Dissociating the reinforcing and sensory properties of medial accumbens shell dopamine.

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Abstract: Electrical stimulation of the medial forebrain bundle vigorously reinforces self-stimulation behaviour, yet rodents perform operant responses to terminate this stimulation. Whether distinct neural substrates underpin the reinforcing and sensory properties of electrical brain stimulation is a question that has hampered the development of efficacious treatment for psychopathology. The accumbens shell emerged as a substrate subserving the reinforcing and therapeutic properties of electrical medial forebrain bundle stimulation. Here, we examine whether there are dissociable reinforcing and sensory properties of medial accumbens shell dopamine and probe the substrates underlying these properties. Using a novel self-stimulation procedure, transgenic DAT-Cre mice expressing channelrhodopsin-II in ventral tegmental area dopamine neurons were trained to hold-down a lever to engage, and then release the lever to disengage, optogenetic stimulation of dopaminergic inputs to the medial accumbens shell through an implanted optic fiber. The cumulative and mean duration of hold-downs show divergent frequency responses identifying dissociable reinforcing and sensory properties of dopamine's actions in the medial accumbens shell. At higher stimulation frequencies the cumulative duration of hold-downs grows, whereas the mean duration of hold-downs wanes. Dopamine agonists reduced the cumulative duration of self-stimulation hold-downs, but only a D1 agonist produced this reduction through decreases in the mean duration of hold-downs, which were lengthened with a D2 antagonist. Intense dopamine activity within the medial accumbens shell may achieve negative valence, disengaging behaviour through augmented neurotransmission at D1 receptors which proposes an adjuvant pharmacotherapy to enhance the treatment of binge eating and alcohol use disorder with electrical accumbens stimulation.

Key words: Dopamine, Ventral Striatum, Addiction, Optogenetics, Valence, Reward

1. Introduction.

Soon after it was discovered that electrical stimulation of the medial forebrain bundle vigorously reinforced self-stimulation behaviour in rodents [1] was it realized that electrical brain stimulation could treat psychopathology [2]. The insight that electrical brain stimulation engaged psychological processes was fodder for speculation about its sensory properties: Did this stimulation substitute for natural goals [3], positive memories [4], or utility [5] – generate hunger [6], reward or drive [7]? A parallel, search for the neural substrates recruited by electrical stimulation of the medial forebrain bundle spurred [8], ever complicated by the swath of cells and transgressing fibers characterizing this large bundle [9,10]. An imprecise, but indelible, link between substrate and psychology was established.

A description of the neural substrates underlying the reinforcing and therapeutic properties of electrical brain stimulation focused on an area of the ventral striatum called the nucleus accumbens. Diverse patterns of electrical medial forebrain bundle stimulation that supported self-stimulation similarly activated ventral tegmental area dopamine neurons [11] and downstream release of dopamine in the accumbens, but not dorsal striatum [12,13]. Further, electrical stimulation of the accumbens, but not dorsal striatum, reduced pathological feeding and drug seeking [14,15]. Considerable clinical and preclinical evidence identifies electrical stimulation of the accumbens as a promising treatment for alcohol use [16,17] and binge eating disorder [18,19]. Thus, the reinforcing and therapeutic actions of electrical brain stimulation are, at least partially, subserved by the accumbens.

Electrical stimulation indiscriminately recruits substrates near the electrode, permitting speculation about separate substrates subserving distinct consequences. It was suggested that electrical stimulation of the medial forebrain bundle activates substrates subserving positive, negative, and mixed sensory properties [20] because rodents will press a lever to terminate this stimulation [21,22]. A separation between the stimulation parameters that modulate latencies to engage, and disengage, electrical stimulation of the medial forebrain bundle suggests that separate neural substrates underpin disparate sensory properties of this stimulation [23,24]. The recruitment of dissociable neural substrates reconciles the seemingly incompatible actions of electrical stimulation to vigorously reinforce self-stimulation behaviour while also mitigating pathological food and alcohol consumption.

Optogenetic stimulation of ventral tegmental area dopamine neurons robustly support self-stimulation, particularly at high stimulation frequencies, and early reports suggested that these neurons underpinned the reinforcing properties of electrical medial forebrain bundle stimulation [25–31]. Intriguingly, when mice could choose between operant responses that delivered brief or prolonged high frequency optogenetic stimulation of ventral tegmental area dopamine neurons, brief bursts of stimulation were preferred [32]. The preferential activation of ventral tegmental area dopamine neurons for brief durations is akin to behavioural responses that terminate ongoing electrical stimulation of the medial forebrain bundle. Differently, however, the substrate serving to engage and disengage optogenetic self-stimulation of ventral tegmental area dopamine neurons is unequivocal.

Within the accumbens, the medial shell subregion critically underpins the reinforcing properties of medial forebrain bundle stimulation. Optogenetic stimulation of dopaminergic inputs to [33], or cells within [34,35], the medial accumbens shell support self-stimulation and elevating dopamine in the medial accumbens shell amplifies the reinforcing efficacy of medial forebrain bundle stimulation [36]. Further, dopamine levels in the accumbens shell closely, but imperfectly, track stimulation parameters that maximally reinforce behaviour [37]. Thus, the reinforcing properties of medial forebrain bundle stimulation are partially attributable to elevations in accumbens shell dopamine which occurs through transsynaptic activation of ventral tegmental area dopamine neurons [28,38,39].

Selective recruitment of genetically defined and input-specific substrates could clarify speculation about separable or overlapping substrates subserving the sensory and reinforcing properties of electrical brain stimulation. Further, controlling an unambiguous substrate that supports self-stimulation could elucidate the therapeutic mechanisms of accumbal electrical brain stimulation. To this end, we developed a novel temporally constrained self-stimulation procedure wherein mice hold-down a lever to engage, and then release the lever to disengage, optogenetic stimulation

of dopaminergic inputs to the medial accumbens shell. By varying the frequency with which dopamine inputs to the medial accumbens shell are stimulated and modulating neurotransmission at dopamine type-1 (D1) and type-2 (D2) receptors with pharmacology, we begin to dissociate and identify substrates that subserve the reinforcing, sensory, and therapeutic properties of electrical brain stimulation.

Here, we provide evidence that the positive and negative sensory properties of electrical medial forebrain bundle stimulation arise through dopamine's actions at D1 and D2 receptors in the medial accumbens shell. The reinforcing properties of medial accumbens shell dopamine, inferred from cumulative time spent holding down the active lever, appear more strongly influenced by neurotransmission at D2 receptors. Differently, the sensory properties of medial accumbens shell dopamine, inferred from the mean duration of individual hold-downs, appear more strongly influenced by neurotransmission at D1 receptors. We argue that accumbens shell dopamine disengages self-stimulation behaviour through amplified neurotransmission at D1 receptors, which may underpin the capacity of electrical accumbens stimulation to treat alcohol use [16,17], and binge eating disorder [18,19].

2. Materials and methods.

2.1. Subjects.

Subjects were twelve experimentally naïve DAT-Cre (7F, 5M) C57BL/6 mice (25-30 g, 8 weeks old; bred in-house) that were single or pair-housed in standard shoe-box cages (31 cm x 16.5 cm x 12 cm) under a reversed 12-hour light/dark cycle (Lights ON 9 AM, OFF 9 PM) with unrestricted access to standard chow and water. All procedures were conducted in the dark phase and in accordance with the Canadian Council on Animal Care and the McGill Animal Care Committee.

2.2. Surgery.

DAT-Cre transgenic mice were anesthetized with ketamine (Ventoquinol, 100mg/kg) and xylazine (Bayer, 10mg/kg) before being secured in a stereotaxic apparatus. The viral construct AAV5-DIO-CaMKIIa-hChR2(H134R)-eYFP (Neurophotonics, 5.0×10^{12} GC/ml) was microinfused using a Nanoject II injector and glass micropipette (Drummond Scientific, 3-000-203-G/X, 10 μm tip) in the ventral tegmental area (10° angle, AP 1.14 mm, ML ±0.35 mm, DV -3.75 to -2.75 mm; 5 min infusion, 10 min diffusion). In the same surgery optic fibers (200 μm) were implanted bilaterally in the NAc (10° angle, AP 1.5 mm, ML ±1.4 mm, DV -4.32 mm). Mice were left to recover for 2-3 months in their home-cage allowing sufficient viral expression in dopaminergic terminals.

2.3. Behavioral apparatus.

Behavioral training occurred in six conditioning chambers (ENV-307W-CT; Med-Associates Inc.), enclosed in sound-attenuating, fan-ventilated (ENV-025F) melamine cubicles (42.5 x 62.5 x 42 cm). Each chamber had a stainless-steel bar floor, paneled aluminum sidewalls, and a clear polycarbonate rear wall, ceiling, and front door. The upper left wall of the chamber featured a central white house-light (ENV-215M). The right wall contained two retractable levers (ENV-312-3) located on each side of a central fluid-port (ENV-303LP2-3). All experimental events were controlled and recorded using Med PC-V software.

2.4. Temporally constrained self-stimulation.

Mice received 40 self-stimulation training sessions (36 min) consisting of trials (30s; 48 trials/session) initiated and terminated with the extension and retraction, respectively, of active and inactive levers. In each trial, the active lever was pseudorandomly assigned to deliver pulsed laser stimulation (5 ms; 473 nm; 10 mW) at 2.5, 10, or 40 Hz such that one third of all trials represented each frequency. Trials occurred around a variable time inter-trial interval that was drawn from an exponential distribution from 2-18 s with a mean duration of 10 s. When mice performed hold-downs by depressing the active lever, pulsed laser-light was delivered through an implanted optic fiber to the medial accumbens shell, which continued so long as the lever remained depressed and terminated when the lever was released. Inactive lever hold-downs had no consequence.

After the 40 training sessions, the contributions of neurotransmission at D1 and D2 receptors to self-stimulation of medial accumbal dopamine inputs was tested. Prior to each test session, which were otherwise similar to training sessions, mice received an intraperitoneal injection of Raclopride (D2 antagonist, 0.03 mg/kg), Quinpirole (D2 agonist, 0.125 mg/kg), SCH23390 (D1 antagonist, 0.02 mg/kg), SKF38393 (D1 agonist, 0.25 mg/kg) or vehicle (saline). There were

three sessions for each treatment (15 total) that occurred according to a Latin-square design. Retraining sessions without injections intervened each test session to mitigate potential treatment carryover effects.

