Title

Postnatal development attunes olfactory bulb mitral cells to high frequency signaling

Abbreviated Title

Postnatal development of mitral cell intrinsic properties

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Conflict of Interest

The authors declare no competing financial interests.
Mitral cells (MCs) are a major class of principal neurons in the vertebrate olfactory bulb, conveying odor-evoked activity from the peripheral sensory neurons to olfactory cortex. Previous work has described the development of MC morphology and connectivity during the first few weeks of postnatal development. However, little is known about the postnatal development of MC intrinsic biophysical properties. To understand stimulus encoding in the developing olfactory bulb, we have therefore examined the development of MC intrinsic biophysical properties in acute slices from postnatal day 7 to 35 (P7-P35) mice. Across development, we observed systematic changes in passive membrane properties and action potential waveforms consistent with a developmental increase in sodium and potassium conductances. We further observed developmental decreases in hyperpolarization-evoked membrane potential sag and firing regularity, extending recent links between MC sag heterogeneity and firing patterns. We then applied a novel combination of statistical analyses to examine how the evolution of these intrinsic biophysical properties specifically influenced the representation of fluctuating stimuli by MCs. We found that immature MCs responded to frozen fluctuating stimuli with lower firing rates, lower spike-time reliability, and lower between-cell spike-time correlations than more mature MCs. Analysis of spike-triggered averages revealed that these changes in spike timing were driven by a developmental shift from broad integration of inputs to more selective detection of coincident inputs. Consistent with this shift, generalized linear model fits to MC firing responses demonstrated an enhanced encoding of high frequency stimulus features by mature MCs.

KEYWORDS
development, intrinsic biophysical properties, stimulus encoding, generalized linear model, olfaction
INTRODUCTION

Early in brain development, neuronal morphology, excitability, and synaptic connectivity dynamically evolve from day-to-day (e.g., see Hinds and Hinds, 1976; McCormick and Prince, 1987; Zhu, 2000; Zhang, 2004; Oswald and Reyes, 2008; Doischer et al., 2008; Okaty et al., 2009; Dietz et al., 2011; Ehrlich et al., 2012; Hovis et al., 2012; Dufour et al., 2014). Understanding the developmental trajectory of these changes can provide insight into the relationship between single cell properties and the circuits that these neurons make up (Kepecs and Fishell, 2014). Characterizing neuronal development is of particular importance in areas of the brain, such as the olfactory system, that are critically involved in early postnatal learning (for review, see Wilson and Sullivan, 1994). Here, we investigate developmental changes in the intrinsic biophysical properties of mitral cells (MCs), a major class of principal neurons in the olfactory bulb, and we determine how these changes influence the ability of MCs to encode physiological stimuli.

MCs make synapses with several neuron types in the olfactory bulb and receive inputs that have a range of temporal features. Specifically, MC firing is strongly modulated at theta frequencies by respiration-coupled input from peripheral sensory neurons and glomerular layer interneurons (Gerkin et al., 2013) and at beta and gamma frequencies by GABAergic input in the external plexiform layer (for review, see Kay, 2014). Sensory information in the olfactory bulb is then collectively encoded by the precise spatiotemporal firing pattern of MCs, which emerges from the interaction between the temporal features of MC inputs and MC intrinsic biophysical properties (Padmanabhan and Urban, 2010; Tripathy et al., 2013). A systematic investigation of the interaction between MC intrinsic biophysical properties and stimuli with varying temporal features, within the context of postnatal developmental, will thus provide critical insight into how odor information is processed in the developing olfactory bulb.

In this study, we quantify MC biophysical properties in acute brain slices prepared from mice between P7 and P35, a period of dynamic change in MC synaptic properties (De Saint Jan and Westbrook, 2007; Dietz et al., 2011) and final refinement of MC lateral dendrite morphology (Blanchart et al., 2006). We find that the intrinsic biophysical properties of MCs, including both sub- and suprathreshold properties, show profound changes that are consistent with an acceleration of MC signaling across development. To better understand how these developmental changes regulate sensory processing in the olfactory bulb, we then examine the response of MCs to physiological fluctuating stimuli. Critically, our results directly show that postnatal development broadly enhances MC encoding of high frequency (>30 Hz) stimulus features. Our study thus strongly suggests that the developmental increase in odor-evoked gamma frequency activity in the olfactory bulb (Fletcher et al., 2005) emerges, in part, through the postnatal attunement of MCs to high frequency signaling.
MATERIALS AND METHODS

Slice preparation. All experiments were completed in compliance with the guidelines established by the Institutional Animal Care and Use Committee of Carnegie Mellon University. P7 to P35 C57/Bl6 mice of both sexes were used. Olfactory bulbs were dissected from mice that were deeply anesthetized with isoflurane. 300 µm-thick coronal sections were cut in ice-cold Ringer’s solution using a vibratome (VT1200S; Leica, Nussloch, Germany). The Ringer’s solution contained (in mM): 119 NaCl, 11 glucose, 2.5 KCl, 26.2 NaHCO₃, 1 NaH₂PO₄, 1 MgCl₂, and 2.5 CaCl₂. Slices were then incubated in Ringer’s solution warmed to 37°C for at least 30 min before recording.

