Disruption of cortical dopaminergic modulation impairs preparatory activity and delays licking initiation

Abbreviated title: Dopaminergic modulation of licking initiation

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Number of pages: 39

Number of figures: 7

Number of words:

Abstract: 174

Introduction: 581

Discussion: 1467

Conflicts of interest:

The authors declare that no competing interests exist**.**

Acknowledgements:

The authors would like to acknowledge Dr. Arianna Maffei, Dr. Craig Evinger, Dr. Joshua L. Plotkin, past and present members of the Fontanini and Maffei's laboratories for their feedback and insightful comments. This work has been supported by the Thomas Hartman Foundation for Parkinson Research.

ABSTRACT

Dysfunction of motor cortices is thought to contribute to motor disorders such as Parkinson's disease (PD). However, little is known on the link between cortical dopaminergic loss, abnormalities in motor cortex neural activity and motor deficits. We address the role of dopamine in modulating motor cortical activity by focusing on the anterior lateral motor cortex (ALM) of mice performing a cued-licking task. We first demonstrate licking deficits and concurrent alterations of spiking activity in ALM of mice with unilateral depletion of dopaminergic neurons (i.e., mice injected with 6-OHDA into the medial forebrain bundle). Hemi-lesioned mice displayed delayed licking initiation, shorter duration of licking bouts, and lateral deviation of tongue protrusions. In parallel with these motor deficits, we observed a reduction in the prevalence of cue responsive neurons and altered preparatory activity. Acute and local blockade of D1 receptors in ALM recapitulated some of the key behavioral and neural deficits observed in hemi-lesioned mice. Altogether, our data show a direct relationship between cortical D1 receptor modulation, cue-evoked and preparatory activity in ALM, and licking initiation.

SIGNIFICANCE STATEMENT

The link between dopaminergic signaling, motor cortical activity and motor deficits is not fully understood. This manuscript describes alterations in neural activity of the anterior lateral motor cortex (ALM) that correlate with licking deficits in mice with unilateral dopamine depletion or with intra-ALM infusion of dopamine antagonist. The findings emphasize the importance of cortical dopaminergic modulation in motor initiation. These results will appeal not only to researchers interested in cortical control of licking, but also to a broader audience interested in motor control and dopaminergic modulation in physiological and pathological conditions. Specifically, our data suggest that dopamine deficiency in motor cortex could play a role in the pathogenesis of the motor symptoms of Parkinson's disease.

INTRODUCTION

Dysfunction of motor cortices, which are important for movement planning, initiation and execution, has been suggested to play a role in the motor symptoms of Parkinson's disease (PD) (Lindenbach and Bishop, 2013). Studies on motor cortices of human patients and animal models of PD revealed abnormalities in preparatory activity, excitability, excitation/inhibition balance and oscillatory dynamics (Doudet et al., 1990; Ridding et al., 1995; Goldberg et al., 2002; Escola et al., 2003; Lefaucheur, 2005; Pasquereau and Turner, 2011; Pasquereau et al., 2015). However, it is unclear whether abnormal patterns of motor cortical activity are secondary to dysfunction of the basal ganglia or whether they result from disruption of local dopaminergic modulation. Midbrain dopaminergic neurons project to the striatum and motor cortex. While dopaminergic innervation to the striatum has been studied extensively for its modulatory role on motor initiation and execution, studies on dopaminergic innervation to the motor cortex have been more limited and focused mostly in its role in synaptic plasticity and motor skill learning (Molina-Luna et al., 2009; Guo et al., 2015). To date, little is known about the direct link between loss of dopaminergic signaling in the motor cortex, alterations of motor cortical single unit activity, and corresponding motor deficits.

Here, we investigate the role of motor cortex dopaminergic transmission in movement initiation and execution. We focus on the anterior lateral motor cortex (ALM) of mice engaged in a cued-licking task. Licking was chosen because it is an innate motor behavior whose cortical control is well-studied. In rodents, licking is regulated by a central pattern generator circuit in the brainstem, which is under the control of the motor cortex (Travers et al., 1997). ALM plays an important role in the planning and execution of licking (Komiyama et al., 2010; Guo et al., 2014; Li et al., 2015; Inagaki et al., 2018), as reflected by the presence of neurons whose firing rates are modulated

before the onset of licking (defined as "preparatory" neurons) (Guo et al., 2014; Li et al., 2015; Chen et al., 2017; Inagaki et al., 2018). In addition, this area appears to be responsible for controlling the direction of tongue movements, as unilateral optogenetic silencing of ALM can introduce a directional bias towards the ipsilateral side (Guo et al., 2014; Li et al., 2015). Although ALM has been studied for its involvement in controlling normal licking, how lack of dopaminergic signaling impacts activity and function of this region remains unknown.

The experiments described here rely on behavioral training, pharmacology, and electrophysiological recordings to study licking deficits and related abnormalities of ALM neural activity in the context of unilateral dopamine depletion (i.e., unilateral injection of 6-OHDA into the medial forebrain bundle). This manipulation has been classically used to model some of the features of PD (Lundblad et al., 2004; Thiele et al., 2012; Jagmag et al., 2016). First, we show that mice with unilateral dopamine depletion display delayed licking initiation, shorter duration of licking bouts, and deviated tongue protrusions compared to control mice. Next, we report changes in cue responses and preparatory activity for neurons in ALM of 6-OHDA lesioned mice. Finally, we perform local pharmacological blockade of dopaminergic receptors to determine the contribution of cortical dopaminergic deficit in ALM to the electrophysiological and behavioral alterations seen in 6-OHDA lesioned mice.

Using licking as a model behavior, our data show motor deficits and abnormalities in neural activity associated with unilateral dopamine depletion. The results demonstrate the importance of cortical dopaminergic modulation for motor initiation and for modulating preparatory activity.

MATERIALS AND METHODS

Experimental subjects

The experiments were performed on adult male mice (C57BL/6, 12-20 weeks old, Charles River). Mice were group housed and maintained on a 12 h light/dark cycle with *ad libitum* access to food and water unless otherwise specified. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Stony Brook University, and complied with university, state, and federal regulations on the care and use of laboratory animals.

Surgical procedures for 6-OHDA injections in the medial forebrain bundle

Mice were anesthetized with isoflurane (1-1.5%) in oxygen (1 L/min). Once fully anesthetized, mice were placed on a stereotaxic apparatus. The scalp was cut open to expose the skull and a hole was drilled above the medial forebrain bundle (MFB, anterior-posterior: -1.2 mm, medial-lateral: 1.3 mm, dorsal-ventral: -4.75 mm). In a first group of mice (referred hereafter as 6-OHDA lesioned), 3.5 µg 6-OHDA dissolved in 1 µl 0.02% ascorbic acid (vehicle, prepared from sterile saline) was unilaterally injected into the MFB. A second group of mice (sham-lesioned mice, referred hereafter as control) underwent the same surgical procedure but received 1 µl vehicle injection into the MFB. To prevent dehydration, mice were monitored daily and subcutaneously injected with 1 mL lactated ringer's solution after the surgery as needed. In addition, food pellets soaked in 15% sucrose were placed on the floor of cages to facilitate eating (Francardo et al., 2011).

Behavioral screening of lesion: cylinder test

Two to three weeks after the MFB lesion surgery, mice were placed into a clear plastic cylinder. Mice could freely explore the cylinder, rearing and touching the cylinder wall with their forepaws.

The behavior during the first 3 min in the cylinder was videotaped and analyzed. The number of wall touches with the ipsilateral or contralateral forepaw was counted and used to calculate the forepaw preference. Only lesioned mice with less than 40% usage of contralateral forepaw for touching the cylinder wall were used for further experiments (Lundblad et al., 2004).

Surgical procedures for implanting electrodes, infusion cannula, and electrode-cannula assemblies

2-4 weeks after the lesion surgery, 6-OHDA lesioned and control mice were anesthetized with an intraperitoneal injection of a mixture of ketamine (70 mg/kg) and dexmedetomidine (1 mg/kg) and placed on a stereotaxic apparatus. The scalp was incised to expose the skull. For electrode implantation, 1 mm craniotomies were performed above both anterior lateral motor cortices (ALM, anterior-posterior: 2.4 mm, medial-lateral: ±1.5 mm) and two holes were drilled above visual cortex on both hemispheres for inserting ground wires (silver wire). A linear array of 16 electrodes (formvar-insulated nichrome wire, catalog no. 761000, A-M System, Sequim, WA) was bilaterally implanted into ALM (dorsal-ventral: -0.8 - -1 mm). For infusion cannula implantation, naïve mice were used instead, and a 1 mm craniotomy was performed on left ALM. A 26-gauge guide cannula with a dummy (0.5 mm projection) was inserted into ALM (dorso-ventral: -700 μm). To record single units after local D1 receptor blockade, a group of naïve mice was unilaterally implanted in ALM with a custom-built ensemble containing 8 tetrodes (Item No. PX000004, Sandvik-Kanthal, Hallstahammar, Sweden) around an infusion guide cannula (26 gauge). Electrodes, cannulae or electrode-cannula assemblies and a head bolt (for the purpose of head restraint) were cemented to the skull with dental acrylic. Mice were allowed to recover from surgery for a week before starting water restriction regimen.

150

151 **Cued-licking paradigm**

152 Following recovery, mice were started on a water restriction regime, with 1.5 ml water daily
153 one week before training. Weight was monitored and maintained at > 80% of the standard weight
154 for age, strain and sex. In the first phase of training, mice were habituated to restraint. During brief
155 restraint sessions, a spout containing a drop of sucrose (200 mM) was moved close to the animal
156 to encourage licking. Once the mouse started to reliably lick the spout, session duration was
157 increased and training in the cued-licking paradigm began. For each trial, a movable spout
158 containing a drop of sucrose (~3 µl, 200 mM) moved in front of the mouth of the animal 1 s after
159 the onset of an auditory cue (200 ms, 2k Hz, 70 dB). The spout remained in place for 2 s to allow
160 the mouse to lick and access the sucrose solution before retracting. The inter-trial interval was 10
161 s. An infrared beam (940 nm, powered by a fiber-coupled LED, Thorlabs, Newton, NJ) was put in
162 front of the mouth of the mouse such that each lick could be detected. Orofacial movements were
163 also recorded with a videocamera (30 Hz frame rate) synchronized with the data acquisition
164 software (CinePlex, Plexon, Dallas, TX).