2.5. Histology.

At the end of the experiment, mice were deeply anesthetized with an injection of sodium pentobarbital (Euthanyl™, 270 mg/kg) and perfused with a 4% paraformaldehyde 0.1 M phosphate-buffered saline solution. Brains were extracted from the skull and cryoprotected in a 4% paraformaldehyde 30% sucrose solution for 1-2 days before being frozen at -80°C. Brain tissue was sliced into 50 μm sections on a Lecia™ cryostat, thaw-mounted on microscope slides, and cover-slipped with a MOWIOL + DAPI solution. Tissue was then imaged using a Leica™ epifluorescent microscope to verify viral expression in the ventral tegmental area and locate tracts from optical fiber implants in the medial accumbens shell.

2.6. Data and Statistical Analysis.

MED-PC V software controlled and recorded the timing of all experimental events including the initiation and termination of lever hold-downs. The cumulative and mean hold-down durations, as well as the number of hold-downs, per trial, were principal variables of interest. Data were analyzed using repeated measures analysis of variance (RMANOVA) in Prism™ v9.5.1 or SPSS v27. Conditioning sessions were blocked into groups of five (training) or three (test) sessions to ensure that enough hold-downs occurred to adequately calculate mean active and inactive hold-down duration. All analyses were conducted on raw data, although difference scores are sometimes shown to highlight significant interactions. Two mice did not perform a hold-down at one set of frequency-treatment conditions during the test phase and their data was excluded from test analyses. Where post-hoc t-tests were used to decompose significant interactions, the reported p-

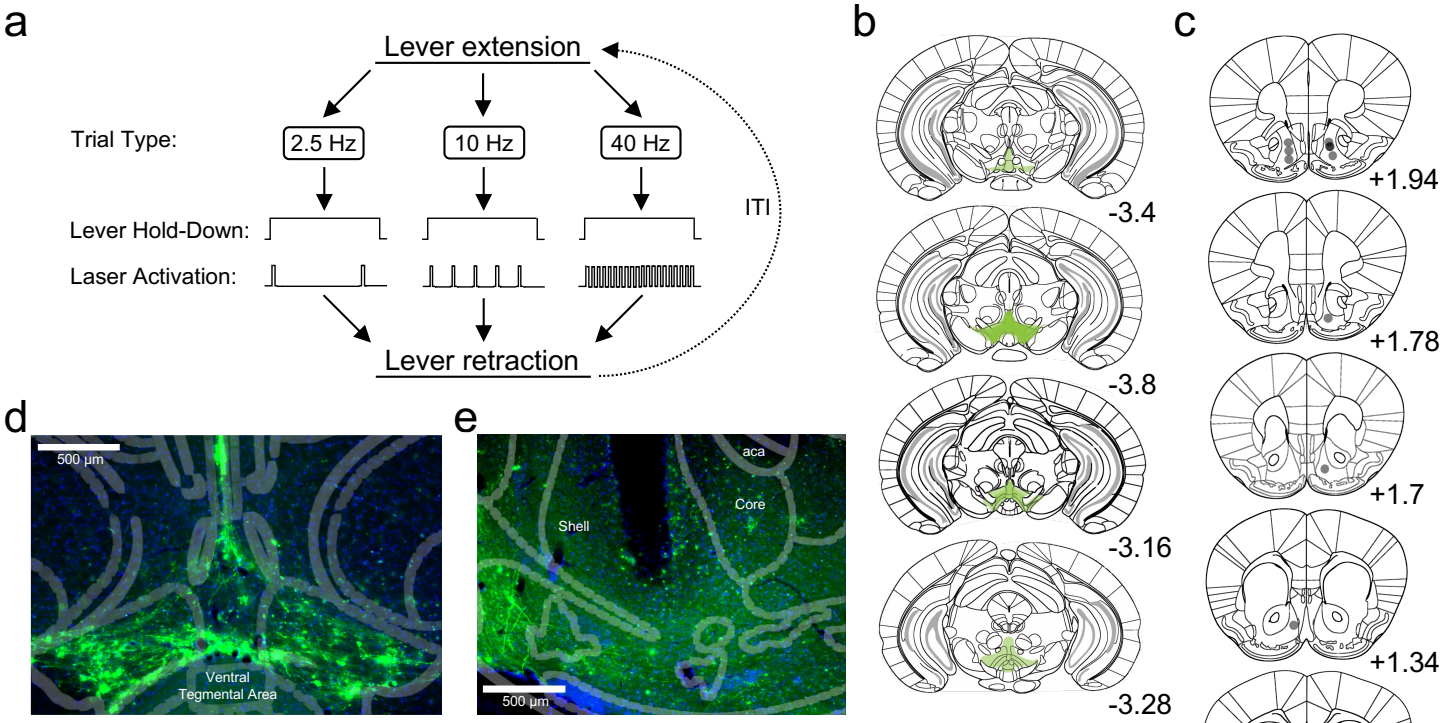

Fig. 1 | Temporally constrained self-stimulation of dopaminergic inputs from the ventral tegmental area to the medial accumbens shell. a, Active and inactive levers were inserted into the conditioning chamber for 30 s trials (48 trials/session) which were separated by intertrial intervals (ITI; ~10 s). Active lever hold-downs triggered the delivery of patterned laser light (473 nm) to the medial accumbens shell at 2.5, 10, or 40 Hz which terminated when the lever was released. **b**, DAT-Cre mice (n=12, 7F, 5M) received microinfusion of the viral construct AAV2/9-EF1a-fDIO-hChR2(H134R)-eYFP in the ventral tegmental area and **c**, implantation of an optical fibre in the medial accumbens shell which are shown on modified panels from the atlas of Franklin and Watson (2007). Representative microscopy (5x) images with overlaid anatomical delineations showing viral expression (green) and nuclei (DAPI, blue) in the **d**, ventral tegmental area and **e**, medial accumbens shell.

values are Bonferroni-corrected and multiplicity-adjusted [40]. All analyses used α=.05.

3. Results.

3.1. *Acquisition of temporally constrained self-stimulation of dopaminergic inputs to the medial accumbens shell.*

DAT-Cre mice were trained (**Fig 1a**) to hold-down an active lever to optogenetically stimulate ventral tegmental area dopaminergic inputs to the medial accumbens shell (**Fig 1b-e**) during 30 s trials. Depression of the active lever engaged optogenetic stimulation until the lever was released; inactive lever hold-downs had no consequence. Across training blocks of five sessions [Session, $F_{(7, 77)}$=14.392, p<.001], mice increased the cumulative time spent holding down the active, but not inactive lever [Lever x Session, $F_{(7, 77)}$=14.332, p<.001; Lever, $F_{(1, 11)}$=34.470, p<.001], particularly so for higher frequency trials [Lever x Frequency x Session, $F_{(14, 154)}$=2.323, p=.006; Frequency x Session, $F_{(14, 154)}$=1.011, p=.445; Lever x Frequency, $F_{(2, 22)}$=4.470, p=.023; Frequency, $F_{(2, 22)}$=6.042, p=.008; **Fig 2a**]. A selective, and frequency-dependent increase in the cumulative time spent holding down the active lever is consistent with dopamine in the medial accumbens shell reinforcing self-stimulation behaviour, however this increase was not reflected in the mean duration of hold-downs. While mice learned to lengthen the mean duration of active, but not inactive, lever hold-downs across sessions [Lever x Session, $F_{(7, 77)}$=4.509, p<.001; Lever, $F_{(1, 11)}$=37.598, p<.001; Session, $F_{(7, 77)}$=4.125, p=.001] the trajectory of this lengthening was not frequency-dependent [Lever x Frequency x Session, $F_{(14, 154)}$=.301, p=.993; Frequency x Session, $F_{(14, 154)}$=.671, p=.8; Lever x Frequency, $F_{(2, 22)}$=2.390, p=.115; Frequency, $F_{(2, 22)}$=1.802, p=.188; **Fig 2b**]. Consistently, an increase in the cumulative, but not mean, duration of active lever hold-downs was accompanied by a selective increase in the number of active lever hold-downs across sessions [Lever x Session, $F_{(7, 77)}$=8.541, p<.001; Lever, $F_{(1, 11)}$=20.890, p=.001; Session, $F_{(7, 77)}$=8.126, p<.001] which was pronounced during higher frequency trials [Lever x Frequency x Session, $F_{(14, 154)}$=2.679, p=.002; Frequency x Session, $F_{(14, 154)}$=1.088, p=.373; Lever x Frequency, $F_{(2, 22)}$=5.376, p=.013; Frequency, $F_{(2, 22)}$=3.450, p=.05; **Fig 2c**]. During training, the cumulative duration and number of hold-downs were averaged across trials to allow comparison against an intuitive total trial duration of 30 s, however, the frequency-dependent increase in these measures that emerged for the active lever across training was also captured in the total number of hold-downs performed [Lever x Frequency x Session, $F_{(14, 154)}$=1.971, p=.023; Frequency x Session, F(14, 154)=1.818, p=.04; Lever x

