitude was assessed on the first and last day of drug administration as described above. Thereafter, PPI testing was accomplished 1, 2, 3, and 4 weeks after termination of daily treatment following challenge with the same dose (0.0 or 0.1 mg/kg sc) received previously.

Another group of rats received repeated drug treatment once daily for 28 consecutive days with ropinirole HCl (0.0 or 0.1 mg/kg sc) in 0.9% sterile saline vehicle. Startle amplitude was assessed on the first and last day of drug administration in both groups as described above. Twenty-eight days later, during which time all subjects were handled daily, rats that previously received 0.1 mg/kg ropinirole received the same challenge dose while rats that previously received vehicle treatment received either 0.0 or 0.1 mg/kg of ropinirole, followed by PPI testing. Brains were removed on the 29th day after termination of drug treatment and processed for CREB and phosphoCREB immunohistochemistry as described above.

Experiment IV

Following habituation and normalization of groups, rats underwent stereotaxic surgery for adeno-associated viral vector-mediating gene transfer of CREB or mCREB, in which Ser¹³³ is replaced with alanine to prevent phosphorylation, co-expressed with enhanced green fluorescent protein (eGFP), or eGFP alone bilaterally in the NAc. The next day, PPI was assessed 10 min after an injection of 0.9% sterile saline (1.0 ml/kg, sc) to examine the effect of surgery as described above.

Three weeks after stereotaxic surgery, rats received quinpirole HCl (0.0 or 0.1 mg/kg, sc.; Sigma-RBI, St. Louis, MO) in 0.9% sterile saline followed by PPI testing 10 min later. Twenty-four h later, rats underwent subcutaneous implantation of custom-made control or quinpirole pellets, which released quinpirole HCl (0.0 or approximately 0.1 mg/kg/day), into the dorsal neck. All rats received PPI testing following saline injection (1.0 ml/kg, sc) 1 and 28 days after pellet implantation. PPI was re-assessed the next day 10 min after challenge with quinpirole HCl (0.0 or 0.1 mg/kg sc).

Fabrication of pellets—Pellets were fabricated through solvent casting and compression-molding of 75:25 poly-lactide-co-glycolide (Surmodics; Birmingham, AL) co-precipitated with quinpirole as previously described (18). Quinpirole pellets were composed of 20% drug and released approximately 0.035 mg/day of quinpirole, while control pellets contained the polymer alone.

AAV vector construction and stereotaxic injections—Adeno-associated viral vectors were produced using Stratagene's helper-free system to add an eGFP tag to the N-terminus of CREB and mCREB, as described previously (19,20). See Supplement: Methods and Materials for details.

Rats were anesthetized and placed into a stereotaxic frame, a sterile incision was made through the dermis, a bore hole was placed into the skull, and a Hamilton microsyringe was lowered into the NAc at the following coordinates: +1.2 mm anterior to bregma, ± 1.3 mm lateral, and 7.1 mm from the pial surface. AAV was infused at a rate of 0.01 $\mu\text{l}/\text{min}$ for 10 min on each side, after which the needle remained in place for 5 min.

Immunolabeling—Thirty days after pellet implantation (the day after the final PPI challenge), rats were anesthetized and decapitated, brains were rapidly frozen in 2-methylbutane and stored at -80°C prior to processing. A unilateral 2.0 mm wide micropunch (1.0 mm deep) of the NAc, corresponding to 1.6 mm anterior to bregma (21) was collected in a -20°C cryostat, and tissues were processed for western blotting. Levels of CREB and phosphoCREB (pCREB) were determined and normalized relative to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). See Supplement: Methods and Materials for details.

Brains from which micropunches were taken were sectioned by cryostat, sections were mounted on slides and stored at -35°C prior to immunohistochemical processing. Sections were post-fixed with 4% paraformaldehyde in PBS and then processed for immunohistochemical quantification of phosphoCREB as described above.

