CHAPTER SIXTEEN

Secondary motor cortex: Broadcasting and biasing animal's decisions through long-range circuits

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Abstract

Medial secondary motor cortex (MOs or M2) constitutes the dorsal aspect of the rodent medial frontal cortex. We previously proposed that the function of MOs is to link antecedent conditions, including sensory stimuli and prior choices, to impending actions. In this review, we focus on the long-range pathways between MOs and other cortical and subcortical regions. We highlight three circuits: (1) connections with visual and auditory cortices that are essential for predictive coding of perceptual inputs; (2) connections with motor cortex and brainstem that are responsible for top-down, context-dependent modulation of movements; (3) connections with retrosplenial cortex, orbitofrontal cortex, and basal ganglia that facilitate reward-based learning. Together, these long-range circuits allow MOs to broadcast choice signals for feedback and to bias decision-making processes.

1. Introduction

The secondary motor cortex in rodents, denoted as MOs in the Allen Mouse Brain Atlas (Wang, Ding, et al., 2020) or M2 in the Paxinos and Franklin's Atlas (Paxinos & Franklin, 2004), is a large region, likely consisting of multiple sub-regions serving different functions. In particular, the medial portion of MOs constitutes the most dorsal aspect of the medial frontal cortex (Fig. 1). It has been labeled with various names including medial agranular cortex (AGm), medial precentral cortex (PrCm), frontal orienting field (FOF), and frontal eye field MOs domain (MOs-fef). Other studies have referred to this region based on its spatial location, calling it the dorsomedial prefrontal cortex (dmPFC) or medial motor cortex (MM). The medial MOs has poorly defined boundaries with neighboring regions such as anterior cingulate cortex (ACAd) and vibrissa motor cortex (vM1). Furthermore, medial MOs contains features similar to those found in the supplementary motor cortex, premotor cortex, and frontal eye field of primates (Barthas & Kwan, 2017; Wise, 2008).

In a previous article (Barthas & Kwan, 2017), we summarized evidence from anatomical, lesion, and electrophysiological studies to propose that the main function of medial MOs is to link antecedent conditions to actions. Antecedent conditions could be sensory inputs including visual and auditory stimuli, but could also be other actions for chaining motor sequences and maintaining choice history information for decision-making. The goal of this book chapter is to build on the previous article and discuss the several dozens of new studies on MOs that have emerged over the last several years. The new studies have focused on long-range interactions between MOs and

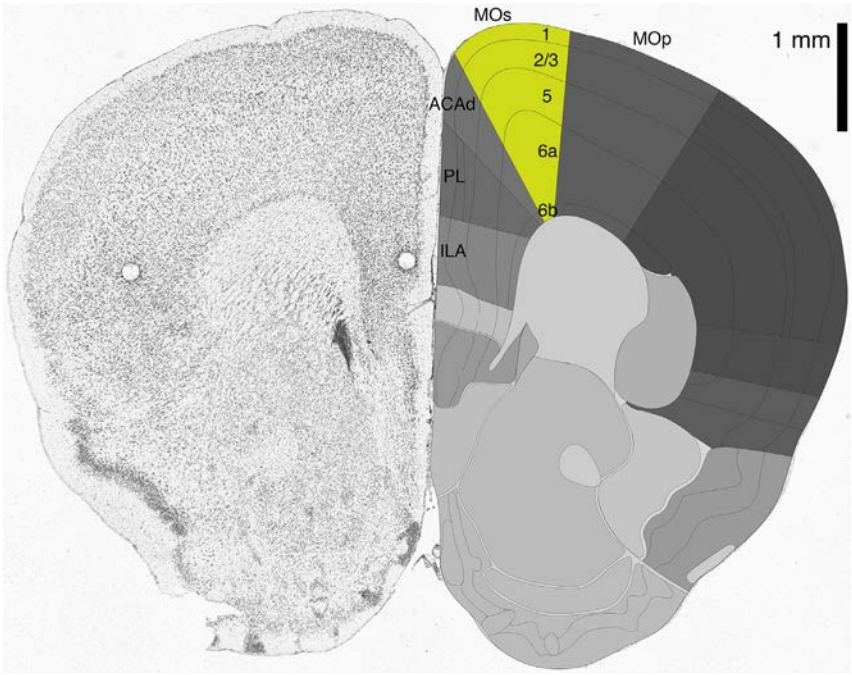

Fig. 1 The medial secondary motor cortex of the mouse. A coronal section of the frontal cortex at around +1.5 mm anterior of bregma for a 56 postnatal day old, C57BL/6J mouse, from the Allen Mouse Brain Atlas. Left half, Nissl stain. Right half, regions demarcated and named based on the Allen Mouse Common Brain Coordinate Framework (Wang, Ding, et al., 2020). MOs, secondary motor area. ACAd, anterior cingulate cortex, dorsal part. PL, prelimbic area. IL: infralimbic area.

other cortical or subcortical brain regions. These long-range connections are extensive, heterogeneous, and complex. Often, they are reciprocal and topographically organized. Overall, if MOs is indeed crucial for generating actions based on antecedents, then the novel findings on long-range circuits are beginning to illuminate how action signals are transmitted downstream to broadcast motor intent and bias the animal's decisions.

A common theme in the new studies is that the involvement of medial MOs is more pronounced in awake animals during active behavior. For example, as we will discuss below, there is evidence that MOs conveys locomotor information for predictive sensory processing and injects biases into action selection for decision-making. Its contributions therefore are less about explicit motor actions, but more about motivated behaviors. As such, instead of thinking of the region as the 'secondary motor cortex' with the

word *motor* immediately emphasizing movement generation, thinking of medial MOs as the 'secondary motivation cortex' may be a more apt name that encompasses its broad range of behavioral functions.

