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Research article

Neurobehavioral correlates of dopamine agonist-induced eye-blinking in the marmoset monkey

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ABSTRACT

Eye-blinking has been used to catalog dopaminergic receptor subtype activation in several mammalian species. In this study, the dissimilar effects of directly-acting D_1 and D_2 agonists and an indirectly-acting non-selective agonist (SKF-82958, quinelorane, cocaine, respectively) on eye-blinking were confirmed in marmosets. Subsequently, functional magnetic resonance imaging (fMRI) was used to examine their effects on functional connectivity (FC) between the dopamine-rich putamen and other brain regions. Results indicate that SKF-82958 produced dose-dependent increases in blinking, with the highest dose (0.3 mg/kg) yielding > 9-fold increases over baseline values. In contrast, the highest dose of quinelorane (0.001 mg/kg) reduced blink rates to ~30 % of baseline. Following the highest dose of cocaine (5.6 mg/kg), only limited (~20 %) and short-lived (~20-min) decreases in eye-blinking were observed. In fMRI studies, cocaine induced transient FC increases between putamen and striatal regions, whereas the D_1 and D_2 agonists induced distinct temporal dynamics and region-specific changes in putamen FC. SKF-82958 strengthened putamen FC with motor and sensory regions and reduced FC with visual and cerebellar regions. Conversely, quinelorane reduced putamen connectivity with motor and sensory areas and strengthened FC with regions associated with visual and emotional regulation. These effects in marmosets align with previous outcomes and show that dopamine receptor-subtype activation produce distinct patterns of FC between the putamen and brain regions that play key integrative roles in eye-blinking and other behavior. These findings support eye-blinking as a non-invasive cross-species indicator of dopaminergic subtype activation that can be used to enhance our understanding of dopamine-related dysfunction in neuropsychiatric disorders.

1. Introduction

Numerous studies, dating from Carlsson's [1] early work with L-DOPA in reserpinized subjects, have linked ocular movement and spontaneous blinking with dopaminergic actions. It is now widely accepted that eye-blink rates serve as a non-invasive marker of dopamine-related abnormalities in certain neurological disorders and, as well, of treatment response [2]. For example, patients with Parkinson's disease, characterized by dopamine depletion, exhibit reduced blinking [3,4], whereas individuals with Tourette's syndrome [5,6] or Huntington's disease [7,8], associated with heightened dopaminergic activity, often demonstrate elevated blinking rates. Eye blinking also has been used as a marker of dopamine function in schizophrenia [9,10] and, though less extensively, other psychiatric disorders including depression [11,12], attention-deficit hyperactivity disorder [13], panic disorder [14], and anorexia nervosa [15]. Taken together, these clinical observations among heterogeneous conditions promote eye blinking as a useful indicator of dopamine function.

Studies in laboratory animals have provided further support for the idea that spontaneous eye-blinking can serve as an index of dopamine function. For example, eyeblink rates in nonhuman primates can be significantly decreased by treatment with MPTP, with the degree of effect directly related to the degree of ensuing Parkinsonism and dopamine depletion in the caudate nucleus [16,17]. Additionally, rates of spontaneous eye-blinking in nonhuman primates can be predictably and reliably altered by dopamine receptor agonists and antagonists. Importantly, previous findings show that dopamine receptor-subtype (i.e., D_1-family and D_2-family [18]) ligands dissimilarly regulate eye-blinking behavior [19–21]. Thus, D_1, but not D_2, agonists can produce dose-related increases in blink rates, an effect that can be blocked by D_1

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antagonists or D_2 agonists. Of interest, indirect dopamine agonists do not readily increase blink rate, perhaps reflecting the combined stimulation of both D_1 and D_2 receptors by increased levels of synaptic dopamine. While this research has identified the differing roles of dopamine receptor subtypes in the regulation of eye-blinking behavior, the neural circuitry that may underlie these differing dopaminergic actions has not yet been clarified.

