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1 **Disruption of cortical dopaminergic modulation delays licking initiation**
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5 Ke Chen[1,2], Roberto Vincis[1], Alfredo Fontanini[1,2]
6
7 [1] Department of Neurobiology and Behavior and [2] Graduate Program in Neuroscience, State
8 University of New York at Stony Brook, Stony Brook, NY, 11794, USA
9
10 Corresponding authors: Ke Chen and Alfredo Fontanini
11 Department of Neurobiology and Behavior
12 Room 545, Life Science Building
13 SUNY at Stony Brook
14 Stony Brook, 11794, NY, USA.
15 Tel: +1 631 632 3242
16 Email: alfredo.fontanini@stonybrook.edu or ke.chen@stonybrook.edu
17
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20 **ABSTRACT**

21 Parkinson's disease (PD) is a neurodegenerative disorder affecting motor control. Dysfunction
22 of motor cortices has been suggested to contribute to the motor symptoms of PD. However, little
23 is known on the link between cortical dopaminergic loss, abnormalities in neural activity and motor
24 deficits. We address this issue by focusing on the anterior lateral motor cortex (ALM) of mice
25 performing a cued-licking task. We first demonstrate licking deficits and concurrent alterations of
26 spiking activity in ALM of hemi-parkinsonian mice. Hemi-parkinsonian mice displayed delayed
27 licking initiation, shorter duration of licking bouts, and lateral deviation of tongue protrusions. In
28 addition, we observed a reduction in cue responsive neurons and altered preparatory activity. Acute
29 and local blockade of D1 receptors in ALM recapitulated some of the behavioral and neural deficits
30 observed in hemi-parkinsonian mice. Our data show a direct relationship between cortical D1
31 receptor modulation, cue-evoked and preparatory activity in ALM, and licking initiation.

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32 **INTRODUCTION**

33 Dysfunction of motor cortices, which are important for movement planning, initiation and
34 execution, has been suggested to play a role in the motor symptoms of Parkinson's disease (PD)
35 (Lindenbach and Bishop, 2013). Studies on motor cortices of human patients and animal models
36 of PD revealed abnormalities in preparatory activity, excitability, excitation/inhibition balance and
37 oscillatory dynamics (Doudet et al., 1990; Ridding et al., 1995; Goldberg et al., 2002; Escola et
38 al., 2003; Lefaucheur, 2005; Pasquereau and Turner, 2011; Pasquereau et al., 2015). However,
39 several questions regarding the role of motor cortices in the pathogenesis of PD symptoms remain
40 unanswered. First, it is unclear whether abnormal patterns of motor cortical activity are secondary
41 to dysfunction of the basal ganglia or whether they result from disruption of local dopaminergic
42 modulation. Related to this is the question of the causal role of specific motor cortical
43 abnormalities in generating some of the parkinsonian symptoms. In fact, little is known about the
44 direct link between loss of dopaminergic signaling in the cortex, alterations of motor cortical single
45 unit activity, and motor deficits.

46 Here, we investigate the role of motor cortex dopaminergic transmission in movement initiation
47 and execution focusing on the anterior lateral motor cortex (ALM) of mice engaged in a cued-
48 licking task. We choose licking because it is an innate motor behavior whose cortical control is
49 well-studied. Licking in rodents is regulated by a central pattern generator circuit in the brainstem,
50 which is under the control of the motor cortex (Travers et al., 1997). ALM plays an important role
51 in the planning and execution of licking (Komiyama et al., 2010; Guo et al., 2014; Li et al., 2015;
52 Inagaki et al., 2018), as reflected by the presence of neurons whose firing rates are modulated
53 before the onset of the movement (defined as "preparatory" neurons) (Guo et al., 2014; Li et al.,
54 2015; Chen et al., 2017; Inagaki et al., 2018). In addition, this area appears to be responsible for

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55 controlling the direction of tongue movements, as optogenetic silencing of ALM can introduce a
56 directional bias towards the ipsilateral side (Guo et al., 2014; Li et al., 2015). Although ALM has
57 been studied for its involvement in controlling normal licking, how lack of dopaminergic signaling
58 impacts activity and function of this region remains unknown.

59 The experiments described here rely on behavioral training, pharmacology, and
60 electrophysiological recordings to study licking deficits and related abnormalities of ALM neural
61 activity in hemi-parkinsonian mice. First, we show that hemi-parkinsonian mice (i.e., mice injected
62 with 6-OHDA into the medial forebrain bundle) display delayed licking initiation, shorter duration
63 of licking bouts, and deviated tongue protrusion compared to control animals. Next, we report
64 changes in ALM neurons cue responses and preparatory activity in the PD model. Finally, we
65 perform local pharmacological blockade of dopaminergic receptors to determine the contribution
66 of cortical dopaminergic deficit in ALM to the electrophysiological and behavioral alterations seen
67 in hemi-parkinsonian mice.

68 Using licking as a model behavior, our data show motor deficits and abnormalities in neural
69 activity associated with parkinsonism, and further demonstrate the importance of dopaminergic
70 signaling in ALM for licking initiation and for modulating preparatory activity.

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72 **RESULTS**

73 Unilateral injections of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB)
74 of mice were used to model PD. 6-OHDA causes a unilateral depletion of dopaminergic fibers in
75 the striatum and loss of dopaminergic neurons in ventral tegmental area (VTA) and substantia
76 nigra pars compacta (SNc) (**Figure 1A** and **1B**) (Lundblad et al., 2004; Thiele et al., 2012). The
77 effectiveness of the lesion was assessed by comparing the number of weight bearing wall touches
78 between the ipsilateral and contralateral forelimbs with a cylinder test (**Figure 1C**) (Schallert et
79 al., 2000; Lundblad et al., 2002). Lesioned mice show a lower percentage of touches with the
80 contralateral forelimb compared to intact mice (Lundblad et al., 2004). In accordance with the
81 literature (Lundblad et al., 2004; Lundblad et al., 2005), we defined mice as hemi-parkinsonian
82 (and as such eligible for this study) if they showed less than 40% usage of the contralateral paw
83 compared to control (**Figure 1D**). We confirmed the loss of dopaminergic fibers with histological
84 staining.

85

86 **Licking deficits in hemi-parkinsonian mice**

87 To assess for possible deficits in licking behaviors, hemi-parkinsonian mice (n = 7) and vehicle
88 injected control mice (n = 9) were trained to lick a spout to receive a drop of sucrose 1 s after an
89 anticipatory auditory cue (**Figure 2A**). **Figure 2B** and **2C** show raster plots of licks from control
90 and hemi-parkinsonian mice, respectively. We analyzed the latency and duration of licking bouts
91 (**Figure 2D**). The latency of bout initiation was significantly longer in lesioned mice compared to
92 controls (2.40 ± 0.08 s vs 1.06 ± 0.04 s, $t_{(14)} = 15.78$, $p < 0.001$) (**Figure 2E).** The bout duration
93 was shorter in lesioned mice relative to controls (1.05 ± 0.06 s vs 1.70 ± 0.062 s, $t_{(14)} = -7.24$, $p <$
94 0.001) (**Figure 2F**). The inter-lick interval, however, was not significantly affected (hemi-

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95 parkinsonian vs control: 138.8 ± 4.1 ms vs 144.8 ± 4.5 ms, $t_{(14)} = -1$, $p = 0.336$). In addition to the
96 timing, we also assessed the direction of tongue movements during licking via analysis of videos
97 of the orofacial region (**Figure 2G**). The direction of tongue movements was quantified by
98 calculating the angle between the axis of symmetry of the tongue and the midline of the mouth
99 (see methods). A positive angle indicated a directional bias toward the side ipsilateral to the lesion,
100 whereas a negative angle indicated a contralateral bias. Hemi-parkinsonian mice showed a positive
101 licking angle that was significantly different from that observed in control mice (27.9 ± 5.8 deg vs
102 -0.6 ± 1.0 deg, Welch's t-test, $t_{(6)} = -4.82$, $p < 0.01$).
103 Altogether, these results demonstrate that hemi-parkinsonian mice have a longer latency to
104 initiate a lick, a shorter duration of licking bouts, and a directional bias of the tongue toward the
105 side ipsilateral to the lesion.
106

107 **Changes in cue responses and preparatory activity in ALM of hemi-parkinsonian mice**