2. Organization of long-range circuits

The foundations for our current understanding of connections to and from the medial frontal cortex, including medial MOs, ACAd, prelimbic area (PL), and infralimbic area (ILA), were built on tried-and-true anterograde tract-tracing (Sesack, Deutch, Roth, & Bunney, 1989) and retrograde labeling studies (Gabbott, Warner, Jays, Salway, & Busby, 2005). Scaling up the methods and systematic analyses of big data produced extensive databases for cortico-cortical (Oh et al., 2014; Zingg et al., 2014), cortico-striatal (Hintiryan et al., 2016), and cortico-thalamic (Hunnicutt et al., 2014) connectivity. Therefore, in bulk where many axons are mapped at once, detailed information is available for medial MOs. Based on early neuroanatomical tracing studies and large databases, we noted differences in long-range connectivity between MOs and the neighboring ACAd and primary motor cortex (MOp) (Barthas & Kwan, 2017). It should also be mentioned that there is no detectable sex difference in the connectivity pattern of MOs afferents (Billeh et al., 2016).

Recent efforts have focused on tracing a single neuron's axon including all of the collaterals. Although this approach was not new even for medial MOs (Kita & Kita, 2012), large-scale studies based on automated methods can map axonal collaterals of thousands of single neurons, revealing striking diversity in the projection patterns (Winnubst et al., 2019). Specifically, a single cortical neuron is likely to send axonal collaterals to numerous brain regions, and few neurons share the same exact set of efferent targets. In this regard, MOs is no exception to the principle. In one study, efferents from 80 pyramidal cells including 36 intratelencephalic (IT) neurons in the mouse MOs were mapped in a brain-wide manner (Lin et al., 2018). Individual MOs IT neurons had extensive axonal collaterals, with axonal length of single neurons reaching as much as 300 mm. Given the remarkable axonal length, unsurprisingly each neuron had terminal fields in a variety of brain regions and the axonal trajectory was distinct between neurons.

Characterization of inputs can be performed at single-cell resolution and with monosynaptic specificity using rabies viruses (Wickersham et al., 2007) [although see caveats, (Svoboda, 2019)]. The trans-synaptic tracing approach gave clues into the connectivity principles for distinct inputs impinging on

MOs and ACAd (Zhang et al., 2016). Luo and colleagues used a similar approach to map long-range inputs into glutamatergic and GABAergic neurons in MOs and MOp (Luo et al., 2019). Consistent with bulk tracing (Zingg et al., 2014), MOs was found to receive inputs from neurons in the medial subnetwork such as orbitofrontal cortex (ORB), retrosplenial cortex (RSP), visual cortex (VIS), and auditory cortex (AUD) (Fig. 2). By contrast, MOp received inputs primarily from somatosensory regions. Glutamatergic and GABAergic neurons in MOs had similar proportions of inputs from the various regions.

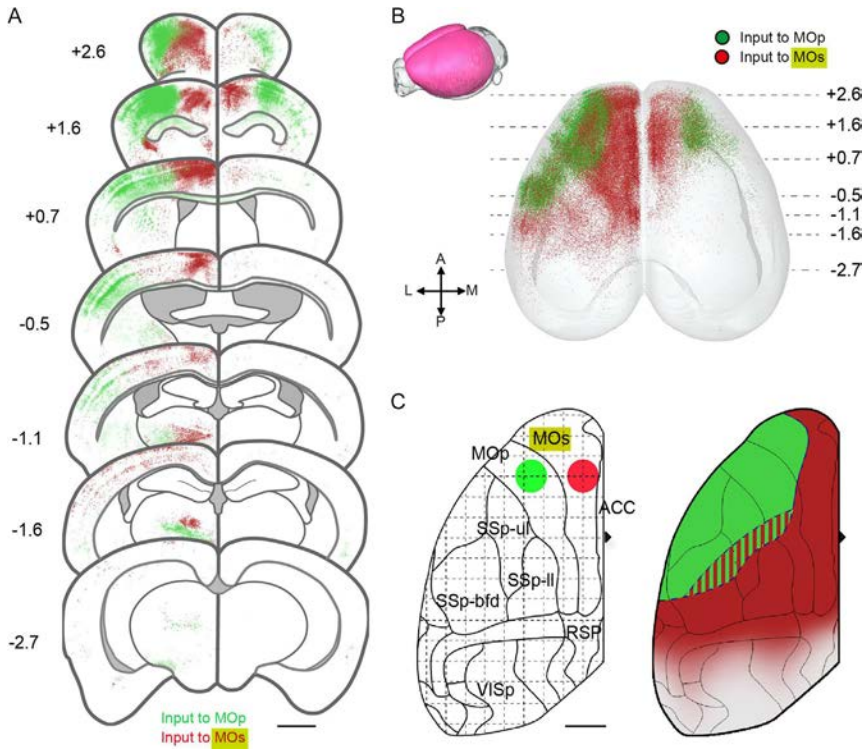

Fig. 2 Long-range cortical connections with the secondary motor cortex. (A) Coronal sections showing cortical neurons that send long-range connections to the primary motor cortex (MOp, in green) and secondary motor cortex (MOs, red). Each dot is a neuron. Scale bar = 1 mm. (B) Three-dimensional visualization. (C) Cartoon illustrating the input cortical regions areas projecting to MOp (green), MOs (red), or both (green and red stripes). The green and red circles indicate the injection sites. Scale bar = 1 mm. *Adapted from Luo, P., Li, A., Zheng, Y., Han, Y., Tian, J., Xu, Z., et al. (2019). Whole brain mapping of long-range direct input to glutamatergic and gabaergic neurons in motor cortex.* Frontiers in Neuroanatomy, 13, *44*.

Among neurons that send long-range projections, less is known about their microcircuit organization within MOs. In MOp, a series of elegant studies by Shepherd and colleagues have delineated directional preferences for connectivity between subpopulations of cortico-striatal, cortico-spinal, and cortico-thalamic pyramidal neurons (Anderson, Sheets, Kiritani, & Shepherd, 2010; Kiritani, Wickersham, Seung, & Shepherd, 2012; Yamawaki & Shepherd, 2015). Similar microcircuit motifs may be present for projection neurons in MOs. Although the organization is not fully tested, findings from current studies are at least consistent with this view. For example, Kawaguchi and colleagues found that crossed-corticostriatal cells innervate corticopontine cells unidirectionally with depressing synapses in MOs (Kawaguchi, 2017). Moreover, corticopontine cells frequently had reciprocal connections with short-term facilitating synapses in MOs.