Electrophysiology. Slices were continuously superfused in Ringer’s solution warmed to 35°C while recording. Electrodes with a final resistance of less than 8 MΩ were used for whole cell current-clamp recordings. Electrodes were filled with the following solution (in mM): 130 potassium gluconate, 10 HEPES, 2 MgCl₂, 2 MgATP, 2 Na₂ATP, 0.3 GTP, 4 NaCl. MCs were recognized in coronal slices by somatodendritic shape and laminar position. Neurobiotin (0.2%; Vector Labs, Burlingame, CA, USA) was included in a subset of recordings to verify morphological integrity of targeted neurons. 9 of 9 MCs examined for morphological integrity (1 P7, 1 P10, 2 P14, 2 P27, 1 P29, 2 P34) exhibited intact, single apical dendritic tufts, consistent with the developmental retraction of supernumerary apical dendritic tufts early in postnatal development, between P5 and P10 (Malun and Brunjes, 1996; Lin et al., 2000; Blanchart et al., 2006). The remaining MCs in our dataset were not subjected to morphological analysis, and thus likely contain at least some MCs with truncated apical and/or lateral dendrites. Of note, however, such dendritic truncation has previously been found to minimally impact the intrinsic biophysical properties of MCs (e.g., see: Salin et al., 2001; Pimentel and Margrie, 2008). MCs were held at their resting membrane potential (−58.6 ± 5.5 mV) throughout all recordings. MCs were typically silent at rest under our recording conditions, consistent with previous reports (e.g., see Chen and Shepherd, 1997; Desmaisons et al., 1999). Pipette capacitance was neutralized and series resistance was compensated. Ionotropic synaptic antagonists (10 µM CNQX, 25 µM APV, 10 µM bicuculline) were included in the Ringer’s solution in a subset of recordings to evaluate the influence of ongoing synaptic transmission on intrinsic biophysical properties. Data were pooled for parameters exhibiting no significant difference in the absence or presence of synaptic antagonists. The postnatal development of MC synaptic activity was not further considered, but has been extensively examined elsewhere (e.g., see: De Saint Jan and Westbrook, 2007; Dietz et al., 2011). Reported voltages were not corrected for the liquid junction potential. Recordings were filtered at 4 kHz and digitized at 10 kHz using a MultiClamp 700A amplifier (Molecular Devices, Sunnyvale, CA, USA) and ITC-18 acquisition board (Instrutech, Mineol, NY, USA) controlled by custom software written in Igor Pro (WaveMetrics, Lake Oswego, OR, USA).
Stimulus generation and data analyses. For each MC, we first recorded its response to a series of 2 s-step currents (ranging from −50 to 450 pA) to measure passive membrane properties, action potential properties, excitability, and firing regularity. Input resistance was calculated using the membrane potential change evoked by the −50 pA step current. The membrane time constant was calculated by fitting a single exponential function to the first 400 ms of the membrane potential response to the offset of a −50 pA step current. Both input resistance and the membrane time constant were averaged from ≥3 trials. Firing regularity for each MC was characterized by the interspike interval (ISI) coefficient of variation (CV_{ISI}) calculated from spike trains with ~30 Hz firing rate (30.6 ± 13.3 Hz). Action potential properties were calculated from the first action potential evoked by the weakest suprathreshold step current input per MC (i.e., “rheobase”) and averaged over 3–8 trials. The first action potentials were typically well isolated from subsequent action potentials, thus minimizing the influence of any spike train-dependent effects (e.g., spike-frequency adaptation from M-type potassium currents) on our measures of action potential and afterhyperpolarization (AHP) properties. Action potential threshold was defined as the membrane potential during an action potential at which the third order derivative of the membrane potential reached its first peak (Henze and Buzsáki, 2001). Action potential rising and falling slopes were calculated as the maximum and minimum rate of membrane potential change, respectively, during the mean action potential waveform (Fig. 3Ci). The AHP peak was defined as the action potential threshold subtracted from the minimum membrane potential reached following an action potential (Fig. 3Di). The AHP peak time was defined as the time interval between the point at which the membrane potential dropped below action potential threshold and the point at which the AHP peak was measured (Fig. 3Di).

Next, we measured hyperpolarization-evoked membrane potential sag by injecting a series of 2 s-hyperpolarizing step currents (ranging from 0 to −300 pA). Membrane potential sag was quantified similar to previous investigations (Angelo and Margrie, 2011; Burton and Urban, 2014). Briefly, the initial voltage response induced by hyperpolarizing current injection was measured either from the minimal voltage reached for MCs exhibiting a “positive sag” (e.g., Fig. 2A), or from the voltage at 120 ms after the initiation of the step current injection for MCs exhibiting a “negative sag”. The steady state voltage response was calculated as the mean value of the last 100 ms of the membrane potential response to the hyperpolarizing step current. The sag amplitude for each MC was then measured as the initial voltage subtracted from the steady state voltage for whichever step current injection yielded a steady state voltage closest to −90 mV. Cells with “positive sag” thus exhibited a transient response that was more hyperpolarized than the steady state response, consistent with the expression of a hyperpolarization-activated current (I_h) that reverses at −32 mV.
(Angelo and Margrie, 2011). In contrast, cells with “negative sag” exhibited a gradual hyperpolarization due to an apparent lack of $I_h$ (Angelo and Margrie, 2011).

Finally, we recorded each MC’s response to frozen fluctuating stimuli ($\sigma = 50$ pA) mimicking physiological synaptic input across ≥50 trials. Fluctuating stimuli were generated by convolving white noise with an alpha function ($\tau = 3$ ms). The DC offset of the fluctuating current was adjusted from 100-400 pA to achieve comparable firing rates across all MCs ($35.4 \pm 8.4$ Hz), and only MCs with stable trial-to-trial firing rates (yielding a within-cell firing rate standard deviation of $3.0 \pm 3.1$ Hz across ≥50 trials per MC) were used for subsequent analyses. The spike-triggered average (STA) for each MC was calculated by averaging the fluctuating stimulus preceding each action potential by the MC’s maximum ISI. The STA slope was calculated as the maximum slope of the final depolarizing phase of the STA, while the integration time was calculated as the temporal duration of the final depolarizing phase of the STA (Fig. 6A inset). Rhythmic fluctuating stimuli mimicking physiological sniff-coupled synaptic input were generated by convolving event times generated via an inhomogeneous Poisson process (rate plotted in Fig. 5A) with an alpha function ($\tau = 3$ ms) (e.g., see Oswald and Urban, 2012). Spike-time correlations were calculated from binary spike trains binned at 1 ms (except where otherwise noted).

All analyses, statistical tests, and modeling were performed in MATLAB (MathWorks, Natick, MA, USA). Values are reported as mean ± standard deviation, unless otherwise noted. The maturation of intrinsic biophysical properties across postnatal development was examined using linear and multiple linear regression, except where noted. Both linear and exponential relationships were considered, but in all instances exponential relationships yielded a negligible improvement in the variance explained and are thus not shown.

**Generalized linear models.** Fluctuating stimulus-evoked spike trains of 48 MCs (P7-P35) were fit with generalized linear models (GLMs), as previously described (Tripathy et al., 2013). Each fit GLM yielded a prediction of a single MC’s firing based on a filter of the stimulus, a spike history function, and a baseline firing rate. Each MC was described by a single GLM using ten stimulus filter parameters, ten spike history filter parameters, and one baseline firing rate parameter. For each MC, GLM parameters were fit using the first 1.8 s of each 2 s-long spike train and then averaged over the first 50 trials recorded. The fit GLM was then evaluated by predicting the MC’s firing response to the last 0.2 s of the fluctuating stimulus and comparing the resulting prediction to the experimentally recorded firing. Specifically, a total of 50 spike trains were simulated for each MC, corresponding to the 50 spike trains recorded for each neuron. We then calculated peristimulus time histograms (PSTHs) for the simulated and recorded trials, and measured the
correlation coefficient between the simulated PSTH and recorded PSTH to provide a single value evaluating model performance. Similar results were obtained when GLMs were fit to a random 90% of trials and evaluated against the remaining 10% of trials (data not shown).