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

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118 either to the cue or to the bout initiation, and categorized neurons as cue responsive and/or
119 preparatory depending on whether their firing changed shortly after the cue and/or just before
120 licking (see methods). **Figure 3A** shows raster plots and PSTHs for two representative cue-
121 responsive neurons from control mice: one excited and one suppressed by the auditory cue. We
122 found that 41.7% of neurons (73 of 175 units) from control mice changed their firing rates within
123 500 ms from the onset of the cue, while only 14.3% of neurons (12 of 84 units) from the ipsilateral
124 side and 19.5% of neurons (15 of 77 units) from the contralateral side of hemi-parkinsonian mice
125 were cue responsive (**Figure 3B**). The differences in the proportion of cue responsive neurons
126 among these three groups were significant (Pearson's χ^2 test, $\chi^2_{(2)} = 25.48$, $p < 0.001$). Specifically,
127 the proportion of cue-responsive neurons in the ipsilateral and contralateral side in hemi-
128 parkinsonian was similar (Pearson's χ^2 test, $\chi^2_{(1)} = 0.449$, Bonferroni adjusted $p = 1$), and
129 significantly reduced from that observed in control mice (Pearson's χ^2 test, control vs ipsilateral,
130 $\chi^2_{(1)} = 18.14$, Bonferroni adjusted $p < 0.001$; control vs contralateral, $\chi^2_{(1)} = 10.67$, Bonferroni
131 adjusted $p < 0.01$).
132 A large fraction of cue responsive neurons was also preparatory (90.4%, 66 of 73 units from
133 control; 88.9%, 24 of 27 units from lesioned mice). However, not all preparatory neurons showed
134 modulation of their activity by the onset of the cue: 52.2% (72 of 138) of the units from control
135 and 78.9% (90 of 114) from lesioned animals did not show modulation by the cue. This difference
136 indicates that, in a subset of neurons, preparatory activity started longer than 500 ms after the cue,
137 thus closer to licking onset. **Figure 3C** shows raster plots and PSTHs of two representative neurons
138 with preparatory activity recorded in control mice: the activity of one of the neurons is increased
139 and that of the other neurons is suppressed before the initiation of a licking bout. In total, the
140 percentage of neurons showing preparatory activity was 78.9% (138/175) in control, 66.7% (56/84)

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141 in the ispsilateral side and 75.3% (58/77) in the contralateral side of hemi-parkinsonian mice
142 (**Figure 3D**). Although the proportion of preparatory responses was similar across groups
143 (Pearson's χ^2 test, $\chi^2_{(2)} = 4.50$, $p = 0.105$), there were significant differences in the ratio of
144 excitatory and inhibitory responses (Pearson's χ^2 test, $\chi^2_{(2)} = 16.06$, $p < 0.001$). Specifically, the
145 ipsilateral side from hemi-parkinsonian mice had a significantly different proportion of excitatory
146 and inhibitory responses compared to control (ipsilateral side: 67.9% [38/56] excitatory, 32.1%
147 [18/56] inhibitory; control: 38.4% [53/138] excitatory, 62.6% [85/138] inhibitory; Pearson's χ^2
148 test, $\chi^2_{(1)} = 17.72$, Bonferroni adjusted $p < 0.001$) and compared to the contralateral side (ipsilateral
149 side: see above; contralateral side: 36.2% [21/58] excitatory, 63.8% [37/58] inhibitory; Pearson's
150 χ^2 test, $\chi^2_{(1)} = 10.20$, Bonferroni adjusted $p < 0.01$).
151 Altogether, these results show that unilateral 6-OHDA lesions produce alterations in the
152 proportion of cue responsive neurons and changes in the balance between excitation and inhibition
153 for preparatory activity.
154
155 **Slower onset of preparatory responses in ALM of hemi-parkinsonian mice**
156 Given the high prevalence of preparatory responses in our experimental conditions, we further
157 analyzed them to extract possible differences in their time course. Since preparatory activity in
158 ALM has been shown to be important for planning tongue-related movements (Guo et al., 2014;
159 Li et al., 2015; Inagaki et al., 2018), it is reasonable to expect that the slow onset of licking
160 observed with dopamine depletion may relate to changes in the latency of preparatory activity.
161 **Figure 4A** and **4B** show raster plots and PSTHs of four representative neurons with preparatory
162 responses aligned to the onset of the cue: two from control mice (**Figure 4A**, left: excitatory, right:
163 inhibitory) and two from ipsilateral side of hemi-parkinsonian mice (**Figure 4B**, left: excitatory,

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164 right: inhibitory). **Figure 4C** and **4D** displays the normalized responses (auROC, see methods) for
165 all the preparatory neurons recorded from both hemispheres of control and lesioned mice. Visual
166 inspection of the population activity suggests that the onset of preparatory firing may be delayed
167 in hemi-parkinsonian mice. This suggestion is corroborated by population PSTHs shown in **Figure**
168 **4E**. The latency of preparatory activity was directly quantified using a change point (CP) analysis
169 approach (see methods). Response latency differed across conditions (Kruskal-Wallis Test, $H_{(2)}$ =
170 30.68, $p < 0.001$). While neurons in the ipsilateral and contralateral side of hemi-parkinsonian
171 mice showed preparatory responses with comparable latencies (0.82 ± 0.06 s vs 0.70 ± 0.05 s, n =
172 56 and 58 respectively, *post hoc* Tukey HSD test, $p = 0.184$), for both groups the latency was
173 longer than that in control mice (ipsilateral side vs control: 0.82 ± 0.06 s vs 0.46 ± 0.03 s, n = 56
174 and 131 respectively, *post hoc* Tukey HSD test, $p < 0.001$; contralateral side vs control, 0.70 ±
175 0.05 s vs 0.46 ± 0.03 s, n = 58 and 131 respectively, *post hoc* Tukey HSD test, $p < 0.01$) (**Figure**
176 **4F** and **4G**).

177 To investigate preparatory activity relative to the onset of movement, we re-aligned spikes to
178 the initiation of a licking bout (**Figure 5A** and **5B**). Visual inspection of population PSTHs
179 suggests a possible difference in the latency of preparatory activity relative to licking initiation
180 (**Figure 5C**). Indeed, CP analysis revealed significant differences across conditions (Kruskal-
181 Wallis test, $H_{(2)} = 12.33$, $p < 0.01$) (**Figure 5D** and **5E**). Although there were no significant
182 differences in the onset of preparatory activity relative to the initiation of licking between the
183 control and contralateral side of hemi-parkinsonian mice (-0.73 ± 0.04 s vs -0.75 ± 0.07 s, n = 131
184 and 56 respectively, *post hoc* Tukey HSD test, $p = 1$), the onset of preparatory activity in the
185 ipsilateral ALM of hemi-parkinsonian mice was significantly closer to the initiation of licking
186 when compared to that in control mice (-0.51 ± 0.06 s vs -0.73 ± 0.04 s, n = 54 and 131 respectively,

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187 *post hoc* Tukey HSD test, $p < 0.01$) and contralateral ALM of hemi-parkinsonian mice (-0.51 ±
188 0.06 s vs -0.75 ± 0.07 s, n = 54 and 56 respectively, *post hoc* Tukey HSD test, $p < 0.05$).
189 Altogether, neural recordings in hemi-parkinsonian mice show that unilateral dopamine
190 depletion induces changes in cue responsiveness and preparatory activity. There are fewer cue
191 responsive neurons in lesioned animals. While the incidence of preparatory neurons was not
192 affected, 6-OHDA lesions altered the balance between excitation/inhibition and the timing of
193 preparatory activity.
194

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

196 The results described above demonstrate significant alterations of neural activity in ALM
197 following unilateral 6-OHDA lesions. Are these changes epiphenomenal or indicative of a
198 contribution of ALM to the licking deficits observed in hemi-parkinsonian mice? To determine the
199 link between dopaminergic modulation in ALM and licking deficits, we unilaterally and acutely
200 infused D1 or D2 receptor antagonists into ALM of a new cohort of unlesioned mice (naïve) trained
201 to perform the cued-licking paradigm. Infusion of a D1 receptor antagonist (SCH23390
202 hydrochloride, 5 µg/µl) significantly increased the latency of bout initiation (1.18 ± 0.05 s vs 1.47
203 ± 0.03 s, $n = 7$, paired t-test, $t_{(6)} = -6.64$, $p < 0.01$) (**Figure 6A**), and reduced the duration of licking
204 bouts (1.85 ± 0.09 s vs 1.09 ± 0.13 s, $n = 7$, paired t-test, $t_{(6)} = 9.62$, $p < 0.01$) compared to control
205 saline-infused mice (**Figure 6B**). The licking angle, however, was not significantly affected
206 (SCH23390 vs saline: 3.6 ± 0.8 deg vs 1.0 ± 1.1 deg, $n = 7$, paired t-test, $t_{(6)} = 1.61$, $p = 0.158$)
207 (**Figure 6C**). Differently, ALM infusion of a D2 antagonist (raclopride tartrate salt, 5 µg/µl) did
208 not significantly affect the latency of bout initiation (raclopride vs saline: 1.16 ± 0.05 s vs 1.15 ±
209 0.04 s, $n = 9$, paired t-test, $t_{(8)} = 0.20$, $p = 0.85$) (**Figure 6D**), licking bout duration (raclopride vs

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210 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 6E**) or
211 licking angle (raclopride vs saline: 0.1 ± 0.8 deg vs 0.5 ± 1.1 deg, $n = 6$, paired t-test, $t_{(8)} = 0.55$, p
212 $= 0.60$) (**Figure 6F**).