165

166 **Electrophysiological recordings in control and 6-OHDA lesioned mice**

167 Multiple single units were recorded via a multichannel acquisition processor (Plexon) in mice
168 performing the cued-licking paradigm. Neural signals were amplified, bandpass (300-8000 Hz)
169 filtered, and digitized at 40k Hz. Single units were isolated by threshold detection and a waveform
170 matching algorithm and were further sorted offline through principal component analysis using
171 Offline Sorter (Plexon).

172

D1/D2 receptor antagonist infusion in ALM

Thirty to forty minutes before a testing session, mice previously trained in the cued-licking paradigm were briefly anesthetized with 1% isoflurane and a 33-gauge inner cannula (0.5 mm projection) was inserted into the guide cannula. 0.5 µl of a solution of either the D1 receptor antagonist (5 µg/µl SCH23390 hydrocloride, Sigma-Aldrich, St. Louis, MO), the D2 antagonist (5 µg/µl raclopride tartrate salt, Sigma-Aldrich) or sterile saline (0.9%) was unilaterally infused into ALM at 0.25 µl/min using a syringe pump (11 plus, Harvard Apparatus, Holliston, MA).

D1 receptor antagonist infusion in ALM and electrophysiological recordings

After recovery from the surgery for at least a week, mice were water restricted and trained to perform the cued-licking paradigm. Testing started after 8-12 days of training. Thirty to forty minutes before a testing and electrophysiological recording session, mice were head restrained and a 33-gauge inner cannula (0.5 mm projection) was inserted into the guide cannula. 0.5 µl of a solution of either the D1 receptor antagonist (5 µg/µl SCH23390 hydrocloride, Sigma-Aldrich) or sterile saline (0.9%) were infused into ALM at 0.25 µl/min using a syringe pump (11 plus, Harvard Apparatus). Single units were recorded and sorted offline as described above. Each session of saline infusion was followed, on the day after, by a session with D1 receptor antagonist infusion. Each mouse underwent 1-2 sessions of saline and SCH23390 infusion.

Data analysis

Data analysis was performed using Neuroexplorer (Plexon) and custom written scripts in MATLAB (MathWorks, Natick, MA).

Analysis of licking behavior. The analog trace from the infrared beam (and its breaking by the tongue) was used for analyzing licking behaviors. A licking event was detected whenever the trace crossed a fixed threshold. A bout was defined as a train of at least three consecutive licks with an inter-lick interval shorter than 500 ms (Davis and Smith, 1992). Only licking bouts within 4 s after the auditory cue were used for the analysis. In the case of two licking bouts occurred in the same trial, only the first licking bout was used for analysis. Video analysis of the oral region was used to extract the angle of tongue protrusions at each lick. Licking angle was defined as an angle between the midline of the protruded tongue and the midline of the mouse chin.

Analysis of single unit. Single unit spike timestamps were aligned to either the onset of the auditory cue or the licking bout initiation. Perievent rasters of individual units were used to construct peristimulus time histograms (PSTHs, bin size is 100 ms). For analyzing population PSTHs, the firing rate of each neuron was normalized using area under the receiver operating characteristic curve (auROC) method (Cohen et al., 2012; Gardner and Fontanini, 2014). This method normalizes firing rate to a value between 0 and 1, in which 0.5 represents baseline firing rate, value > 0.5 or < 0.5 represents increased or decreased firing rate compared to the baseline, respectively. Population PSTH was calculated by averaging auROC across each unit.

Analysis of cue response. PSTHs of single units were aligned to onset of cue. Activity after onset of cue was assessed by examining firing activity in a 500 ms window after cue onset. Firing rates within each bin (bin size is 100 ms) in the 500 ms window after cue onset were compared to baseline (1 s before the auditory cue) with a Wilcoxon rank sum test ($p < 0.05$) and a correction for multiple comparison (Šidák correction).

Analysis of preparatory response. PSTHs of single units were aligned to bout initiation. Activity preceding licking (i.e., preparatory activity) was assessed by examining firing rates in a

500 ms window before bout initiation. Firing rates within each bin (bin size is 100 ms) in the 500 ms window before bout initiation were compared to baseline (1 s before the auditory cue) with a Wilcoxon rank sum test ($p < 0.05$) and a correction for multiple comparison (Šidák correction). Units with significantly increased firing rate before bout initiation were defined as "excitatory preparatory" units, where units with significantly decreased firing rate before bout initiation were deemed as "inhibitory preparatory". The latency of preparatory activity of each neuron was computed based on "change point" (CP) analysis (Jezzini et al., 2013; Liu and Fontanini, 2015; Vincis and Fontanini, 2016).To calculate latency of preparatory activity relative to the cue or bout initiation, we aligned spikes to cue onset or bout initiation and computed the cumulative distribution (CDF) of spike occurrence across all trials in the time interval starting 2 s before and ending 4 s after the cue or bout initiation, respectively. A sudden change of firing rate caused a correspondent change of the slope of CDF and the occurrence of a CP. The timing of the first significant CP was defined as the latency of preparatory activity. For analysis of latency relative the cue onset, neurons without CP (8/307) or neurons with first CP (2/307) occurring later than 3s after the cue were excluded for the analysis. For analysis of latency relative to the licking initiation, neurons without CP (6/307) or neurons with first CP (11/307) occurring after the licking initiation were excluded.

Histological staining for verification of lesions and electrode/canula positioning

Mice were deeply anesthetized with an intraperitoneal injection of a mixture of ketamine/dexmedetomidine at 2-3 times the anesthetic dose and were intracardially perfused with PBS followed by 4% paraformaldehyde. The brain was further fixed with 4% paraformaldehyde overnight and cryoprotected with 30% sucrose for 3 days. The brain was eventually cut with a

241 cryostat into 50 µm or 80 µm coronal slices. For visualizing electrode and canula tracks, 80 µm
242 slices were stained with Hoechst 33342 (1:5000 dilution, H3570, ThermoFisher, Waltham, MA)
243 using standard techniques. For immunostaining of tyrosine hydroxylase, 50 µm slices were first
244 incubated for 1 h with blocking solution (a mixture of 5% BSA, 5% normal goat serum and 0.02%
245 Triton-X in PBS) and were then incubated overnight at 4 °C with primary antibody (rabbit anti-
246 tyrosine hydroxylase, 1:1000 dilution, ab112, abcam, Cambridge, United Kingdom). Slices were
247 washed with PBS, incubated for 4h at 4 °C with secondary antibody (Alexa Fluor 594 goat anti-
248 rabbit IgG, 1:500 dilution, R37117, ThermoFisher), and finally stained with Hoechst 33342.
249

RESULTS

We unilaterally injected 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) of mice to deplete dopaminergic neurons. 6-OHDA causes a unilateral depletion of dopaminergic fibers in the striatum and loss of dopaminergic neurons in ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) (**Figure 1A** and **1B**) (Lundblad et al., 2004; Thiele et al., 2012). The effectiveness of the lesion was assessed by comparing the number of weight bearing wall touches between the ipsilateral and contralateral forelimbs with a cylinder test (**Figure 1C**) (Schallert et al., 2000; Lundblad et al., 2002). Lesioned mice show a lower percentage of touches with the contralateral forelimb compared to intact mice (Lundblad et al., 2004). In accordance with the literature (Lundblad et al., 2004; Lundblad et al., 2005), we screened mice with motor deficits and included them in the study only if they showed less than 40% usage of the contralateral paw compared to control (**Figure 1D**). We confirmed the loss of dopaminergic neurons and fibers with histological staining.

Licking deficits with dopamine depletion

To assess for possible deficits in licking behaviors, 6-OHDA lesioned mice (n = 7) and vehicle injected control mice (n = 9) were trained to lick a spout to receive a drop of sucrose 1 s after an anticipatory auditory cue (**Figure 2A**). **Figure 2B** and **2C** show raster plots of licks from control and 6-OHDA lesioned mice, respectively. We analyzed the latency and duration of licking bouts (**Figure 2D**). The latency of bout initiation was significantly longer in lesioned mice compared to controls (2.40 ± 0.08 s vs 1.06 ± 0.04 s, $t_{(14)} = 15.78$, $p = 2.6 \times 10^{-10}$) (**Figure 2E).** The bout duration was shorter in lesioned mice relative to controls (1.05 ± 0.06 s vs 1.70 ± 0.062 s, $t_{(14)} = -7.24$, $p = 4.3 \times 10^{-6}$) (**Figure 2F**). The inter-lick interval, however, was not significantly affected (6-OHDA

lesion vs control: 138.8 ± 4.1 ms vs 144.8 ± 4.5 ms, $t_{(14)} = -1$, $p = 0.336$). In addition to the timing, we also assessed the direction of tongue movements during licking via analysis of videos of the orofacial region (**Figure 2G**). The direction of tongue movements was quantified by calculating the angle between the axis of symmetry of the tongue and the midline of the mouth (see methods). A positive angle indicated a directional bias toward the side ipsilateral to the lesion, whereas a negative angle indicated a contralateral bias. 6-OHDA lesioned mice showed a positive licking angle that was significantly different from that observed in control mice (27.9 ± 5.8 deg vs -0.6 ± 1.0 deg, Welch's t-test, $t_{(6)} = -4.82$, $p = 0.003$).

Altogether, these results demonstrate that mice with unilateral dopamine depletion have a longer latency to initiate a lick, a shorter duration of licking bouts, and a directional bias of the tongue toward the side ipsilateral to the lesion.