*Stimulus reconstruction.* We performed Bayesian decoding of both individual and population MC spike trains to reconstruct fluctuating stimuli (e.g., see Tripathy et al., 2013). MC populations were generated by randomly selecting 5-cell groups from immature (P7-P11, n=15) or mature (P20-P35, n=15) MCs. The magnitude-squared coherence between the original and reconstructed stimuli was calculated using Welch’s method. To test the performance of mature and immature MCs in encoding different frequency components of the stimulus, the mean coherence in low frequency (0-10 Hz), beta frequency (10-30 Hz), and gamma frequency (30-100 Hz) bands were compared by t-tests with Bonferroni correction for multiple comparisons. Results shown for between-cell correlations, GLM fits, and stimulus reconstruction were obtained using MC data collected in the absence of synaptic antagonists. Equivalent results were obtained when including MC data collected in the presence of synaptic antagonists (data not shown).
RESULTS

Postnatal development of MC subthreshold membrane properties

To investigate how the encoding of stimuli by MCs changes with respect to postnatal development, we characterized the intrinsic biophysical and stimulus response properties of MCs from P7 to P35. We first studied the development of passive membrane properties, which regulate how neurons respond to and integrate subthreshold synaptic inputs. MC input resistance and membrane time constant both significantly decreased over the targeted age range (Fig. 1B, C; input resistance decreased 2.2 MΩ/day; membrane time constant decreased 1.1 ms/day), similar to most neuron types across the mammalian brain (Tripathy et al., 2015) and consistent with previous analyses of MC input resistance (Maher et al., 2009; Dietz et al., 2011). In contrast, membrane capacitance remained stable across the targeted age range (Fig. 1D), consistent with previous findings over a narrower age range (Burton and Urban, 2014). This result suggests that the observed changes in input resistance and membrane time constant reflect qualitative changes in MC ion channel expression, rather than a gross change in MC size (which is strongly tied to MC membrane capacitance – see Burton and Urban, 2014). We additionally observed no change in resting membrane potential across development (Fig. 1E).

Of note, values recorded for membrane capacitance were moderately but significantly larger in the presence of ionotropic synaptic antagonists (Table 1; see Materials and Methods). However, membrane capacitance exhibited weak or no significant relationship with postnatal development in the absence or presence of synaptic antagonists (Fig. 1D). Moreover, given that: 1) recordings in the absence or presence of synaptic antagonists were obtained from separate cells, and 2) that no consistent difference was observed in either input resistance or membrane time constant between MCs recorded in the absence or presence of synaptic antagonists (Table 1), we expect that this influence of synaptic antagonists on membrane capacitance likely reflects a sampling effect.

Postnatal development of MC excitability and firing regularity

We next examined the development of MC excitability and firing regularity. MCs exhibited heterogeneous firing rate-current (FI) curves (Fig. 1G), consistent with our previous observations (Padmanabhan and Urban, 2010; Burton et al., 2012; Burton and Urban, 2014), with no significant dependence on developmental age. MCs likewise exhibited heterogeneity in firing regularity, including MCs with highly regular firing as well as MCs with irregular “stuttering” of action potential clusters (Fig. 1F), consistent with previous findings from narrower developmental periods (Padmanabhan and Urban, 2010; Angelo and Margrie, 2011; Burton and Urban, 2014). Across development, firing irregularity of MCs, as measured by CV_{ISI}, significantly increased
(Fig. 1H), consistent with previous findings (Burton and Urban, 2014). In particular, the fraction of MCs exhibiting $CV_{ISI} > 0.6$ was markedly enriched in older MCs (Fig. 1H).

Several recent studies have also observed substantial heterogeneity in hyperpolarization-evoked membrane potential sag among MCs, and have further described a relationship between sag, excitability, and firing regularity (Angelo and Margrie, 2011; Angelo et al., 2012; Burton and Urban, 2014). Given the apparent link between hyperpolarization-evoked membrane potential sag and MC firing regularity (Angelo and Margrie, 2011), we next examined the developmental trajectory of sag in MCs. Most MCs in our dataset exhibited a positive sag amplitude across a large range of membrane potentials (Fig. 2B,C), consistent with the widespread expression of $I_h$ and the underlying hyperpolarization-activated cation nonselective (HCN) channels in MCs (Santoro et al., 2000; Angelo et al., 2012) and the distribution of sag amplitudes previously observed in mouse MCs (Angelo et al., 2012; Burton and Urban, 2014). Across postnatal development, the magnitude of these sag amplitudes gradually decreased, with a significant negative correlation between postnatal age and sag amplitude (Fig. 2C). Consistent with previous results (Angelo and Margrie, 2011; Burton and Urban, 2014) and the observed developmental increase in firing irregularity (Fig. 1H) and the decrease in sag amplitude (Fig. 2C), we found that irregularly firing MCs ($CV_{ISI} > 0.6$) had zero or negative sag amplitudes and thus lacked functional $I_h$. Further, across all MCs and developmental stages, we observed a strong negative trend between sag amplitude and $CV_{ISI}$ (data not shown; linear regression, $r = -0.23$, $p = 0.068$, $n=62$).

Thus far, we have examined the age-dependent changes in individual biophysical properties of MCs, and observed significant but overall modest correlation values. Specifically, sag amplitude exhibited the greatest age-dependence change, with age accounting for 26% of the variance in sag amplitude. While modest, these correlation values are consistent with the substantial degree of biophysical diversity among MCs, even within relatively narrow age ranges, that we and others have observed (Padmanabhan and Urban, 2010, 2014; Angelo and Margrie, 2011; Burton et al., 2012; Tripathy et al., 2013; Tucker et al., 2013; Burton and Urban, 2014). It is also possible that the postnatal development of MC physiology manifests in coordinated changes across multiple properties, such that a multivariate analysis of several properties may better explain MC postnatal development (e.g., see Dufour et al., 2014). To begin to explore this possibility, we tested whether multilinear regression of input resistance, membrane time constant, and sag amplitude could better predict age than any individual passive membrane property alone. This multilinear regression model provided a highly significant ($p=1.7 \times 10^{-6}$) but still modest correlation with age ($r=0.56$), with sag amplitude surprisingly proving to be the only significant factor ($p=5.0 \times 10^{-3}$; input resistance, $p=0.13$; membrane time constant, $p=0.055$). Consistent with this finding, sag amplitude significantly correlated with input resistance (Fig. 2D)
and membrane time constant (Fig. 2E) across development. Our results thus suggest that changes in HCN channel expression and/or conductance is a critical component of the postnatal development of MC passive membrane properties and firing regularity.

**Postnatal development of MC action potential properties**

In addition to subthreshold membrane properties, excitability, and firing regularity, the intrinsic biophysical properties reflected in the action potential waveform can also critically contribute to stimulus encoding. Therefore, we next characterized the developmental trajectory of MC action potential properties. MCs exhibited broadly distributed action potential thresholds, peaks, and durations (full width at half maximum amplitude), as well as broadly distributed AHP amplitudes at each age (Fig. 3). Despite this heterogeneity, however, we observed a systematic shift in the average action potential waveform across development (Fig. 3A). Specifically, action potential duration substantially decreased from P7 to P35 (Fig. 3Biii) – an effect likely to have significant consequences on calcium entry at MC neurotransmitter release sites (e.g., see King and Meriney, 2005). Moreover, a strong trend existed for hyperpolarization of the action potential threshold across development (Fig. 3Bi). No consistent relationship existed between age and the absolute peak voltage of the action potential (Fig. 3Bii), the height of the action potential (i.e., the difference in action potential peak and threshold; data not shown; linear regression, r=0.10, p=0.40; n=67) or the AHP peak amplitude (Fig. 3Biv).