213 These data demonstrate that acute, unilateral blockade of D1 dopaminergic signaling in ALM
214 of naïve mice reproduces the behavioral impairments in licking initiation and duration observed in
215 hemi-parkinsonian mice, but not the ipsilateral bias in licking direction.

216

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

218 To identify the neural correlates of the licking deficits observed after acute, local D1 receptor
219 blockade, we infused SCH23390 (or saline) unilaterally into ALM of mice performing the cued-
220 licking paradigm and recorded single unit activity from the same side of the cortex. Unilateral
221 infusion of D1 receptor antagonist significantly reduced the proportion of cue responsive neurons
222 compared to saline infusions (SCH23390: 11.4% [5/44]; saline: 34.3% [12/35]; Pearson's χ^2 test,
223 $\chi^2_{(1)} = 4.78$, $p < 0.05$) (**Figure 7A**). Infusion of SCH23390 did not change the overall prevalence
224 of neurons with preparatory activity (SCH23390: 72.7% [32/44], saline: 65.7% [23/35], Pearson's
225 χ^2 test, proportion: $\chi^2_{(1)} = 0.182$, $p = 0.669$), nor the relative proportion of excitatory and inhibitory
226 response compared to control (SCH23390: 56.2% [18/32] excitatory, 43.8% [14/32] inhibitory;
227 saline: 47.8% [11/23] excitatory, 52.2% [12/23] inhibitory; Pearson's χ^2 test, proportion: $\chi^2_{(1)} =$
228 0.12, $p = 0.731$) (**Figure 7B** and **7C**). D1 receptors blockade did, however, affect the latency of
229 preparatory activity, as suggested by visual inspection of population PSTHs (**Figure 7C, 7D and**
230 **7G**). Quantification of the latency of preparatory activity relative to the cue revealed that D1
231 receptor antagonist infusion in ALM delayed its onset compared to control infusions (0.8 ± 0.09 s
232 vs 0.44 ± 0.06 s, n = 21 and 31 respectively, Wilcoxon rank-sum test, W = 414, $p < 0.01$) (**Figure**

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233 **7E and 7F**). To compare the timing of preparatory activity relative to the onset of movement, we
234 re-aligned spikes to the initiation of a licking bout. SCH23390 moved the onset of preparatory
235 spiking closer to the initiation of licking compared to control (-0.52 ± 0.08 s vs -0.77 ± 0.10 s, n =
236 20 and 29 respectively, Wilcoxon rank-sum test, W = 403, $P < 0.05$) (**Figure 7H and 7I**).

237 Altogether, these results show that acute, intra-ALM infusion of a D1 receptor antagonist not
238 only reproduces the slower licking initiation, but also recapitulates the reduction of cue responsive
239 neurons and the slower onset of preparatory activity observed in hemi-parkinsonian mice.
240 Interestingly, neither lateral deviation of the tongue, not changes in the proportion of excitatory
241 and inhibitory responses were observed in animals infused with the antagonist.

242

243 **DISCUSSION**

244 The results presented here provide behavioral, pharmacological and electrophysiological
245 evidence regarding how dysfunction of ALM neural activity relates to licking deficits in hemi-
246 parkinsonian mice. Hemi-parkinsonian mice trained to perform a cued-licking task showed
247 delayed licking initiation, shorter duration of licking bouts and deviated tongue protrusion
248 compared to controls. Single unit recordings revealed that unilateral dopamine depletion affects
249 neural activity in ALM in several ways. First, it reduces the numbers of neurons activated by an
250 anticipatory cue. Second, it changes the ratio between excitatory and inhibitory preparatory
251 activity preceding movement, leading to more excitatory and fewer inhibitory modulations in the
252 lesioned hemisphere of hemi-parkinsonian mice. Finally, unilateral dopamine depletion resulted
253 in delayed preparatory activity compared to controls. To determine whether disruption of cortical
254 dopaminergic modulation directly caused licking deficits, we locally infused D1 or D2 receptor
255 antagonists in ALM of unlesioned mice. Acutely antagonizing D1 receptors in ALM produced

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256 delayed licking initiation and shorter licking bouts. Single unit recordings after intra-ALM D1
257 blockade demonstrated that the behavioral deficits were associated with a reduction in the
258 prevalence of cue responsive neurons and a delay in preparatory activity. Neither the lateral
259 deviation of the tongue, nor the changes in the proportion of excitatory and inhibitory preparatory
260 responses were mimicked by the infusion. Altogether, our data show a direct relationship between
261 D1 receptor dopaminergic signaling in ALM, cue-evoked and preparatory activity and deficits in
262 licking. More generally, these results emphasize the importance of cortical dopaminergic
263 transmission in the genesis of some of the key symptoms of PD.
264
265 **Licking in Parkinson's Disease**
266 Previous studies showed that unilateral 6-OHDA lesion of the MFB in rats significantly reduced
267 tongue force and slightly increased the duration of pressing time during a tongue pressing test
268 (Ciucci et al., 2011; Nuckolls et al., 2012). However, these experiments relied on a complex task
269 in which rats were trained to press a disk with their tongue, and did not investigate neither natural
270 licking nor its latency of onset. Here, we studied tongue movements in the context of simple cued-
271 licking paradigm. Hemi-parkinsonian mice displayed slower licking initiation, shorter bout
272 duration (i.e. fewer licks per bout) and deviated tongue protrusion. The lesion did not affect inter-
273 licking interval, demonstrating that the speed of each lick was not an issue in our animals.
274 These motor deficits could reflect an inability to control movement initiation, execution and
275 termination. It is possible that some of the deficits observed in our task may be secondary to
276 impairments in learning (Wise, 2004). For instance, delayed licking could derive from a reduced
277 ability to associate a predictive cue with a reward. According to this view, longer latency to initiate
278 movement would emerge from a weaker associative strength of the anticipatory signal or from the

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279 lack of anticipatory dopaminergic signaling. While we do not exclude underlying learning deficits
280 in hemi-parkinsonian mice, the results from acute unilateral infusions of D1 receptor antagonists
281 in ALM emphasize the importance of real-time dopaminergic activity in the cortex in initiating
282 movement.
283 ALM is known to regulate the direction of movement. Unilateral silencing of ALM activity,
284 either with pharmacological or optogenetic approaches, causes deviation of the tongue on the
285 ipsilateral side (Li et al., 2015). However, our local pharmacological manipulations demonstrate
286 that this effect cannot be produced by acute, unilateral intra-ALM impairments in dopaminergic
287 transmission. Hence, tongue deviation in hemi-parkinsonian mice may result either from the
288 effects of chronic unilateral disruption of ALM dopaminergic transmission, or by deficits that
289 initiate in other nodes of the cortico-striatal loop (Von Voigtlander and Moore, 1973).
290 Altogether, our experiments establish active licking in mice as a model for studying PD-related
291 motor deficits, and point to the importance of D1 receptor signaling in ALM for mediating
292 initiation and termination of tongue movements.
293
294 **Motor cortex and Parkinson's Disease**
295 Motor cortical activity is abnormal in PD patients and in animal models of PD (Lindenbach and
296 Bishop, 2013). Changes in general excitability, excitation/inhibition balance, and timing have been
297 described in the motor cortex during movement preparation or execution (Escola et al., 2003;
298 Lindenbach and Bishop, 2013; Pasquereau et al., 2015). Our results on ALM fit with the existing
299 literature and significantly extend it.
300 We showed that unilateral 6-OHDA lesion of the medial forebrain bundle impacts activity in
301 the ALM. There was a significant reduction in the proportion of neurons whose firing rates showed