Changes in cue responses and preparatory activity in ALM after dopamine depletion

Evidence from the literature points at the anterior lateral motor cortex (ALM) as the area responsible for modulating licking and controlling licking direction (Komiyama et al., 2010; Guo et al., 2014; Li et al., 2015; Li et al., 2016; Chen et al., 2017; Inagaki et al., 2018). To assess possible deficits in neural activity associated with dopamine depletion, we bilaterally recorded single units from ALMs of control (175 single units; n = 9 mice) and 6-OHDA lesioned mice (161 single units; n = 7 mice) engaged in the cued-licking paradigm described above. Units recorded from both hemispheres of control mice were pooled together. Units from 6-OHDA lesioned mice were analyzed separately depending on whether they were recorded on the side ipsilateral or contralateral to the site of the 6-OHDA lesion. We focused on firing rate modulations occurring in the interval from the onset of the cue to the initiation of licking bouts. We aligned neural activity

296 either to the cue or to the bout initiation, and categorized neurons as cue responsive and/or
297 preparatory depending on whether their firing changed shortly after the cue and/or just before
298 licking (see methods). **Figure 3A** shows raster plots and PSTHs for two representative cue-
299 responsive neurons from control mice: one excited and one suppressed by the auditory cue. We
300 found that 41.7% of neurons (73 of 175 units) from control mice changed their firing rates within
301 500 ms from the onset of the cue. Differently, only 14.3% of neurons (12 of 84 units) from the
302 ipsilateral side, and 19.5% of neurons (15 of 77 units) from the contralateral side, of 6-OHDA
303 lesioned mice were cue responsive (**Figure 3B**). The differences in the proportion of cue
304 responsive neurons among these three groups were significant (Pearson's χ^2 test, $\chi^2_{(2)} = 25.48$, p
305 $= 2.6 \times 10^{-6}$). Specifically, the proportion of cue-responsive neurons in the ipsilateral and
306 contralateral side in 6-OHDA lesioned mice was similar (Pearson's χ^2 test, $\chi^2_{(1)} = 0.449$,
307 Bonferroni adjusted $p = 1$). However, it was significantly reduced from that observed in control
308 mice (Pearson's χ^2 test, control vs ipsilateral, $\chi^2_{(1)} = 18.14$, Bonferroni adjusted $p = 6.2 \times 10^{-5}$;
309 control vs contralateral, $\chi^2_{(1)} = 10.67$, Bonferroni adjusted $p = 0.003$).

310 A large fraction of cue responsive neurons was also preparatory (90.4%, 66 of 73 units from
311 control; 88.9%, 24 of 27 units from lesioned mice). However, not all preparatory neurons showed
312 modulation of their activity by the onset of the cue: 52.2% (72 of 138) of the units from control
313 and 78.9% (90 of 114) from lesioned animals did not show modulation by the cue. This difference
314 indicates that, in a subset of neurons, preparatory activity started longer than 500 ms after the cue,
315 thus closer to licking onset. **Figure 3C** shows raster plots and PSTHs of two representative neurons
316 with preparatory activity recorded in control mice: the activity of one of the neurons is increased
317 and that of the other neurons is suppressed before the initiation of a licking bout. In total, the
318 percentage of neurons showing preparatory activity was 78.9% (138/175) in control, 66.7% (56/84)

in the ipsilateral side and 75.3% (58/77) in the contralateral side of 6-OHDA lesioned mice (**Figure 3D**). Although the proportion of preparatory responses was similar across groups (Pearson's χ^2 test, $\chi^2_{(2)} = 4.50$, $p = 0.105$), there were significant differences in the ratio of excitatory and inhibitory responses (Pearson's χ^2 test, $\chi^2_{(2)} = 16.06$, $p = 3.2 \times 10^{-4}$). Specifically, neurons in the ipsilateral side of 6-OHDA lesioned mice showed a significantly larger proportion of excitatory responses when compared to neurons from control mice (ipsilateral side: 67.9% [38/56] excitatory, 32.1% [18/56] inhibitory; control: 38.4% [53/138] excitatory, 62.6% [85/138] inhibitory; Pearson's χ^2 test, $\chi^2_{(1)} = 17.72$, Bonferroni adjusted $p = 0.001$), and from the contralateral side of 6-OHDA lesioned mice (ipsilateral side: see above; contralateral side: 36.2% [21/58] excitatory, 63.8% [37/58] inhibitory; Pearson's χ^2 test, $\chi^2_{(1)} = 10.20$, Bonferroni adjusted $p = 0.004$).

Altogether, these results show that unilateral 6-OHDA lesions produce alterations in the proportion of cue responsive neurons and changes in the ratio of excitatory and inhibitory responses for preparatory activity.

Slower onset of preparatory responses in ALM after dopamine depletion

Given the high prevalence of preparatory responses in our experimental conditions, we further analyzed them to extract possible differences in their time course. Since preparatory activity in ALM is important for planning tongue-related movements (Guo et al., 2014; Li et al., 2015; Inagaki et al., 2018), it is reasonable to expect that the slow onset of licking observed with dopamine depletion may relate to changes in the latency of preparatory activity. **Figure 4A** and **4B** show raster plots and PSTHs of four representative neurons with preparatory responses aligned to the onset of the cue: two from control mice (**Figure 4A**, left: excitatory, right: inhibitory) and two from ipsilateral side of 6-OHDA lesioned mice (**Figure 4B**, left: excitatory, right: inhibitory).

342 **Figure 4C** and **4D** display the normalized responses (auROC, see methods) for all the preparatory
343 neurons recorded from both hemispheres of control and lesioned mice. Visual inspection of the
344 population activity suggests that the onset of preparatory firing may be delayed in 6-OHDA
345 lesioned mice. This suggestion is corroborated by population PSTHs shown in **Figure 4E**. The
346 latency of preparatory activity was directly quantified using a change point (CP) analysis approach
347 (see methods). Response latency differed across conditions (Kruskal-Wallis Test, $H_{(2)} = 30.68$, p
348 $= 2.2 \times 10^{-7}$). While neurons in the ipsilateral and contralateral side of 6-OHDA lesioned mice
349 showed preparatory responses with comparable latencies (0.82 ± 0.06 s vs 0.70 ± 0.05 s, n = 56
350 and 58 respectively, *post hoc* Tukey HSD test, $p = 0.184$); the latency in both groups was longer
351 than in control mice (ipsilateral side vs control: 0.82 ± 0.06 s vs 0.46 ± 0.03 s, n = 56 and 131
352 respectively, *post hoc* Tukey HSD test, $p = 4.2 \times 10^{-7}$; contralateral side vs control, 0.70 ± 0.05 s
353 vs 0.46 ± 0.03 s, n = 58 and 131 respectively, *post hoc* Tukey HSD test, $p = 0.003$) (**Figure 4F**
354 and **4G**).

355 To investigate preparatory activity relative to the onset of movement, we re-aligned spikes to
356 the initiation of a licking bout (**Figure 5A** and **5B**). Visual inspection of population PSTHs
357 suggests a possible difference in the latency of preparatory activity relative to licking initiation
358 (**Figure 5C**). Indeed, CP analysis revealed significant differences across conditions (Kruskal-
359 Wallis test, $H_{(2)} = 12.33$, $p = 0.002$) (**Figure 5D** and **5E**). There were no significant differences in
360 the onset of preparatory activity relative to the initiation of licking between neurons in control
361 mice and in the contralateral side of 6-OHDA lesioned mice (-0.73 ± 0.04 s vs -0.75 ± 0.07 s, n =
362 131 and 56 respectively, *post hoc* Tukey HSD test, $p = 1$). However, the onset of preparatory
363 activity in neuron from ipsilateral ALM in 6-OHDA lesioned mice was significantly closer to the
364 initiation of licking when compared to that in control mice (-0.51 ± 0.06 s vs -0.73 ± 0.04 s, n =

365 54 and 131 respectively, *post hoc* Tukey HSD test, $p = 0.002$), and contralateral ALM of 6-OHDA
366 lesioned mice (-0.51 ± 0.06 s vs -0.75 ± 0.07 s, n = 54 and 56 respectively, *post hoc* Tukey HSD
367 test, $p = 0.01$).

368 Altogether, neural recordings in 6-OHDA lesioned mice show that unilateral dopamine
369 depletion induces changes in cue responsiveness and preparatory activity. There are fewer cue
370 responsive neurons in lesioned animals. While the incidence of preparatory neurons was not
371 affected, 6-OHDA lesions altered the balance between excitation/inhibition and delayed the timing
372 of preparatory activity.

373

374 **D1 but not D2 receptor antagonism in ALM slows licking initiation**

375 The results described above demonstrate significant alterations of neural activity in ALM
376 following unilateral 6-OHDA lesions in the MFB. Are these changes epiphenomenal or indicative
377 of a contribution of ALM to the licking deficits observed in 6-OHDA lesioned mice? To determine
378 the link between dopaminergic modulation in ALM and licking deficits, we unilaterally and
379 acutely infused D1 or D2 receptor antagonists into ALM of a new cohort of unlesioned mice (naïve)
380 trained to perform the cued-licking paradigm. Infusion of a D1 receptor antagonist (SCH23390
381 hydrochloride, 5 µg/µl) significantly increased the latency of bout initiation (1.18 ± 0.05 s vs 1.47
382 ± 0.03 s, $n = 7$, paired t-test, $t_{(6)} = -6.64$, $p = 5.6 \times 10^{-4}$) (**Figure 6A**) and reduced the duration of
383 licking bouts (1.85 ± 0.09 s vs 1.09 ± 0.13 s, $n = 7$, paired t-test, $t_{(6)} = 9.62$, $p = 7.2 \times 10^{-5}$) when
384 compared to control, saline-infused, mice (**Figure 6B**). The licking angle, however, was not
385 significantly affected (SCH23390 vs saline: 3.6 ± 0.8 deg vs 1.0 ± 1.1 deg, $n = 7$, paired t-test, $t_{(6)}$
386 $= 1.61$, $p = 0.158$) (**Figure 6C**). Differently, ALM infusion of a D2 antagonist (raclopride tartrate
387 salt, 5 µg/µl) did not significantly affect the latency of bout initiation (raclopride vs saline: 1.16 ±

388 0.05 s vs 1.15 ± 0.04 s, $n = 9$, paired t-test, $t_{(8)} = 0.20$, $p = 0.85$) (**Figure 6D**), licking bout duration
389 (raclopride vs saline: 1.66 ± 0.11 s vs 1.65 ± 0.08 s, $n = 9$, paired t-test, $t_{(8)} = 0.09$, $p = 0.93$) (**Figure**
390 **6E**) or licking angle (raclopride vs saline: 0.1 ± 0.8 deg vs 0.5 ± 1.1 deg, $n = 6$, paired t-test, $t_{(8)} =$
391 0.55, $p = 0.60$) (**Figure 6F**).