Genetics likely play a role in shaping long-range and local connectivity, however neural activity could also be a powerful factor in sculpting neuronal networks. Ren and colleagues examined columnar microcircuits of excitatory neuron clones that are sister cells in primary somatosensory cortex (SSp) and primary visual cortex (VISp), and characterized their presynaptic partners in the medial frontal cortex (Ren, Li, Lin, Bergami, & Shi, 2019). Interestingly, like the sister cells, the presynaptic partners were organized vertically and preferentially synapse on each other. The authors went on to show that formation of these microcircuits in the frontal cortex depended on the clonal relationship between the sister cells and required synaptic communication from the sensory cortex. The interpretation is that the assembly of specific microcircuits in frontal cortex relies on the reciprocal microcircuit-to-microcircuit communication between frontal and sensory cortices.

3. Circuits for perceptual behavior

3.1 Behavioral engagement during perceptual tasks

During perceptual tasks, MOs is often seen to respond after initial activity in sensory cortices. For example, in a whisker-based, delayed-response task, Gilad and colleagues reported stimulus-driven activation in a subdivision of posterior parietal cortex (PTLp), which then transitioned into activity in the frontomedial area (Gilad, Gallero-Salas, Groos, & Helmchen, 2018). The response in medial frontal cortex was specific to trials in which animals made limb movements, body stretching, or vigorous whisking during stimulus presentation, but absent for trials when animals were still.

This could be because animals are using an active versus passive strategy to solve the discrimination problem, although alternatively active trials might reflect heightened arousal. The involvement of MOs was not unique to tactile stimuli, because neural responses in the medial frontal cortex was present in an auditory version of the task (Gallero-Salas et al., 2020). Locations of frontal responses overlapped for the two tasks, with auditory and tactile responses observed in anterior and posterior parts of medial frontal cortex respectively.

Likewise, visually evoked cortical activity was observed to emerge in VIS, followed by response in medial MOs (Musall, Kaufman, Juavinett, Gluf, & Churchland, 2019; Salkoff, Zagha, McCarthy, & McCormick, 2020; Zatka-Haas, Steinmetz, Carandini, & Harris, 2020). The frontal cortical response is dependent on behavioral engagement. Take for instance the study by Salkoff and colleagues. In a go/no-go task, the authors detected calcium transients in MOs during hit trials, but not in miss trials when the same visual stimuli were presented (Salkoff et al., 2020). Causal perturbations corroborate with the imaging results. Muscimol-mediated inactivation of MOs increased response time and partially weakened discriminability (Salkoff et al., 2020). Optogenetic inactivation of MOs reduced the probability of choosing contralateral option, but had no effect during no-go trial (Zatka-Haas et al., 2020). Altogether, these studies point to the involvement of MOs in perceptual behavior selectively during situations when animals are engaged in the task.

3.2 Processing of sensory stimuli into choices

What is the function of MOs in perceptual tasks? In a previous article (Barthas & Kwan, 2017), we posited that a major function of MOs is to flexibly link antecedent conditions, including sensory inputs, to actions. MOs is active when the animals are engaged, presumably more so for complex and demanding tasks. This precondition is illustrated by a study by Siniscalchi and colleagues, who developed a task that switches between multiple auditory-motor mapping rules (Siniscalchi, Phoumthipphavong, Ali, Lozano, & Kwan, 2016). That is, depending on the rule, for the same auditory stimulus, the animals had to respond differently to gain reward. Bilateral inactivation of MOs led to preservation and impaired flexibility, specifically for the more difficult switches from non-conditional responding (e.g., always choose left) to conditional actions (e.g. depending on stimuli, choose left or right), while sparing the simpler rule switches. Further support for a role of MOs in

adaptive sensory-motor selection came from a new study (Wang, Liu, & Yao, 2020). Using a task where animals had to categorize visual stimulus, but with the categorization boundary changing in blocks of trials within a session, Wang and colleagues found that decisions are influenced by the history of sensory stimuli, which was diminished following bilateral inactivation of MOs. Together, the results highlight the role of MOs in an uncertain sensory environment where the animals need to adjust cue-action associations.

The mechanisms for how sensory cues are associated with choice signals remain poorly understood. Local GABAergic neurons may contribute to this process. In an auditory go/no-go task, during delay period between the stimulus and choice, optogenetic activation of somatostatin- (SST) or parvalbumin-expressing (PV) interneurons would increase the number of false alarms (Kamigaki & Dan, 2017). Intriguingly, activation of vasoactive intestinal peptide-expressing (VIP) interneurons had the opposite effect of reducing false alarms to improve performance. The impact of these GABAergic signals onto pyramidal neurons, which presumably are responsible for the sensorimotor coupling, is not fully known.

3.3 Circuit with visual cortex

Medial MOs has reciprocal connections with visual cortical regions including VISp and higher visual areas (Itokazu et al., 2018; Zhang et al., 2016; Zingg et al., 2014). In terms of laminar specificity, MOs neurons in layer 5 mainly target pyramidal neurons in layer 2/3 (through axons terminating in superficial layer 1) and layer 6 (through axons terminating in layer 6) in VISp, as well as GABAergic interneurons including all of the PV, SST, and VIP subtypes in VISp (Leinweber, Ward, Sobczak, Attinger, & Keller, 2017). There is topographic organization, at least in the macroscale, with posterior MOs and ACAd projecting to medial VISp, versus anterior MOs and ACAd projecting to anterior VISp. Projections from MOs to higher visual areas are localized to rostrolateral portions of VIS (Itokazu et al., 2018).

Numerous recent studies provided solid evidence that the MOs-to-VISp connection is crucial for providing feedback to predict optic flow. As an animal moves, the visual scene shifts and the movement should be proactively subtracted for accurate perception (e.g., for a predator to estimate the speed of a prey during chase). Leinweber and colleagues imaged neural activity in MOs and ACAd axons in VISp in a head-fixed mouse as it navigates a 2D virtual environment (Leinweber et al., 2017). Calcium transients in the

frontal cortical axons contained motor-related signals that could be used for estimating optic flow. Moreover, optogenetic stimulation of MOs axons over VISp caused turning behavior—consistent with disrupting optic flow. The corollary discharge available for predictive coding in the sensory cortex is not limited to locomotion, because other motions such as eye movements also involves feedback from MOs (Itokazu et al., 2018). The specifics for how the predicting coding occur and how feedback shapes activity dynamics in VIS is less known. Conceivably, feedback axons could sculpt excitatory dynamics through their actions on GABAergic neurons (Zhang et al., 2014), which in turn produce gain modulation in the sensory cortex to adapt for the environment (Ferguson & Cardin, 2020).