The use of marmoset monkeys (*Callithrix jacchus*) in neuropsychiatric and pharmacological studies offer significant advantages for investigating dopaminergic function. These include anatomical and functional similarities with humans, particularly in cortical and behavioral features [22–26] as well as similarities between marmoset and human ocular structures and visual systems [27,28]. Marmosets have also been extensively utilized for neuroimaging studies [29–32], allowing researchers to assess the brain's responses to pharmacological manipulations. The present studies were designed to systematically replicate and extend the work of Kotani et al. [33], who showed that dopamine D_1 receptor agonists increased blink counts in marmosets, whereas D_2 receptor agonists decreased them. Additionally, neuroimaging was utilized in the present study to identify drug-induced changes in putamen functional connectivity (FC), providing a means for relating drug-induced changes in neural responses and corresponding alterations in eye-blinking behavior. The putamen represents a region of interest due to its high density of both D_1- and D_2-like dopamine receptors [34,35] and its established role in the regulation of repetitive motor behaviors, including eye blinking, which may serve as an accessible behavioral marker of dopaminergic function in both healthy individuals and those with neurological disorders [9]. Consistent with this, converging evidence highlights the putamen as a key striatal site involved in the dopaminergic modulation and control of blinking behavior. Functional MRI studies in individuals with Tourette syndrome, for example, have shown that greater activation of the right putamen, along with prefrontal regions, is associated with better inhibitory control of semi-involuntary movements such as eye blinks, underscoring the contribution of fronto-striatal circuits to motor suppression [36]. Pharmacological and PET studies further link spontaneous blink rate to striatal dopamine receptor function: reduced blink rate in cocaine users reflects dopaminergic hypoactivity [37], while methylphenidate-induced changes in blink rate correlate with both D_1 and D_2 receptor availability in the putamen and caudate [38]. Together, these findings emphasize that dopaminergic signaling within the putamen is central to regulating spontaneous and controlled blinking behaviors.

2. Methods

2.1. Animals

Three adult male common marmosets (*Callithrix jacchus*) were individually housed in a temperature- and humidity-controlled environment with a 12-hour light/dark cycle (lights on at 7 am). Subjects had ad libitum access to water in their home cage and were maintained at approximate free-feeding weights with nutritionally balanced portions of LabDiet® New World Primate Diet and ZuPreem® Soft Marmoset Diet. Fresh fruit, mealworms, and environmental enrichment were provided daily. All subjects had a previous experimental history engaging in touchscreen-based cognitive tasks [39] but were drug-naïve at the beginning of the present studies. The experimental protocol for this study was approved by the Institutional Animal Care and Use Committee at McLean Hospital. Subjects were maintained in a facility licensed by the U.S. Department of Agriculture and in accordance with guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources [40].

2.2. Blink count measurement

Over the course of several weeks, subjects were acclimated to resting comfortably on their haunches and in a prone position within a custom-designed (L: 27 cm, W: 12 cm, H: 12 cm) 3D printed (ABS plastic) chair and helmet, reduced in size for marmoset body types but modeled after MRI-compatible restraint devices designed for neuroimaging in squirrel monkeys (see [41] for additional details and schematics). The helmet was mounted to the chair body with plastic screws and was lined with padding to limit motion and optimize comfort. Blink counts were monitored using a high-definition digital video camera (Canon VIXIA HF R80) placed approximately 1 m from the apparatus. Session recordings started immediately following vehicle or drug administration (see 2.4) and continued throughout the 60-minute observational session. Blinks were defined as a rapid, complete closure and reopening of the eyelids, and were manually counted by a trained observer reviewing the video footage and blinded to treatment condition. To quantify baseline blink count for comparison with blink counts following drug administration, blink counts were averaged across four 60-minute observational sessions following treatment with vehicle (saline). A within-subject design was used such that each subject received vehicle and three doses of all three drugs in a mixed order, with at least a one-week washout period between drug administration to prevent carryover effects. Approximately 4 weeks after all dose-response determinations were completed, the dose of each drug that produced peak effects on blink counts was studied further during 30-minute neuroimaging sessions using the following protocol.