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302 modulation by the cue predicting the arrival of the spout. This result is consistent with the
303 hypothesis of hypo-activation of motor cortex in PD and with recordings from MPTP-treated
304 monkeys showing fewer cue responsive neurons in lesioned animals compared to controls (Escola
305 et al., 2003). Although the total number of neurons changing their firing rates just before licking
306 (i.e., preparatory neurons) was not affected by unilateral 6-OHDA lesion, we observed alterations
307 in the ratio of excitatory and inhibitory modulations. Changes in excitation and inhibition were
308 described in motor cortices of PD patients using paired-pulse transcranial magnetic stimulation
309 (Lefaucheur, 2005; Lindenbach and Bishop, 2013). Our comparison of excitatory and inhibitory
310 preparatory activity revealed a reduction in the proportion of neurons inhibited and an increase in
311 the proportion of neurons excited prior to movement, a result consistent with the decrease of
312 GABAergic tone observed in PD patient (Ridding et al., 1995). Finally, in addition to the changes
313 described above, we observed deficits in the timing of preparatory activity. Preparatory activity in
314 6-OHDA lesioned mice had a longer latency from the cue compared to control mice, consistent
315 with the delayed onset of the licking initiation observed after 6-OHDA lesion. Unilateral dopamine
316 depletion affected the timing of preparatory activity also when spiking was aligned to the onset of
317 licking. In lesioned animals, preparatory modulations in firing rates appeared less spread in time
318 and more closely clustered toward the onset of the movement relative to control mice. While the
319 specific abnormalities differ depending on the cortical area examined and the type of lesions,
320 changes in timing of neural activity were also observed in primate models of PD and in human PD
321 patients (Doudet et al., 1990; Pasquereau et al., 2015). Specifically, in a reaction time task, PD
322 patients showed a longer latency in initiating movement paralleled by a slower buildup of neuronal
323 activation over the motor cortex (Dick et al., 1989; Mazzoni et al., 2012).

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324 The results from acute D1 receptor blockade experiments provide very important information
325 regarding the relationship between firing abnormalities in the cortex and licking deficits. They
326 demonstrate that the reduction of cue responsive neurons and the delaying of preparatory activity
327 in ALM can be sufficient to generate changes in motor systems leading to delayed licking.
328 Furthermore, the lack of changes in balance between excitatory and inhibitory preparatory activity
329 is evidence that this abnormality has limited causal role with regard to licking timing, and perhaps
330 is more involved in tongue deviation (a symptom not present after local manipulations of ALM).
331 Altogether, our results show changes in ALM activity consistent with those described in PD
332 patients and validate the study of ALM control of licking as a model for understanding the cortical
333 involvement in PD.
334

335 **Dopaminergic modulation of cortical activity**

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

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347 self-paced movement initiation (Jin and Costa, 2010; Howe and Dombeck, 2016; da Silva et al.,
348 2018) and with experiments showing that optogenetic manipulation of transient dopaminergic
349 activity can causally affect movement initiation (da Silva et al., 2018). In addition, our results on
350 D1 receptor modulation of licking initiation dovetail nicely with data showing the importance of
351 D1, but not D2, dopaminergic signaling in prefrontal cortex for the temporal control of action
352 (Narayanan et al., 2012).

353 Our experiments clearly point at ALM D1 receptors as important in licking initiation and in
354 modulating cue responses and preparatory activity in ALM. How activation of D1 receptors
355 contribute to the patterns of activity observed in the ALM of mice performing a cued-licking
356 paradigm remains to be seen and will be the subject of future investigations.
357

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358 **MATERIALS AND METHODS**

359 **Experimental subjects**

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

365

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

367 Mice were anesthetized with isoflurane (1-1.5%) in oxygen (1 L/min). Once fully anesthetized,
368 mice were placed on a stereotaxic apparatus. The scalp was cut open to expose the skull and a hole
369 was drilled above the medial forebrain bundle (MFB, anterior-posterior: -1.2 mm, medial-lateral:
370 1.3 mm, dorsal-ventral: -4.75 mm). In a first group of mice (referred hereafter as 6-OHDA lesioned
371 or hemi-parkinsonian), 3.5 μg 6-OHDA dissolved in 1 μl 0.02% ascorbic acid (vehicle, prepared
372 from sterile saline) was unilaterally injected into the MFB. A second group of mice (sham-lesioned
373 mice, referred hereafter as control) underwent the same surgical procedure but received 1 μl
374 vehicle injection into the MFB. To prevent dehydration, mice were monitored daily and
375 subcutaneously injected with 1 mL lactated ringer's solution after the surgery as needed. In
376 addition, food pellets soaked in 15% sucrose were placed on the floor of cages to facilitate eating
377 (Francardo et al., 2011).

378

379 **Behavioral screening of lesion: cylinder test**

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380 Two to three weeks after the MFB lesion surgery, mice were placed into a clear plastic cylinder.
381 Mice could freely explore the cylinder, rearing and touching the cylinder wall with their forepaws.
382 The behavior during the first 3 min in the cylinder was videotaped and analyzed. The number of
383 wall touches with the ipsilateral or contralateral forepaw was counted and used to calculate the
384 forepaw preference. Only lesioned mice with less than 40% usage of contralateral forepaw for
385 touching the cylinder wall were used for further experiments (Lundblad et al., 2004).
386

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

389 2-4 weeks after the lesion surgery, 6-OHDA lesioned and control mice were anesthetized with
390 an intraperitoneal injection of a mixture of ketamine (70 mg/kg) and dexmedetomidine (1 mg/kg)
391 and placed on a stereotaxic apparatus. The scalp was incised to expose the skull. For electrode
392 implantation, 1 mm craniotomies were performed above both anterior lateral motor cortices (ALM,
393 anterior-posterior: 2.4 mm, medial-lateral: ±1.5 mm) and two holes were drilled above visual
394 cortex on both hemispheres for inserting ground wires (silver wire). A linear array of 16 electrodes
395 (formvar-insulated nichrome wire, catalog no. 761000, A-M System, Sequim, WA) was bilaterally
396 implanted into ALM (dorsal-ventral: -0.8 - -1 mm). For infusion cannula implantation, naïve mice
397 were used instead, and a 1 mm craniotomy was performed on left ALM. A 26-gauge guide cannula
398 with a dummy (0.5 mm projection) was inserted into ALM (dorso-ventral: -700 μm). To record
399 single units after local D1 receptor blockade, a group of naïve mice was unilaterally implanted in
400 ALM with a custom-built ensemble containing 8 tetrodes (Item No. PX000004, Sandvik-Kanthal,
401 Hallstahammar, Sweden) around an infusion guide cannula (26 gauge). Electrodes, cannulae or
402 electrode-cannula assemblies and a head bolt (for the purpose of head restraint) were cemented to

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403 the skull with dental acrylic. Mice were allowed to recover from surgery for a week before starting
404 water restriction regimen.
405
406 **Cued-licking paradigm**
407 Following recovery, mice were started on a water restriction regime, with 1.5 ml water daily
408 one week before training. Weight was monitored and maintained at > 80% of the standard weight
409 for age, strain and sex. In the first phase of training, mice were habituated to restraint. During brief
410 restraint sessions, a spout containing a drop of sucrose (200 mM) was moved close to the animal
411 to encourage licking. Once the mouse started to reliably lick the spout, session duration was
412 increased and training in the cued-licking paradigm began. For each trial, a movable spout
413 containing a drop of sucrose (~3 μl, 200 mM) moved in front of the mouth of the animal 1 s after
414 the onset of an auditory cue (200 ms, 2k Hz, 70 dB). The spout remained in place for 2 s to allow
415 the mouse to lick and access the sucrose solution before retracting. The inter-trial interval was 10
416 s. An infrared beam (940 nm, powered by a fiber-coupled LED, Thorlabs, Newton, NJ) was put in
417 front of the mouth of the mouse such that each lick could be detected. Orofacial movements were
418 also recorded with a videocamera (30 Hz frame rate) synchronized with the data acquisition
419 software (CinePlex, Plexon, Dallas, TX).
420
421 **Electrophysiological recordings in control and hemi-parkinsonian mice**
422 Multiple single units were recorded via a multichannel acquisition processor (Plexon) in mice
423 performing the cued-licking paradigm. Neural signals were amplified, bandpass (300-8000 Hz)
424 filtered, and digitized at 40k Hz. Single units were isolated by threshold detection and a waveform