We additionally measured the temporal dynamics of membrane potential change during the action potential rising and falling phases (Fig. 3Ci). The magnitude of the action potential rising and falling slopes significantly increased across postnatal development (Fig. 3C), suggesting a developmental increase in the functional expression of both voltage-gated sodium and potassium channels (for review, see Bean, 2007). The faster falling slope was additionally accompanied by faster AHP kinetics (Fig. 3D), consistent with a recent report of shorter AHP durations in mature vs. immature rat MCs (Duméniel et al., 2015). Specifically, AHPs reached their peak amplitude noticeably faster across development (Fig. 3Dii, iii). The faster AHP kinetics of older MCs are likely linked to the enhanced propensity of older MCs to fire clusters of high frequency action potentials (Fig. 1H; Golomb et al., 2007; Stiefel et al., 2013), similar to the rapid kinetics of olfactory bulb tufted cell AHPs (Burton and Urban, 2014).

Interestingly, MC action potentials exhibited a significantly slower falling slope and longer duration in the presence of ionotropic synaptic antagonists (Table 1). This broadening of the action potential waveform may reflect the blockade of rapidly recruited recurrent inhibition from a subset of external plexiform layer interneurons (Huang et al., 2013; Kato et al., 2014), which would act to accelerate the repolarization of MC
membrane potentials. Importantly, action potential duration decreased and the magnitude of action potential falling slope increased across postnatal development regardless of the presence or absence of synaptic antagonists, thus identifying the acceleration of action potential waveforms as a fundamental feature of postnatal MC maturation. Consistent with this finding, multilinear regression of action potential duration, rising slope, falling slope, and AHP peak timing significantly predicted MC age \( (r=0.74; \ p=8.7\times10^{-13}) \), with falling slope the only significant factor \( (p=8.8\times10^{-4}; \ \text{duration}, \ p=0.60; \ \text{rising slope}, \ p=0.61; \ \text{AHP peak timing}, \ p=0.15) \).

**Postnatal development of MC spike-time correlations**

So far, we have demonstrated that several intrinsic biophysical properties of MCs systematically change during postnatal development. In the second half of our study, we explored how these developmental changes collectively shape the ability of individual MCs and MC populations to encode stimuli. We first examined how reliably specific stimulus features evoked MC firing. Each MC was injected with a constant step current (100-400 pA) overlaid with a frozen fluctuating current. This stimulus was designed to both mimic the spectral properties of physiological synaptic input to MCs (Galán et al., 2008) and to evoke MC firing within physiological beta/gamma frequency ranges (Fig. 4A; see Materials and Methods). Consistent with previous findings (Padmanabhan and Urban, 2010), MCs across all developmental stages exhibited heterogeneous firing responses to the frozen fluctuating stimulus (Fig. 4A). To examine the postnatal development of within-cell spike-time reliability and between-cell spike-time correlations, we calculated the pairwise correlation between the resulting spike trains from all recorded MCs (Fig. 4B). As expected, self-correlation values (i.e., within-cell correlations across trials) across postnatal development were significantly higher in the presence of ionotropic synaptic antagonists (Fig. 4C; Table 1), consistent with the blockade of probabilistic spontaneous and recurrent synaptic input. Importantly, however, self-correlation values significantly increased from P7 to P35 independent of ongoing synaptic activity (Fig. 4C). This increase in within-cell spike-time reliability further proved robust across a large range of temporal bin sizes used to calculate the correlations (Fig. 4D) and also held when stimulus properties better reflected the spectral characteristics of physiological sniff-coupled inputs (Fig. 5). In addition, the between-cell spike-time correlation (i.e., the fast timescale synchronization of MCs in response to an identical input) also increased across development (Fig. 4F). This developmental increase in both within-cell and between-cell spike-time correlations suggests that mature MCs may convey stimulus-dependent information with higher fidelity to downstream brain regions than immature MCs (Giridhar et al., 2011).

Of note, even though immature MCs were injected with higher step current amplitudes to achieve comparable firing rates across all MCs \( (35.4 \pm 8.4 \ \text{Hz}) \), MC firing rates nevertheless significantly increased
across development in response to the fluctuating stimulus (Fig. 4E). This finding suggests that the increased within-cell and between-cell spike-time correlations observed in mature MCs emerged, at least partially, from the ability of fluctuating stimuli to more effectively drive firing in mature MCs than in immature MCs (de la Rocha et al., 2007). The firing rate dependence of spike-time correlations is minimal for high input correlations, however (Tchumatchenko et al., 2010), suggesting that the developmental changes in MC spike timing that we observed in response to an identical fluctuating current may also reflect a developmental shift in stimulus feature selectivity (see below).

Postnatal development of MC stimulus encoding

The developmental changes observed in MC intrinsic biophysical properties are likely to significantly influence which features of incoming stimuli that MC activity encodes. To directly examine the stimulus feature selectivity of MCs across development, we calculated the STA for each MC in our dataset. The STA specifically describes the optimal current waveform triggering an action potential (Bryant and Segundo, 1976; Mainen and Sejnowski, 1995; Forger et al., 2011), and thereby reflects the intrinsic dynamical states of the neuron and its various ion channels that are relevant to action potential firing (e.g., see Fricker and Miles, 2000; Svirskis et al., 2002; Slee et al., 2005; Mease et al., 2013). Moreover, the STA provides a tractable, intuitive measure of the collective integrative and resonant properties of the neuron (Hong et al., 2012; Ratté et al., 2013; Das and Narayanan, 2014) and its ability to encode information (Padmanabhan and Urban, 2010).