2.3. Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) scans were acquired using a 9.4 Tesla horizontal bore magnet system (Varian Direct Drive, Varian Inc, Palo Alto, CA, USA). An 11.6 cm inner diameter gradient (Resonance Research Institute, Billerica, MA) was used with maximum gradient strength of 40 G/cm; a custom-made surface coil was used for data capture. On scan days, subjects were transported in a covered cart from the vivarium to an animal preparation room within the scanner suite. After approximately 30-minutes of acclimation, subjects were sedated with ketamine (10 mg/kg, intramuscular) for anesthesia induction, intubated, and then maintained on 1–1.2 % isoflurane gas throughout the scanning procedures. A circulating warm-water blanket and fleece wrap were used to maintain body temperature. Monkeys were scanned in the prone position in an MR-compatible monkey cradle. Vital signs including heart rate, respiration rate, body temperature, and oxygen saturation (SPO2) were monitored and maintained throughout the procedure by trained technical staff.

2.4. MRI data acquisition

All MRI data were acquired at a 9.4 T/400 horizontal bore MR system (Agilent Technologies, Santa Clara, CA, USA) running Vnmrj software (Version 3.2 A). A 116 mm inner diameter gradient insert was used with maximum gradient strength of 460 mT/m (Resonance Research, Billerica, MA). A custom-made surface coil designed for marmoset brain scans was used. The image protocol included one anatomic scan (multi-slice fast spin echo) with the following parameters: TR = 4009.28 ms, TE = 20 ms, fast spin echo factor = 8, 40 axial slices, slice thickness = 1 mm, in-plane acquisition matrix = 64 × 64, FOV = 48 mm, flip angle = 90°, 4 averages, acquisition time = 4 min 25 s, and one resting state fMRI scan (gradient-echo EPI): TR = 1500 ms, TE = 12 ms, 54 contiguous axial slices, slice thickness = 0.75 mm, in-plane acquisition matrix = 64 × 64, FOV = 48 mm, flip angle = 60°, 1600 volumes, acquisition time = 40 min 6 s. Image preprocessing was conducted using the FMRIB Software Library (FSL, Centre for Functional MRI of the Brain, University of Oxford, UK). During preprocessing, the orientation and field of view were adjusted to align with the reference T1-weighted (T1w)

image. The first five volumes were discarded to allow for magnetization equilibrium. Head motion correction was performed using MCFLIRT, followed by slice timing correction. The data were then spatially smoothed with a Gaussian kernel of 2 mm full width at half maximum (FWHM) and temporally filtered with a high-pass filter of 100 s. Registration to the Paxinos marmoset brain atlas was performed using the JIP toolbox (Joe's Image Program, Massachusetts General Hospital, Harvard University, MA, USA). A region-of-interest (ROI) seed (6 mm^3) was manually defined and drawn on the reference image at the level of the bilateral caudal portion of the putamen (see Fig. 2). Prior fMRI studies in humans have demonstrated functional organization of the putamen with caudal regions more closely associated with higher-order motor control [42,43].

2.5. Drugs

The dopamine D_1 full agonist SKF-82958 hydrobromide was purchased from MedChemExpress (Monmouth Junction, NJ), the D_2 full agonist quinelorane hydrochloride was purchased from Tocris Bioscience (Bristol, UK), and the indirect non-selective monoaminergic agonist cocaine hydrochloride was purchased from Sigma Pharmaceuticals (St. Louis, MO). All drugs were dissolved in 0.9 % saline solution and administered in volumes of 0.3 mL or less via intramuscular injection. Drug doses (0.03, 0.1, 0.3 mg/kg SKF-82958; 0.0001, 0.00032, 0.001 mg/kg quinelorane; 1, 3.2, 5 mg/kg cocaine) are expressed in terms of their free base weight.