392 These data demonstrate that acute, unilateral blockade of D1, but not D2, dopaminergic
393 signaling in ALM of naïve mice reproduces the behavioral impairments in licking initiation and
394 duration observed in 6-OHDA lesioned mice, but not the ipsilateral bias in licking direction.

395

396 **Blockade of dopamine D1 receptor in ALM affects cue responses and preparatory activity**

397 To identify the neural correlates of licking deficits observed after acute, local D1 receptor
398 blockade, we infused SCH23390 (or saline) unilaterally into ALM of mice performing the cued-
399 licking paradigm, and recorded single unit activity from the same side of the cortex. Unilateral
400 infusion of D1 receptor antagonist significantly reduced the proportion of cue responsive neurons
401 compared to saline infusions (SCH23390: 11.4% [5/44]; saline: 34.3% [12/35]; Pearson's χ^2 test,
402 $\chi^2_{(1)} = 4.78$, $p = 0.029$) (**Figure 7A**). Infusion of SCH23390 did not change the overall prevalence
403 of neurons with preparatory activity (SCH23390: 72.7% [32/44], saline: 65.7% [23/35], Pearson's
404 χ^2 test, proportion: $\chi^2_{(1)} = 0.182$, $p = 0.669$), nor the relative proportion of excitatory and inhibitory
405 response compared to control (SCH23390: 56.2% [18/32] excitatory, 43.8% [14/32] inhibitory;
406 saline: 47.8% [11/23] excitatory, 52.2% [12/23] inhibitory; Pearson's χ^2 test, proportion: $\chi^2_{(1)} =$
407 0.12, $p = 0.731$) (**Figure 7B** and **7C**). D1 receptors blockade did, however, affect the latency of
408 preparatory activity, as suggested by visual inspection of population PSTHs (**Figure 7C, 7D and**
409 **7G**). Quantification of the latency of preparatory activity relative to the cue revealed that D1
410 receptor antagonist infusion in ALM delayed its onset compared to control infusions (0.8 ± 0.09 s

vs 0.44 ± 0.06 s, n = 21 and 31 respectively, Wilcoxon rank-sum test, W = 414, p = 0.008) (**Figure 7E and 7F**). To compare the timing of preparatory activity relative to the onset of movement, we re-aligned spikes to the initiation of a licking bout. SCH23390 moved the onset of preparatory spiking closer to the initiation of licking compared to control (-0.52 ± 0.08 s vs -0.77 ± 0.10 s, n = 20 and 29 respectively, Wilcoxon rank-sum test, W = 403, P = 0.0496) (**Figure 7H and 7I**).

Altogether, these results show that acute intra-ALM infusion of a D1 receptor antagonist not only reproduces the slower licking initiation, but also recapitulates the reduction of cue responsive neurons and the slower onset of preparatory activity observed in 6-OHDA lesioned mice. Interestingly, neither lateral deviation of the tongue, nor changes in the proportion of excitatory and inhibitory responses were observed in animals infused with the antagonist.

DISCUSSION

The results presented here provide behavioral, pharmacological and electrophysiological evidence showing a link between dopaminergic transmission in ALM, dysfunction of ALM neural activity and licking deficits. 6-OHDA lesioned mice trained to perform a cued-licking task showed delayed licking initiation, shorter duration of licking bouts and deviated tongue protrusion compared to controls. Single unit recordings revealed that unilateral dopamine depletion affects neural activity in ALM in several ways. First, it reduces the number of neurons activated by an anticipatory cue. Second, it changes the ratio between excitatory and inhibitory preparatory activity preceding movement, leading to more excitatory and fewer inhibitory modulations in the lesioned hemisphere of 6-OHDA lesioned mice. Finally, unilateral dopamine depletion results in delayed preparatory activity compared to controls. To determine whether disruption of cortical dopaminergic modulation directly caused licking deficits, we locally infused D1 or D2 receptor

antagonists in ALM of unlesioned mice. Acutely antagonizing D1 receptors in ALM produced delayed licking initiation and shorter licking bouts. Single unit recordings after intra-ALM D1 blockade demonstrated that the behavioral deficits were associated with a reduction in the prevalence of cue responsive neurons and a delay in preparatory activity. Neither the lateral deviation of the tongue, nor the changes in the proportion of excitatory and inhibitory preparatory responses were reproduced by the infusion. Altogether, our data show a direct relationship between D1 receptor dopaminergic signaling in ALM, cue-evoked and preparatory firing and deficits in licking. More generally, these results suggest that cortical dopaminergic transmission may play a role in the genesis of some of the key symptoms of PD.

Licking behavior after dopamine depletion

Patients with PD suffer from orolingual dysfunction, including tongue tremor and tongue weakness. Previous studies aimed at understanding how dopamine depletion affects tongue movement showed that unilateral 6-OHDA lesion of the MFB in rats significantly reduced tongue force and slightly increased the duration of pressing time during a tongue pressing test (Ciucci et al., 2011; Nuckolls et al., 2012). However, these experiments relied on a complex task in which rats were trained to press a disk with their tongue, and did not investigate natural licking or its latency of onset. Here, we studied tongue movements in the context of simple cued-licking paradigm. 6-OHDA lesioned mice displayed slower licking initiation, shorter bout duration (i.e. fewer licks per bout) and deviated tongue protrusion. The lesion did not affect inter-licking interval, demonstrating that the speed of each lick was not an issue in our animals.

These motor deficits could reflect an inability to control movement initiation, execution and termination. It is possible that some of the deficits observed in our task may be secondary to

457 impairments in learning (Wise, 2004). For instance, delayed licking could derive from a reduced
458 ability to associate a predictive cue with a reward. According to this view, longer latency to initiate
459 movement would emerge from a weaker associative strength of the anticipatory signal or from the
460 lack of anticipatory dopaminergic signaling. While we do not exclude underlying learning deficits
461 in 6-OHDA lesioned mice, the results from acute unilateral infusions of D1 receptor antagonists
462 in ALM emphasize the importance of real-time dopaminergic activity in the cortex in initiating
463 movement.

464 ALM is known to regulate the direction of movement. Unilateral silencing of ALM activity,
465 either with pharmacological or optogenetic approaches, causes deviation of the tongue on the
466 ipsilateral side (Li et al., 2015). However, our local pharmacological manipulations demonstrate
467 that this effect cannot be produced by acute, unilateral intra-ALM impairments in dopaminergic
468 transmission. Hence, tongue deviation in 6-OHDA lesioned mice may result either from the effects
469 of chronic unilateral disruption of ALM dopaminergic transmission, or by deficits that initiate in
470 other nodes of the cortico-striatal loop (Von Voigtlander and Moore, 1973).

471 Altogether, our experiments establish active licking in mice as a model for studying motor
472 deficits in the context of dopamine depletion, and point to the importance of D1 receptor signaling
473 in ALM for mediating initiation and termination of tongue movements.

474

475 **Motor cortex and Parkinson's Disease**

476 Motor cortical activity is abnormal in PD patients and in animal models of PD (Lindenbach and
477 Bishop, 2013). Changes in general excitability, excitation/inhibition balance, and timing have been
478 described in the motor cortex during movement preparation or execution (Escola et al., 2003;

Lindenbach and Bishop, 2013; Pasquereau et al., 2015). Our results on ALM fit with the existing literature and significantly extend it.

We showed that unilateral 6-OHDA lesion of the medial forebrain bundle impacts activity in the ALM. There was a significant reduction in the proportion of neurons whose firing rates showed modulation by the cue predicting the arrival of the spout. This result is consistent with the hypothesis of hypo-activation of motor cortex in PD and with recordings from MPTP-treated monkeys showing fewer cue responsive neurons in lesioned animals compared to controls (Escola et al., 2003). Although the total number of neurons changing their firing rates just before licking (i.e., preparatory neurons) was not affected by unilateral 6-OHDA lesion, we observed alterations in the ratio of excitatory and inhibitory modulations. Changes in excitation and inhibition were described in motor cortices of PD patients using paired-pulse transcranial magnetic stimulation (Lefaucheur, 2005; Lindenbach and Bishop, 2013). Our comparison of excitatory and inhibitory preparatory activity revealed a reduction in the proportion of neurons inhibited and an increase in the proportion of neurons excited prior to movement, a result consistent with the decrease of GABAergic tone observed in PD patient (Ridding et al., 1995). Finally, in addition to the changes described above, we observed deficits in the timing of preparatory activity. Preparatory activity in 6-OHDA lesioned mice had a longer latency from the cue compared to control mice, consistent with the delayed onset of the licking initiation observed after 6-OHDA lesion. Unilateral dopamine depletion affected the timing of preparatory activity also when spiking was aligned to the onset of licking. These changes in timing of neural activity were also observed in primate models of PD and in human PD patients (Doudet et al., 1990; Pasquereau et al., 2015). Specifically, in a reaction time task, PD patients showed a longer latency in initiating movement paralleled by a slower buildup of neuronal activation over the motor cortex (Dick et al., 1989; Mazzoni et al., 2012).

The results from acute D1 receptor blockade experiments provide very important information regarding the relationship between firing abnormalities in the cortex and licking deficits. They demonstrate that the reduction of cue responsive neurons and the delaying of preparatory activity in ALM can be sufficient to generate changes in motor systems leading to delayed licking. Furthermore, the lack of changes in balance between excitatory and inhibitory preparatory activity is evidence that this abnormality has limited causal role with regard to licking timing, and perhaps is more involved in tongue deviation (a symptom not present after local manipulations of ALM).

Altogether, our results show changes in ALM activity consistent with those described in PD patients and validate the study of ALM control of licking as a model for understanding the cortical involvement in PD.

Dopaminergic modulation of cortical activity

Motor cortices receive direct dopaminergic innervation from the midbrain and dopaminergic inputs are known to play an important role in cortical plasticity and motor skill learning (Gaspar et al., 1991; Molina-Luna et al., 2009; Hosp et al., 2011; Guo et al., 2015). Dopamine exerts its function through five different receptors which are grouped into D1-like and D2-like receptors (Jaber et al., 1996). While both D1 and D2 receptors in motor cortex are important for modulating cortical plasticity and motor skill learning (Molina-Luna et al., 2009; Guo et al., 2015), here we show that dopaminergic signaling via D1, but not D2, receptors in ALM is required for modulating licking initiation and maintenance. This discrepancy may reflect the multiple functions of dopaminergic modulation in cortex. Our results indicate that acute D1 receptor signaling in ALM plays a role in modulating licking initiation and the timing of preparatory activity. This suggestion is consistent with recent findings showing transient activation of dopaminergic neurons before

525 self-paced movement initiation (Jin and Costa, 2010; Howe and Dombeck, 2016; da Silva et al.,
526 2018) and with experiments showing that optogenetic manipulation of transient dopaminergic
527 activity can causally affect movement initiation (da Silva et al., 2018). In addition, our results on
528 D1 receptor modulation of licking initiation dovetail nicely with existing literature in primates
529 (Sawaguchi, 1995) and with data showing the importance of D1, but not D2, dopaminergic
530 signaling in prefrontal cortex for the temporal control of action (Narayanan et al., 2012).