Results

PPI disruption following acute quinpirole or ropinirole administration

Experiment 1 demonstrates that acute treatment with the D_2 -like receptor agonist, quinpirole, significantly ($p \leq 0.001$) disrupted PPI in a dose-dependent manner (Fig. 1). For example, escalating doses of 0.05, 0.1 and 0.3 mg/kg reduced PPI by 17, 30, and 40%, respectively, compared to vehicle treatment. Higher doses of quinpirole caused significantly greater reduction in PPI than did the lowest dose. Acute ropinirole also produced significant ($p \leq 0.001$) PPI disruption (Fig. 1). However, the magnitude of ropinirole-induced PPI disruption was similar (25, 33 and 34% reduction after 0.05, 0.1 and 0.5 mg/kg, respectively) after all but the highest dose (46% reduction after 1.0 mg/kg). Neither mean acoustic startle response to pulse only trials (Supplement: Table S1) nor responses in the presence of ambient noise alone (data not shown) were altered by acute quinpirole or ropinirole treatment.

PPI attenuation and CREB phosphorylation following repeated ropinirole administration

In Experiment 2, the initial ropinirole treatment reduced PPI at all doses compared to baseline PPI, while saline treatment had no effect. In contrast, repeated ropinirole treatment significantly increased PPI compared to day 1 treatment levels (Fig. 2), indicating that complete recovery of PPI occurred with repeated ropinirole treatment at all doses. Neither mean acoustic startle response to pulse only trials (Supplement: Table S2) nor responses in the presence of ambient noise alone (data not shown) were altered by repeated ropinirole treatment. There was, however, a significant main effect on startle response to pulse only trials over time regardless of treatment; repeated saline treatment increased pulse response by 125%, while average pulse response to repeated ropinirole treatment increased by 116%.

Immunohistochemical data revealed that the phosphorylation state of CREB was altered by repeated ropinirole treatment in the NAc, but not in the caudatoputamen. CREB phosphorylation was moderate in NAc core and shell neurons following saline treatment, and increased after treatment with higher ropinirole doses in the NAc (Fig. 3). For example, repeated ropinirole treatment at a dose of 0.1 mg/kg increased phosphoCREB labeling by 90% in the NAc core ($p \leq 0.05$) and by 73% in the NAc shell ($p \leq 0.05$) compared to

repeated saline treatment. However, repeated ropinirole treatment had no significant effect on the number of phosphoCREB immunoreactive profiles in either the dorsolateral (DL) or medial (Med) caudatoputamen. Repeated ropinirole treatment also had no significant effect on the number of CREB immunoreactive profiles in the caudatoputamen (Supplement: Table S3).

Sustained effect of repeated ropinirole administration

Ropinirole disrupted PPI upon initial administration (day 1), but a challenge with ropinirole on the 28th day of treatment was without effect (Fig. 4). Moreover, ropinirole challenge had no significant effect 1, 2, 3 or 4 weeks after repeated treatment compared to baseline or saline challenge (Supplement: Figure S1). Furthermore, when ropinirole challenge was given 28 days after the termination of repeated administration in the absence of additional weekly challenges, there was no significant effect compared to baseline (Fig. 4). Neither mean acoustic startle response to pulse only trials (Supplement: Table S4), nor responses in the presence of ambient noise alone (data not shown) were altered by termination of ropinirole treatment.

Immunohistochemical data revealed that phosphorylation of CREB decreased slightly with acute ropinirole challenge compared to saline, however this effect was not statistically significant. Twenty-nine days after termination of repeated ropinirole treatment, CREB phosphorylation did not differ significantly compared to saline in the NAc or caudatoputamen (Figs. 5 and 6). Furthermore, there was no significant change in the number of CREB immunoreactive profiles in the striatum 29 days after termination of repeated ropinirole treatment (Supplement: Table S5).

mCREB prevents PPI recovery after chronic quinpirole administration

Intra-NAc AAV infusions had no effect on PPI 24 hours after surgery in any group compared to baseline (Fig. 7). Subsequent quinpirole challenge significantly reduced PPI by 30, 40 and 46% compared to baseline ($p \leq 0.05$) in AAV-eGFP-, AAV-mCREB-eGFP and AAV-CREB-eGFP-treated groups, respectively, however there was no effect of saline challenge. After 28 days of quinpirole pellet implantation, PPI fully recovered in rats expressing eGFP alone and in those overexpressing CREB, as demonstrated by significantly increased PPI upon quinpirole challenge compared to preimplantation levels (Fig. 7). In contrast, PPI remained disrupted after quinpirole pellet implantation and upon quinpirole challenge in rats overexpressing mCREB.