2.6. Statistical analysis

Blink counts were summed across twelve 5-minute bins to examine the time course of effect and expressed graphically as group mean (±SEM). Blink counts were analyzed statistically using repeated measures ANOVA to assess the effect of each drug and dose during different time intervals using a Greenhouse-Geisser correction. Post-hoc comparisons using Dunnett's test were conducted to determine statistical significance in differences between drug-treated and control blink counts. Statistical analyses were performed using GraphPad Prism 10 (Boston, MA). Statistical analysis of neuroimaging data was conducted within the FMRIB Software Library as follows. A total of 1600 images were divided into four 10-minute time bins, each containing 400 images. The first time bin represented the pre-infusion period, while the subsequent three time bins (Post1 [0–10 min], Post2 [10–20 min], and Post3 [20–30 min]) corresponded to post-infusion periods and allowed for an assessment of changes in brain connectivity over time as a result of drug treatment. FC with the putamen during each of the post-infusion time bins was compared to connectivity during the pre-infusion time bin at the group level using paired *t*-tests. Significance was set at $p < 0.05$ for behavioral and neuroimaging analyses.

3. Results

3.1. Blinking

The effects of treatment with saline and doses of SKF-82958, quinelorane, and cocaine on blinking are presented in Fig. 1. An average of 52.9 (±13.2) blinks were counted in the first 5 min after saline administration, after which the value decreased and remained constant at approximately 25 blinks in each time bin throughout the remainder of the session. As shown in Fig. 1a, the D_1-selective agonist, SKF-82958, produced dose-dependent increases in blink counts across the 60-minute observational period. The highest dose of 0.3 mg/kg resulted in the greatest increase, with counts peaking sharply to > 300 blinks in the first 5-minute bin and gradually declining thereafter. The 3-fold lower dose (0.1 mg/kg) had lesser effects but still increased blink counts to approximately 230 blinks in the first 5-minute bin, whereas the lowest dose of SKF-82958 (0.03 mg/kg), while least effective, still nearly

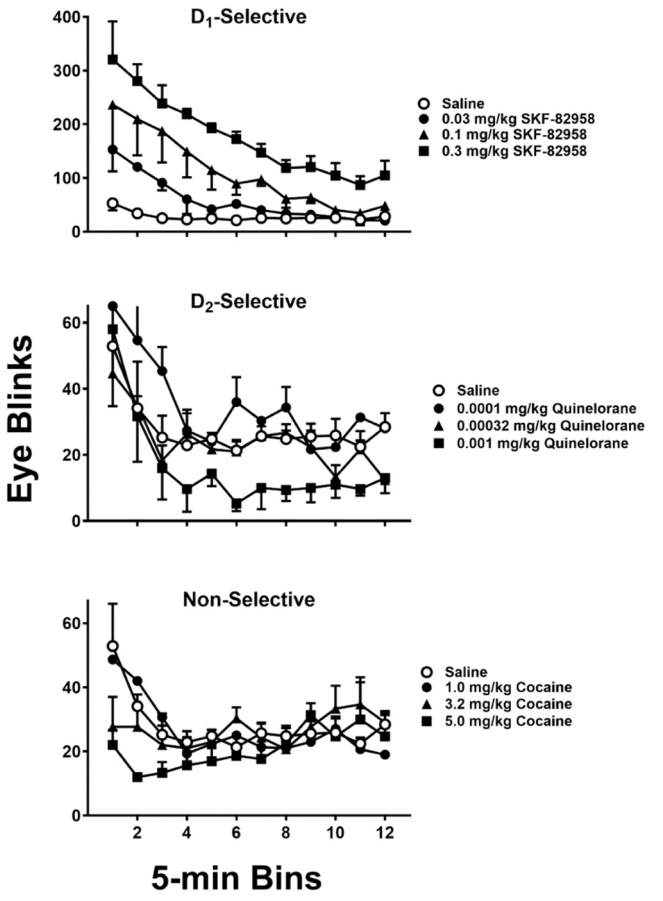

Fig. 1. Effects of SKF-82958 (a), quinelorane (b), and cocaine (c) on eye blink counts in marmosets. Data represent mean (±SEM) eye blinks across 5-minute bins over the course of the 60-minute observational session following administration of saline or one of three doses of each drug. Note differences in y-axis scale for SKF-82958 compared to quinelorane and cocaine.

tripled blink counts over baseline levels. The effects of SKF-82958 were relatively short-lived, and only the highest dose maintained elevated blinking above baseline values throughout the session (approximately 100 blinks in the last bin). Statistical analysis confirmed dose-dependency in the effects of SKF-82958, revealing a significant main effect of dose ($F(3,8) = 17.25$; $p < 0.001$) and time ($F(1.72,13.77) = 18.87$; $p < 0.001$). A significant dose-by-time interaction was also observed ($F(33,88) = 2.20$; $p < 0.01$) further supporting the temporal dynamics of the dose-dependent increases.