Consistent with previous results, we observed a large diversity of STA waveforms among MCs at each developmental stage (Padmanabhan and Urban, 2010; Burton et al., 2012). Within this diversity, however, we also observed a systematic change in the STA despite no significant change in the total charge transferred (i.e., the area under the STA waveform). In particular, the final depolarizing phase preceding action potential firing increased in both amplitude (data not shown; linear regression, r = 0.3, p = 0.037) and slope (Fig. 6B) across development. Likewise, STAs became more biphasic across development, exhibiting a shorter integration of depolarizing inputs (Fig. 6C) and a more pronounced negative peak (Fig. 6D) prior to action potential initiation. These developmental changes in STA shape were independent of ongoing synaptic transmission (Fig. 6; Table 1) and collectively suggest a stronger dependence of firing on coincidence detection in mature MCs (Hong et al., 2012; Ratté et al., 2013). Indeed, we observed a strong correlation between MC STA slope and within-cell spike-time reliability (Fig. 6E; linear regression, r = 0.76, p = 4.7×10^{-11}), confirming a developmental shift in MCs from broad integration of inputs to more selective coincidence detection and enhanced spike-time reliability.
The changes observed in MC spike-timing and STA shape across postnatal development interestingly parallel the changes observed in CA1 pyramidal cells upon entering a high-conductance state (Prescott et al., 2006, 2008; Hong et al., 2012). It is thus possible that the decrease in input resistance observed in MCs across postnatal development can explain the qualitative change in MC STA shape. Surprisingly, however, we found no significant correlation between MC input resistance and STA slope (linear regression, $r=0.13$, $p=0.30$), integration time ($r=0.17$, $p=0.16$) or negative peak ($r=0.024$, $p=0.84$) across development. Mechanistically, decreasing input resistance changes stimulus feature selectively in pyramidal cells by depolarizing action potential threshold, allowing a nonlinear increase in perithreshold-activated M-type potassium conductance that hyperpolarizes the membrane potential and strongly inhibits spiking, thereby transforming pyramidal cells from integrators to coincidence detectors (Prescott et al., 2006, 2008). While MCs express negligible M-type conductance and their action potential threshold tends to hyperpolarize, rather than depolarize, across development, MCs do express a perithreshold-activated A/D-type potassium conductance responsible for their irregular stuttering of action potential clusters (Balu et al., 2004). The change in MC STA shape may thus largely derive from a development increase in A/D-type potassium channel conductance. Consistent with this hypothesis, MCs exhibited greater stuttering (as measured by CV ISI) across development (Fig. 1H), and CV ISI significantly correlated with STA slope (linear regression, $r=-0.55$, $p=1.7\times10^{-6}$), integration time ($r=-0.54$, $p=1.9\times10^{-6}$), and negative peak ($r=-0.26$, $p=0.036$). Thus, the developmental shift in MC stimulus feature selectivity likely emerges from a distinct biophysical – but common dynamical – mechanism as the shift in pyramidal cell stimulus feature selectively when entering a high conductance state.

To further extend our understanding of the input-output relationship in MCs, we next turned to an approach that allows physiological data to constrain models that provide important insight into the capabilities of single neurons or populations of neurons to encode stimuli. The probabilistic GLM approach (e.g., see Paninski et al., 2007; Pillow et al., 2008; Tripathy et al., 2013) allows each MC’s recorded firing response to the fluctuating current stimulus to be fit to a GLM incorporating a stimulus filter, spike history effects, and a constant bias term (see Materials and Methods). The stimulus filter characterizes how the probability of firing changes as a function of the stimulus and is similar to the STA of a neuron. The spike history filter describes how the probability of firing varies as a function of time since the last action potential. For example, refractory period effects are reflected in negative regions of the spike history filter, while positive regions reflect rebound and burst firing. Each fit GLM thereby provided a complete statistical description of the relationship between input current and output spike train for a single MC, and moreover enabled us to simulate MC firing responses to any arbitrary stimulus (Fig. 7A).
The fluctuating stimulus-evoked spike trains of MCs were well modeled by GLMs, with correlations between experimental and GLM-predicted PSTHs greater than 0.7, and most of them greater than 0.9 (Fig. 7B). Within this range of high performance, some immature MCs interestingly exhibited comparatively lower model performance (<0.8), suggesting that immature MCs may exhibit less stable stimulus selectivity across trials than more mature MCs. Less stable stimulus selectivity may be one factor contributing to the lower spike-time reliability observed in immature versus mature MCs (Figs. 4,5). However, we emphasize that MCs across all developmental stages exhibited high correlations (>0.7) between experimental and GLM-predicted PSTHs (Fig. 7B). Importantly, this strong model performance was specifically observed using a subset of data not used during model fitting, thus ensuring that the fit GLMs accurately captured the stimulus-encoding properties of each MC without over-fitting to a specific stimulus structure. Moreover, GLM performance was independent of variations in spike-time reliability across MC development (data not shown; linear regression, p=0.60). Therefore, the GLM strategy provided a viable approach for further investigating specific changes in MC stimulus encoding across postnatal development.

Across development, GLM spike history filters significantly decreased in both peak gain and peak timing (Fig. 7Cii,Ciii), and tended to develop a second peak at short times post-stimulus (Fig. 7Ci). The developmental decrease in spike history filter gain may arise from the strong developmental decline in sag amplitude (Fig. 2), which underlies rebound firing in many neuron types across the brain, while the developmental decrease in spike history filter peak timing and emergence of a second peak is consistent with the increased tendency of mature MCs to exhibit irregular stuttering of high frequency action potential clusters (Fig. 1; Burton and Urban, 2014). In turn, GLM stimulus filters significantly increased in both peak gain and slope across development (Fig. 7D), directly paralleling the developmental trajectory of MC STAs (Fig. 6). Collectively, these results thus reinforce the conclusion that MC coding evolves from broad temporal integration into resonance and selective coincidence detection across development.

We next assessed how well MCs from different developmental stages were able to encode stimuli by performing stimulus decoding from their spike trains. Specifically, we used a Bayesian decoder to generate a predicted stimulus from the spike trains of individual MCs and populations of age-matched MCs, and then compared predicted (i.e., reconstructed) stimuli to the original stimulus used to evoke MC firing (Fig. 8A; Tripathy et al., 2013). Overall, stimulus reconstruction by mature individual MCs (P20-35) proved significantly more accurate than stimulus reconstruction by immature MCs (P7-11) (Fig. 8B). Interestingly, however, there was a broad range of encoding efficiency across both immature and mature MCs (Fig. 8B), consistent with the substantial degree of heterogeneity in MC response properties that we and others have
observed (Padmanabhan and Urban, 2010, 2014; Angelo and Margrie, 2011; Burton et al., 2012; Tripathy et al., 2013; Tucker et al., 2013; Burton and Urban, 2014).

To better resolve which components of the stimulus are more efficiently reconstructed by mature MCs, we calculated the spectral coherence between the predicted and actual stimuli. This analysis revealed that mature individuals MCs were more effective in encoding high frequency (>30 Hz) components of the stimulus than immature MCs (Fig. 8C). Indeed, examination of the mean coherence within low (0-10 Hz), beta (10-30 Hz), and gamma (30-100 Hz) frequency bands – each of which is critically involved in olfaction (for review, see Kay, 2014) – demonstrated that mature MCs were significantly better at encoding gamma, but not beta or low, frequency components than immature MCs (Fig. 8C). This result directly parallels our earlier observations of: 1) a transition from monophasic (i.e., lower frequency composition) to biphasic (i.e., higher frequency composition) STAs (Fig. 6), and 2) an acceleration of GLM stimulus filters and spike history effects (Fig. 7) across development. Consistent with previous observations (Padmanabhan and Urban, 2010; Tripathy et al., 2013), small 5-cell populations of heterogeneous MCs exhibited uniformly more efficient stimulus encoding than any individual mature or immature MC (compare Fig. 8C,D). Even within these small heterogeneous populations, however, groups of mature MCs were still substantially more effective than groups of immature MCs in encoding high frequency components of the stimulus (Fig. 8D). Similar to our results with individual MCs, populations of mature MCs exhibited significantly higher mean coherence within the gamma frequency band than populations of immature MCs, while no difference was observed within the low frequency band (Fig. 8D). Of note, populations of mature MCs also exhibited modestly but significantly higher mean coherence within the beta frequency band than populations of immature MCs. Collectively, these results thus establish the preferential enhancement of high frequency stimulus encoding as a robust feature of postnatal MC development.
DISCUSSION