There was no effect of pellet surgery on PPI as demonstrated by all groups receiving control pellets, however, PPI was reduced compared to baseline 24 hours after implantation of quinpirole-containing pellets by 29, 31, and 24% in AAV-eGFP-, AAV-mCREB-eGFP and AAV-CREB-eGFP-treated groups, respectively (Table 1). Neither mean acoustic startle response to pulse trials nor responses in the presence of ambient noise was altered by viral or quinpirole treatment (data not shown).

CREB phosphorylation and total CREB protein in the NAc after chronic quinpirole treatment

Western blot analyses revealed that both CREB and mCREB overexpression consistently enhanced CREB immunolabeling in NAc tissue compared to eGFP groups, although the extent of this effect did not reach significance (Supplement: Table S6). AAV-mCREB infusion resulted in 53–57% induction, while AAV-CREB infusion produced 22–33% enhancement of regional CREB labeling. Chronic quinpirole treatment consistently, but non-significantly, elevated phosphoCREB immunolabeling in NAc tissue compared to blank

pellet implantation by 46, 51 and 57% in AAV-eGFP-, AAV-mCREB-eGFP- and AAV-CREB-eGFP-treated groups, respectively.

Immunohistochemical data indicate that chronic quinpirole treatment altered CREB phosphorylation in the NAc, but not in the caudatoputamen (Fig. 8). PhosphoCREB labeling increased significantly ($p \leq 0.05$) after treatment with chronic quinpirole in AAV-eGFP-, AAV-mCREB-eGFP- and AAV-CREB-eGFP-treated groups by 65, 58 and 94%, respectively in the NAc core, and by 28, 32 and 47%, respectively in the NAc shell.

Discussion

Recovery of sensorimotor gating deficits by chronic D₂-like receptor agonist treatment

Acute administration of selective D₂-like receptor agonists reduces PPI, an indication of sensorimotor gating deficits (6,9,22). Here we show that two such compounds, the D₂/D₃-selective agonists quinpirole and ropinirole, significantly reduce PPI in drug-naïve rats. Both compounds produced equipotent PPI disruption at a dose of 0.05 mg/kg. Quinpirole produced dose-dependent PPI disruption, while the magnitude of ropinirole-induced PPI disruption did not increase further except after a very high dose (1.0 mg/kg). Ropinirole obtained from tablets also was shown to reduce PPI, however this effect was not dose-dependent (6). Thus, ropinirole may have a moderate effect on PPI across a broader range of doses.

In contrast to acute administration, repeated ropinirole treatment reverses sensorimotor gating deficits, as observed previously following repeated quinpirole treatment (9). After 28 days of treatment, ropinirole-mediated PPI disruption significantly recovered at all doses (0.05 – 0.5 mg/kg). The recovery of sensorimotor gating in response to repeated exposure to a D₂-like receptor agonist suggests that tolerance occurred. In fact, repeated exposure to a non-selective dopamine agonist, apomorphine, or the indirect dopamine agonists, amphetamine (23) or cocaine (7) also demonstrates attenuation of the disruptive effects upon repeated treatment. Our results using selective direct agonists support the hypothesis that such behavioral tolerance is due to a selective action on D₂-like receptors or their signaling cascades.

These studies also reveal that both intermittent daily treatment (Fig. 2) and continuous subcutaneous treatment (Fig. 7) with a D₂-like agonist produced PPI recovery. Therefore, this is likely a compensatory response to steady state drug exposure achieved with both treatment paradigms.

Mean acoustic startle response to pulse only trials was not altered by repeated ropinirole treatment, however there was a significant increase in all groups over time. This may be due to daily handling and injections, as it also was observed in saline vehicle-treated rats. Although increased startle response to pulse only trials may alter the calculated percent PPI, the magnitude of pulse response increased even more after repeated saline than ropinirole treatment. Thus, the effect of repeated ropinirole treatment on PPI recovery is likely due to altered processing of PPI in forebrain circuits.

CRE activation in the NAc is necessary for PPI recovery

Previously, we and others demonstrated that in contrast to its acute effects, chronic quinpirole treatment increased phosphoCREB expression in the NAc (12,13). The present results reveal that repeated ropinirole treatment (0.1 and 0.5 mg/kg/day) increased phosphoCREB labeling in the NAc by 80–90% (Fig. 3). CREB is an important transcription factor associated with memory and learning, as well as with behavioral plasticity after long-term drug administration (24). For example, chronic cocaine upregulates NAc dynorphin

expression via indirect stimulation of dopamine D₁-like receptors, enhanced cAMP signaling and CREB phosphorylation (25). Increased CREB phosphorylation observed in the NAc following repeated ropinirole administration may regulate gene expression, which could be responsible for the induction and maintenance of PPI recovery.