As presented in Fig. 1b, the highest dose (0.001 mg/kg) of the D_2-selective agonist, quinelorane, gradually decreased blink counts in the first third of the test session, with values dropping to 9.67 (±6.89) in the 15–20-minute bin and remaining constant throughout the rest of the session. The intermediate dose of quinelorane (0.00032 mg/kg) produced a milder and less consistent decrease in blink counts whereas the lowest dose (0.0001 mg/kg) was without effect, compared to saline control. Statistical analysis confirmed these observed reductions, revealing a significant main effect of dose ($F(3,8) = 4.60$; $p < 0.05$) and time ($F(1.60,12.79) = 7.94$; $p < 0.01$). No significant dose-by-time interaction was observed ($p = 0.98$), indicating that the pattern of decrease did not vary substantially across time.

Fig. 1c shows blink counts following cocaine administration which, unlike SKF-82958 and quinelorane, did not have consistent effects on blinking. A clear dose-related decrease in blink counts was observed within the first 20 min of the session. Blink counts following the lowest dose of 1.0 mg/kg (140.7 [±7.6]) approximated baseline levels (135.1 [±7.5]) during the first 20 min, whereas the intermediate (3.2 mg/kg)

and high dose (5 mg/kg) lowered blink counts to 98.3 (±3.3) and 63.0 (±2.4) blinks during the first 20 min, respectively. However, this effect did not persist throughout the session, and no systematic effects of cocaine were evident beyond the initial 20-minute period. Statistical analysis confirmed no significant main effect of dose ($F(3,8) = 1.26$; $p = 0.35$) or time ($F(2.51,20.04) = 2.71$; $p = 0.08$), and no dose-by-time interaction ($p = 0.17$).

3.2. Putamen functional connectivity

Whole brain differences in putamen FC over time following administration of SKF-82958, quinelorane, and cocaine are shown in Fig. 2 and detailed in Supplemental Table 1. Following the administration of SKF-82958, stronger FC with putamen was observed in the first time bin, primarily within local striatal regions, including the left putamen, as well as in regions associated with interoceptive processes (dysgranular insula) and sensory processing (auditory cortex). Conversely, weaker connectivity with putamen was found in the globus pallidus, which regulates voluntary movement, the amygdala, involved in emotional regulation, and the claustrum, which integrates motor, sensory, and limbic information (p's < 0.05). In the second time bin, putamen FC remained stronger with regions associated with stimulant-like effects such as primary motor cortex, caudate, and retrospinal cortex/hippocampus. Weaker FC was found between putamen and areas associated with processing and integrating visual information such as medial and lateral intraparietal areas and V3, as well as with the hippocampal/amygdala/entorhinal complex and cerebellum. Unexpectedly, the third time bin revealed widespread changes in putamen FC predominantly reflecting increases relative to the pre-infusion period. Primary motor cortex connectivity with putamen remained stronger from the second time bin through the third. Other areas including thalamus (anterior portion), medial superior temporal regions, parietal area PFG and PG, temporo-parietal-occipital area and primary visual cortex, and caudate (anterior and dorsal portions) also showed stronger connectivity with putamen compared to the pre-infusion period.