One caveat with the aforementioned experiments is that they were performed on head-fixed mice. To overcome this limitation, Guitchounts and colleagues recorded from neurons in layer 2/3 of VISp in freely moving rats equipped with a head-mounted inertial measurement unit (Guitchounts, Masis, Wolff, & Cox, 2020). The VISp neurons encoded the direction of head orientation in light, and more importantly, also in dark conditions. The head orientation-related signal in VISp rose before movement onset, suggesting that it was predictive in nature, and was substantially reduced following lesion of MOs with ibotenic acid. Therefore, extending the idea that MOs is transmitting signals for optic flow prediction, these results further suggest that feedback signals broadcasted from MOs to VIS contain a rich multitude of movement and postural information. As there is increasing recognition that vision should be studied in context and as part of a sensorimotor loop (Froudarakis et al., 2019; Gomez-Marin & Ghazanfar, 2019), this connection between MOs and VIS should be an important pathway towards understanding naturalistic visual processing.

3.4 Circuit with auditory cortex

Auditory cortical regions including primary auditory area (AUDp) and anterior auditory field innervate the medial part of MOs (Nakata, Takemoto, & Song, 2020; Zhang et al., 2016). The connection is reciprocal, because MOs axons extend to auditory cortex, projecting ipsilaterally to superficial and deep cortical layers (Nelson et al., 2013).

Activation of MOs by auditory stimuli depends on the behavioral state of the animal. In one clear demonstration, voltage-sensitive dye imaging revealed robust activation of medial frontal cortex by an auditory stimulus in awake mice (Mohajerani et al., 2013). This response was absent in anesthetized

animals. Single-unit recordings showed spiking responses to auditory stimulus in the rat medial agranular cortex (Handa, Takekawa, Harukuni, Isomura, & Fukai, 2017). Additionally, even when frontal cortical neurons do not overtly change firing rate, they may still encode information about the auditory stimulus via other means such as spiking timing (Insanally et al., 2019).

The primary effect of feedback from MOs on auditory cortical areas is suppression. Aside from excitatory synapses onto pyramidal neurons, MOs axons innervate PV interneurons in AUDp which mediate powerful inhibition on the spiking activity of pyramidal neurons (Nelson et al., 2013). Indeed, optogenetic activation of MOs axonal terminals in AUD reduced spontaneous and stimulus-evoked activity in auditory cortical neurons in mice (Schneider, Nelson, & Mooney, 2014). In a follow-up study, Schneider and colleagues tested whether this suppression can be specific to certain auditory stimuli. The rationale is that, in order to detect sounds from the external environment, it is advantageous for animals to suppress sounds generated from their own movements (Schneider, Sundararajan, & Mooney, 2018). Using a clever acoustic virtual reality system, they found that MOs connected preferentially to auditory cortical interneurons that respond to the frequency of the movement-related sound, such that interneurons can be selectively recruited during locomotion to mediate the filtering. Thus, not only can MOs suppress neural activity in sensory cortex, but the strength and specificity of the predictive coding scheme is shaped by experience.

4. Circuits for adaptive movements

4.1 Higher order control of movements

One of the more consistent findings for medial MOs is that inactivating the region has minimal impact on simple motor movements. For example, bilateral inactivation of MOs with muscimol led to no detectable change in lick response time (Siniscalchi et al., 2016). Some studies reported subtle effects. In a delay response task, perturbation of MOs during the delay period prompted the mouse to take a more passive stance, but overall had little effect on discrimination performance (Gilad et al., 2018). In cases when movements were altered, the deficits were mild. Kawai and colleagues lesioned the entire motor cortex including both MOp and MOs in rats (Kawai et al., 2015). In a skilled task that requires pressing a lever in quick succession within a time gap, lesion had no effect on well-trained rats. There were only transient behavioral impairments immediately after the lesion.

Whereas execution is intact, MOs appears to be important for learning new movements, chaining motor sequence, and timing self-paced movements. Naïve rats with motor cortex lesion failed to master the lever-pressing task with temporal gap (Kawai et al., 2015). Moreover, for rats that learned the timing, subsequent lesion rendered them unable to adjust to learn a new timing. These findings echo an earlier demonstration that animals with dorsomedial frontal lesions had difficulty figuring out motor sequence and could not reverse learned sequences (Ostlund, Winterbauer, & Balleine, 2009). The defects in motor learning and sequencing may be explained by a role for MOs in controlling the stochastic variability of action timing (Makino et al., 2017; Murakami, Shteingart, Loewenstein, & Mainen, 2017). Variability should be controlled for learning, because animals need to initially stray from the previously learned timing, and then subsequently hone in on the new timing. By contrast, mismanaging variability is less harmful to execution, as animals can still perform the action on average.

Results of causal perturbations emphasized that MOs can orchestrate the activity of other cortical regions (Allen et al., 2017; Kondo & Matsuzaki, 2020; Makino et al., 2017). In one such study, Makino and colleagues studied mice as they learned a lever pressing task (Makino et al., 2017). Using a suite of modern imaging methods and sophisticated analytical methods, they showed that learning was associated with a temporal compression of sequential activation of cortical regions. Granger analysis highlighted causality from MOs; there was an activity stream emanating from MOs that predicted the activity of other regions in expert mice. Importantly, muscimol inactivation of MOs disrupted the learned, compressed temporal sequence of activity. To summarize, multiple lines of evidence indicate that MOs exerts top-down modulation on MOp and other cortical regions for the learning of new movements and for the generation of timed or sequential actions.