In order to determine whether sensorimotor gating adaptation depends on increased CREB phosphorylation in the NAc, we used adeno-associated virus-mediated gene transfer to induce prolonged overexpression of mCREB in the NAc. We show here that mCREB overexpression during chronic treatment blocked PPI recovery in response to quinpirole challenge, even though chronic treatment increased neuronal labeling of phosphoCREB in the NAc but not in the caudatoputamen. By contrast, chronic quinpirole treatment induced complete PPI recovery after eGFP or CREB overexpression, with similar selective regional increases of phosphoCREB in the NAc. mCREB dimerizes with endogenous phosphoCREB and, although resulting dimers can bind to cAMP response elements, they are functionally inactive. mCREB thereby acts as a dominant negative inhibitor of CREB-mediated transcription (26). Thus, chronic quinpirole treatment induces selective NAc CREB phosphorylation which appears to be necessary for PPI recovery.

Viral-mediated mCREB overexpression in the NAc is known to enhance the rewarding effects of cocaine and intracranial self-stimulation, and to produce antidepressant-like effects (26–28). These behavioral effects generally coincide with altered dynorphin expression in the NAc, implicating NAc neurons expressing predominantly D₁-like receptors. In contrast, PPI is regulated by D₂-like receptors in the NAc (29), and PPI recovery is produced by chronic treatment with selective D₂/D₃ receptor agonists, suggesting that NAc neurons expressing predominantly D₂-like receptors are preferentially involved.

It should be noted that while mCREB overexpression blocked PPI recovery in response to quinpirole challenge, PPI response to continuous subcutaneous quinpirole treatment was attenuated over time (Table 1). The lower dose of quinpirole released by subcutaneous pellets was sufficient to cause initial PPI disruption which was reduced over time. Nevertheless, the larger challenge dose still produced disruption suggesting that PPI recovery was not achieved.

Long-lasting effects of repeated D₂-like agonist treatment

Our results reveal that recovery of sensorimotor gating is present for at least 28 days after the termination of repeated ropinirole treatment in the presence or absence of weekly challenges which produced no PPI deficit. This suggests that PPI recovery is long-lasting and independent of weekly “priming” injections. Furthermore, NAc phosphoCREB labeling is elevated immediately after termination of repeated treatment, but not 28 days later even though PPI recovery remains. Thus, NAc phosphoCREB is necessary to induce PPI recovery, but it need not be present to maintain PPI recovery. This suggests that activation of CREB might induce transcriptional regulation of NAc protein expression whose effect on PPI is then sustained. We showed previously that D₂-like receptor-G protein function is unaffected by repeated agonist treatment, but cAMP-dependent protein kinase activity increased (9,13). Other proteins might be altered in such a way as to offset D₂-like receptor action. Alternatively, transcriptional regulation might induce plasticity of NAc neuronal connections or function with long-term effects. While it might seem paradoxical that repeated treatment with a D₂-like receptor agonist can reverse sensorimotor gating deficits, the resulting CREB activation in critical brain circuits, as is present following antipsychotic treatments (30) which also reverse PPI disruption (31), may underlie this persistent neuroadaptive behavioral response.

In humans, some direct dopamine agonists, such as apomorphine, induce intolerable nausea (32). By contrast, ropinirole is better tolerated and has clinical efficacy for other dopaminergic disorders (14). Our data reveal that ropinirole has a greater effective dose range than quinpirole and that repeated ropinirole treatment leads to PPI recovery which is long-lasting even without further treatment. Both drugs appear to utilize the same mechanism of selective regional CREB phosphorylation in NAc neurons to induce PPI recovery after chronic steady-state pharmacotherapy. A prolonged release formulation of ropinirole that provides steady-state pharmacokinetics in a single daily dose (33) is now available (34,35). Our results suggest that this prolonged release formulation of ropinirole might be efficacious for treating sensorimotor gating deficits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. PPI disruption by acute quinpirole or ropinirole

Animals were challenged with quinpirole (0.0, n=36; 0.05, n=12; 0.1, n=12; or 0.3 mg/kg, n=12) or ropinirole (0.0, n=36; 0.05, n=6; 0.1, n=10; 0.5, n=9; or 1.0 mg/kg, n=10) 10 min prior to PPI testing. Percent PPI (mean ± SEM) are mean values collapsed across three prepulse intensities. There was a significant effect of quinpirole dose ($F_{11,204} = 4.399$, $p \leq 0.001$) and ropinirole dose ($F_{14,297} = 2.383$, $p \leq 0.005$) using one-way ANOVA. * - $p \leq 0.05$ compared to 0.0 mg/kg dose; † - $p \leq 0.05$ compared to 0.05 mg/kg dose.