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425 matching algorithm and were further sorted offline through principal component analysis using
426 Offline Sorter (Plexon).
427

428 **D1/D2 receptor antagonist infusion in ALM**

429 Thirty minutes before a testing session, mice previously trained in the cued-licking paradigm
430 were briefly anesthetized with 1% isoflurane and a 33-gauge inner cannula (0.5 mm projection)
431 was inserted into the guide cannula. 0.5 µl of a solution of either the D1 receptor antagonist (5
432 µg/µl SCH23390 hydrocloride, Sigma-Aldrich, St. Louis, MO), the D2 antagonist (5 µg/µl
433 raclopride tartrate salt, Sigma-Aldrich) or sterile saline (0.9%) was unilaterally infused into ALM
434 at 0.25 µl/min using a syringe pump (11 plus, Harvard Apparatus, Holliston, MA).
435

436 **D1 receptor antagonist infusion in ALM and electrophysiological recordings**

437 After recovery from the surgery for at least a week, mice were water restricted and trained to
438 perform the cued-licking paradigm. Testing started after 8-12 days of training. Thirty minutes
439 before a testing and electrophysiological recording session, mice were head restrained and a 33-
440 gauge inner cannula (0.5 mm projection) was inserted into the guide cannula. 0.5 µl of a solution
441 of either the D1 receptor antagonist (5 µg/µl SCH23390 hydrocloride, Sigma-Aldrich) or sterile
442 saline (0.9%) were infused into ALM at 0.25 µl/min using a syringe pump (11 plus, Harvard
443 Apparatus). Single units were recorded and sorted offline as described above. Each session of
444 saline infusion was followed, on the day after, by a session with D1 receptor antagonist infusion.
445 Each mouse underwent 1-2 sessions of saline and SCH23390 infusion.
446

447 **Data analysis**

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

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

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

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

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471 *Analysis of preparatory response.* PSTHs of single units were aligned to bout initiation.
472 Activity preceding licking (i.e., preparatory activity) was assessed by examining firing rates in a
473 500 ms window before bout initiation. Firing rates within each bin (bin size is 100 ms) in the 500
474 ms window before bout initiation were compared to baseline (1 s before the auditory cue) with a
475 Wilcoxon rank sum test ($p < 0.05$) and a correction for multiple comparison (Šidák correction).
476 Units with significantly increased firing rate before bout initiation were defined as "excitatory
477 preparatory" units, where units with significantly decreased firing rate before bout initiation were
478 deemed as "inhibitory preparatory". The latency of preparatory activity of each neuron was
479 computed based on "change point" (CP) analysis (Jezzini et al., 2013; Liu and Fontanini, 2015;
480 Vincis and Fontanini, 2016).To calculate latency of preparatory activity relative to the cue or bout
481 initiation, we aligned spikes to cue onset or bout initiation and computed the cumulative
482 distribution (CDF) of spike occurrence across all trials in the time interval starting 2 s before and
483 ending 4 s after the cue or bout initiation, respectively. A sudden change of firing rate caused a
484 correspondent change of the slope of CDF and the occurrence of a CP. The timing of the first
485 significant CP was defined as the latency of preparatory activity. For analysis of latency relative
486 the cue onset, neurons without CP (8/307) or neurons with first CP (2/307) occurring later than 3s
487 after the cue were excluded for the analysis. For analysis of latency relative to the licking initiation,
488 neurons without CP (6/307) or neurons with first CP (11/307) occurring after the licking initiation
489 were excluded.
490

491 **Histological staining for verification of lesions and electrode/canula positioning**

492 Mice were deeply anesthetized with an intraperitoneal injection of a mixture of
493 ketamine/dexmedetomidine at 2-3 times the anesthetic dose and were intracardially perfused with

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494 PBS followed by 4% paraformaldehyde. The brain was further fixed with 4% paraformaldehyde
495 overnight and cryoprotected with 30% sucrose for 3 days. The brain was eventually cut with a
496 cryostat into 50 µm or 80 µm coronal slices. For visualizing electrode and canula tracks, 80 µm
497 slices were stained with Hoechst 33342 (1:5000 dilution, H3570, ThermoFisher, Waltham, MA)
498 using standard techniques. For immunostaining of tyrosine hydroxylase, 50 µm slices were first
499 incubated for 1 h with blocking solution (a mixture of 5% BSA, 5% normal goat serum and 0.02%
500 Triton-X in PBS) and were then incubated overnight at 4 °C with primary antibody (rabbit anti-
501 tyrosine hydroxylase, 1:1000 dilution, ab112, abcam, Cambridge, United Kingdom). Slices were
502 washed with PBS, incubated for 4h at 4 °C with secondary antibody (Alexa Fluor 594 goat anti-
503 rabbit IgG, 1:500 dilution, R37117, ThermoFisher), and finally stained with Hoechst 33342.

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507 and insightful comments. This work has been supported by The Thomas Hartman Foundation for
508 Parkinson's Research.

509

510 **Competing interest:**

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

512

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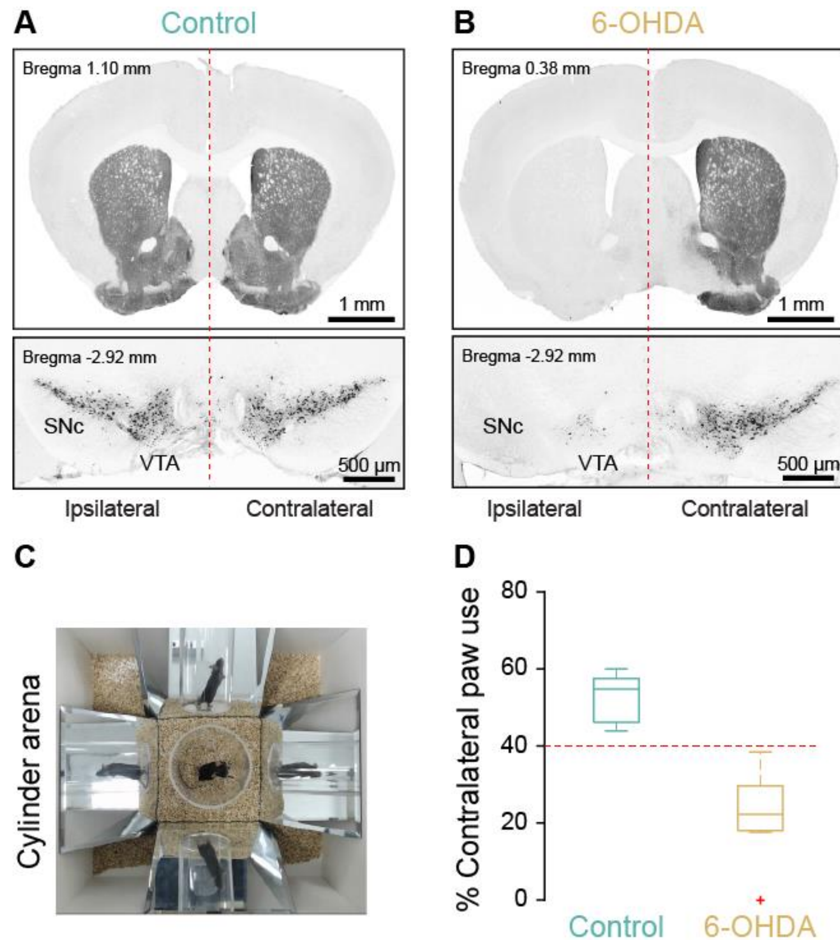
513 **FIGURE LEGEND**



514

515 **Figure 1.** Confirmation of lesion and hemi-parkinsonism after unilateral 6-OHDA injections in
516 MFB**. A and B**, Representative tyrosine hydroxylase (TH) immunofluorescence staining showing
517 dopaminergic fibers in striatum (top panel) and dopaminergic neurons in SNc and VTA (bottom
518 panel) in a control mouse (**A**) and in a hemi-parkinsonian mouse (**B**). Vertical dashed red lines
519 indicate the midline of the brain. **C**, A representative snapshot of a unilateral 6-OHDA lesioned
520 mouse performing the cylinder test. **D**, Boxplots of percentage of contralateral paw usage during
521 the cylinder test in control (n = 9, blue) and screened hemi-parkinsonian mice (n = 7, brown).