531 Our experiments clearly point at ALM D1 receptors as important in licking initiation and in
532 modulating cue responses and preparatory firing in ALM. How activation of D1 receptors
533 contribute to the patterns of activity observed in the ALM of mice performing a cued-licking
534 paradigm remains to be seen and will be the subject of future investigations.

535

REFERENCE

Chen T-WW, Li N, Daie K, Svoboda K (2017) A Map of Anticipatory Activity in Mouse Motor Cortex. Neuron 94:866-8790000.

Ciucci MR, Russell JA, Schaser AJ, Doll EJ, Vinney LM, Connor NP (2011) Tongue force and timing deficits in a rat model of Parkinson disease. Behavioural Brain Research 222:315-320.

Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N (2012) Neuron-type-specific signals for reward and punishment in the ventral tegmental area. Nature 482:85-88.

da Silva J, Tecuapetla F, Paixão V, Costa RM (2018) Dopamine neuron activity before action initiation gates and invigorates future movements. Nature 554:244.

Davis JD, Smith GP (1992) Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. Behavioral neuroscience 106:217-228.

Dick JP, Rothwell JC, Day BL, Cantello R, Buruma O, Gioux M, Benecke R, Berardelli A, Thompson PD, Marsden CD (1989) The Bereitschaftspotential is abnormal in Parkinson's disease. Brain : a journal of neurology 112 (Pt 1):233-244.

Doudet DJ, Gross C, Arluison M, Bioulac B (1990) Modifications of precentral cortex discharge and EMG activity in monkeys with MPTP-induced lesions of DA nigral neurons. Experimental Brain Research 80:177-188.

Escola L, Michelet T, Macia F, Guehl D, Bioulac B, Burbaud P (2003) Disruption of information processing in the supplementary motor area of the MPTP-treated monkeyA clue to the pathophysiology of akinesia? Brain 126:95-114.

Francardo V, Recchia A, Popovic N, Andersson D, Nissbrandt H, Cenci MA (2011) Impact of the lesion procedure on the profiles of motor impairment and molecular responsiveness to L-DOPA in the 6-hydroxydopamine mouse model of Parkinson's disease. Neurobiol Dis 42:327-340.

Gardner M, Fontanini A (2014) Encoding and Tracking of Outcome-Specific Expectancy in the Gustatory Cortex of Alert Rats. The Journal of Neuroscience 34:13000-13017.

Gaspar P, Duyckaerts C, Alvarez C, Javoy-Agid F, Berger B (1991) Alterations of dopaminergic and noradrenergic innervations in motor cortex in Parkinson's disease. Annals of neurology 30:365-374.

Goldberg JA, Boraud T, Maraton S, Haber SN, Vaadia E, Bergman H (2002) Enhanced synchrony among primary motor cortex neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primate model of Parkinson's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:4639-4653.

Guo L, Xiong H, Kim J-I, Wu Y-W, Lalchandani RR, Cui Y, Shu Y, Xu T, Ding JB (2015) Dynamic rewiring of neural circuits in the motor cortex in mouse models of Parkinson's disease. Nature Neuroscience 18:1299-1309.

Guo ZV, Li N, Huber D, Ophir E, Gutnisky D, Ting JT, Feng G, Svoboda K (2014) Flow of Cortical Activity Underlying a Tactile Decision in Mice. Neuron 81:179-194.

576 Hosp JA, Pekanovic A, Rioult-Pedotti MS, Luft AR (2011) Dopaminergic Projections from
577 Midbrain to Primary Motor Cortex Mediate Motor Skill Learning. The Journal of
578 Neuroscience 31:2481-2487.
579 Howe MW, Dombeck DA (2016) Rapid signalling in distinct dopaminergic axons during
580 locomotion and reward. Nature 535:505-510.
581 Inagaki HK, Inagaki M, Romani S, Svoboda K (2018) Low-dimensional and monotonic
582 preparatory activity in mouse anterior lateral motor cortex. The Journal of
583 Neuroscience:3152-3117.
584 Jaber M, Robinson SW, Missale C, Caron MG (1996) Dopamine receptors and brain function.
585 Neuropharmacology 35:1503-1519.
586 Jagmag SA, Tripathi N, Shukla SD, Maiti S, Khurana S (2016) Evaluation of Models of
587 Parkinson's Disease. Frontiers in Neuroscience 9:503.
588 Jezzini A, Mazzucato L, Camera G, Fontanini A (2013) Processing of Hedonic and Chemosensory
589 Features of Taste in Medial Prefrontal and Insular Networks. The Journal of Neuroscience
590 33:18966-18978.
591 Jin X, Costa RM (2010) Start/stop signals emerge in nigrostriatal circuits during sequence learning.
592 Nature 466:457-462.
593 Komiyama T, Sato TR, O'Connor DH, Zhang Y-X, Huber D, Hooks BM, Gabitto M, Svoboda K
594 (2010) Learning-related fine-scale specificity imaged in motor cortex circuits of behaving
595 mice. Nature 464:1182-1186.
596 Lefaucheur J-P (2005) Motor cortex dysfunction revealed by cortical excitability studies in
597 Parkinson's disease: influence of antiparkinsonian treatment and cortical stimulation.
598 Clinical Neurophysiology 116:244-253.
599 Li N, Daie K, Svoboda K, Druckmann S (2016) Robust neuronal dynamics in premotor cortex
600 during motor planning. Nature 532.
601 Li N, Chen T-W, Guo ZV, Gerfen CR, Svoboda K (2015) A motor cortex circuit for motor
602 planning and movement. Nature 519:51-56.
603 Lindenbach D, Bishop C (2013) Critical involvement of the motor cortex in the pathophysiology
604 and treatment of Parkinson's disease. Neuroscience & Biobehavioral Reviews 37:2737-
605 2750.
606 Liu H, Fontanini A (2015) State Dependency of Chemosensory Coding in the Gustatory Thalamus
607 (VPMpc) of Alert Rats. The Journal of Neuroscience 35:15479-15491.
608 Lundblad M, Picconi B, Lindgren H, Cenci MA (2004) A model of l-DOPA-induced dyskinesia
609 in 6-hydroxydopamine lesioned mice: relation to motor and cellular parameters of
610 nigrostriatal function. Neurobiology of Disease 16.
611 Lundblad M, Andersson M, Winkler C, Kirik D, Wierup N, Cenci MA (2002) Pharmacological
612 validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's
613 disease. European Journal of Neuroscience 15:120-132.

614 Lundblad M, Usiello A, Carta M, Håkansson K, Fisone G, Cenci MA (2005) Pharmacological
615 validation of a mouse model of l-DOPA-induced dyskinesia. Experimental Neurology
616 194:66-75.
617 Mazzoni P, Shabbott B, Cortés J (2012) Motor Control Abnormalities in Parkinson's Disease.
618 Cold Spring Harbor Perspectives in Medicine 2.
619 Molina-Luna K, Pekanovic A, Röhrich S, Hertler B, Schubring-Giese M, Rioult-Pedotti M-S, Luft
620 AR (2009) Dopamine in Motor Cortex Is Necessary for Skill Learning and Synaptic
621 Plasticity. PLoS ONE 4.
622 Narayanan NS, Land BB, Solder JE, Deisseroth K, DiLeone RJ (2012) Prefrontal D1 dopamine
623 signaling is required for temporal control. Proceedings of the National Academy of
624 Sciences 109:20726-20731.
625 Nuckolls AL, Worley C, Leto C, Zhang H, Morris JK, Stanford JA (2012) Tongue force and tongue
626 motility are differently affected by unilateral vs bilateral nigrostriatal dopamine depletion
627 in rats. Behavioural brain research 234:343-348.
628 Pasquereau B, Turner RS (2011) Primary motor cortex of the parkinsonian monkey: differential
629 effects on the spontaneous activity of pyramidal tract-type neurons. Primary motor cortex
630 of the parkinsonian monkey: differential effects on the spontaneous activity of pyramidal
631 tract-type neurons.
632 Pasquereau B, DeLong MR, Brain TRS (2015) Primary motor cortex of the parkinsonian monkey:
633 altered encoding of active movement. Primary motor cortex of the parkinsonian monkey:
634 altered encoding of active movement.
635 Ridding MC, Inzelberg R, Rothwell JC (1995) Changes in excitability of motor cortical circuitry
636 in patients with Parkinson's disease. Annals of neurology 37:181-188.
637 Sawaguchi T. (1995) The Role of Dopamine in Frontal Motor Cortical Functions of Monkeys. In:
638 Kimura M., Graybiel A.M. (eds) Functions of the Cortico-Basal Ganglia Loop. Springer,
639 Tokyo
640 Schallert T, Fleming, Leasure JL, Tillerson JL, Bland ST (2000) CNS plasticity and assessment of
641 forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation,
642 parkinsonism and spinal cord injury. Neuropharmacology 39:777-787.
643 Thiele SL, Warre R, Nash JE (2012) Development of a Unilaterally-lesioned 6-OHDA Mouse
644 Model of Parkinson's Disease. Journal of Visualized Experiments.
645 Travers JB, Dinardo LA, Karimnamazi H (1997) Motor and premotor mechanisms of licking.
646 Neuroscience and biobehavioral reviews 21:631-647.
647 Vincis R, Fontanini A (2016) Associative learning changes cross-modal representations in the
648 gustatory cortex. eLife 5.
649 Von Voigtlander PF, Moore KE (1973) Turning behavior of mice with unilateral 6-
650 hydroxydopamine lesions in the striatum: effects of apomorphine, L-DOPA, amanthadine,
651 amphetamine and other psychomotor stimulants. Neuropharmacology 12:451-462.
652 Wise RA (2004) Dopamine, learning and motivation. Nature Reviews Neuroscience 5.
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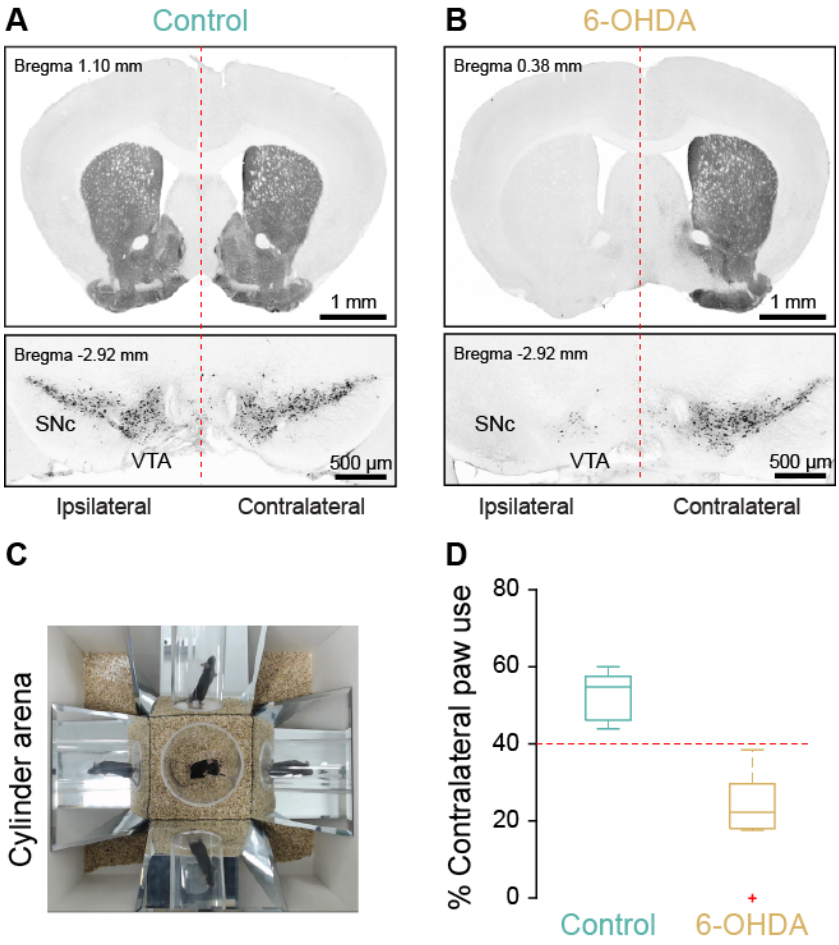
654 **FIGURE LEGEND**