Quinelorane administration produced rapid and widespread reductions in putamen FC. Regions with weaker FC to putamen were those associated with reward function, such as striatal areas, accumbens, and septum, as well as several regions involved in sensory and visual information processing (prostriate area, primary visual cortex, temporal areas V3 and V4). Stronger connectivity with putamen was found in cerebellum, brainstem, superior colliculus, and subregions of V3 and V4. Interestingly, many of the initial decreases either dissipated or, in several regions, changed direction during the second time bin. For example, stronger FC of putamen was found with somatosensory areas, primary visual cortex, superior colliculus, V4, primary motor cortex, caudate (dorsal), and parietal area PFG. Additionally, piriform cortex, amygdala, hippocampal formation, temporal areas TE1, TE2, and TE3 showed stronger functional connectivity with putamen. Decreased connectivity with putamen was primarily in cerebellum and several visual regions (see Supplemental Table 1). The changes in putamen FC during the second time bin were largely preserved in the third time bin, with increased connectivity between putamen and temporal regions, piriform cortex, striatal regions, and primary motor and visual cortices and decreased connectivity of putamen with cerebellum and visual regions.

Changes in putamen FC following cocaine administration were time-dependent and primarily involved increased connectivity between the putamen and reward-related brain regions. For instance, during the first time bin after cocaine administration, putamen FC increased with both cortical and subcortical regions, including the right primary motor cortex, putamen, caudate, and amygdala. FC with putamen was also increased with cortical regions associated with processing various aspects of rewarding stimuli, such as areas 30, 35, 36, 23, and 24, as well as the entorhinal cortex. Conversely, a small cluster showing decreased putamen FC was observed in area 3b and the left primary motor cortex; all p's < 0.05. These changes were transient, however, as putamen FC was not different from the pre-infusion period during the second nor third post-infusion time bin.

4. Discussion

The present findings align closely with previous research on dopaminergic modulation of eye-blinking in rodents, marmosets, and other nonhuman primate species [19–21,33,44,45]. Data from the present and these earlier studies consistently show that selective D_1 receptor activation leads to dose-related increases in blink rates whereas selective D_2 receptor activation results in decreased blink rates. These data further support the utility of eye-blinking as a cross-species marker for D_1 and D_2 receptor activity in relevant brain regions. Thus, the dose-dependent increase in blinking produced by D_1 agonists (e.g., SKF-82958 in the present study) and, conversely, dose-dependent reductions in blinking rates produced by D_2 receptor agonists (e.g., quinelorane in the present study) are consistent with excitatory and inhibitory roles, respectively, ascribed to D_1 and D_2 receptors. The indirect and non-selective monoaminergic agonist cocaine showed only a transient and moderate decrease in this study, perhaps due to its simultaneous activation of both D_1 and D_2 receptors, yielding a less pronounced change in blinking behavior. As cocaine is a non-selective monoaminergic drug, it is possible that its indirect serotonergic and noradrenergic actions also may have played a role in its effects on blinking. However, there is currently no evidence with directly-acting serotonergic or noradrenergic ligands to support this possibility.

Importantly, these results in marmosets, demonstrating dissociable effects of D_1- and D_2-selective agonists on both eye-blinking behavior and putamen FC, complement and extend previous findings in humans. Using PET, Demiral et al. [38] showed that blink rate correlates with D_1 and D_2 receptor availability in the striatum, including the putamen, particularly following dopaminergic stimulation with methylphenidate. Consistent with this, the present results reveal that direct activation of D_1 receptors (SKF-82958) markedly increased blinking and strengthened putamen connectivity with motor and sensory regions, whereas D_2 receptor activation (quinelorane) decreased blinking and produced an opposite connectivity pattern. Together, these findings underscore that dopaminergic modulation of eye-blinking involves receptor subtype-specific mechanisms within the putamen that influence both molecular signaling and large-scale network dynamics.

Building on this, the present findings highlight distinct temporal dynamics and region-specific differences associated with D_1- (SKF-82958) and D_2- (quinelorane) receptor activation in the putamen. These differences may underlie variations in blinking, particularly through mechanisms involving motor control, sensory processing, and visual integration. For instance, SKF-82958 appears to facilitate activation of motor and sensory processing areas while reducing visual and cerebellar connectivity with putamen, yielding increased motor drive and reduced visual integration (i.e., responsivity to stimuli) that may mediate heightened blinking. In contrast, quinelorane appears to initially reduce sensory and motor connectivity of the putamen but, over time, gradually strengthens connectivity with visual and emotional regulation connectivity. This may lead to a more modulated pattern of blinking characterized by delayed motor activation. It should be noted that blinks were not recorded during the fMRI sessions, and future studies will be needed to directly examine the relationship between putamen FC and blinking behavior. Regardless, these findings demonstrate, for the first time, that selective dopamine agonists induce bi-directional changes in putamen FC within brain regions previously implicated in blinking behavior.