4.2 Neural representation of actions and postures

Action—whether the animal made a specific movement—is robustly encoded by neurons in MOs, although the signal is not unique to MOs and widely represented in most of the brain (Allen et al., 2017; Musall et al., 2019; Steinmetz, Zatka-Haas, Carandini, & Harris, 2019). Based on an elegant set of analyses, Musall and colleagues captured a wide array of movements, and dissociated influence of instructed movements, which is licking for their task, versus uninstructed movements, which include whisking, facial movements, pupil diameter, hindpaws, and other motions. They

found, surprisingly, that uninstructed movements correlate strongly with activity in many cortical regions. In fact, incorporating uninstructed movements into their model was more effective than using task variables in terms of capturing the variance of single-cell activity in MOs. The cortex-wide representation of action could be observed at the level of single neurons. Using a novel method for cellular-resolution imaging over large area of the dorsal cortex, Kauvar and colleagues identified representation of actions in numerous regions in a three-option lick-to-target odor task (Kauvar et al., 2020). Despite the widespread representation, it is worth noting that the action- and choice-related signals in MOs are among the earliest to emerge relative to other brain regions (Musall et al., 2019; Sul, Jo, Lee, & Jung, 2011).

Not all movements are the same, and there is evidence that MOs may be particularly sensitive to different types of postures and naturalistic behaviors. In a study that tracked freely foraging rats in three dimensions, neurons in MOs and PTLp exhibited tuning curves for postures of the animal (Mimica, Dunn, Tombaz, Bojja, & Whitlock, 2018). Spikes in MOs neurons were related to the pitch, azimuth, and roll of the head, as well as neck, back, or combinations of these features. The most prominently encoded feature in medial MOs was head-related movements, consistent with the earlier discussion on the role of feedback for predictive coding in sensory cortex (Guitchounts et al., 2020). The representation of a broad array of postural information may contribute to the distinct neural activity patterns observed in MOs during naturalistic behaviors such as grasping, eating, grooming, rearing, and turning (Tombaz et al., 2020).

4.3 Circuit with primary motor cortex

In a series of studies, Kawaguchi and colleagues mapped the recurrent connections between MOs and MOp (Kawaguchi, 2017; Ueta, Hirai, Otsuka, & Kawaguchi, 2013; Ueta, Otsuka, Morishima, Ushimaru, & Kawaguchi, 2014). In MOs, the MOp-projecting pyramidal cells are distributed mainly in lower layer 2/3 and upper layer 5, including commissural neurons and cortico-thalamic projection neurons, but not corticospinal neurons that lay in deep layer 5. These afferents to MOp preferentially terminate in upper layer 1 of MOp. There is some topography in the projections: lateral-medial axis in MOs aligns with the rostral-caudal axis of MOp (Ueta et al., 2014).

How do frontal cortical signals influence MOp? In rats, recording from neurons in the dorsomedial prefrontal cortex and MOp uncovered a coupling

between the two regions during the delay period in a delayed lever-pressing task (Narayanan & Laubach, 2006). The inter-areal coordination is consistent with a top-down control on the neural ensembles in the motor cortex to inhibit inappropriate responding. In another study, recording from mice running different types of ladder wheels and carefully visualizing the kinematics of joints and muscles, Omlor and colleagues found that MOs is responsible for context-dependent modulation of neural representation of joint movements in MOp (Omlor et al., 2019). Together, these studies illustrate a modulatory role for MOs on MOp, which is to provide contextual information for adjusting movements.

4.4 Circuit with brainstem

Anterograde tracing of the axon emanating from MOs have shown that MOs and the neighboring vM1 project to various subcortical targets (Fig. 3), including nuclei in the brainstem (Grinevich, Brecht, & Osten, 2005; Kita & Kita, 2012). How different frontal cortical locations maps onto motor output and their relations to brainstem connections were explored recently in a rigorous study by Mercer Lindsay and colleagues (Mercer Lindsay et al., 2019). They systematically tested focal activation of MOs via optogenetics and measured motor outputs, including forelimb, jaw, nose, and vibrissae movements, as well as more ethologically relevant actions, such as bringing forelimb to mouth, using electromyography and high-speed videography. Tracing backwards from muscle-innervating motor neurons, they identified spinal trigeminal pars oralis (SpVO) and spinal trigeminal interpolaris rostralis (SpVlr) as trigeminal premotor nuclei in the medulla portion of the brainstem, which received axonal collaterals from MOs. The implication of the results is that neighboring neurons in MOs can send parallel pathways to pools of neurons in premotor brainstem nuclei to initiate complex movements.

5. Circuits for decision-making

5.1 Choice, outcomes, and choice history

In the previous section, we discussed a potential role for MOs in learning new movements. However, learning for animals in the laboratory is in essence a process of optimizing action plans based on the outcomes of prior actions. Namely, rewarded choices should be repeated, whereas mistakes should be avoided. Accordingly, numerous studies have shown that neurons