522

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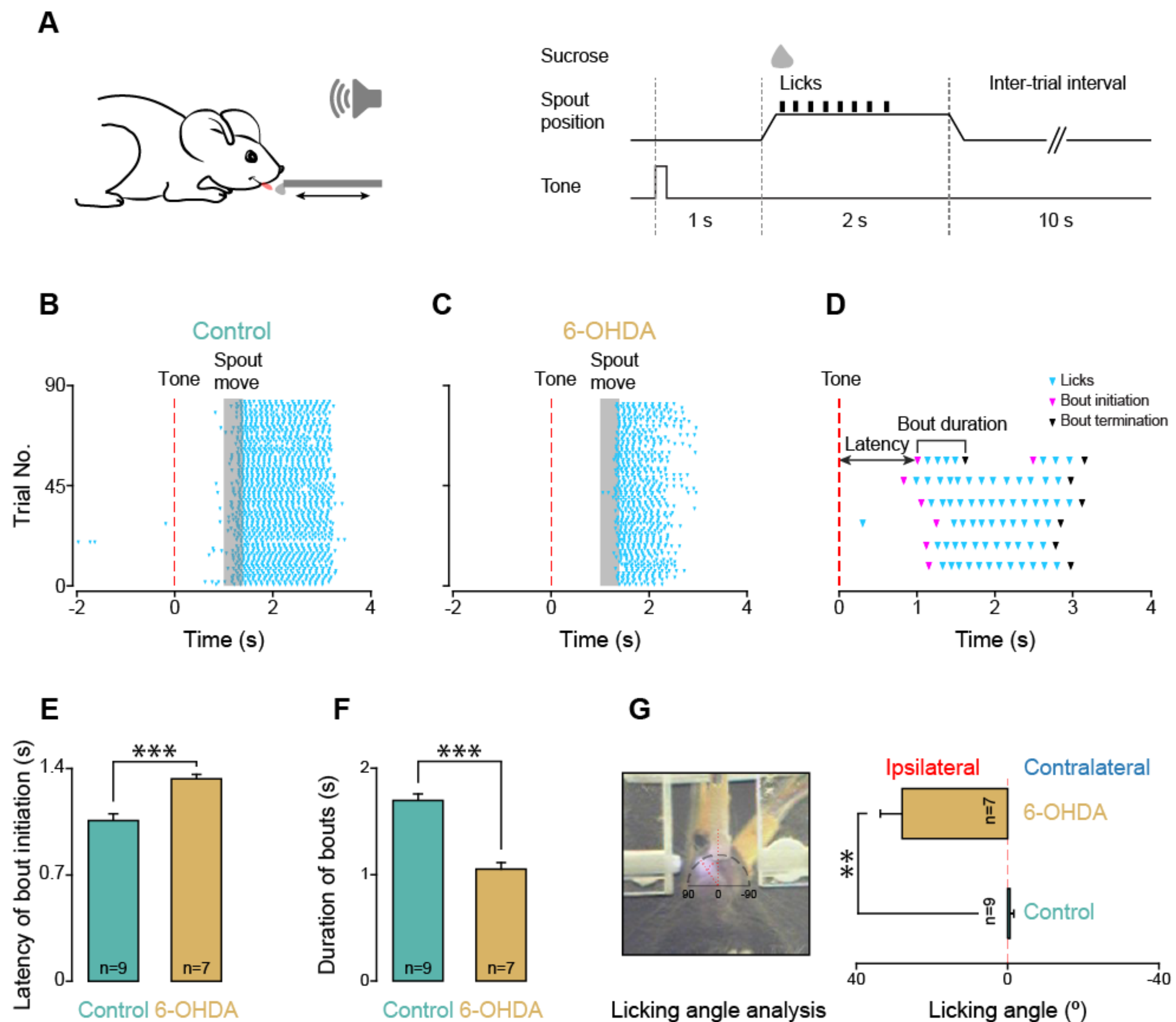

523

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

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532 is defined as the latency of the first lick of a licking bout after tone onset. Cyan triangles represent
533 each individual lick. Magenta triangles highlight the first lick of a licking bout (bout initiation)
534 and black triangles highlight the last lick of a licking bout. **E and F**, Average values of latency of
535 bout initiation (**E**) and duration of licking bouts (**F**) in control (n = 9 mice, blue) and hemi-
536 parkinsonian (n = 7 mice, brown) mice (**E and F**, t-test, *** $p < 0.001$). Error bars represent SEM.
537 **G**, *Left panel*, a presentative snapshot showing a hemi-parkinsonian mouse extending the tongue
538 towards the licking spout. Note that the tongue protrudes on the right compared to the midline of
539 the chin; *Right panel*: average values of the angles of tongue protrusion during licking in control
540 (n = 9 mice, blue) and hemi-parkinsonian (n = 7 mice, brown) mice (Welch's corrected t-test, **p
541 <0.01). Error bars represent SEM.
542

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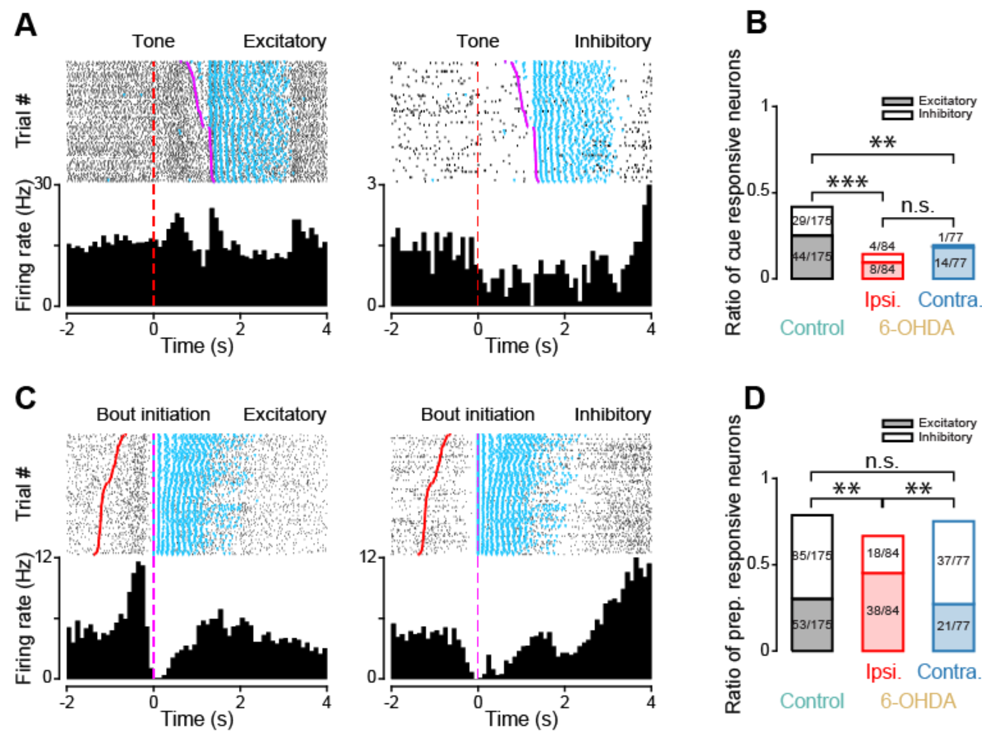

543

544 **Figure 3.** Cue responses and preparatory activity in ALM. **A,** Raster plots and PSTHs of neural

545 activity recorded from two representative ALM neurons modulated by the cue within 500 ms from

546 its onset. Dashed red vertical lines (time 0) indicate the onset of the auditory cue. Cyan markers

547 represent each individual lick. Magenta markers represent the onset of each licking bout. Black

548 ticks in raster plots represent individual action potentials. **B**, Proportion of cue responsive neurons

549 in control mice (black) as well as ipsilateral (red) and contralateral (blue) sides of hemi-

550 parkinsonian mice (*post hoc* pairwise Pearson's χ^2 test with Bonferroni correction, *** p<0.001,

551 ** p<0.01, n.s. indicates not significant). **C,** Raster plots and PSTHs of neural activity recorded

552 from two other ALM neurons modulated within 500 ms before licking bout initiation. Dashed