The study of spontaneous eye-blinking behavior and brain FC has potential implications for understanding and characterizing neuropsychiatric disorders associated with dopaminergic dysfunction [2]. Elevated eye-blinking, as observed here with D_1 activation, may serve as a behavioral indicator of conditions like schizophrenia, where dopamine dysregulation often results in motor hyperactivity [9,10]. Conversely,

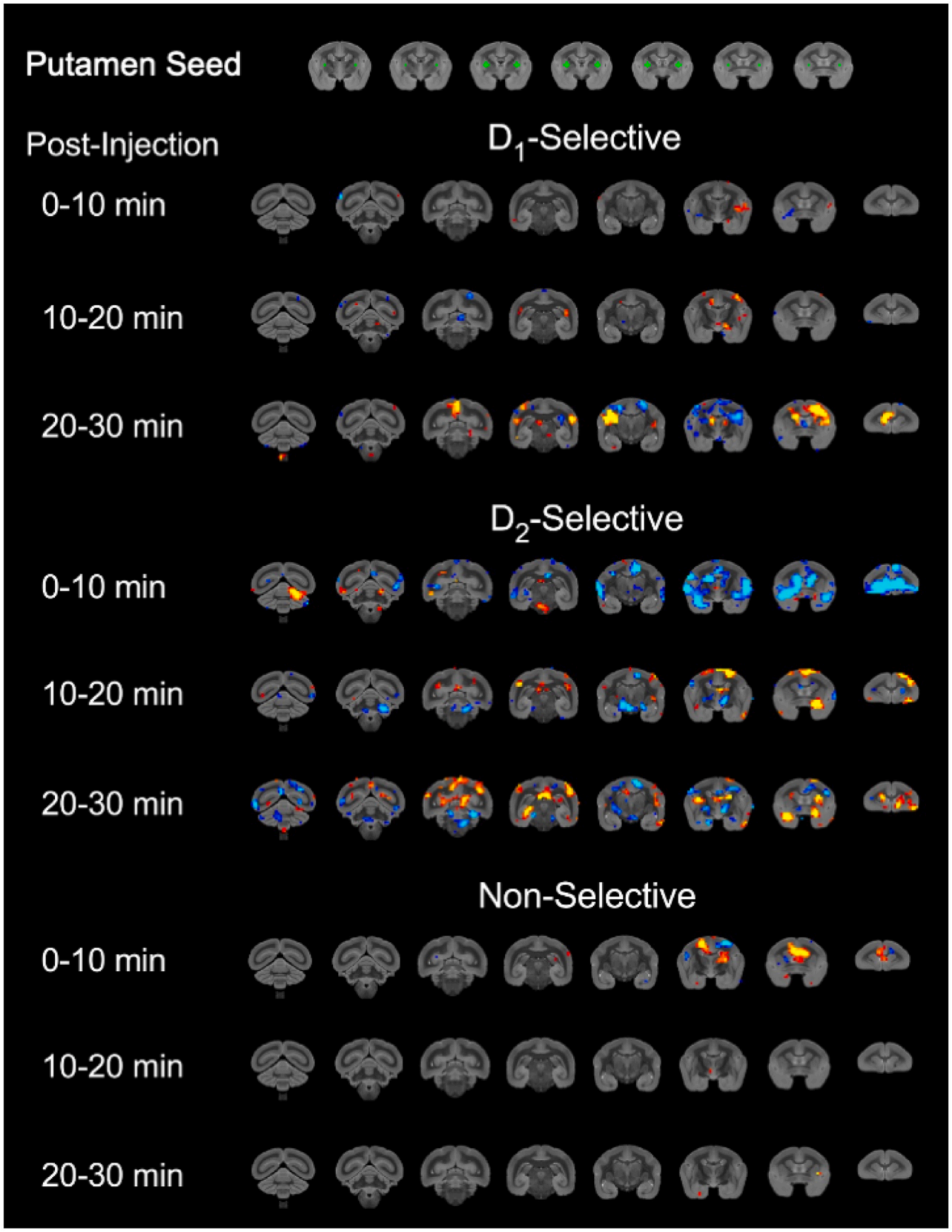

Fig. 2. Top row shows the location of the ROI seed (in green) used for analysis. Effects of SKF-82958 (D_1-Selective), quinelorane (D_2-Selective), and cocaine (Non-Selective) on putamen FC in marmosets. Drug post-injection period > pre-drug baseline is red/yellow; post-injection period < pre-drug baseline is shown in blue. Additional details in Supplemental Table 1 and in text.

the reduction in blinking, as observed here with D_2 activation, resembles deficits in Parkinson's disease, where dopamine depletion and the reduction in dopamine receptor stimulation leads to suppressed motor behaviors, including reduced blink frequency [3,46]. Beyond motor disorders, our findings also may be relevant to the role of dopamine subtypes in affective disorders like depression. Although depression is generally linked to reduced dopamine activity, compensatory mechanisms such as upregulation of postsynaptic dopamine receptors or reduced dopamine transporter density might lead to increased blink rates, as seen with elevated D_1 activity [47]. The link between blink modulation and dopaminergic pathways could also provide a behavioral marker for assessing changes in dopamine function in response to therapeutic interventions.

This study has several limitations that should be addressed in future research. First, our analytic focus on the putamen was guided by prior literature identifying this structure as a key brain region in the dopaminergic modulation of blinking. However, other regions with a high density of dopamine receptors, such as the thalamus and cortical areas involved in striatal-cortical regulation of blinking behavior, should also be examined in future work to provide a more comprehensive, whole-brain characterization of dopaminergic network dynamics underlying this behavior. Second, only adult male marmosets were studied, potentially limiting the generalizability of our findings. While neither age nor sex differences in the dopaminergic regulation of blinking have been documented in marmosets, previous studies in other species suggest there may be age-dependent [36,48] and