Sensory experience in the vertebrate olfactory system is encoded by the precise spatiotemporal patterns of firing in MCs, a major class of principal neurons in the main olfactory bulb (for review, see Friedrich, 2006; Bathellier et al., 2010). These spatiotemporal patterns of MC firing emerge from the interaction between MC synaptic inputs and the intrinsic biophysical properties of MCs. To begin to understand the sensory processing underpinning early postnatal olfactory-guided behavior, we have thus systematically characterized MC intrinsic biophysical and stimulus-response properties across a broad developmental range over which MCs receive and process sensory-evoked input from peripheral sensory neurons (Malun and Brunjes, 1996).

Across postnatal development, we observed a gradual decline in both MC membrane time constant and input resistance. This finding, together with the acceleration of the MC action potential waveform, suggests a developmental increase in the functional expression of sodium and potassium channels (for review, see Bean, 2007). In contrast, we observed a gradual decline in MC membrane potential sag across development, suggesting a change in subunit composition, subcellular localization, and/or decrease in expression of HCN channels (for review, see Robinson and Siegelbaum, 2003). This loss of rebound current, together with an acceleration of MC AHPs, correlated with a developmental transition of MCs from regular firing of action potentials to irregular stuttering of high frequency action potential clusters. Concurrent with this change in firing pattern, MCs additionally exhibited an increased trial-to-trial spike-time reliability and greater between-cell spike-time synchrony in response to physiological fluctuating currents across development. This enhanced spike-time precision arose from a developmental transition in stimulus feature selectivity from broad integration of inputs over a relatively long time window to more selective coincidence detection and high frequency resonance, as reflected by a qualitative change in STA waveform from monophasic to biphasic and an enhanced ability of mature MCs to encode high frequency (>30 Hz) components of the fluctuating stimuli.

Our results thus collectively suggest that normal postnatal development of MCs acts to attune MCs to high frequency components of network activity within the olfactory bulb. This attunement specifically includes both faster MC output speeds, such as by accelerated AP waveforms and an increased propensity to fire high frequency action potential clusters, as well as greater selectivity for faster synaptic inputs, reflecting coincident transmission from multiple presynaptic partners. In other words, MCs increase their ability to both respond to and generate high frequency signals across postnatal development. Interestingly, this developmental program strongly parallels the postnatal development of cortical fast-spiking interneurons (Doischer et al., 2008; Okaty et al., 2009), which exhibit similar stuttering of high frequency action potential
clusters and are likewise critical for generating gamma frequency network rhythms (for review, see Hu et al., 2014).

What is the physiological relevance of this developmental attunement of MCs to high frequency signaling? Different frequency components of network activity within the olfactory bulb emerge from synaptic interactions in distinct lamina. Theta frequency components emerge from respiration-coupled sensory input in the glomerular layer (for review, see Wachowiak, 2011), which is further modulated by intrinsically bursting external tufted cells (Hayar et al., 2004a,b; Liu and Shipley, 2008) that coordinate glomerulus-wide long-lasting depolarizations (Carlson et al., 2000; Gire and Schoppa, 2009; De Saint Jan et al., 2009). In turn, gamma frequency components are strongly driven by synaptic interactions in the external plexiform layer between MCs and granule cells (Neville and Haberly, 2003; Friedman and Strowbridge, 2003; Lagier et al., 2004; Schoppa, 2006; Bathellier et al., 2006; Lepousez and Lledo, 2013; Fukunaga et al., 2014) and also likely external plexiform layer interneurons (Hamilton et al., 2005; Huang et al., 2013, Kato et al., 2013; Miyamichi et al., 2013). Critically, the glomerular layer activity of each MC is confined to a single odorant receptor-specific glomerulus, while lateral EPL interactions engage MCs across multiple glomeruli (e.g., see Dhawale et al., 2010). Our results thus suggest that immature MCs are likely to be preferentially attuned to theta frequency-modulated glomerular layer activity, while mature MCs are attuned to gamma frequency-modulated EPL activity. Consequently, immature MC activity will strongly reflect direct sensory input, encoding first-order sensory information (e.g., odor identity), while mature MC activity will be shaped by interglomerular interactions to encode more complex, second-order sensory information (e.g., odor context). Indeed, several studies have demonstrated that, mature MC activity progressively decorrelates from glomerular layer sensory inputs following the onset of odor exposure (Bathellier et al., 2008; Cury and Uchida, 2010; Kato et al., 2012; Adam et al., 2014).

The developmental attunement of MCs to high frequency activity and complex, second-order sensory information is well supported by in vivo electrophysiological and behavioral data. In particular, immature rodents exhibit theta and weak beta, but not gamma frequency activity in the olfactory bulb while navigating a simple olfactory discrimination task (Fletcher et al., 2005). While this absence of odor-evoked gamma frequency activity in the immature rodent was previously attributed to the late integration of olfactory bulb interneurons (Fletcher et al., 2005), we now show that the intrinsic biophysical properties of mature MCs themselves likely also contributes to gamma frequency signaling in the mature olfactory bulb. Indeed, consistent with a direct role of mature MCs in gamma frequency signaling, rhythmic activation of mature MCs alone generates a resonant peak in the olfactory bulb local field potential at gamma, but not beta or theta, frequencies (Lepousez and Lledo, 2013), directly paralleling the ability of mature, but not immature,
cortical fast-spiking interneurons to drive gamma frequency activity (Doischer et al., 2008; Cardin et al., 2009). Strikingly, gamma frequency activity in the mature olfactory bulb is driven by difficult, but not simple, olfactory discrimination tasks that may require complex, second-order olfactory information (Beshel et al., 2007), and perturbation of gamma frequency activity selectively impairs difficult odor discrimination (Lepousez and Lledo, 2013). This suggests that postnatal development of MC intrinsic biophysical properties may enhance select features of olfactory processing – specifically, the high frequency precision of olfactory bulb activity necessary for difficult odor discrimination.
REFERENCES


Figure 1. Postnatal development of passive membrane properties, excitability, and firing regularity. A, Examples of reconstructed MC morphologies from a P7 animal (upper) and a P29 animal (lower). B-E, Input resistance (B; linear regression, r=−0.32, p=0.009, n=67) and membrane time constant (C; linear regression, r=−0.45, p=0.0001, n=67) significantly decreased across postnatal development, while membrane capacitance (D; linear regression, r=−0.15, p=0.24, n=67; in the absence of synaptic antagonists: r=−0.30, p=0.040, n=48; in the presence of synaptic antagonists: r=0.18, p=0.47, n=19) and resting membrane potential (E; linear regression, r=−0.073, p=0.56, n=67) remained stable. Colored circles in B-E,H indicate parameters of representative cells in F. For B-E,H, solid circles indicate MCs recorded in the absence of synaptic antagonists, while closed circles indicate MCs recorded in the presence of synaptic antagonists. F, Representative firing responses evoked by constant step current injections in a regular firing MC (blue) and in an irregular firing MC (magenta). G, FI curves of immature (P7-11; gray) and mature (P20-30; black) MCs did not significantly differ (2-way ANOVA, p=0.99) H, MCs fired more irregularly across development (linear regression, r=0.49, p=3.0×10⁻⁵, n=67).