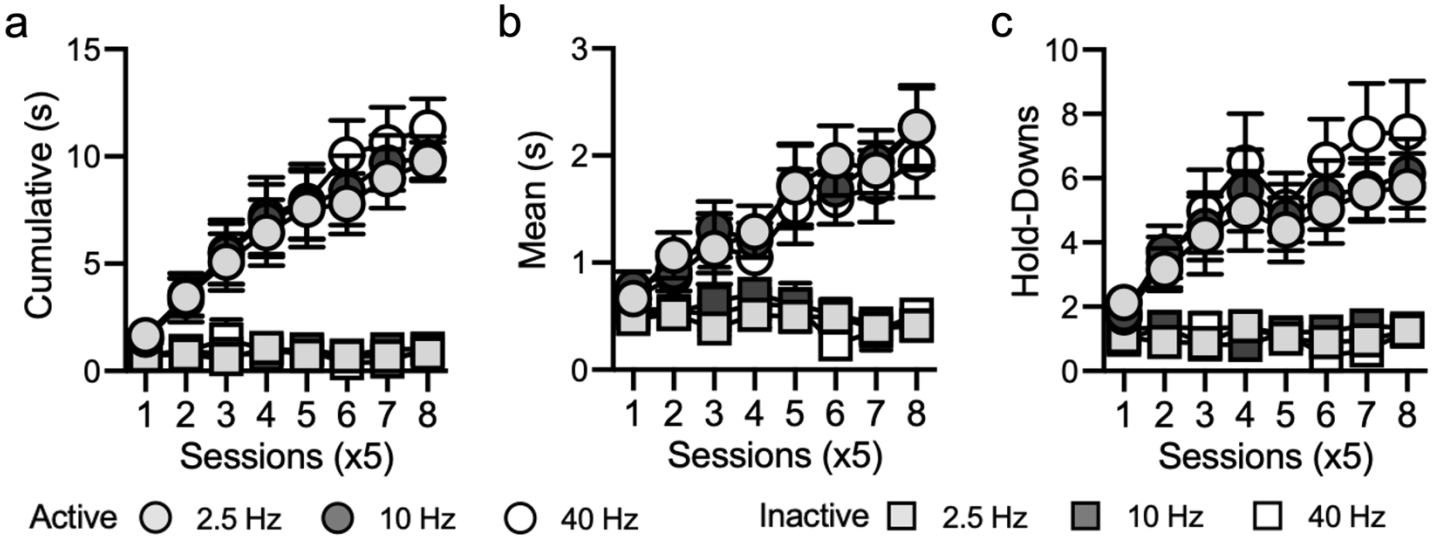

Fig. 2 | Acquisition of temporally-constrained self-stimulation of dopaminergic inputs to the medial accumbens shell is frequency-dependent. a, Across blocks of 5 sessions, mice increased the cumulative time spent holding down the active lever during 30 s trials whereas the cumulative time spent holding down the inactive lever remained low. Higher-frequency trials came to support more cumulative active hold-down time than did lower-frequency trials [Lever x Frequency x Session, $F_{(14, 154)}$=2.323, p=.006]. **b**, The mean duration of active, but not inactive, lever hold-downs increased over sessions [Lever x Session, $F_{(7, 77)}$=4.509, p<.001]. **c**, Mice increased the number of active, but not inactive, lever hold-downs performed during 30 s trials across sessions particularly so for high-frequency relative to low-frequency trials [Lever x Frequency x Session, $F_{(14, 154)}$=2.679, p=.002]. Averaged data are mean ± s.e.m.

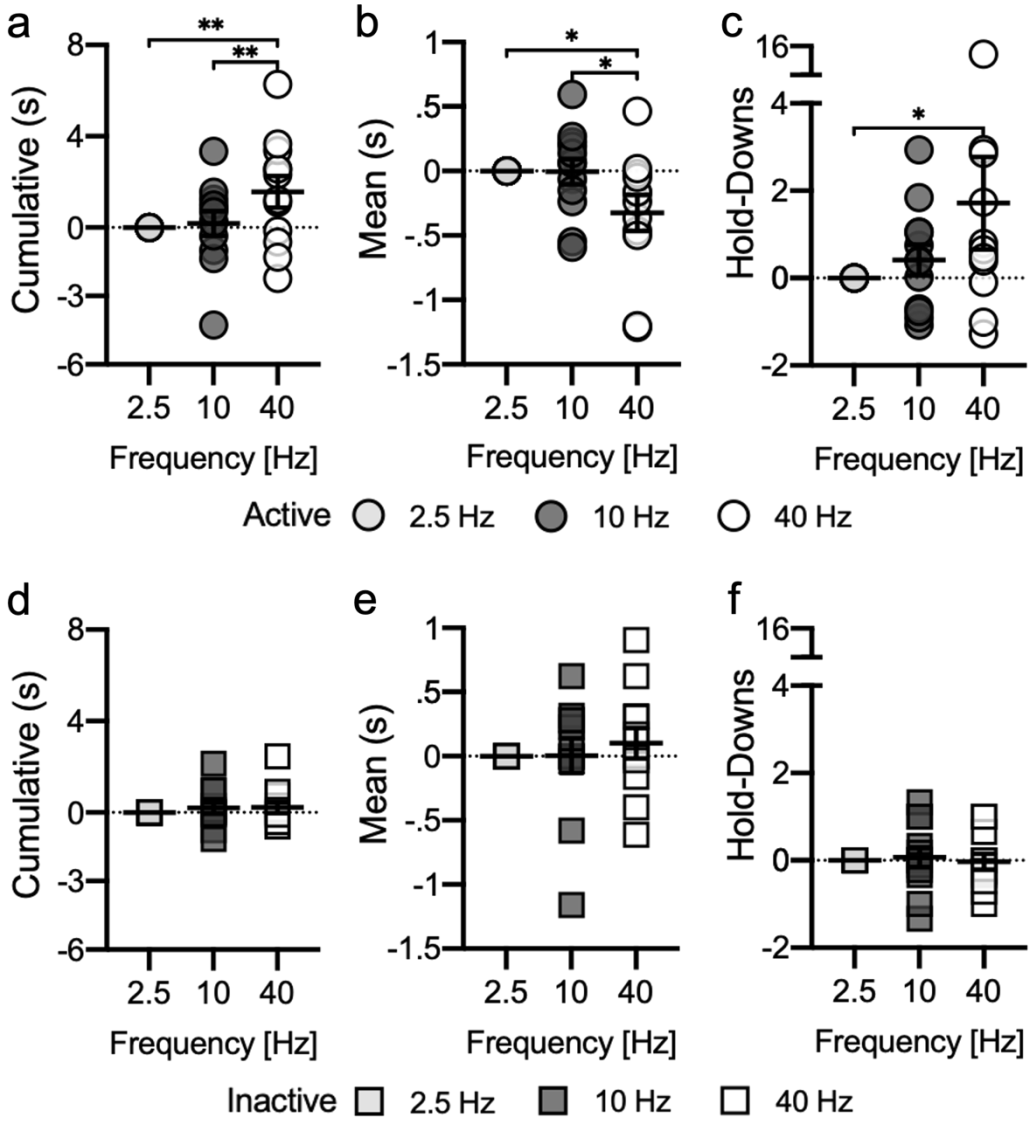

Fig. 3 | Cumulative and mean duration of self-stimulation hold-downs show divergent frequency responses at the end of training. a, Mice spent significantly more cumulative time holding-down the active lever during 40 Hz trials than during 10 [$t_{(11)}$=3.367, p=.0083] or 2.5 [$t_{(11)}$=3.79, p=.003] Hz trials [Lever x Frequency, $F_{(2, 22)}$=3.594, p=.044]. **b**, Differently, the mean duration of individual hold-downs, were briefer at 40 Hz than at 10 [$t_{(11)}$=3.022, p=.0188] or 2.5 [$t_{(11)}$=3.061, p=.0172] Hz [Lever x Frequency, $F_{(2, 22)}$=5.3, p=.0132]. **c**, Consistently, mice performed more hold downs during 40 Hz trials than during 2.5 Hz trials [$t_{(11)}$=2.993, p=.020; Lever x Frequency, $F_{(2, 22)}$=2.607, p=.0963]. The **d**, cumulative **e**, mean and **f**, number of inactive lever hold-downs did not vary across stimulation frequencies. Data are shown as within-subjects differences from the lowest stimulation frequency (2.5 Hz). Averaged data are mean ± s.e.m. Symbols represent data from individual mice. Asterisks indicate Bonferroni-corrected and multiplicity-adjusted post-hoc comparisons p<.01**, p<.05*.

Session, $F_{(7, 77)}$=8.349, p<.001; Lever x Frequency, $F_{(2, 22)}$=3.805, p=.038; Session, $F_{(7, 77)}$=8.317, p<.001; Frequency, $F_{(2, 22)}$=3.736, p=.04; Lever, $F_{(1, 11)}$=14.280, p=.003; **Fig S1a**].

3.2. Cumulative and mean duration of self-stimulation hold-downs show divergent frequency responses.

During the last block of training sessions when self-stimulation behaviour had stabilized, the cumulative and mean duration of active lever hold-downs showed divergent trajectories across stimulation frequencies. The cumulative duration of active, relative to inactive, lever hold-downs increased [Lever x Frequency, $F_{(2, 22)}$=3.594, p=.044; Lever, $F_{(1, 22)}$=72.57, p<.0001; Frequency, $F_{(2, 22)}$=3.789, p=.0385; **Fig 3a**] whereas the mean duration of active, relative to inactive, lever hold-downs decreased across stimulation frequencies [Lever x Frequency, $F_{(2, 22)}$=5.3, p=.0132; Lever, $F_{(1, 22)}$=17.54, p=.0015; Frequency, $F_{(2, 22)}$=.9435, p=.045; **Fig 3b**]. Specifically, the cumulative active lever hold-down duration during 40 Hz trials was significantly longer than during 10 Hz [$t_{(11)}$=3.367, p=.0083] or 2.5 Hz [$t_{(11)}$=3.79, p=.003] trials; 2.5 and 10 Hz trials did not differ [$t_{(11)}$=.4339, p>.999]. The upward trajectory of cumulative active lever hold-down duration across stimulation frequencies is consistent with the reinforcing properties of medial accumbens shell dopamine. Differently, the mean duration of hold-downs when the active lever was armed with 40 Hz stimulation was briefer than when the active lever was armed with 10 Hz [$t_{(11)}$=3.022, p=.0188] or 2.5 Hz [$t_{(11)}$=3.061, p=.0172]; 2.5 and 10 Hz trials did not differ [$t_{(11)}$=.03845, p>.999]. The decrease in mean active lever hold-down duration at higher stimulation frequencies is consistent with the emergence of a negatively valenced sensory property of medial accumbens shell dopamine. Although the requisite interaction was not observed [Lever x Frequency, $F_{(2, 22)}$=2.607, p=.0963; Frequency, $F_{(2,}$