553 magenta vertical lines (time 0) indicate the onset of the bout initiation. Cyan markers represent

554 each individual lick. Red markers represent the onset of the cue. Black ticks in raster plots represent

555 each action potential. **D**, Proportion of preparatory responsive neurons in control mice (black) as

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556 well as ipsilateral (red) and contralateral (blue) sides of hemi-parkinsonian mice (*post hoc* pairwise

557 Pearson's χ^2 test with Bonferroni correction, ** p <0.01, n.s. indicates not significant).

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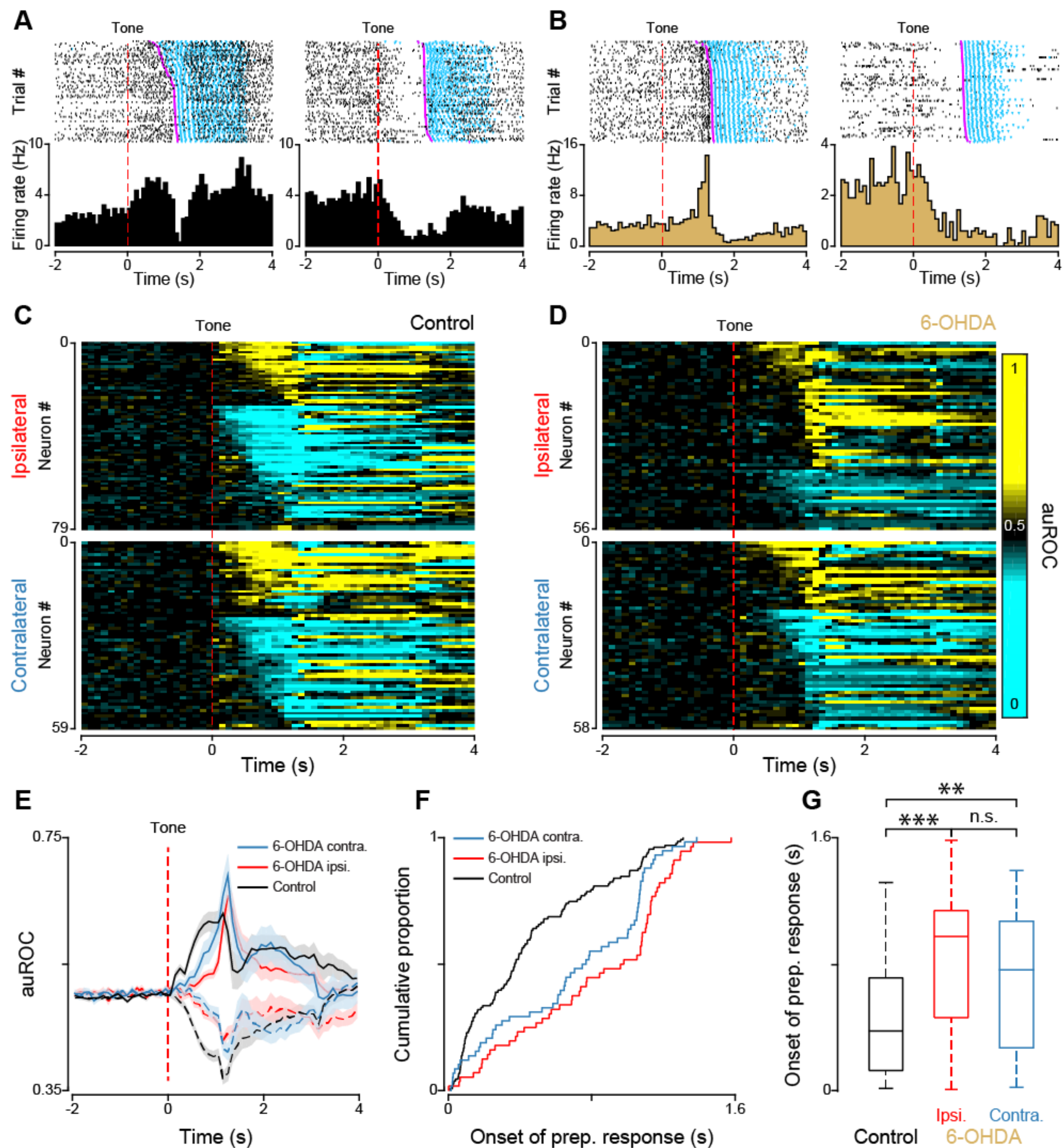

558

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

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564 onset of each licking bout. Black vertical ticks in raster plots represent action potentials. **C and D**,
565 Population plots of all ALM neurons recorded from ipsilateral and contralateral sides in control
566 (**C**) and hemi-parkinsonian (**D**) mice. Each row represents a neuron and the color of each square
567 along the x axis represents the normalized (auROC) firing rate within each 100 ms bin. Dashed
568 red vertical lines (time 0) indicate the onset of the auditory cue. **E**, Population PSTHs of excitatory
569 and inhibitory preparatory responses from control mice (black; data from ipsilateral and
570 contralateral ALM were pulled together), ipsilateral (red) and contralateral (blue) sides of hemi-
571 parkinsonian mice. The dashed red vertical line (time 0) indicates the onset of the auditory cue.
572 The shadow area around each curve represents the corresponding SEM. **F and G**, Cumulative
573 distributions (**F**) and boxplots (**G**) for the latency of preparatory activity relative to the cue onset
574 in control (black), ipsilateral (red) and contralateral (blue) sides of hemi-parkinsonian mice
575 (Kruskal-Wallis test, post hoc Tukey HSD test, ** $p<0.01$, *** $p<0.001$, n.s. indicates not
576 significant).
577

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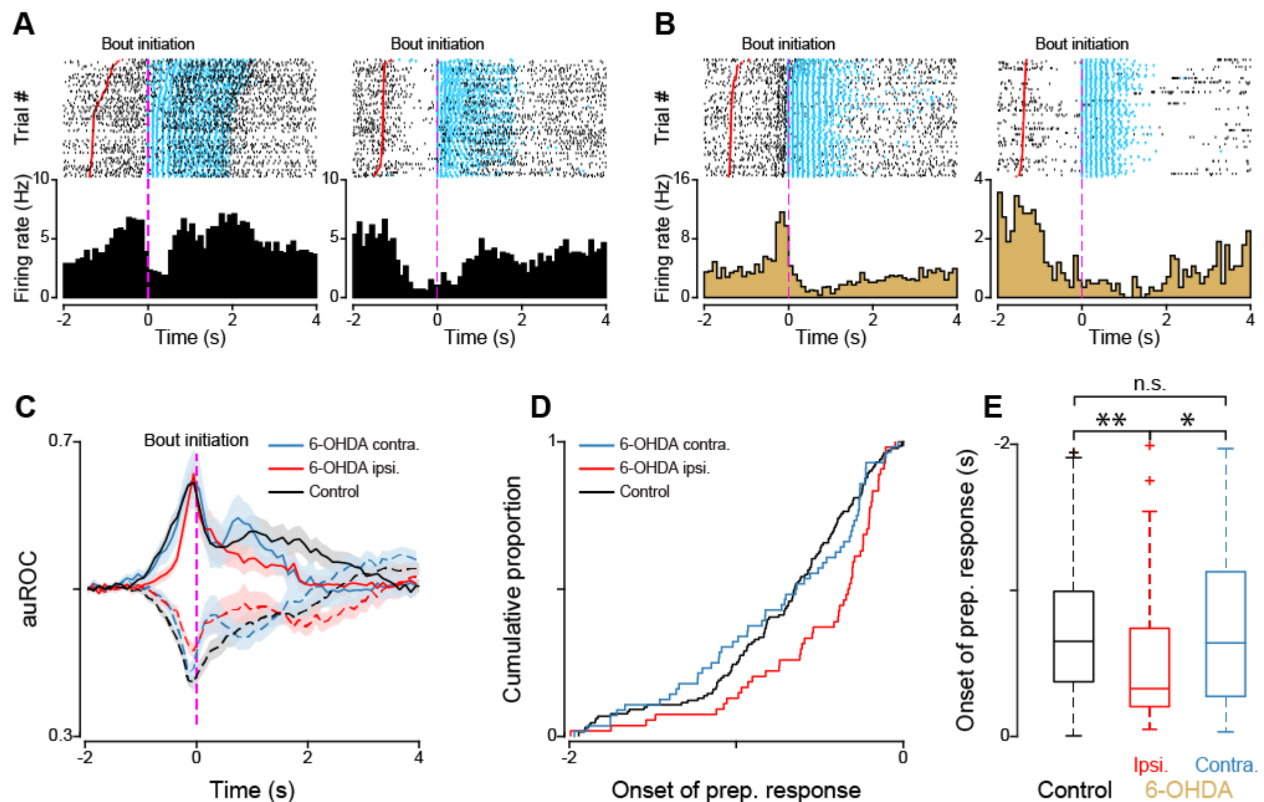