655

656 **Figure 1.** Confirmation of lesion and motor deficits after unilateral 6-OHDA injections in MFB**.**
657 **A and B**, Representative tyrosine hydroxylase (TH) immunofluorescence staining showing
658 dopaminergic fibers in striatum (top panel) and dopaminergic neurons in SNc and VTA (bottom
659 panel) in a control mouse (**A**) and in a 6-OHDA lesioned mouse (**B**). Vertical dashed red lines
660 indicate the midline of the brain. **C**, A representative snapshot of a unilateral 6-OHDA lesioned
661 mouse performing the cylinder test. **D**, Boxplots of percentage of contralateral paw usage during
662 the cylinder test in control (n = 9, blue) and screened 6-OHDA lesioned mice (n = 7, brown).

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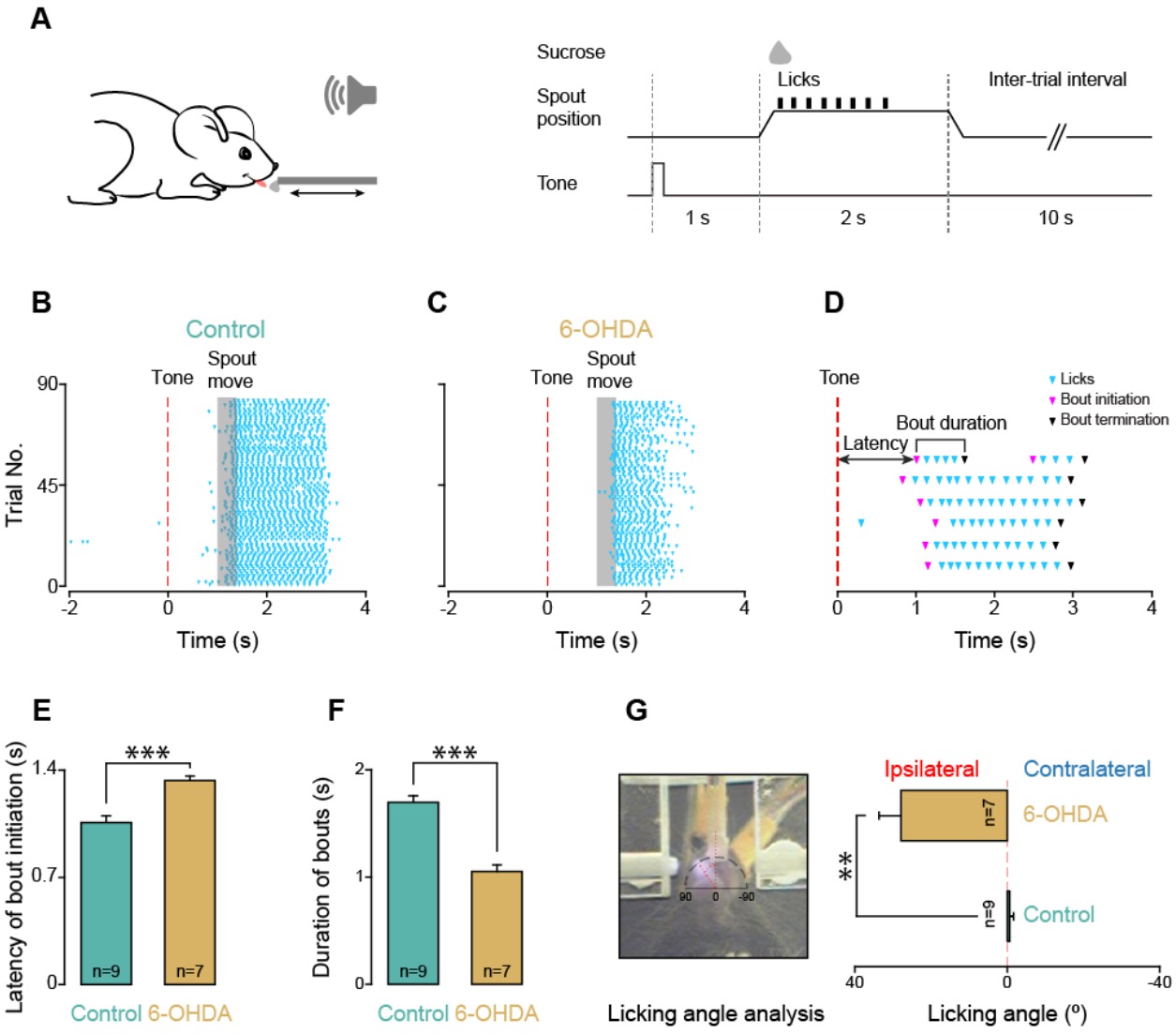


Figure 2. Licking deficits in 6-OHDA lesioned mice**. A,** *Left panel*: sketch showing a head-fixed mouse licking a spout to obtain sucrose. *Right panel*: schematic diagram of the experimental design for each trial. **B and C**, Representative raster plots of licking recorded from a control mouse (**B**) and a unilateral 6-OHDA lesioned (**C**) mouse performing the cued-licking paradigm. Dashed red vertical lines (time 0) indicate the onset of the auditory cue. Cyan triangles represent each individual lick. The gray shaded area highlights the movement of the spout. **D**, Representative raster plot of licking demonstrating bout analysis. A licking bout is defined as a train of at least three consecutive licks with an inter-lick interval shorter than 500 ms. Latency of bout initiation

673 is defined as the latency of the first lick of a licking bout after tone onset. Cyan triangles represent
674 each individual lick. Magenta triangles highlight the first lick of a licking bout (bout initiation)
675 and black triangles highlight the last lick of a licking bout. **E and F**, Average values of latency of
676 bout initiation (**E**) and duration of licking bouts (**F**) in control (n = 9 mice, blue) and 6-OHDA
677 lesioned (n = 7 mice, brown) mice (**E and F**, t-test, *** $p < 0.001$). Error bars represent SEM. **G**,
678 *Left panel*, a presentative snapshot showing a 6-OHDA lesioned mouse extending the tongue
679 towards the licking spout. Note that the tongue protrudes on the right compared to the midline of
680 the chin; *Right panel*: average values of the angles of tongue protrusion during licking in control
681 (n = 9 mice, blue) and 6-OHDA lesioned (n = 7 mice, brown) mice (Welch's corrected t-test, **p
682 <0.01). Error bars represent SEM.
683