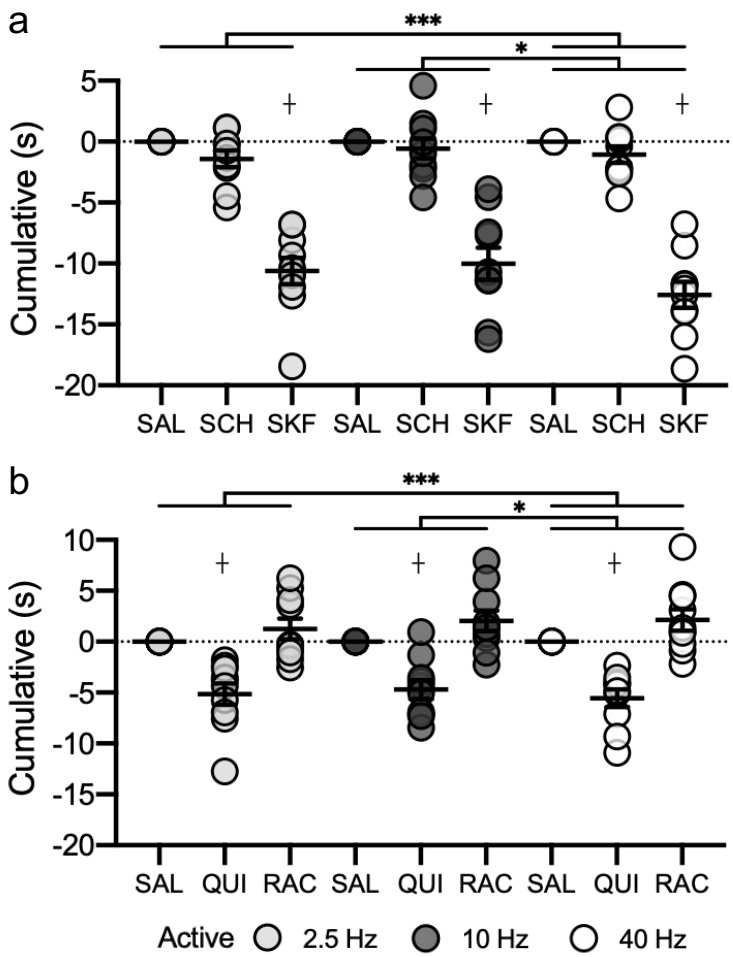

Fig. 4 | D1 and D2 receptor agonists reduce the cumulative duration of active lever hold-downs. Mice performed longer cumulative active lever hold-downs during 40 Hz than 10 Hz [$t_{(9)}$=3.313, p=.0116] or 2.5 Hz [$t_{(9)}$=4.543, p=.0008] trials [Frequency, $F_{(2, 18)}$=11.04, p=.0007]. Simple main effects reveal that the **a**, D1 agonist SKF38393 [SKF; $t_{(9)}$=12.94, p<.0001] and **b**, D2 agonist quinpirole [QUI; $t_{(9)}$=6.013, p<.00001] reduced the cumulative duration of hold-downs irrespective of stimulation frequency [Treatment, $F_{(72, 30)}$=73.3, p<.00001]. The D1 and D2 antagonists, SCH23390 [SCH; $t_{(9)}$=1.193, p>.9999] and Raclopride [RAC; $t_{(9)}$=2.118, p=.4118], did not affect cumulative hold-down duration. Data are shown as within-subjects differences from the Saline (SAL) treatment condition. Averaged data are mean ± s.e.m. Symbols represent data from individual mice. Asterisks indicate Bonferroni-corrected and multiplicity-adjusted post-hoc comparisons p<.001***, p<.05*. Crosses indicate similarly corrected simple effects of treatment relative to saline p<.0001┼.

$_{22)}$=1.928, p=.1692; **Fig 3c**] more active than inactive lever hold-downs were performed [Lever, $F_{(1, 22)}$=19.40, p<.0011] and a corrected post-hoc comparison revealed that more active lever hold-downs occurred during 40 Hz than 2.5 Hz trials [$t_{(11)}$=2.993, p=.0201]. Neither the cumulative duration [all $t_{(11)}$<.5358, p>.999; **Fig 3d**], mean duration [all $t_{(11)}$<.9620, p>.999; **Fig 3e**], or number [all $t_{(11)}$<.1787, p>.999; **Fig 3f**] of inactive lever hold-downs varied across stimulation frequencies.

During the first block of training sessions, the cumulative duration [Lever x Frequency, $F_{(2, 22)}$=.2794, p=.7589; Lever, $F_{(1, 22)}$=4.065, p<.0695; Frequency, $F_{(2, 22)}$=.1069, p=.8991], and mean duration [Lever x Frequency, $F_{(2, 22)}$=.3291, p=.7231; Lever, $F_{(1, 22)}$=2.442, p<.1464; Frequency, $F_{(2, 22)}$=.2892, p=.7517], of active and inactive lever hold-downs were similar to one another and across stimulation frequencies. The number of active and inactive lever hold-downs did not differ across frequencies [Lever x Frequency, $F_{(2, 22)}$=.9508, p=.4017; Frequency, $F_{(2, 22)}$=.7957, p=.4638] but more active than inactive lever hold-downs were performed [Lever, $F_{(1, 22)}$=7.124, p<.0218; **Fig S2a-f**]. The lack of stimulation frequency and lever effects at the beginning of training confirm that changes in the cumulative and mean duration of active lever hold-downs at the end of training are not inherent to the motoric aspects of holding down a lever.

3.3. Neurotransmission at D1 and D2 receptors determines the reinforcing and sensory properties of medial accumbens dopamine.

At test, the impact of modulating neurotransmission at dopamine receptors on temporally constrained self-stimulation of dopamine inputs to the medial accumbens shell was examined using pharmacology. The D1 agonist SKF38393 (.25 mg/kg) and D2 agonist Quinpirole (.125 mg/kg) were used to augment neurotransmission, whereas the D1 antagonist SCH23390 (.02 mg/kg) and the D2 antagonist Raclopride (.03 mg/kg) were used to blunt neurotransmission at dopamine receptors. Doses of dopamine receptor agonists and antagonists were chosen because they were extensively shown to not affect locomotor behaviour in mice [41–52].

During test sessions, the cumulative duration of active lever hold-downs varied across stimulation frequencies [Frequency, $F_{(2, 18)}$=11.04, p=.0007] and treatments [Treatment, $F_{(4, 36)}$=73.3, p<.00001], but these factors did not interact [Frequency x Treatment, $F_{(8, 72)}$=1.302, p=.2564; **Fig 4a-b**]. As during training, the cumulative duration of active lever hold-downs was elevated during 40 Hz trials relative to 10 Hz [$t_{(9)}$=3.313, p=.0116] or 2.5 Hz [$t_{(9)}$=4.543, p=.0008; **Fig S3a-b**] trials. Augmenting neurotransmission with the D1 agonist SKF38393 [$t_{(9)}$=12.94, p<.0001] reduced the cumulative duration of active lever hold-downs whereas blunting neurotransmission with the D1 antagonist SCH23390 had no effect [$t_{(9)}$=1.193, p>.9999; **Fig 4a**]. Augmenting neurotransmission with the D2 agonist quinpirole reduced the cumulative duration of active lever hold-

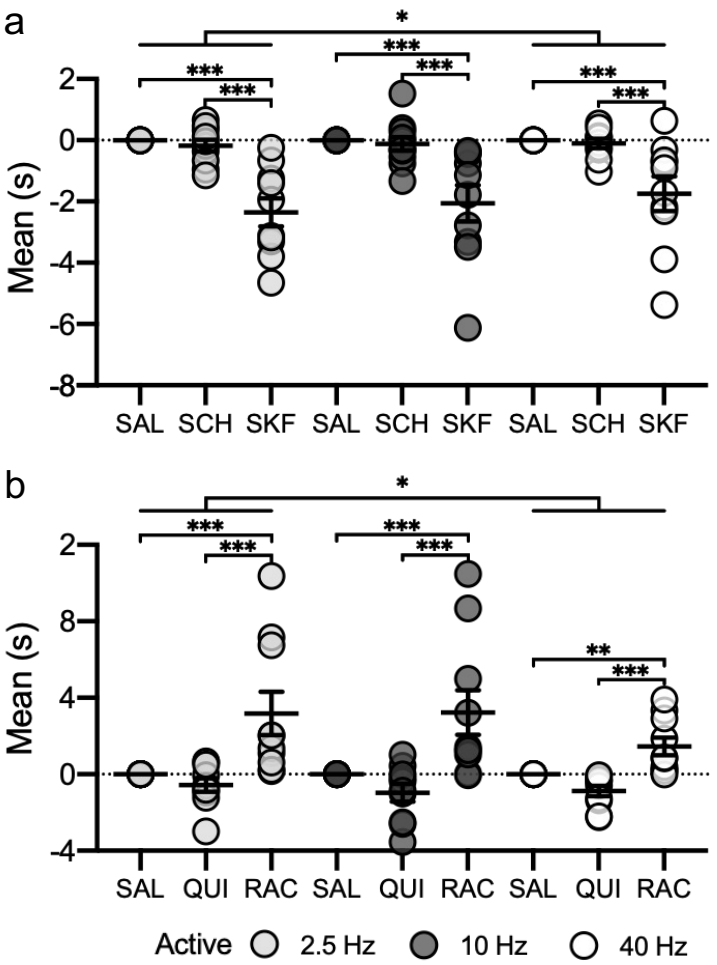

Fig. 5| The mean duration of self-stimulation hold-downs are modulated by neurotransmission at D1 and D2 receptors. The mean duration of active lever hold-downs were briefer at 40 Hz than at 2.5 Hz [$t_{(9)}$=3.359, p=.0105] and modulated by the D1 agonist SKF38393 and the D2 antagonist Raclopride in a frequency-dependent manner [Frequency x Treatment, $F_{(8, 72)}$=4.948, p=.0002]. **a**, The D1 agonist SKF38393 (SKF) shortened [2.5 Hz, $t_{(9)}$=6.22, p<.0001; 10 Hz, $t_{(9)}$=6.041, p<.0001; 40 Hz, $t_{(9)}$=5.125, p<.0001] whereas **b**, the D2 antagonist Raclopride (RAC) lengthened [2.5 Hz, $t_{(9)}$=11.01, p<.0001; 10 Hz, $t_{(9)}$=12.32, p<.0001; 40 Hz, $t_{(9)}$=6.826, p=.0002], mean hold-down duration relative to Saline (SAL). The D1 antagonist SCH23390 (SCH) and D2 agonist Quinpirole (QUI) did not affect mean hold-down duration. Data are shown as within-subjects differences from the Saline (SAL) treatment condition. Averaged data are mean ± s.e.m. Symbols represent data from individual mice. Asterisks indicate Bonferroni-corrected and multiplicity-adjusted post-hoc comparisons, p<.001***, p<.01**, p<.05*.

downs [$t_{(9)}$=6.013, p<.00001] whereas blunting neurotransmission with the D2 antagonist raclopride had no effect [$t_{(9)}$=2.118, p=.4118; **Fig 4b**]. Thus, the reinforcing properties of medial accumbens shell dopamine are reduced by augmenting neurotransmission at D1 and D2 receptors.