Figure 2. Recovery of PPI after 28 days of repeated ropinirole treatment

Animals were treated daily for 28 consecutive days with ropinirole (0.0, n=10; 0.05, n=11; 0.1, n=11; or 0.5 mg/kg, n=10). Following baseline testing, PPI was assessed 10 min after drug treatment on days 1 and 28. Percent PPI (mean $\pm$ SEM) are mean values collapsed across three prepulse intensities. There was no significant effect over time of saline vehicle ($F_{8,81} = 1.056$, $p > 0.05$), however, there was a significant effect of ropinirole treatment (0.05 mg/kg: $F_{8,90} = 6.163$, $p \leq 0.005$; 0.1 mg/kg: $F_{8,90} = 6.787$, $p \leq 0.005$; 0.5 mg/kg: $F_{8,90} = 5.220$, $p \leq 0.05$) using repeated measures ANOVA. * - $p \leq 0.05$ compared to baseline PPI within treatment group; † - $p \leq 0.05$ compared to day 1 PPI within treatment group.

Figure 3. CREB phosphorylation increased after repeated ropinirole treatment in the NAc, but not in the caudatoputamen (CP)

Immunohistochemistry was performed on tissue obtained from rats treated for 28 days with ropinirole (0.0, n=10; 0.05, n=11; 0.1, n=11; or 0.5 mg/kg, n=10). Data are expressed as number of nuclear profiles (mean $\pm$ SEM) per mm². There was a significant effect in the NAc core ($F_{3,38} = 4.561$, $p \leq 0.05$) and shell ($F_{3,38} = 3.653$, $p \leq 0.05$), but not in the caudatoputamen (DL CP: $F_{3,38} = 1.837$, $p > 0.05$; Med CP: $F_{3,38} = 0.727$, $p > 0.05$) using repeated measures ANOVA. * - $p \leq 0.05$ compared to 0.0 mg/kg dose within brain region.

Figure 4. Ropinirole challenge 28 days after repeated ropinirole treatment does not disrupt PPI
 Rats were treated daily for 28 consecutive days with ropinirole (0.0, n=7 or 0.1 mg/kg, n=7). Thereafter, PPI testing was accomplished 28 days after termination of daily treatment following challenge with the same dose received previously. Percent PPI (mean $\pm$ SEM) are mean values collapsed across three prepulse intensities. There was a significant effect on Day 1 ($F_{1,40} = 4.442$, $p \leq 0.05$), but not on any other day (Baseline: $F_{1,40} = 0.002$, $p > 0.05$; Day 28: $F_{1,40} = 0.002$, $p > 0.05$; Day 28 after treatment: $F_{1,40} = 0.268$, $p > 0.05$) using repeated measures ANOVA. There was no significant effect over time of saline ($F_{3,200} = 2.207$, $p > 0.05$) or ropinirole treatment ($F_{3,200} = 1.896$, $p > 0.05$). * - $p \leq 0.05$ compared to 0.0 mg/kg dose.

Figure 5. CREB phosphorylation returned to basal levels 28 days after the termination of repeated ropinirole treatment

Immunohistochemistry was performed on tissue obtained from rats following a ropinirole challenge 29 days after termination of daily treatment. Rats that previously received vehicle treatment received either 0.0 or 0.1 mg/kg of ropinirole, while rats that previously received 0.1 mg/kg ropinirole received the same challenge dose ($n=9$ in each group). Data are expressed as number of nuclear profiles (mean $\pm$ SEM) per mm^2 . There was no significant effect in any brain region (NAc Core: $F_{2,24} = 1.548$, $p > 0.05$; NAc Shell: $F_{2,24} = 1.150$, $p > 0.05$; DL CP: $F_{2,24} = 0.704$, $p > 0.05$; Med CP: $F_{2,24} = 1.006$, $p > 0.05$) using repeated measures ANOVA.