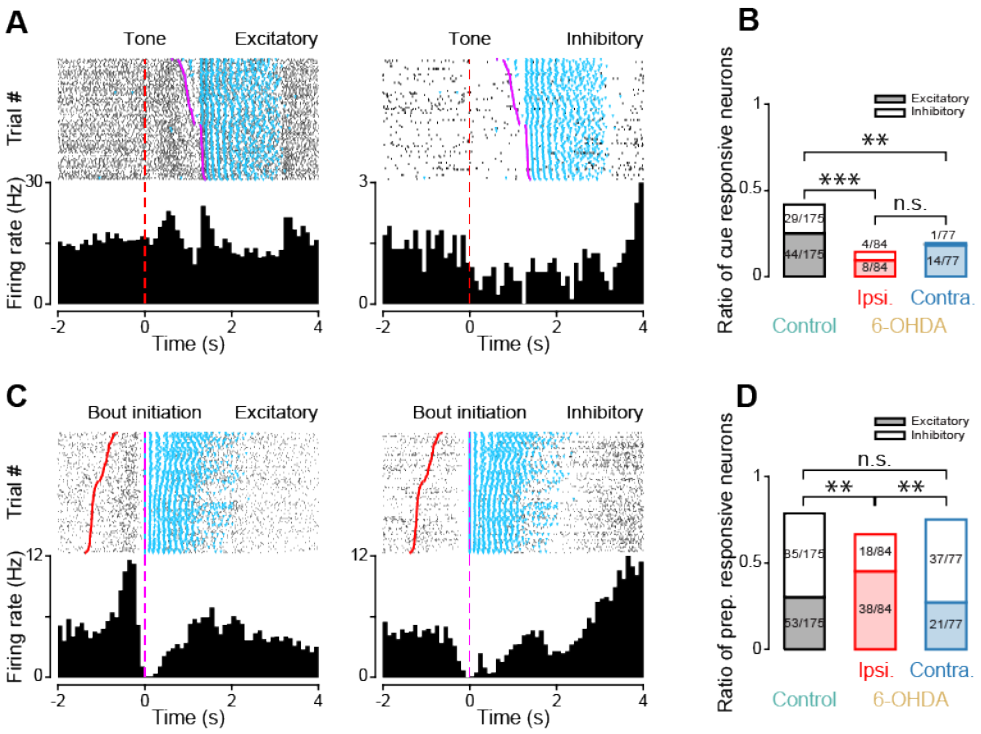


Figure 3. Cue responses and preparatory activity in ALM**. A,** Raster plots and PSTHs of neural activity recorded from two representative ALM neurons modulated by the cue within 500 ms from its onset. Dashed red vertical lines (time 0) indicate the onset of the auditory cue. Cyan markers represent each individual lick. Magenta markers represent the onset of each licking bout. Black ticks in raster plots represent individual action potentials. **B**, Proportion of cue responsive neurons in control mice (black) as well as ipsilateral (red) and contralateral (blue) sides of 6-OHDA lesioned mice (*post hoc* pairwise Pearson's χ^2 test for overall proportion of cue responsive neurons with Bonferroni correction, *** $p<0.001$, ** $p<0.01$, n.s. indicates not significant). **C,** Raster plots and PSTHs of neural activity recorded from two other ALM neurons modulated within 500 ms before licking bout initiation. Dashed magenta vertical lines (time 0) indicate the onset of the bout initiation. Cyan markers represent each individual lick. Red markers represent the onset of the cue. Black ticks in raster plots represent each action potential. **D**, Proportion of preparatory responsive neurons (filled: excitatory responses; empty: inhibitory responses) in control mice (black) as well

as ipsilateral (red) and contralateral (blue) sides of 6-OHDA lesioned mice (*post hoc* pairwise Pearson's χ^2 test for excitation-inhibition ratio of preparatory responses with Bonferroni correction, ** $p < 0.01$, n.s. indicates not significant).

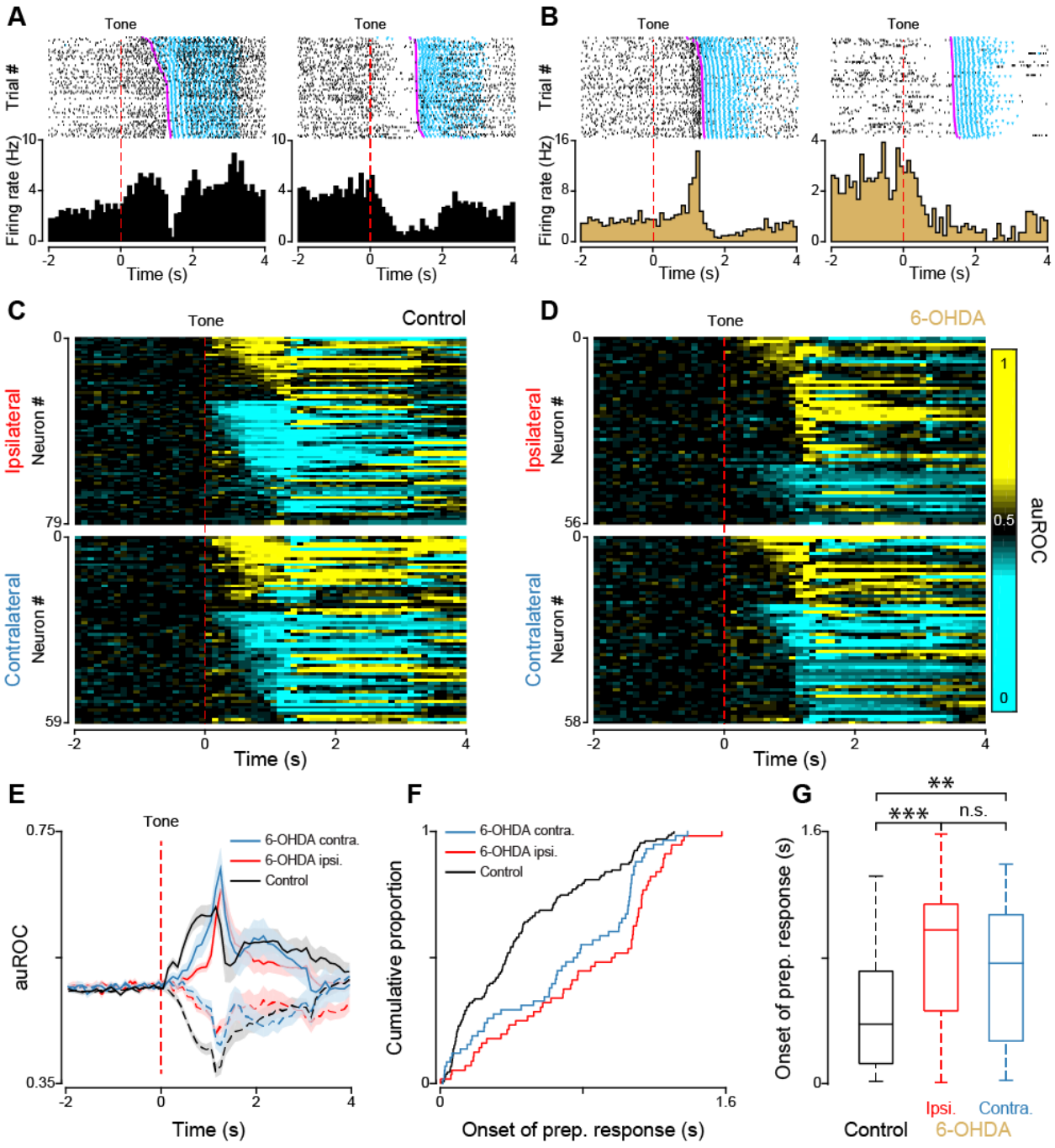


Figure 4. Timing of preparatory activity relative to the onset of the cue in control and 6-OHDA lesioned mice. **A and B**, Raster plots and PSTHs of neural activity recorded from four ALM neurons showing representative excitatory and inhibitory preparatory activity recorded from control (**A**) and 6-OHDA lesioned mice (**B**). Dashed red vertical lines (time 0) indicate the onset of the auditory cue. Cyan markers represent each individual lick. Magenta markers represent the

onset of each licking bout. Black vertical ticks in raster plots represent action potentials. **C and D**, Population plots of all ALM neurons recorded from ipsilateral and contralateral sides in control (**C**) and 6-OHDA lesioned (**D**) mice. Each row represents a neuron and the color of each square along the x axis represents the normalized (auROC) firing rate within each 100 ms bin. Dashed red vertical lines (time 0) indicate the onset of the auditory cue. **E**, Population PSTHs of excitatory and inhibitory preparatory responses from control mice (black; data from ipsilateral and contralateral ALM were pulled together), ipsilateral (red) and contralateral (blue) sides of 6-OHDA lesioned mice. The dashed red vertical line (time 0) indicates the onset of the auditory cue. The shadow area around each curve represents the corresponding SEM. **F and G**, Cumulative distributions (**F**) and boxplots (**G**) for the latency of preparatory activity relative to the cue onset in control (black), ipsilateral (red) and contralateral (blue) sides of 6-OHDA lesioned mice (Kruskal-Wallis test, post hoc Tukey HSD test, ** $p<0.01$, *** $p<0.001$, n.s. indicates not significant).