The mean duration of active lever hold-downs was modulated by treatment [Treatment, $F_{(4, 36)}$=11.33, p<.00001] in a frequency-dependent manner [Frequency x Treatment, $F_{(8, 72)}$=4.948, p=.0002; Frequency, $F_{(2, 18)}$=5.721, p<.0119; **Fig 5a-b**]. As during training, the mean duration of active lever hold-downs was briefer at 40 Hz than 2.5 Hz [$t_{(9)}$=3.359, p=.0105; **Fig S4a-b**]. Augmenting neurotransmission with the D1 receptor agonist SKF38393 reduced the mean duration of active lever hold-downs relative to the D1 antagonist SCH23390 [2.5 Hz, $t_{(9)}$=6.396, p<.0001; 10 Hz, $t_{(9)}$=5.744, p<.0001; 40 Hz, $t_{(9)}$=4.815, p=.0002] and saline [2.5 Hz, $t_{(9)}$=6.22, p<.0001; 10 Hz, $t_{(9)}$=6.041, p<.0001; 40 Hz, $t_{(9)}$=5.125, p<.0001]; this effect was most pronounced at lower stimulation frequencies (**Fig 5a**). Blunting neurotransmission with the D2 receptor antagonist raclopride lengthened the mean duration of active lever hold-downs relative to the D2 agonist quinpirole [2.5 Hz, $t_{(9)}$=9.342, p<.0001; 10 Hz, $t_{(9)}$=9.480, p<.0001; 40 Hz, $t_{(9)}$=4.254, p<.0019] and saline [2.5 Hz, $t_{(9)}$=11.01, p<.0001; 10 Hz, $t_{(9)}$=12.32, p<.0001; 40 Hz, $t_{(9)}$=6.826, p=.0002]; this effect was most pronounced at lower stimulation frequencies (**Fig 5b**). Neither the D1 antagonist SCH23390 [2.5 Hz, $t_{(9)}$=.5261, p>.999; 10 Hz, $t_{(9)}$=.2973, p>.999; 40 Hz, $t_{(9)}$=.3093, p>.999] nor the D2 agonist quinpirole [2.5 Hz, $t_{(9)}$=1.670, p>.999; 10 Hz, $t_{(9)}$=2.841, p=.1754; 40 Hz, $t_{(9)}$=2.573, p=.3646] affected the mean duration of active lever hold-downs relative to saline. Thus, the sensory properties of dopamine in the medial accumbens shell were modulated by augmenting neurotransmission at D1 receptors and blunting neurotransmission at D2 receptors.

The number of active lever hold-downs did not vary across stimulation frequencies [Frequency, $F_{(2, 18)}$=1.773, p=.1983; Frequency x Treatment, $F_{(8, 72)}$=1.471, p=.1829] but was affected by treatment [Treatment, $F_{(4, 36)}$=3.84, p=.0106]. After statistical correction, the reduction in the number of active lever hold-downs relative to saline remained only marginally significant for the D1 agonist SKF28393 [$t_{(9)}$=2.954, p=.055] and other treatments were similar to saline [SCH23390, $t_{(9)}$=.4919, p>.9909; Quinpirole, $t_{(9)}$=1.050, p>.9099; Raclopride, $t_{(9)}$=1.809, p=.7873; **Fig 6a-b**].

The cumulative [Frequency x Treatment, $F_{(8, 72)}$=.8387, p=.5718; Treatment, $F_{(4, 36)}$=2.137, p=.0963; Frequency, $F_{(2, 18)}$=1.374, p=.2785; **Fig S5a-b**] and mean [Frequency x Treatment, $F_{(8, 72)}$=1.108, p=.3679; Treatment, $F_{(4, 36)}$=.8582, p=.4982; Frequency, $F_{(2, 18)}$=1.715, p=.2081; **Fig S6a-b**] duration of inactive lever hold-downs were wholly unaffected by stimulation frequencies and treatments. Differently, while the number of inactive lever hold downs was unaffected by stimulation frequency [Frequency x Treatment, $F_{(8,}$

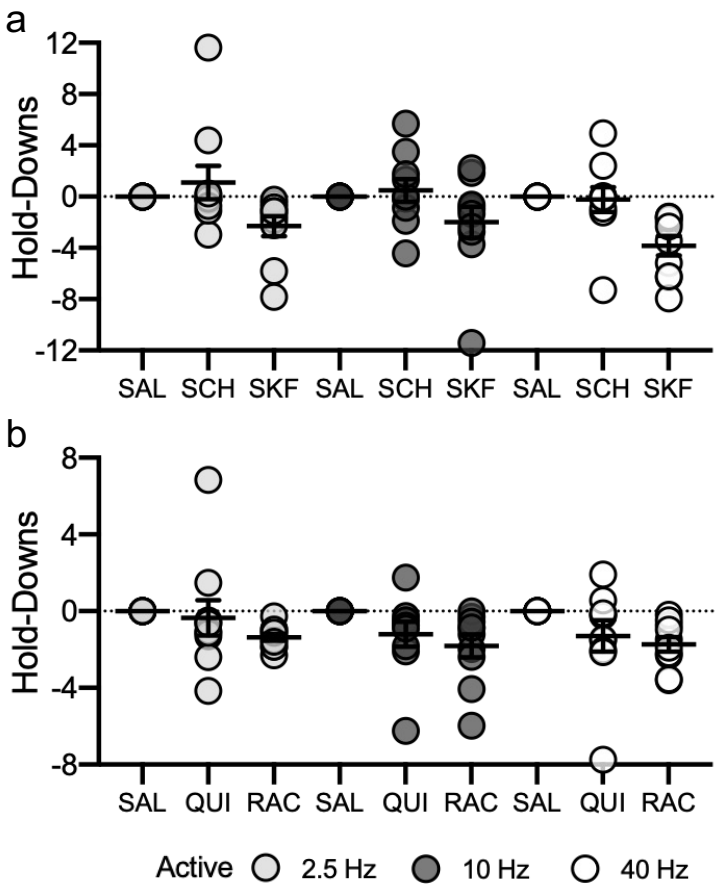

Fig. 6 | The number of self-stimulation hold-downs are unaffected by dopamine receptor agonists and antagonists. Neither **a**, the D1 antagonist SCH23390 (SCH) and D1 agonist SKF38393 (SKF) or **b**, the D2 agonist Quinpirole (QUI) and D2 antagonist raclopride (RAC) affected the number of hold-downs made during trials at any frequency [Frequency x Treatment, $F_{(8, 72)}$=1.471, p=.1829]. Data are shown as within-subjects differences from the Saline (SAL) treatment condition. Averaged data are mean ± s.e.m. Symbols represent data from individual mice.

$_{72)}$=.5677, p=.8009; Frequency, $F_{(2, 18)}$=.1944, p=.8250], there was a significant effect of treatment [Treatment, $F_{(4, 36)}$=9.805, p<.0001]. The D1 agonist SKF38393 elevated the number of inactive lever hold-downs [$t_{(9)}$=3.660, p=.008], whereas other treatments had no effect [SCH23390, $t_{(9)}$=.08864, p>.9999; Quinpirole, $t_{(9)}$=.9229, p>.9999; Raclopride, $t_{(9)}$=2.324, p=.2586; **Fig S7a-b**].

4. Discussion

The medial accumbens shell emerged as a substrate subserving the reinforcing properties of electrical medial forebrain bundle stimulation [36,37] and a target for the treatment of psychopathology [16,53]. Early studies attributed diverse consequences of electrical stimulation to separate substrates within the medial forebrain bundle. Here, by allowing mice to control the duration for which they optogenetically stimulate dopaminergic inputs to the medial accumbens shell, we report behaviour consistent with the emergence of mixed valenced sensory properties. The principal evidence for this claim is that the cumulative and mean duration for which mice hold-down a lever to optogenetically stimulate dopaminergic inputs to the medial accumbens shell show divergent frequency responses. At higher stimulation frequencies the cumulative duration of hold-downs grows, whereas the mean duration of hold-downs wanes. Using pharmacology, we begin identifying substrates subserving the reinforcing and sensory properties of medial accumbens shell dopamine. Dopamine agonists reduced the cumulative duration of self-stimulation hold-downs, but only a D1 agonist produced this reduction through decreases in the mean duration of hold-downs, which were lengthened with a D2 antagonist. An insight gleaned from the modulation of mean hold-down duration with pharmacology is that the reinforcing and sensory properties of medial accumbens shell dopamine are determined by neurotransmission at D1 and D2 receptors. Collectively, these findings are consistent with local substrates subserving the reinforcing and sensory properties of medial accumbens shell dopamine and propose a role for D1 receptors in the mitigation of dopamine-reinforced behaviour like binge consumption of palatable food and alcohol [14,54].

A temporally constrained self-stimulation procedure allowed mice to hold-down a lever to engage, and then release the lever to disengage, optogenetic stimulation of dopaminergic inputs to the medial accumbens shell. As such, two principal measures describe self-stimulation: the cumulative and mean duration of lever hold-downs, both with a maximum possible duration of the 30 s trial length. The cumulative duration of hold-downs is the product of the mean duration and number of hold-downs. We interpret cumulative hold-down duration as a reflection of the reinforcing properties of stimulation, arising either from changes in the mean duration or number of hold-downs, or both. Differently, we interpret the mean duration of hold-downs to reflect the sensory properties of medial accumbens shell dopamine. The brevity of trials, and the pseudorandom selection of stimulation frequency between trials preclude mice from predetermining the duration of hold-downs. Rather, mice decide to terminate a hold-down only after it has begun and in response to the sensory properties of stimulation. Thus, the cumulative and mean duration of self-stimulation hold-downs dissociate

the reinforcing and sensory properties of dopamine release in the medial accumbens shell.