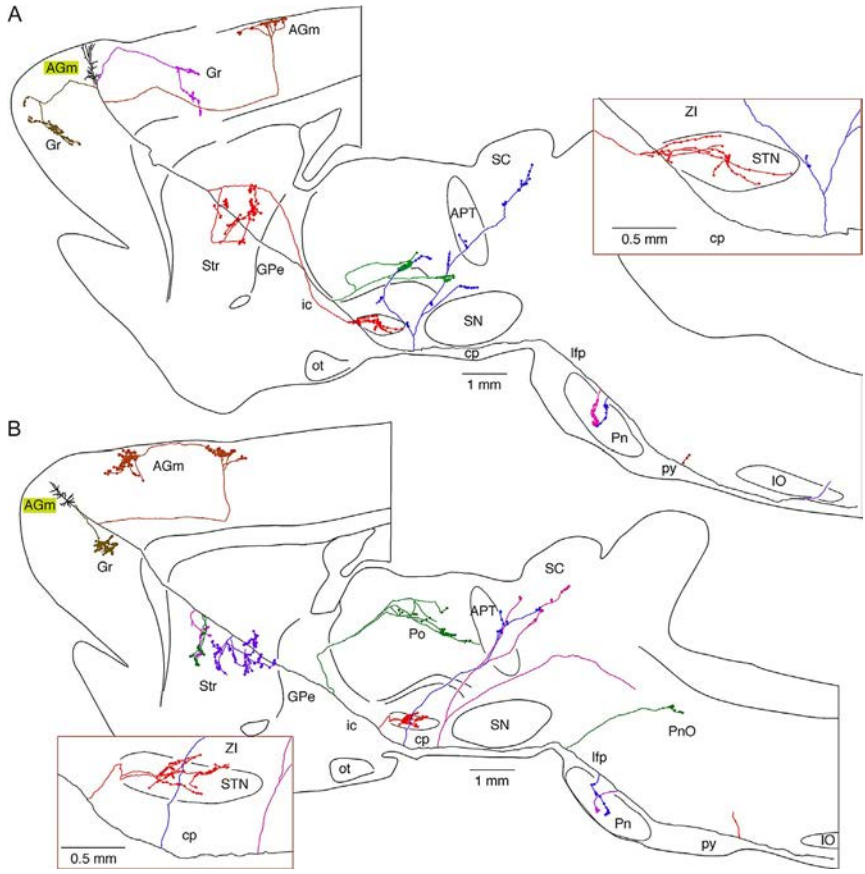

Fig. 3 Long-range subcortical projections from the secondary motor cortex. The axonal field of two pyramidal tract neurons in the rat medial agranular cortex (AGm). The axons emit multiple collaterals to various subcortical sites. Note that both neurons had collaterals innervating the AGm, granular cortex (Gr), and striatum (Str). Also note the differences, for example, in the trajectory of the thalamic collateral. GPe, globus pallidus external segment; ic, internal capsule; ZI, zona incerta; STN, subthalamic nucleus; cp, cerebral peduncle; ot, optic tract; Po, posterior thalamic nucleus; APT, anterior pretectal nucleus; SC, superior colliculus; SN, substantia nigra; lfp, longitudinal fasciculus of the pons; Pn, pontine nucleus; py, medullary pyramid; IO, inferior olive. Adapted from (Kita & Kita, 2012).

in MOs carry signals related to choices (e.g. left versus right in a two-choice task) (Erlich, Bialek, & Brody, 2011; Siniscalchi et al., 2016; Steinmetz et al., 2019; Sul et al., 2011). Moreover, the neural representation for choice in MOs arises early and thus can participate in the decision process ((Sul et al., 2011), although see (Chen, Li, Daie, & Svoboda, 2017)). The choice-related signals are then maintained until at least the next trial

(Jiang, Liu, Zhang, Xie, & Yao, 2019; Scott et al., 2017; Siniscalchi et al., 2016; Sul et al., 2011). These persistent neural signals for chosen actions are modulated by error (Narayanan, Cavanagh, Frank, & Laubach, 2013) and reward (Siniscalchi, Wang, & Kwan, 2019). The enhanced and more persistent representation of rewarded choice in MOs provides a plausible substrate for biasing the animal to repeat that choice in the near future, which could promote reward-guided learning (Siniscalchi et al., 2019).

When MOs is silenced prior to decision during a two-choice perceptual task, the effect is a bias towards the ipsilateral choice at the expense of the contralateral choice (Erlich, Brunton, Duan, Hanks, & Brody, 2015; Guo et al., 2014; Zatka-Haas et al., 2020). However, acute inactivation could have off-target effects. Whether permanent lesions have similar impact on choices and whether MOs participates in higher-order decision-making processes is less understood. Employing a multisensory task involving visual and/or auditory stimuli, Pisupati and colleagues used computational models to fit animal's behavior to make an astute observation that even on easy trials, animals make errors, which could be attributed to exploration (Pisupati, Chartarifsky-Lynn, Khanal, & Churchland, 2019). Unilateral inactivation of MOs alters the lapse rate on one side, suggesting a role in action value encoding. More specifically, the results indicate a selective devaluation of contralateral actions following the loss of MOs.

We do not know much about the neural computations that occur within MOs during decision-making. A challenge is that current decision-making tasks for rodents tend to be simplistic. Consequently, learning-related shaping of neural dynamics can be highly individualized, because an almost countless number of circuit motifs might be sufficient to solve simple tasks (Atilgan & Kwan, 2018). Indeed, empirical measurements of the neural ensemble dynamics in the medial frontal cortex showed diversity across individuals (Kurikawa, Haga, Handa, Harukuni, & Fukai, 2018). It is probable that MOs engages other brain regions for reward-guided learning. In a classical conditioning task where cues are associated probabilistically with rewards, Kondo and colleagues identified the dorsomedial frontal cortex as a hub with causal influence on other cortical regions (Kondo & Matsuzaki, 2020).

5.2 Circuit with retrosplenial cortex

The projection from RSP to MOs is dense and has been characterized in detail (Li, Yamawaki, Barrett, Kording, & Shepherd, 2018; Yamawaki, Radulovic, & Shepherd, 2016). To gain insight into the function of this long-range connection, Olson and colleagues made unit recordings in MOs

of rats navigating a maze with turns and tracks (Olson, Li, Montgomery, & Nitz, 2020). They found neural activity related to turn direction at all of the turn locations in the maze. These choice-related signals were detected just before each turn and then persisted, suggesting contributions to action planning and execution. Moreover, contextual variables, such as progress and route, modulate the choice-related signals. Overall, these observations during navigation agree with earlier reports of early rise and context dependence of choice-related signals in two-choice tasks (Erlich et al., 2011; Siniscalchi et al., 2016; Sul et al., 2011). However, Olson and colleagues noted also that action encoding is still present when a decision is not needed (e.g. a corner to turn for the rat when that turn is the only option). This result appears to suggest a role for MOs in orientation, navigation, and generation of feedback for sensory cortex, rather than a decision-making role—although these functions are not mutually exclusive and can be supported by MOs in parallel. Based on comparing these results to prior studies of RSP in related tasks (e.g., (Alexander & Nitz, 2015)), it was suggested that navigation-related signals may originate from RSP and/or PTLp and propagate to MOs.

5.3 Circuit with orbitofrontal cortex

ORB is thought to be involved in the formation of stimulus-outcome associations for decision-making, although more recently also suggested to signal expected outcomes and maintain cognitive maps (Schoenbaum, Roesch, Stalnaker, & Takahashi, 2009). In a lever-pressing paradigm, chemogenetic attenuation of ORB projection to MOs biased the exploratory use of a novel lever (Schreiner & Gremel, 2018). The idea is that experience-dependent strengthening of the ORB-to-MOs pathway may promote the exploitation of learned associations, and therefore inactivation leads to exploration. Modification of the ORB projection in MOs over longer time scale can also influence decision behaviors. In a rule learning task, mice were trained to discriminate between odor options (Johnson, Peckler, Tai, & Wilbrecht, 2016). Longitudinally across learning, the ORB boutons in MOs undergo structural plasticity such that the gain of boutons was correlated to exploiting a learned rule, whereas the loss of bouton loss was related to exploratory behavior. Therefore, both in the short and long timescales, the pathway from ORB to MOs may be important for regulating the balance between exploration and exploitation during decision-making.