sex-related [49] variation in dopamine receptor density and sensitivity that might be expressed in dopamine-related behavioral endpoints. Third, the use of manually counted blink rates may introduce observer variability, although the blinding of treatment conditions helps mitigate this bias. Future studies might capitalize on automated eye-tracking apparatus and recent advances in machine learning for observational studies of drug action in laboratory animals [50] which could allow for a richer characterization of blink architecture. Finally, another limitation of the present work is that neuroimaging was conducted in anesthetized subjects only at the peak dose for each agonist, which restricts our understanding of potential dose-response relationships in the effects of dopamine agonists on FC in awake marmosets. While peak dose and anesthetized imaging were selected for feasibility, a more comprehensive examination of dose-dependent neural activation in awake subjects would allow for exploring the direct relationship between blinking and its neural correlates without potential anesthetic-related confounds and, in turn, reveal additional insights into how varying levels of dopaminergic stimulation influence the putamen and other motor regions. Despite these limitations, the current study provides a framework for exploring the broader neuropharmacological networks underlying blinking behavior and, more generally, a behavioral marker of subtype selective dopamine receptor activation.

CRediT authorship contribution statement

AbdulRahman Abbas: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Oanh T. Luc:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Lei Cao:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Kenroy Cayetano:** Validation, Methodology, Conceptualization. **Jack Bergman:** Writing – review & editing, Methodology, Conceptualization. **Stephen J. Kohut:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Kangas Brian D:** Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

Over the past 3 years, BDK has received sponsored research agreements from BlackThorn Therapeutics, Compass Pathways, Delix Therapeutics, Engrail Therapeutics, Neurocrine Biosciences, and Takeda Pharmaceuticals. No funding from these entities was used to support the current work. All other authors have no conflicts of interest or relevant disclosures.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2025.115939.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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