Across training, mice increased the cumulative duration, mean duration, and number of active relative to inactive lever hold-downs, consistent with the acquisition of temporally constrained self-stimulation. The increase in cumulative duration and number of hold-downs in a frequency-dependent manner demonstrate that high-frequency dopamine activity in the medial accumbens shell is strongly reinforcing. The gradual, but substantial, increase in the mean duration of active, relative to inactive, lever hold-downs during training demonstrates that mice have considerable flexibility in their ability to control the duration of dopamine activity in the accumbens shell. At the end of training, the effects of stimulation frequency were most prominent in the cumulative and mean duration of active lever hold-downs which increased and decreased, respectively, across stimulation frequencies and relative to inactive lever hold-downs. Differently, while the number of active lever hold-downs was elevated on high relative to low frequency trials, this effect was marginally significant with respect to inactive lever hold-downs.

Pronounced effects of stimulation frequency on the cumulative and mean duration, but not number, of active lever hold-downs indicates that behaviour refined such that mice principally varied the duration, rather than frequency, of self-stimulation hold-downs. This is possible because large changes in the cumulative duration of hold-downs could be accomplished by subtle coincident changes in the mean duration and number of hold-downs. The effects of stimulation frequency detected here are smaller than in procedures where rodents control only the number, not duration, of trains of dopamine activity. Importantly, most previous work directly stimulated dopamine cell bodies in the ventral tegmental area [29,32], rather than accumbal inputs, while varying stimulation frequency which may explain this discrepancy. Nevertheless, mice demonstrate sophisticated and considerable (~25%) control over the duration with which dopamine inputs to the medial accumbens shell are stimulated.

At least one previous study reported that mice could not learn to hold-down a lever to self-stimulate dopaminergic cell bodies in the substantia nigra [55], which may relate to an impoverished role of nigral, relative to ventral tegmental area dopamine neurons, in reinforcing behaviour [33,56]. Additionally, nigral projection targets in the dorsal striatum [56] differ from ventral tegmental area dopamine inputs to the medial accumbens shell and likely engage circuitries that serve different functions. Also, the previous attempt to use hold-down duration as a self-stimulation operant may have been unsuccessful because of the high frequency of stimulation used, 50 Hz, which generally supported sub half-second hold-downs, consistent with the disengaging property of high frequency dopamine activity in the current work.

We attribute the decrease in mean hold-down duration during high frequency stimulation trials to the emergence of negatively valenced sensory properties of intense dopamine activity in the medial accumbens shell. This interpretation is incongruent with a paradigm in contemporary neuroscience describing a relationship between striatal cells and motivated behaviour. In the striatum, the activity of neurons expressing dopamine type-1 (D1) receptors is thought to encode positive valence and encourage behaviour, whereas neurons expressing dopamine type-2 (D2) receptors oppose this influence, encoding negative valence when active and discouraging behaviour [57–59]. Thus, input from the ventral tegmental area to the striatum, including the accumbens, is thought to reinforce behaviour because dopamine elevates the activity of D1 neurons and mutes the activity of D2 neurons, serving as a strong positive valence signal. Certainly, we observe increases in the cumulative time spent self-stimulating dopamine inputs to the medial accumbens shell, consistent with the reinforcing and positively valenced sensory properties of driving this substrate. Differently, the mean duration for which mice hold-down a lever to stimulate dopaminergic inputs to the medial accumbens shell wanes at high stimulation frequencies, consistent with the emergence of a negatively valenced sensory property serving to disengage behaviour. It is difficult to reconcile a negatively valenced sensory property owing only to dopamine activity in the medial accumbens shell with conventional views of striatal dopamine and motivation.

Medial accumbens shell dopamine achieving mixed valence may arise from the co-release of glutamate by ventral tegmental area dopamine neurons that innervate the accumbens shell. When mice could nose-poke at five concurrently available apertures armed with 40 Hz optogenetic stimulation of different durations (i.e., 1, 5, 20, 40 s), ventral tegmental area dopamine cell bodies predominantly supported responses for 5 s of stimulation. Differently, a subpopulation of ventral

tegmental area dopamine neurons that co-release glutamate predominantly supported responses for 1 s of stimulation [32] and mice would perform an operant response to terminate noncontingent stimulation of these inputs to the medial accumbens shell [60]. Thus, glutamate co-release from dopaminergic inputs to the medial accumbens shell may contribute to the current behaviour, where high-frequency stimulation supported the briefest mean duration of self-stimulation hold-downs; however, the modulation of this behaviour with dopamine receptor agonists and antagonists disfavours this possibility.

Dopamine has varied and complex actions on inputs to and cells within the ventral striatum, but the consequence of these actions are simple: to reinforce behaviour. This conjecture is supported by decades of preclinical research manipulating and observing dopamine activity during behaviour and particularly the indiscriminate capacity for drugs that elevate dopamine levels to reinforce behaviour [61]. However, much of this work relied on changes in behavioural frequency to describe the reinforcing properties of dopamine. Even still, some findings hinted a mixed valence sensory property of dopamine. For example, psychostimulants paired with food consumption [62,63], even when administered volitionally [64], caused rodents to stop consuming that food in the future despite encouraging food approach [65]. Further, the self-administration of psychostimulants is reduced, not amplified, by local microinfusion of dopamine agonists into the accumbens [66]. These findings point to an inherent role of dopamine receptor activation in disengaging behaviour.

Here, dopamine receptor agonists and antagonists produced dissociable effects on the cumulative and mean durations for which mice self-stimulated dopaminergic inputs to the medial accumbens shell. While dopamine receptor agonists unanimously reduced the cumulative duration of self-stimulation hold-downs, the D1 agonist SKF38393 did so by reducing the mean duration of hold-downs whereas the D2 agonist quinpirole did not affect this measure. Augmenting neurotransmission at D2 receptors with quinpirole diminished the reinforcing properties of medial accumbens shell dopamine, which likely manifested through subtle coincident reductions in the mean duration and number of hold-downs. Although dopamine antagonists did not affect the cumulative duration of self-stimulation hold-downs, blunting neurotransmission at D2 receptors with raclopride enhanced the positively valenced sensory property of medial accumbens dopamine, elongating self-stimulation hold-downs. Differently, augmenting neurotransmission at D1 receptors with SKF38393 shortened self-stimulation hold-downs, consistent with amplification of the negatively valenced sensory property of medial accumbens shell dopamine. The D1 agonist also modestly elevated the number, but not cumulative or mean duration, of inactive lever hold-downs which may be explained by an incentive property acquired by lever appearance [33]. When mice could not endure long active lever hold-downs owing to enhanced negatively valenced sensory properties of accumbens shell dopamine they may have chosen to interact with the inactive lever.

It is satisfying to ascribe psychological processes like reinforcement and valence to the sole actions of neurotransmitters on receptors and inferred changes in cellular activity. The medial accumbens shell, however, is a complex place; densely innervated and home to various receptors on diverse substrates all intricately interacting. Dopamine receptors are expressed not only on striatal cells, but on glutamatergic corticostriatal inputs to the accumbens shell [67,68]. As such, glutamate and dopamine levels in the ventral striatum are interdependent [69–71] and exciting either glutamatergic [34] or dopaminergic [32,72–74] inputs to the NAc shell increases the excitability of downstream D1 and D2 neurons consistent with several unique aspects of striatal physiology [32,67,73,75–79]. While the D1 agonist SKF38393 may have enhanced the negatively valenced sensory property of medial accumbens shell dopamine by elevating the activity of neurons expressing D1 receptors, it is equally likely that the relevant feature of this manipulation was broader reshaping of accumbal physiology. Consistently, recent work [80–83] describes unintuitive responses of striatal neurons to modulation of dopamine neurotransmission. The relationship between input stimulation, the profile of ventral striatal activity that follows, and motivated behaviour is complex, poorly described, and a major hurtle to treating psychopathologies characterized by striatal dysfunction.

Current use of electrical brain stimulation to treat alcohol use [16,17] and binge eating disorder [18,19] target the accumbens with novel electrode designs and low-current stimulation parameters to mitigate off-target effects [53]. Akin to mean hold-down duration in the current work, mice vary the length of their bouts of consummatory behaviour, which become long during binge alcohol [54,84] and palatable food consumption [85,86]. If augmented neurotransmission at D1

receptors shortens the duration for which mice prefer to self-stimulate dopamine inputs to the medial accumbens shell, it may also serve to prematurely disengage pathological consummatory behaviour. Consistently, arrhythmic low-current multi-electrode stimulation of the accumbens selectively reduces the duration, not number, of bouts of alcohol drinking in a preclinical model of alcohol use disorder [54]. The therapeutic effects of electrical accumbens stimulation to shorten bouts of consummatory behaviour may partially arise from mechanisms involving neurotransmission at D1 receptors, and thus D1 agonists may be an adjuvant for certain therapeutic applications of electrical brain stimulation.

5. Conclusion

The current findings run counter to conventional views of striatal dopamine but are well accompanied by recent work diversifying the role of dopamine in psychology [87–95]. Here, using a novel temporally constrained self-stimulation procedure we report behaviour consistent with dissociable reinforcing and sensory properties of medial accumbens shell dopamine. Specifically, intense dopamine activity in the medial accumbens shell is powerfully reinforcing but provokes a negatively valenced sensory property that together produce short, numerous, self-stimulation hold-downs. The reinforcing and sensory properties of medial accumbens shell dopamine are differentially affected by modulating neurotransmission at dopamine receptors. Notably, augmenting neurotransmission at D1 receptors serves to enhance the negatively valenced sensory property, whereas blunting neurotransmission at D2 receptors amplifies the reinforcing property, of medial accumbens shell dopamine. Recent advances in the development of safe and well-tolerated D1 receptor agonists [96] may enhance the therapeutic efficacy of electrical accumbens stimulation for the treatment of binge eating and alcohol use disorder.

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Declarations

The authors have no competing interests to declare.