Figure 6. CREB phosphorylation increased in the NAc immediately following repeated ropinirole, but not four weeks after termination of treatment
Image of phosphoCREB labeling in the NAc core from (A) saline-, (B) acute ropinirole-, or (C) repeated ropinirole-treated rats, or (D) repeated ropinirole-treated rats 29 days after termination of treatment. Anterior commissure is shown at lower left in each illustration. Scale bar: 100 μ m.

Figure 7. mCREB prevents PPI recovery induced by chronic quinpirole treatment

PPI was determined before and 24 hr after intracranial infusion of adeno-associated virus for gene transfer of eGFP alone, mCREB-eGFP (mCREB) or CREB-eGFP (CREB). Rats were challenged with quinpirole (0.0 or 0.1 mg/kg) 3 weeks later. Pellets were then implanted which released quinpirole (0.0 or 0.1 mg/kg/day, n=9 per virus group). PPI was tested 1 and 28 days after pellet implantation, then following quinpirole challenge on day 29 (0.0 or 0.1 mg/kg). Percent PPI (mean $\pm$ SEM) are mean values collapsed across three prepulse intensities. There was no significant effect in the control group ($F_{8,207} = 1.669$, $p > 0.05$), however there was a significant effect of quinpirole treatment (GFP/quinpirole: $F_{8,207} = 3.211$, $p < 0.005$; mCREB/quinpirole: $F_{8,207} = 5.337$, $p < 0.005$; CREB/quinpirole: $F_{8,207} = 4.894$, $p < 0.005$) using repeated measures ANOVA. * - $p \leq 0.05$ compared to post-virus surgery within treatment group; † - $p \leq 0.05$ compared to challenge 1 within treatment group.

Figure 8. CREB phosphorylation increased after chronic quinpirole treatment in the NAc, but not in the caudatoputamen (CP)

Immunohistochemistry was performed on tissue obtained from rats treated for 28 days with blank or quinpirole pellets (0.0 or 0.1 mg/kg/day, n=9 per virus group) after intracranial infusion of adeno-associated virus for gene transfer of eGFP alone, mCREB-eGFP (mCREB) or CREB-eGFP (CREB). Data are expressed as number of nuclear profiles (mean $\pm$ SEM) per mm². There was a significant effect of quinpirole treatment in the NAc core ($F_{5,22} = 15.83$, $p \leq 0.01$) and shell ($F_{5,22} = 7.116$, $p \leq 0.01$), but not in the CP (DL CP: $F_{5,22} = 0.9656$, $p > 0.05$; Med CP: $F_{5,22} = 0.39$, $p > 0.05$) using repeated measures ANOVA. * - $p \leq 0.05$ compared to blank pellet in the same viral treatment group.

Table 1

Effect of viral overexpression and quinpirole treatment on percent prepulse inhibition

	Post-surgery baseline	Pellet Day 1	Pellet Day 28
eGFP/blank pellet	42.9 ± 3.8	45.7 ± 5.3	55.7 ± 3.9
mCREB/blank pellet	41.5 ± 3.5	46.9 ± 3.6	53.6 ± 4.1
CREB/blank pellet	47.3 ± 4.6	47.7 ± 4.1	50.5 ± 4.2
eGFP/Quinpirole pellet	47.3 ± 4	31.8 ± 4.5*	50.9 ± 5.2
mCREB/Quinpirole pellet	44.4 ± 3.7	26.3 ± 5.4*	52.6 ± 3.4
CREB/Quinpirole pellet	40.0 ± 4.1	21.6 ± 5.6*	50.4 ± 5.5

Data are expressed in Newtons as mean ± SEM. There was no significant effect over time of blank pellets, however, quinpirole-containing pellets reduced PPI in all groups on implantation day 1, which normalized by day 28 (eGFP/blank pellet: $F_{2,70} = 2.314$, $p > 0.05$; mCREB/blank pellet: $F_{2,72} = 2.522$, $p > 0.05$; CREB/blank pellet: $F_{2,71} = 0.1678$, $p > 0.05$; eGFP/Quinpirole pellet: $F_{2,78} = 4.856$, $p \leq 0.01$; mCREB/Quinpirole pellet: $F_{2,75} = 10.29$, $p \leq 0.01$; CREB/Quinpirole pellet: $F_{2,71} = 8.096$, $p \leq 0.01$) using repeated measures ANOVA.

* $p \leq 0.01$ compared to baseline PPI.