Figure 2. Postnatal development of hyperpolarization-evoked membrane potential sag. A, Representative membrane potential traces from a P16 MC evoked by hyperpolarizing step current injections (ranging from 0 pA to −300 pA in steps of −50 pA). B, Initial and steady state membrane potentials in response to hyperpolarizing step current injections. C, MC sag amplitude significantly decreased across postnatal development (linear regression, r=−0.51, p=2.1×10⁻⁵, n=62). D-F, Sag amplitude positively correlated with input resistance (D; linear regression, r=0.27, p=0.031 n=62) and membrane time constant (E; linear regression, r=0.39, p=0.0019, n=62), but not membrane capacitance (F; linear regression, r=0.07, p=0.57, n=62). For C-F, solid circles indicate MCs recorded in the absence of synaptic antagonists, while closed circles indicate MCs recorded in the presence of synaptic antagonists.

Figure 3. Postnatal development of action potential waveform properties. A, Mean action potential waveforms color-coded by age, beginning with threshold crossing at 0 ms and extending out 4 ms. B, Developmental profiles of action potential threshold (Bi), peak (Bii), duration (Biii), and AHP peak (Biv). Action potential duration strongly decreased across development (linear regression, r=−0.62, p=1.8×10⁻⁸ n=67 (solid line); in the absence of synaptic antagonists: r=−0.65, p=4.6×10⁻⁷, n=48; in the presence of synaptic antagonists: r=−0.60, p=0.01, n=19), while a strong trend existed toward hyperpolarization of the action potential threshold (linear regression, r=−0.23, p=0.06, n=67). No significant relationship was observed between age and the absolute action potential peak voltage (linear regression, r=−0.12, p=0.32,
n=67) or peak AHP amplitude (linear regression, r=0.14, p=0.27, n=67). C, Developmental profiles of action potential rising (Cii) and falling slopes (Ci\textsubscript{iii}). Both rising slope (linear regression, r=0.40, p=9×10\textsuperscript{-4}, n=67) and falling slope (linear regression, r=−0.72, p=6.9×10\textsuperscript{-12}, n=67 (solid line); in the absence of synaptic antagonists: r=−0.73, p=3.6×10\textsuperscript{-9}, n=48; in the presence of synaptic antagonists: r=−0.81, p=2.2×10\textsuperscript{-5}, n=19) significantly accelerated across development. D, Developmental profile of AHP kinetics. Normalization of AHP amplitudes (Dii; the average AHP of each MC is plotted from the time of AHP onset to 5 ms after the AHP peak time) reveals a marked acceleration of AHP kinetics across development. AHP peak times significantly shortened across development (linear regression, r=−0.45, p=2×10\textsuperscript{-4}, n=67). For B-D, solid circles indicate MCs recorded in the absence of synaptic antagonists, while closed circles indicate MCs recorded in the presence of synaptic antagonists.

Figure 4. Postnatal development of spike-time reliability and between-cell spike-time correlations. A, Spike raster plots (lower) of a P7 MC (blue) and P26 MC (magenta) in response to repeated injections of a frozen fluctuating stimulus (upper). The firing rate and self-correlation of these representative MCs are indicated by corresponding colored circles in C,E. B, Matrix of all pairwise cell-to-cell spike-time correlations, with MCs arranged by age. Only MCs recorded in the absence of ionotropic synaptic antagonists are shown in this matrix; similar results were obtained using all MCs (data not shown). C, MC self-correlation (i.e., spike-time reliability) increased across development (linear regression, r=0.38, p=0.0014, n=67 (solid line); in the absence of synaptic antagonists: r=0.38, p=0.0083, n=48; in the presence of synaptic antagonists: r=0.52, p=0.023, n=19). D, MC self-correlation dependence on temporal bin size used in calculating correlations. Neurons are grouped into four developmental stages, P7–10 (blue, n=13), P11–15 (pink, n=14), P16–20 (red, n=7), and P21–35 (orange, n=14). Self-correlation of the P7-P10 group is significantly smaller than other groups for temporal binning <15ms. Error bars show standard error of the mean. * P<0.05. E, Mean MC firing rate in response to the frozen fluctuating stimulus increased across development (linear regression, r=2×10\textsuperscript{-4}, p=0.43, n=67). F, Between-cell spike-time correlations for the four developmental stages. P7-10 and P11-15 MCs exhibited lower between-cell correlations than P16-20 and P21-35 MCs (T-test, P7-10 vs. P16-20, p=1.4×10\textsuperscript{-10}; P7-10 vs. P21-35, p=8.5×10\textsuperscript{-5}; P11-15 vs. P16-20, p=2.7×10\textsuperscript{-6}; P11-15 vs. P21-25, p=9.7×10\textsuperscript{-4}. Error bars show standard error of the mean. For C,E, solid circles indicate MCs recorded in the absence of synaptic antagonists, while closed circles indicate MCs recorded in the presence of synaptic antagonists. Data used in D,F were collected in the absence of ionotropc synaptic antagonists.

Figure 5. Postnatal development of responses to physiological synaptic input. A, Physiological synaptic inputs (middle) generated by a periodic 2 Hz respiration-like inhomogeneous Poisson process (upper) evoked rhythmic bursts of firing in both immature (lower, blue) and mature (lower, red) MCs. B, MC self-correlation
(i.e., spike-time reliability) in response to physiological synaptic input was significantly higher in mature MCs (linear regression, r=0.62, p=0.002, n=22). Solid circles indicate MCs recorded in the absence of synaptic antagonists, while closed circles indicate MCs recorded in the presence of synaptic antagonists.