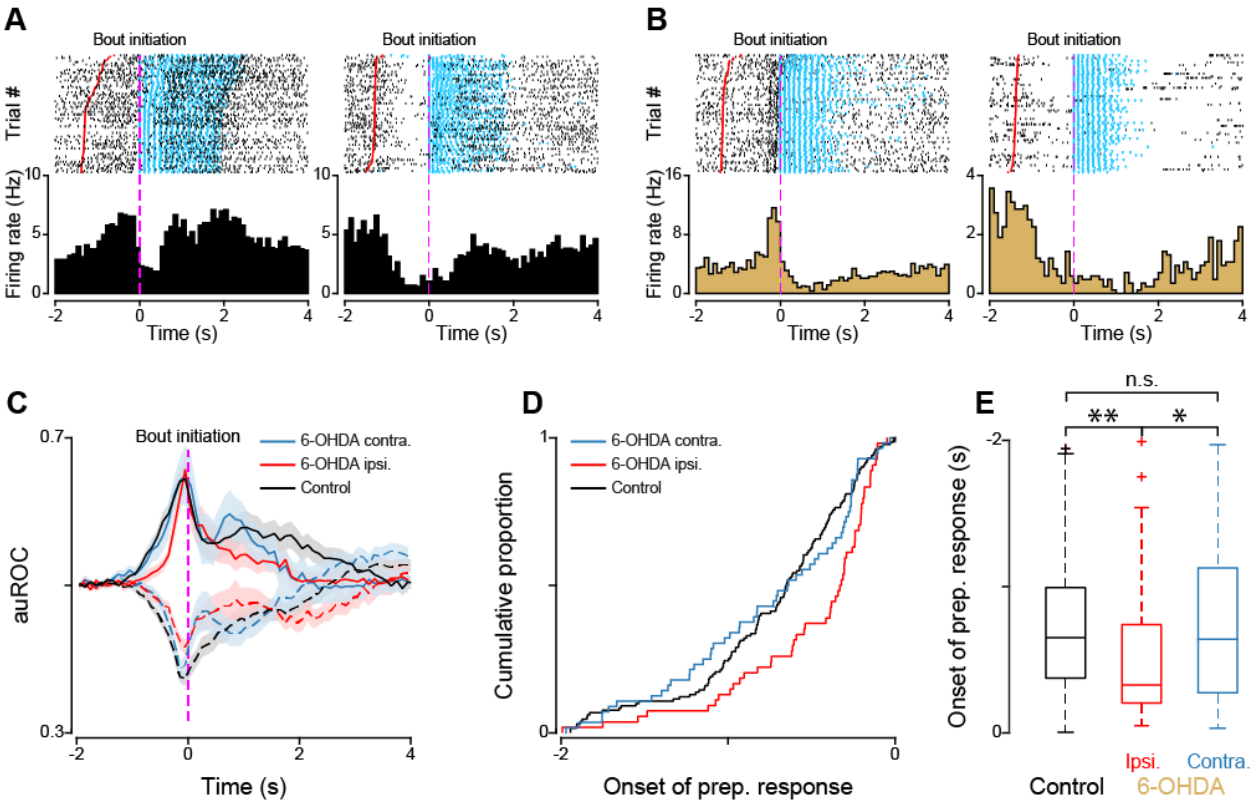


Figure 5. Timing of preparatory activity relative to the onset of a licking bout in control and 6-OHDA lesioned mice. **A and B**, Raster plots and PSTHs of the same ALM neurons shown in Fig. **4A** and **4B**, but re-aligned to licking bout initiation. Dashed magenta vertical lines (time 0) indicate the bout initiation, red markers indicate the onset of the auditory cue, cyan markers represent each individual lick. Black ticks in the raster plots represent individual action potential. **C,** Population PSTHs of excitatory and inhibitory preparatory responses recorded from ALM neurons of control mice (black), ipsilateral (red) and contralateral (blue) sides of 6-OHDA lesioned mice. The dashed magenta vertical line (time 0) indicates the initiation of licking bouts. The shadow area around each curve represents the corresponding SEM. **D and E,** Cumulative distributions (**D**) and boxplots (**E**) for the latency of preparatory activity relative to the bout initiation in control (black), ipsilateral (red) and contralateral (blue) sides of 6-OHDA lesioned mice (Kruskal-Wallis test, post hoc Tukey HSD test, * $p < 0.05$, ** $p<0.01$, n.s. indicates not significant).

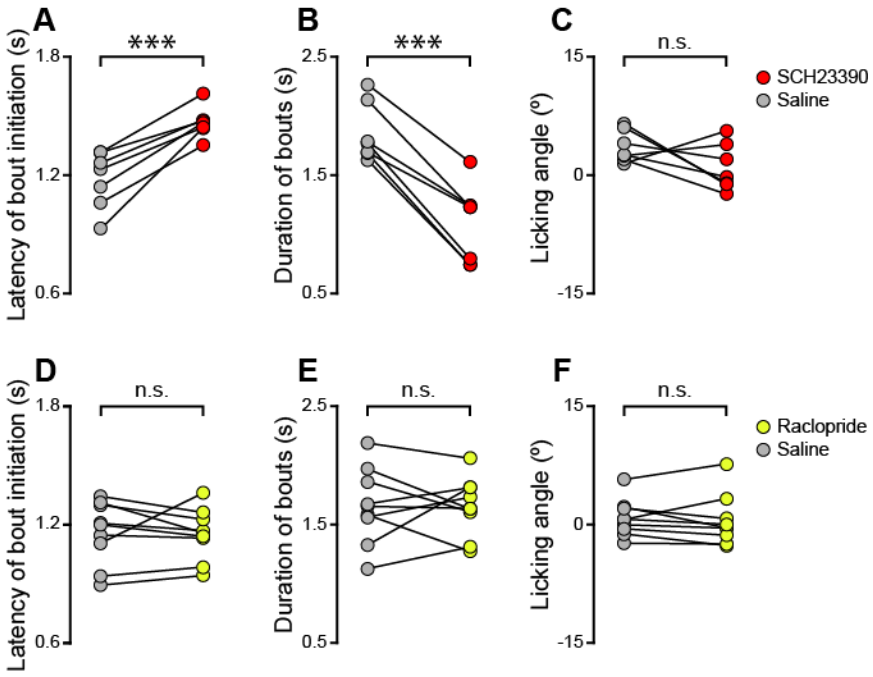
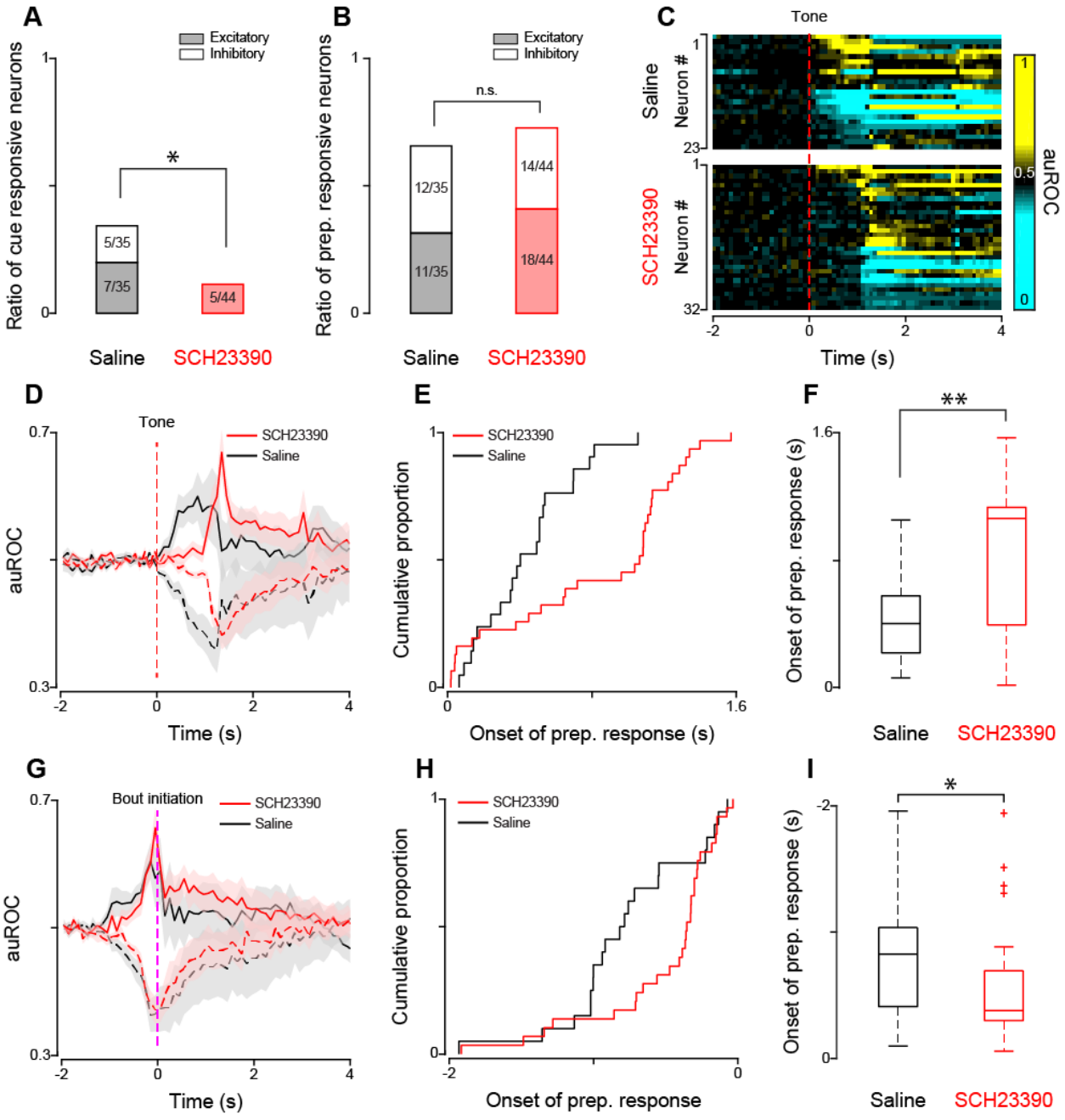


Figure 6. Effects of acute, local infusions of D1 and D2 receptor antagonists in ALM on licking. **A, B and C**, Latency of bout initiation (**A**), duration of licking bouts (**B**) and licking angle (**C**) recorded after unilateral infusion in ALM of saline (gray circles) or the D1 receptor antagonist, SCH23390 (red circles) (n = 7, paired t-test, *** $p<0.001$, n.s. indicates not significant). **D, E and F**, Latency of bout initiation (**D**), duration of bouts (**E**) and licking angle (**F**) recorded after unilateral infusion in ALM of saline (gray circles) or the D2 antagonist, raclopride (yellow circles) (n = 9, paired t-test, n.s. indicates not significant).



744

745 **Figure 7.** Effects of acute, local blockade of D1 receptor on patterns of single neuron activity in

746 ALM. **A and B**, Proportion of neurons with cue responses (**A**) and preparatory responses (**B**)

747 recorded with infusion of saline (black) and infusion of D1 receptor antagonist SCH23390 (red)

748 in ALM (Pearson's χ^2 test, * $p<0.05$, n.s. indicates not significant). ***C*****,** Population plot of

749 preparatory activity in ALM recorded from mice with infusion of saline (top) and D1 receptor

antagonist SCH23390 (bottom). Each row represents a neuron and the color of each square along the x axis represents the normalized (auROC) firing rate within each 100 ms bin. The dashed red vertical line (time 0) indicates the onset of the auditory cue. **D**, Population PSTH of preparatory activity after the infusion of saline (black) and SCH23390 (red). The dashed red vertical line (time 0) indicates the onset of the auditory cue. The shadow area around each curve represents the corresponding SEM. **E and F**, Cumulative distributions (**E**) and boxplots (**F**) for the latency of preparatory activity relative to the cue after the infusion of saline (black) and SCH23390 (red) (Wilcoxon rank-sum test, ** $p<0.01$). **G**, Population PSTH of preparatory activity with the infusion of saline (black) and SCH23390 (red). The dashed red vertical line (time 0) indicates the onset of licking bout initiation. The shadow area around each curve represents the corresponding SEM. **H and I**, Cumulative distributions (**H**) and boxplots (**I**) of the latency of preparatory activity relative to the licking bout initiation with the infusion of saline (black) and SCH23390 (red) (Wilcoxon rank-sum test, * $p<0.05$).