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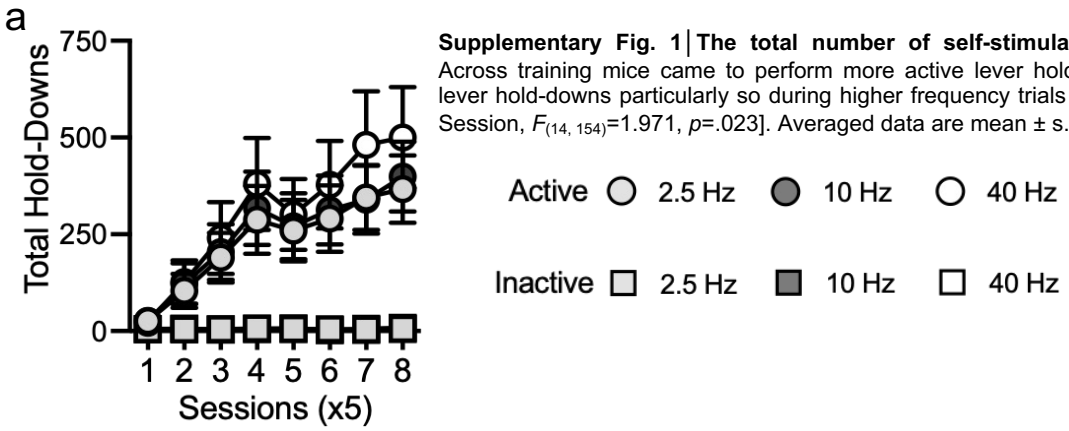
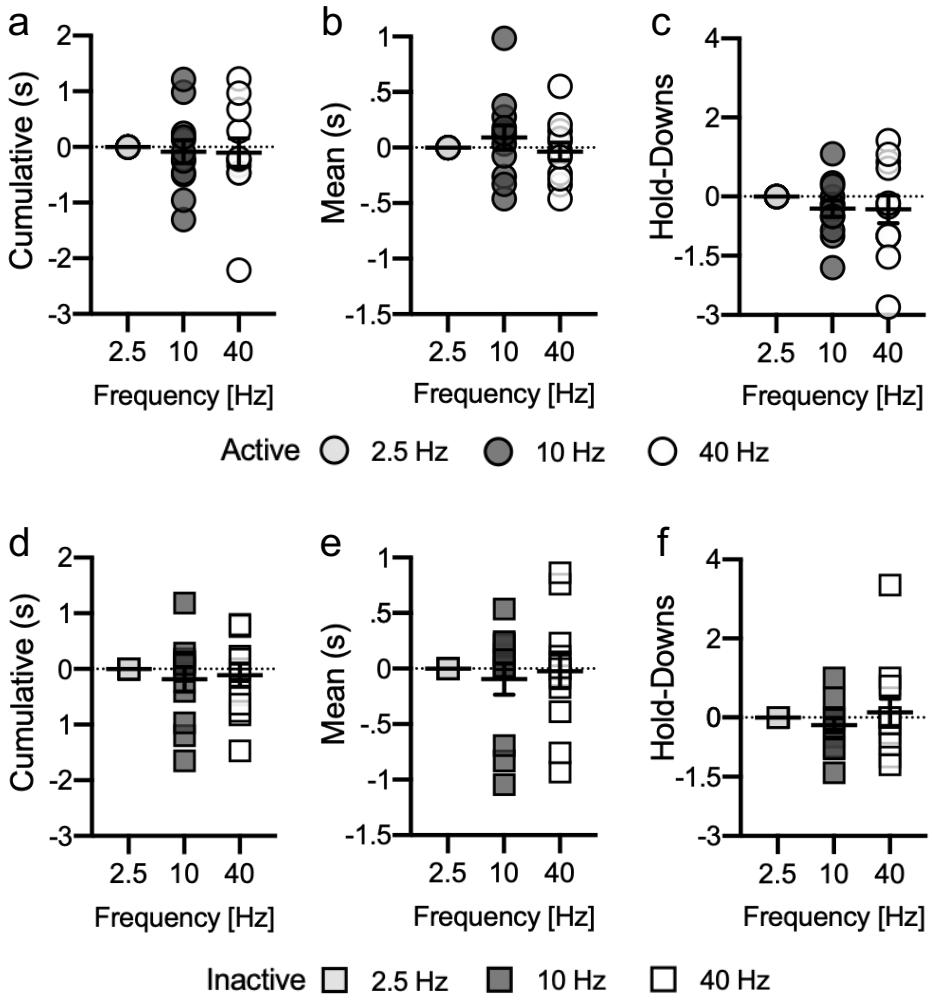


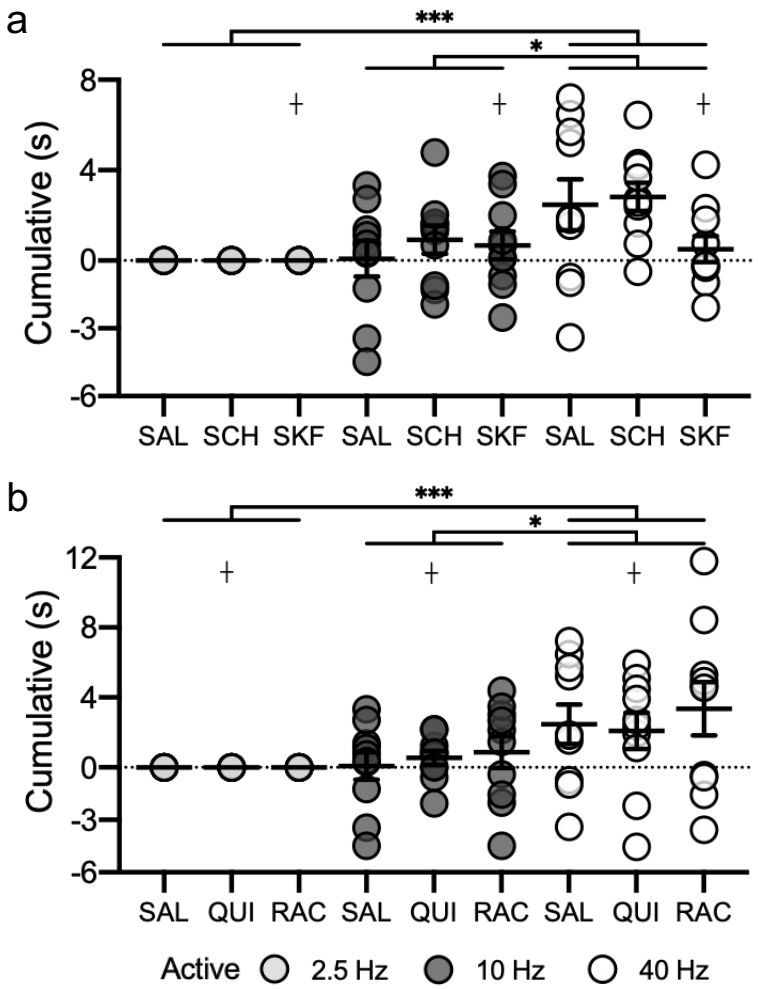
Supplementary Fig. 1 | The total number of self-stimulation hold-downs. a, Across training mice came to perform more active lever hold-downs than inactive lever hold-downs particularly so during higher frequency trials [Lever x Frequency x Session, $F_{(14, 154)}$=1.971, p=.023]. Averaged data are mean ± s.e.m.

Supplementary Figure 1



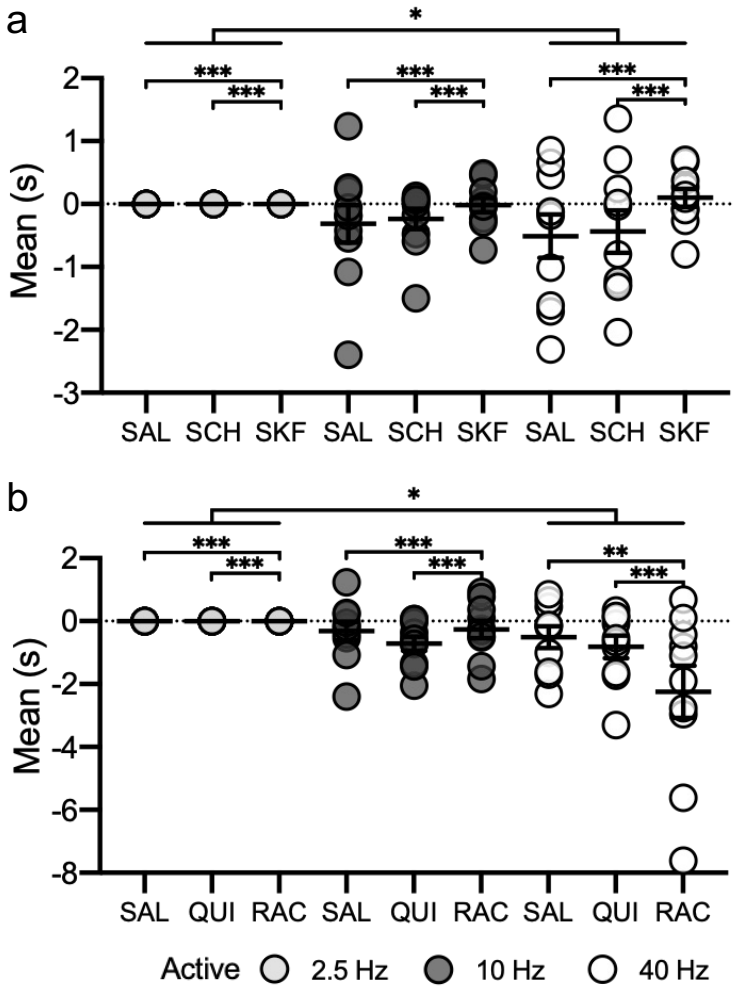
Supplementary Fig. 2 | Frequency dependent self-stimulation of dopamine inputs to the medial accumbens shell absent early in training. The **a**, cumulative **b**, mean and c, number of active lever hold downs were similar to the **d**, cumulative **e**, mean and **f**, number of inactive lever hold downs across all frequencies early in training [Lever x Frequency, All $F<.9508$, $p>.4017$]. Data are shown as within-subjects differences from the lowest stimulation frequency (2.5 Hz). Averaged data are mean ± s.e.m. Symbols represent data from individual mice.