578

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

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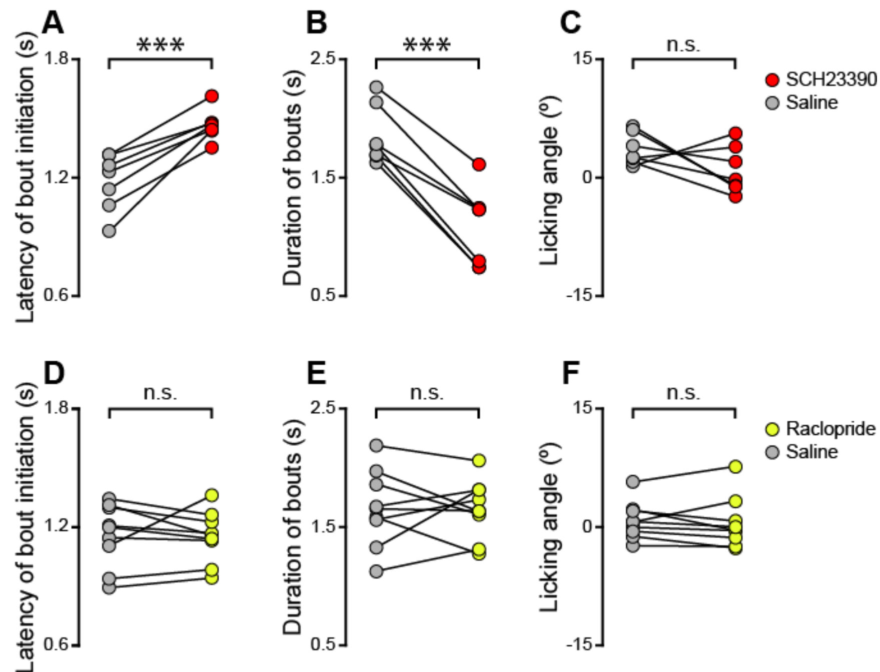

593 **Figure 6.** Effects of acute, local infusions of D1 and D2 receptor antagonists in ALM on licking.

594 **A, B and C**, Latency of bout initiation (**A**), duration of licking bouts (**B**) and licking angle (**C**)

595 recorded after unilateral infusion in ALM of saline (gray circles) or the D1 receptor antagonist,

596 SCH23390 (red circles) (n = 7, paired t-test, *** p<0.001, n.s. indicates not significant). **D, E and**

597 **F**, Latency of bout initiation (**D**), duration of bouts (**E**) and licking angle (**F**) recorded after

598 unilateral infusion in ALM of saline (gray circles) or the D2 antagonist, raclopride (yellow circles)

599 (n = 9, paired t-test, n.s. indicates not significant).

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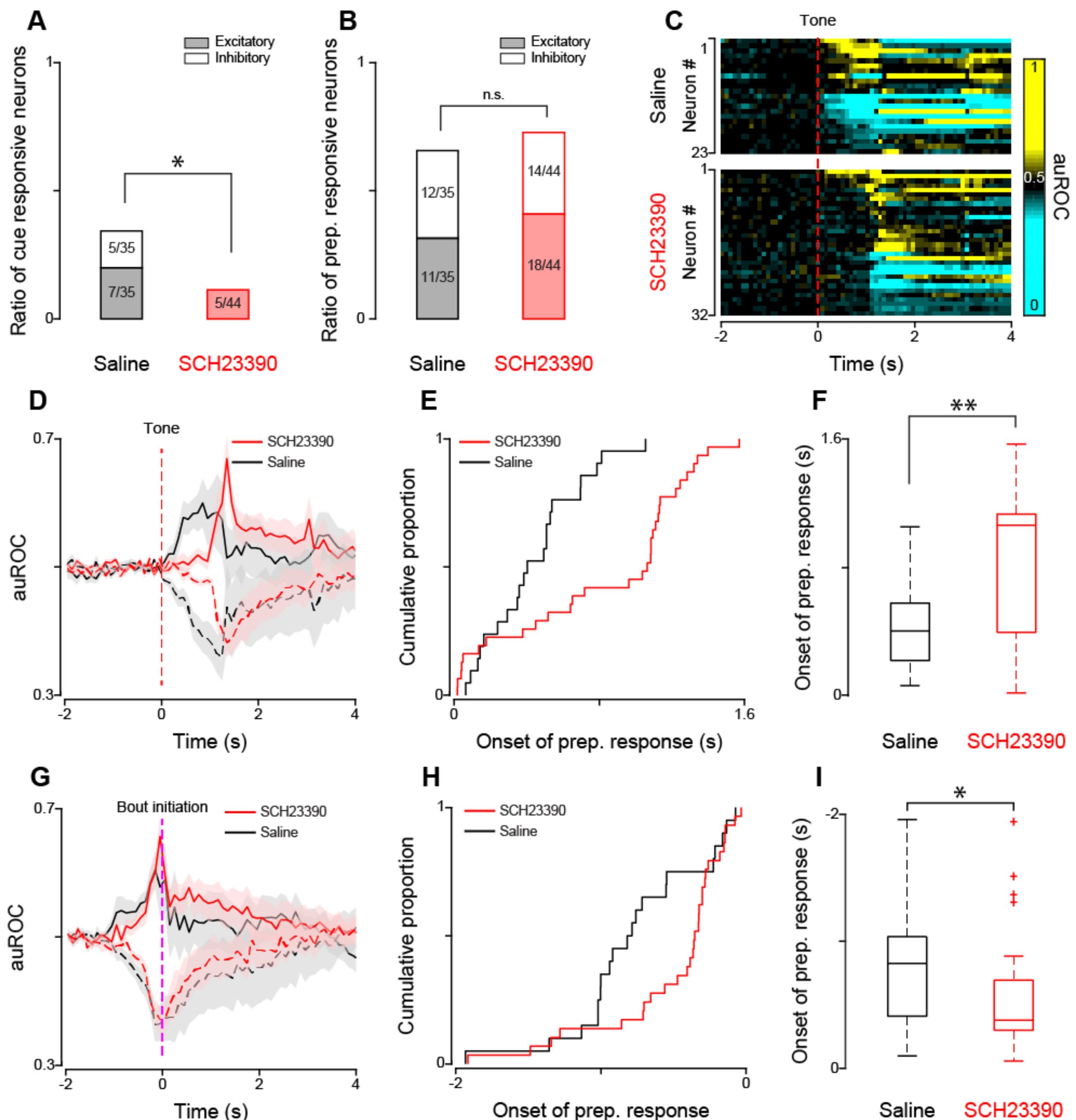

601

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

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

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

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

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

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607 antagonist SCH23390 (bottom). Each row represents a neuron and the color of each square along
608 the x axis represents the normalized (auROC) firing rate within each 100 ms bin. The dashed red
609 vertical line (time 0) indicates the onset of the auditory cue. **D**, Population PSTH of preparatory
610 activity after the infusion of saline (black) and SCH23390 (red). The dashed red vertical line (time
611 0) indicates the onset of the auditory cue. The shadow area around each curve represents the
612 corresponding SEM. **E and F**, Cumulative distributions (**E**) and boxplots (**F**) for the latency of
613 preparatory activity relative to the cue after the infusion of saline (black) and SCH23390 (red)
614 (Wilcoxon rank-sum test, ** $p<0.01$). **G**, Population PSTH of preparatory activity with the
615 infusion of saline (black) and SCH23390 (red). The dashed red vertical line (time 0) indicates the
616 onset of licking bout initiation. The shadow area around each curve represents the corresponding
617 SEM. **H and I**, Cumulative distributions (**H**) and boxplots (**I**) of the latency of preparatory activity
618 relative to the licking bout initiation with the infusion of saline (black) and SCH23390 (red)
619 (Wilcoxon rank-sum test, * $p<0.05$).
620

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