5.4 Circuit with basal ganglia

The basal ganglia circuitry is implicated in reward-guided decision-making, particularly as a substrate for action value encoding and updating (Burton, Nakamura, & Roesch, 2015). MOs has multiple points of entry into the basal ganglia. First, medial MOs projects to dorsomedial striatum (Hintiryan et al., 2016). Ipsilaterally, MOs neurons project to both the patch and matrix compartments of the striatum, with a slight preference for matrix (Smith et al., 2016). Second, there are cortico-pallidal innervations (Abecassis et al., 2020; Karube, Takahashi, Kobayashi, & Fujiyama, 2019). Cortical axons from MOs are found exclusively in the ipsilateral globus pallidus (GP). Intriguingly, these cortical axons preferentially innervate GP neurons that project to striatum, more so than GP neurons that project to the subthalamic nucleus (STN) (Karube et al., 2019).

The third point of entry is a direct projection to STN, which originates from layer 5 corticospinal neurons in MOs (Kita & Kita, 2012). In a new study, Li and colleagues reported that STN-projecting neurons in the dorsomedial frontal cortex are largely distinct from other subpopulations that project to VISp and lateral hypothalamic area (LHA) (Li, Nguyen, Ma, & Dan, 2020). In a go/no-go task, the STN-projecting population has elevated activity when animal inhibited responding correctly. Furthermore, optogenetic stimulation of the MOs-to-STN projection reduced false alarms. The results indicate that the MOs-to-STN projection provides a stopping signal to halt inappropriate response for impulse control, versus the MOs-LHA pathway which acts in the opposite direction to promote responding. This conclusion aligns with the observation that stimulation of MOs terminals in STN led to premature stopping in a virtual stop-and-go task (Adam, Johns, & Sur, 2020).

It should be noted that MOs sends some projections to substantia nigra pars compacta (SNc), which is a site with dopaminergic neurons (Watabe-Uchida, Zhu, Ogawa, Vamanrao, & Uchida, 2012). The connection to SNc does not mean that MOs targets other neuromodulatory centers, because for example there is negligible projection to the ventral tegmental area (VTA) (Watabe-Uchida et al., 2012) or the cholinergic centers in the basal forebrain (Do et al., 2016). The projection to SNc is another potential mechanism for MOs to exert influence on choice behavior and additionally effort-related decision-making, through modulating the dopaminergic tone (Salamone et al., 2018).

6. Other projections

There are a few other prominent projections where the functions are largely unknown. Medial MOs sends extensive callosal axons to MOs in the opposite hemisphere. If MOs in one hemisphere is repeatedly perturbed, the contralateral MOs will adapt, a situation that was observed when mice needed to control eye movements (Sato et al., 2019). This result agrees with findings in the anterior lateral motor cortex (ALM), where interhemispheric connections facilitate recovery from transient perturbations, suggesting that regions in the two hemispheres may carry redundant information for robustness (Li, Daie, Svoboda, & Druckmann, 2016). Indeed, in a more physiological context, the halves of MOs were suggested to work mostly independently with only weak coupling (Scott et al., 2017).

Parietal cortex in rodents is implicated in decision-making and multisensory integration (Choi, Lee, & Lee, 2018), suggesting that recurrent connections between MOs and PTLp could carry both choice- and perceptual-related signals. The dual role might reflect subdivisions within PTLp, which are all connected with MOs: Medial PTLp connects with caudal and rostral MOs, whereas in between, the intermediate rostrocaudal portion of MOs connects with lateral and caudolateral subdivisions of PTLp (Olsen et al., 2019). In one report, the acute inactivation of PTLp-to-MOs neurons altered movement kinematics by reducing trial-to-trial reliability and decreasing peak velocity of a lever press action (Hwang et al., 2019).

There have been progress in mapping of the connection between MOs and various other brain regions including the ACAd (Fillinger, Yalcin, Barrot, & Veinante, 2017, 2018), thalamus (Hunnicutt et al., 2014), superior colliculus (Zhang et al., 2016), and perirhinal cortex (Ueta et al., 2013; Ueta et al., 2019). Other notable connections are between MOs and the ventral portion of the medial frontal cortex, including ILA and PL, as well as the claustrum (Zingg et al., 2014). The functional significance of these connections in perceptual, motor, and decision behaviors remains to be determined.

7. Relevance for mental disorders

7.1 Motor dysfunctions: Obsessive-compulsive disorder and Parkinson's disease

Alterations to corticostriatal circuits have long been implicated in diseases with motor dysfunctions. A few studies have begun to illuminate the role of MOs in mental disorders. Characterizing a *Sapap3* knockout mouse

model for compulsive behaviors, Corbit and colleagues found hyperactive neurons in central striatum (Corbit, Manning, Gittis, & Ahmari, 2019). There was no difference in intrinsic excitability, yet hyperactivity arises because of increased excitatory drive to striatal projection neurons and fast-spiking interneurons. The culprit was a striking 6-fold elevation in the postsynaptic responses when evoking afferents from MOs, due in part to exuberant expression of postsynaptic glutamate receptors. This aberrant increase was selective to MOs and absent for inputs from lateral ORB, suggesting a pathological shift in the balance of MOs versus ORB inputs into the striatum in the mouse model.

In a different study, dopamine-depleted male mice were characterized as a model for Parkinson's disease (Magno et al., 2019). Optogenetic activation of glutamatergic neurons in MOs alleviated some of the motor disturbances, as evident by increased mobility in terms of rotations, distance traveled, and speed. There may even be a restoration of dopaminergic tone—presumably through the SNc pathway (Watabe-Uchida et al., 2012)—because the photostimulation protocol induced an increase of dopamine in basal ganglia.