Supplementary Figure 2



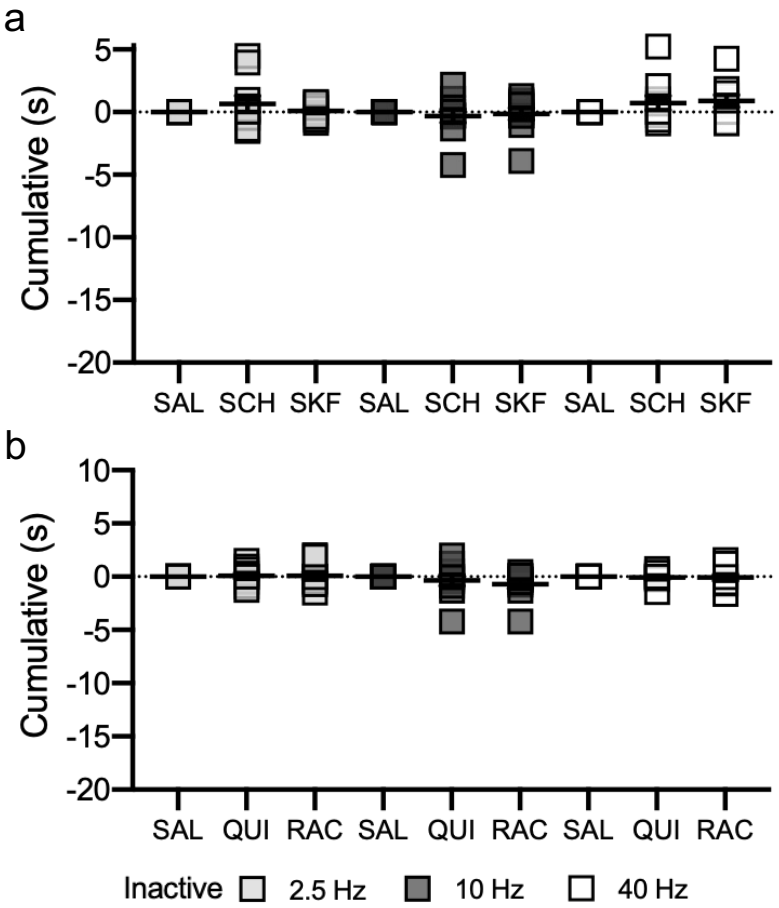
Supplementary Fig. 3 | During treatment with dopamine receptor agonists and antagonists cumulative hold-down duration increased with stimulation frequency. Mice performed longer cumulative active lever hold-downs during 40 Hz than 10 Hz [$t_{(9)}$=3.313, p=.0116] or 2.5 Hz [$t_{(9)}$=4.543, p=.0008] trials [Frequency, $F_{(2, 18)}$=11.04, p=.0007]. Data are shown as within-subjects differences from 2.5 Hz trials. Averaged data are mean ± s.e.m. Symbols represent data from individual mice. Asterisks indicate Bonferroni-corrected and multiplicity-adjusted post-hoc comparisons p<.001***, p<.05*. Crosses indicate similarly corrected simple effects of treatment relative to saline p<.0001┼.

Supplementary Figure 3



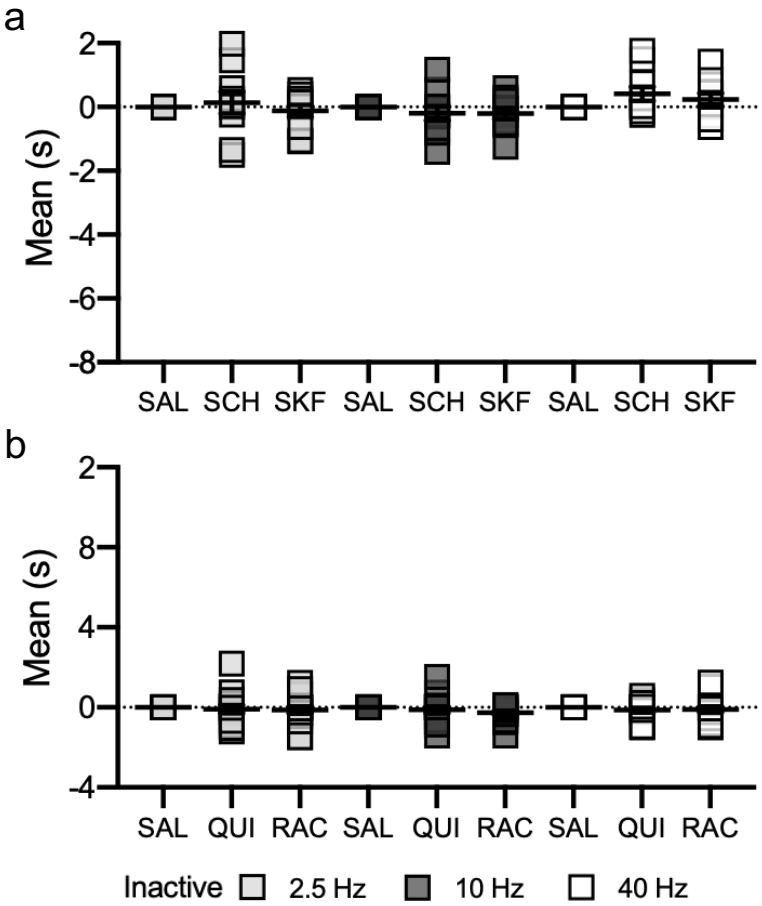
Supplementary Fig. 4 | During treatment with dopamine receptor agonists and antagonists mean hold-down duration decreased with stimulation frequency. The mean duration of active lever hold-downs were briefer at 40 Hz than at 2.5 Hz [$t_{(9)}$=3.359, p=.0105; Frequency, $F_{(2, 18)}$=5.721, p<.0119]. Data are shown as within-subjects differences from 2.5 Hz trials. Averaged data are mean ± s.e.m. Symbols represent data from individual mice. Asterisks indicate Bonferroni-corrected and multiplicity-adjusted post-hoc comparisons, p<.001***, p<.01**, p<.05*.

Supplementary Figure 4



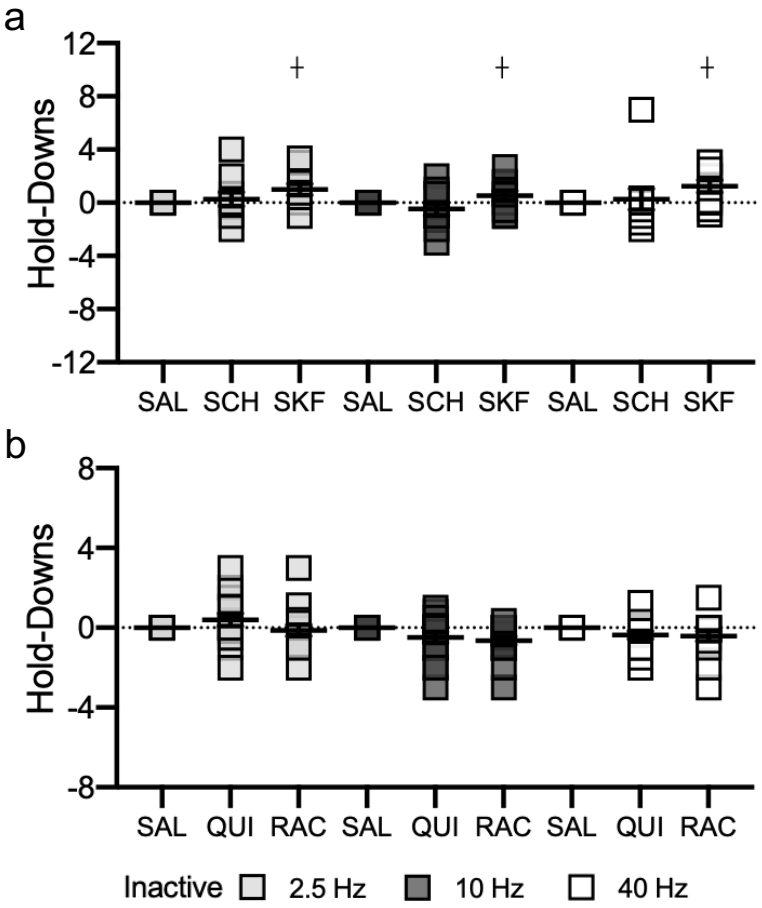
Supplementary Fig. 5 | Cumulative duration of inactive lever-hold downs unaffected by modulating neurotransmission at D1 and D2 receptors. Neither **a**, the D1 antagonist SCH23390 (SCH) and D1 agonist SKF38393 (SKF) or **b**, the D2 agonist Quinpirole (QUI) and D2 antagonist raclopride (RAC) affected the cumulative duration of inactive lever hold-downs made during trials at any frequency [Frequency x Treatment, $F_{(8, 72)}$=.8387, p=.5718]. Data are shown as within-subjects differences from the Saline (SAL) treatment condition. Averaged data are mean ± s.e.m. Symbols represent data from individual mice.

Supplementary Figure 5



Supplementary Fig. 6 | Mean duration of inactive lever-hold downs unaffected by modulating neurotransmission at D1 and D2 receptors. Neither **a**, the D1 antagonist SCH23390 (SCH) and D1 agonist SKF38393 (SKF) or **b**, the D2 agonist Quinpirole (QUI) and D2 antagonist raclopride (RAC) affected the mean duration of inactive lever hold-downs made during trials at any frequency [Frequency x Treatment, $F_{(8,\ 72)}$=1.108, p=.3679]. Data are shown as within-subjects differences from the Saline (SAL) treatment condition. Averaged data are mean ± s.e.m. Symbols represent data from individual mice.

Supplementary Figure 6



Supplementary Fig. 7 | D1 agonist elevated inactive lever hold-downs. The number of inactive lever hold-downs during trials was unaffected by stimulation frequency [Frequency, $F_{(2,\ 18)}$=.1977, p=.825; Frequency x Treatment, $F_{(8,\ 72)}$=.5677, p=.8009] but was attenuated by treatment [Treatment, $F_{(4,\ 36)}$=9.805, p<.0001]. Specifically, the **a**, D1 agonist SKF38393 (SKF) but not the D1 antagonist SCH23390 (SCH) elevated the number of inactive lever hold-downs and neither the **b**, D2 agonist Quinpirole (QUI) nor D2 antagonist raclopride (RAC) affected the number of inactive lever hold-downs. Data are shown as within-subjects differences from the Saline (SAL) treatment condition. Averaged data are mean ± s.e.m. Symbols represent data from individual mice. Crosses indicate Bonferroni-corrected and multiplicity-adjusted simple effects of treatment relative to saline, p=.008┼.

Supplementary Figure 7