**Figure 6.** Postnatal development of STAs. **A,** STAs of P7–10 MCs (black) and P20-30 MCs (blue). Inset: definition of STA slope, integration time, peak, and negative peak (see Materials and Methods). **B,** STA slope increased across development (linear regression, r=0.47, p=0.0001, n=67). **C,** Integration time decreased across development (linear regression, r=−0.43, p=0.0002, n=67). **D,** The negative peak of the STA decreased across development (linear regression, r=−0.31, p=0.01, n=67). **E,** The STA slope strongly predicted within-cell spike-time correlations (linear regression, r=0.78, p=1.2×10−14, n=67). For **B-E,** solid circles indicate MCs recorded in the absence of synaptic antagonists, while closed circles indicate MCs recorded in the presence of synaptic antagonists.

**Figure 7.** Postnatal development of GLM fitting parameters. **A,** Experimentally recorded (black) and GLM predicted (red) spike raster plots (middle) and PSTHs (lower) of a MC (P26, Corr(PSTH\textsubscript{exp} vs. PSTH\textsubscript{GLM}) = 0.96) in response to a frozen fluctuating stimulus (upper). **B,** The correlation between experimentally recorded and GLM predicted PSTHs across development. **C,** Spike history filters (following an action potential at 0 ms) for P7-10 MCs (black) and P20-30 MCs (blue) (Ci), and spike history peak gain (Cii; linear regression, r=−0.56, p=3.8×10^{-5}, n=48) and peak timing (Ciii; linear regression, r=−0.40, p=0.0052, n=48) across development. **D,** Stimulus filters (preceding an action potential at 0 ms) for P7-10 MCs (black) and P20-30 MCs (blue) (Di), and stimulus filter peak gain (Dii; linear regression, r=0.40, p=0.0055, n =48) and slope (Diii; linear regression, r=0.43, p=0.0025, n =48) across development. The slope of the stimulus filter was calculated as for the STA slope (see Materials and Methods). Equivalent results were obtained when MCs with comparatively low (<0.8) correlations between experimentally recorded and GLM predicted PSTHs were omitted from analyses.

**Figure 8.** Postnatal development of stimulus reconstruction. **A,** Representative reconstructions of a fluctuating stimulus (black) by individual MCs (upper) and 5-cell MC populations (P7-11, blue; P20-35, red). **B,** Pearson correlation coefficient between the original input and the stimuli reconstructed by individual MCs. Blue and red points shown group means. Mature individual MCs (n=15) more accurately reconstructed the original stimulus than immature MCs (n=15) (T-test, P=0.008). **C,** **D,** Magnitude-squared coherence between original stimulus and individual MC reconstructions (C) or MC population reconstructions (D). 15 individual MCs and 4 MC populations for each developmental group were analyzed. Dotted curves show coherence of individual MCs (C) and individual MC populations (D). Solid curves show average coherences.
Insets: mean coherence across low (0-10 Hz; “θ”), beta (10-30 Hz; “β”), and gamma (30-100 Hz; “γ”) frequency components of the stimulus. Individual mature MCs were more effective than individual immature MCs in encoding gamma (p=4.0×10^{-4}) but not low (p=0.73) or beta (p=0.08) frequency components. Populations of mature MCs were likewise more effective than populations of immature MCs in encoding gamma (p=2.6×10^{-3}) but not low (p=0.51) frequency components. Populations of mature MCs were also weakly but significantly more effective than populations of immature MCs in encoding beta frequency components (p=6.5×10^{-3}).
Table 1. Influence of spontaneous synaptic transmission on the intrinsic biophysical properties of MCs

<table>
<thead>
<tr>
<th></th>
<th>−CNQX/APV/+CNQX/APV/ Bicuculline</th>
<th>+CNQX/APV/ Bicuculline</th>
<th>p value</th>
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<tr>
<td>Input resistance (MΩ)</td>
<td>128.2 ± 53.4 (48)</td>
<td>121.8 ± 79.7 (19)</td>
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<td>Membrane time constant (ms)</td>
<td>28.1 ± 16.5 (48)</td>
<td>33.4 ± 15.4 (19)</td>
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<td>Membrane capacitance (pF)</td>
<td>235.1 ± 137.1 (48)</td>
<td>330.3 ± 170.0 (19)</td>
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<td>Resting membrane potential (mV)</td>
<td>−58.2 ± 5.5 (48)</td>
<td>−59.6 ± 5.0 (19)</td>
<td>0.35</td>
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<tr>
<td>Firing regularity [CV_{isi}]</td>
<td>0.31 ± 0.33 (48)</td>
<td>0.34 ± 0.42 (19)</td>
<td>0.76</td>
</tr>
<tr>
<td>Sag amplitude (mV)</td>
<td>3.8 ± 4.1 (45)</td>
<td>2.4 ± 1.7 (17)</td>
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<td>Action potential threshold (mV)</td>
<td>−33.8 ± 8.7 (48)</td>
<td>−37.4 ± 10.1 (19)</td>
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<td>Action potential peak (mV)</td>
<td>29.4 ± 9.0 (48)</td>
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<td>Action potential amplitude (mV)</td>
<td>63.2 ± 10.4 (48)</td>
<td>64.1 ± 10.8 (19)</td>
<td>0.74</td>
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<td>Action potential duration (ms)</td>
<td>1.20 ± 0.20 (48)</td>
<td>1.34 ± 0.23 (19)</td>
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<td>Action potential rising slope (mV ms(^{-1}))</td>
<td>175.5 ± 52.3 (48)</td>
<td>179.6 ± 50.5 (19)</td>
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<td>Action potential falling slope (mV ms(^{-1}))</td>
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<td>AHP peak (mV)</td>
<td>−19.5 ± 6.2 (48)</td>
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<td>AHP peak time (ms)</td>
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<td>5.1 ± 1.6 (19)</td>
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<td>Spike-time correlation</td>
<td>0.15 ± 0.08 (48)</td>
<td>0.21 ± 0.10 (19)</td>
<td>7×10(^{-3}) **</td>
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<td>Spike-time correlation [rhythmic inputs]</td>
<td>0.19 ± 0.06 (9)</td>
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<td>STA slope (pA/ms)</td>
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<td>STA integration time (ms)</td>
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<td>STA negative peak (pA)</td>
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<td>−12.3 ± 4.2 (19)</td>
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*p<0.05; **p<0.01; ***p<0.001 (two-tailed unpaired t test). Values reported are mean ± standard deviation (n).
A

100 Hz

50 ms

-0x0

50.7

0.8

0.9

1

age (days)

PSTHexp. vs. PSTHGLM

0.5

1

1.5

50 ms

-40 -20 0

0.2

0.4

0.6

1

gain (a.u.)

ƟŵĞ;ŵƐͿŚŝƐƚŽƌLĮůƚĞƌ

peak gain (a.u.)

ƟŵĞ;ŵƐͿŚŝƐƚŽƌLĮůƚĞƌ

age (days)

ƉĞĂŬƟŵŝŶŐ;ŵƐͿŚŝƐƚŽƌLĮůƚĞƌ

peak timing (ms)

age (days)

AB

Ci Cii Ciii

Di Dii Diii

history filter

stimulus filter