Pre-pulse inhibition (PPI) is an assay of sensorimotor gating and is thought to a translational assay. Although PPI primarily involves thalamic circuits, top-down modulation from the frontal cortex can influence the measure. For example, fear conditioning with the prepulse can enhance subsequent PPI, especially if the prepulse is masked by a background noise. Inactivation of MOs in rats eliminated this attentional enhancement of PPI (Meng, Ding, Chen, & Li, 2020). Furthermore, in mice with a *Shank3* mutation that exhibited frontal cortical defects, animals had deficient PPI (Ali, Shao, et al., 2020). By manipulating GABAergic neurons in MOs to return synaptic excitability to normal levels, PPI performance was restored in mutants to be comparable to control animals. Taken all together, although MOs is traditionally not thought of as a disease-related region, these studies are demonstrating that it underpins motor and sensorimotor dysfunctions.

7.2 Reward processing dysfunctions: Stress-related disorders and depression

Based on the role of MOs in reward-guided decision-making, pathological disruption of the brain region is expected to compromise reward processing, which is a hallmark of stress-related disorders including depression. Indeed, brain-wide mapping of cortical activity alterations in the learned helplessness model for depression identified MOs as one of several cortical regions targeted by stress (Kim et al., 2016). In one recent study, two-photon

calcium imaging was used to characterize longitudinally the impact of social defeat stress on the activity of neurons in MOs and ACAd (Barthas et al., 2020). Acute and chronic stress had significant impact on the spontaneous neural activity. Notably, stress-induced activity changes were distinct between mice that were susceptible and resilient to the stress. The results suggest that reorganization of the neural activity pattern in the medial frontal cortex may contribute to stress resilience.

In contrast to stress, antidepressants act to relieve the symptoms of depression. Subanesthetic ketamine exerts antidepressant effects with a rapid onset and relatively sustained time course. Brain-wide mapping showed that ketamine increases metabolic activity in numerous brain regions including all of the sub-regions of medial frontal cortex: ILA, PL, ACAd, and medial MOs (Duncan, Miyamoto, Leipzig, & Lieberman, 1999; Miyamoto, Mailman, Lieberman, & Duncan, 2001). Given the convergence of stress effects, ketamine action, and role in reward processing in the same region, the medial frontal cortex is a site for investigating the neural mechanisms underlying antidepressant actions. Our lab has characterized the actions of ketamine in MOs and ACAd to show that a single dose of subanesthetic ketamine increases the density of dendritic spines by promoting new spine formation (Phoumthipphavong, Barthas, Hassett, & Kwan, 2016) and elevates dendritic calcium signals that could be the precursor to long-term synaptic plasticity (Ali, Gerhard, et al., 2020).

8. Conclusion: Many circuits, many functions

We reviewed recent studies to highlight long-range connections with MOs that subserve perceptual, motor, and decision-related functions. We divided the functions into three broad categories, although undoubtedly decisions could involve sensory stimuli and would require a motor response, therefore the long-range interactions are expected to occur concurrently in a brain-wide fashion.

One possibility is that distinct subregions within medial MOs subserve the different functions. A challenge to test this possibility is the lack of consensus on the classification and borders of frontal cortical regions (Amarante, Caetano, & Laubach, 2017; Brecht, 2011; Ebbesen et al., 2018; Svoboda & Li, 2018). Instead of arguing about how to divide and name subregions, a concrete path to move forward is to exhaustively map the neural activity in the medial frontal cortex, with exact anatomical coordinates and over a large swath of area. Although several studies have recorded from multiple

regions (e.g., (Siniscalchi et al., 2016; Sul et al., 2011)), arguably the only dense mapping of frontal cortical responses during behavior to date was performed by Chen and colleagues (Chen et al., 2017). By carefully aligning fields of view and densely sampling with two-photon calcium imaging, they measured stimulus, choice, and outcome-related activity from 15,431 neurons in a roughly 1 x 1 mm area spanning the anterior lateral and medial portions of MOs. In a whisker-based tactile task, there was early stimulus-related activity in medial MOs, whereas choice in terms of lick direction arose earliest in deep layers of ALM, and then subsequently spread to medial MOs. The result suggests that, at least for tactile stimuli from whiskers, the genesis of the choice signal may occur in ALM rather than medial MOs. Looking forward, dense recording of neural activity using Neuropixels followed by histological reconstruction of the probe location will be a promising approach (Steinmetz et al., 2019). In a landmark proof-of-principle study, Steinmetz and colleagues searched for the emergence of choice signals in the entire mouse brain and identified set of regions including the medial frontal cortex, as well as midbrain areas including midbrain reticular nucleus, superior colliculus, zona incerta, and parts of basal ganglia.

An alternative possibility is that the multiple functions may be supported by the same neurons in medial MOs. Frontal cortical neurons can exhibit activity with remarkable task dependence, as seen in the differential neural responses when animals have to perform the same tasks using different effectors, such as lever press versus nose-poke (Murakami, Vicente, Costa, & Mainen, 2014), or rotarod versus wheel running (Cao et al., 2015). A strong case for task dependence came from a new study by Pinto and colleagues, in which they tested the involvement of dorsal cortical sites including medial MOs in three tasks with different demands (Pinto et al., 2019). Inactivation of MOs led to significant decrease in performance in memory-guided or accumulating-towers task, but not in visual-guided task, and modeling suggested the region contributes to these tasks in different ways to meet the behavioral demands. The results imply that the function of MOs in behavior may be highly task dependent.

To summarize, we will start by reciting a classic critique by Allen Newell, who declared that 'You can't play 20 questions with nature and win" (Newell, 1973). The idea is that for simple tasks, one may construct a toy computational model to reproduce the observed behavior for each task. However, the models generalize poorly and would fail to solve other tasks, highlighting the difficulty in integrating simple models into a cohesive framework that can broadly predict and mimic behavior. Here, in reviewing

the recent surge of studies of the rodent medial MOs, there is an appreciation for copious amount of new information on the long-range pathways. But given that brain regions are densely interconnected, axonal projections are diverse and heterogeneous, and cortical function can be task-dependent, we arrive at a similar conceptual crossroads as Newell in terms of consolidating these findings to find common grounds. Indeed, many of the long-range pathways may be involved simultaneously and be interacting during more complex behaviors. To overcome the conundrum, future studies that employ naturalistic behaviors or apply rigorous computational models that can dissect the animal's intent will be the most informative and are urgently needed towards uncovering the neural computations in MOs during motivated behaviors.

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