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Research Article

Dopamine Modulates Auditory Responses in the Inferior Colliculus in a Heterogeneous Manner

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ABSTRACT

Perception of complex sounds such as speech is affected by a variety of factors, including attention, expectation of reward, physiological state, and/or disorders, yet the mechanisms underlying this modulation are not well understood. Although dopamine is commonly studied for its role in reward-based learning and in disorders, multiple lines of evidence suggest that dopamine is also involved in modulating auditory processing. In this study, we examined the effects of dopamine application on neuronal response properties in the inferior colliculus (IC) of awake mice. Because the IC contains dopamine receptors and nerve terminals immunoreactive for tyrosine hydroxylase, we predicted that dopamine would modulate auditory responses in the IC. We recorded single-unit responses before, during, and after the iontophoretic application of dopamine using piggyback electrodes. We examined the effects of dopamine on firing rate, timing, and probability of bursting. We found that application of dopamine affected neural responses in a heterogeneous manner. In more than 80 % of the neurons, dopamine either increased (32 %) or decreased (50 %) firing rate, and the effects were similar on spontaneous and sound-evoked activity. Dopamine also either increased or decreased first spike latency and jitter in almost half of the neurons. In 3/28 neurons (11 %), dopamine significantly altered the probability of bursting. The heterogeneous effects of dopamine observed in the IC of awake mice were similar to effects observed in other brain areas. Our findings indicate that dopamine differentially modulates neural activity in the IC and thus may play an important role in auditory processing.

Keywords: mouse, iontophoresis, midbrain, D2 receptors

INTRODUCTION

Dopamine is most commonly studied for its role in reward-based learning and in disorders such as Parkinson's disease, schizophrenia, and addiction (Maia and Frank 2011; Schultz 2013). Multiple lines of evidence suggest that dopamine also plays a role in normal and abnormal auditory processing at a variety of levels between the periphery and the forebrain. For example, dopamine receptors and dopaminergic terminals are present in the cochlea, auditory brainstem, inferior colliculus, thalamus, and forebrain areas (Wamsley et al. 1989; Weiner et al. 1991; Kitahama et al. 1996; Tong et al. 2005; Drescher et al. 2006; Goodson et al. 2009; Kubikova and Kostál 2010; Kubikova et al. 2010; Maison et al. 2012; Hormigo et al. 2012). In addition, dopamine receptor activation modulates auditory responses at multiple levels of the ascending auditory system (Gáborján et al. 1999; Ruel et al. 2001; Leblois et al. 2010; Bender et al. 2012). However, most of these studies have

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focused on the cochlea in mammals and forebrain regions in songbirds. Little is known about how dopamine modulates auditory processing along the ascending auditory system.

The main auditory midbrain nucleus, the inferior colliculus (IC), is a likely locus of dopamine modulation. The IC contains nerve terminals immunoreactive for tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of dopamine (Kitahama et al. 1996; Tong et al. 2005). It also contains dopamine receptors, with the D2-like family predominating (Wamsley et al. 1989; Weiner et al. 1991). In addition, application of the dopamine agonist apomorphine in the IC suppresses prepulse inhibition of the startle reflex, and this is blocked by the D2-receptor antagonist haloperidol (Satake et al. 2012). Taken together, these lines of evidence suggest that dopamine is an important modulator of auditory processing in the IC.

Because the IC receives strong convergence of ascending auditory information and is a hub of information processing (Adams 1979; Brunso-Bechtold et al. 1981; Frisina et al. 1998; Winer and Schreiner 2005), dopamine modulation in the IC could have profound influence on ascending signals to the thalamus and cortex. Evidence supporting dopaminergic neuromodulation comes from observations that neural responses in the IC change with attention (Rinne et al. 2008) and reward-based learning (Metzger et al. 2006). However, the effects of dopamine on the firing properties of individual neurons in the IC are unknown. Thus, in this study, we examined the effects of dopamine on neural responses in the IC of awake mice. We found that dopamine altered neuronal response properties in a heterogeneous manner.

MATERIALS AND METHODS

Auditory responses were recorded from single neurons in the IC of seven awake, restrained CBA/CaJ mice. All mice were female and between 3 and 12 months old. The CBA/CaJ strain exhibits normal hearing sensitivity into its second year of life (Willott 1986). Animals were housed with same-sex littermates on a reversed 12-h light/dark schedule. All mice had *ad libitum* access to food and water. All care and procedures were in accordance with the guidelines of the National Institutes of Health and were approved by the Washington State University Institutional Animal Care and Use Committee.

Surgical Procedures

To fix the head in a stable and consistent position for electrophysiological recordings in the IC, we mounted a headpost onto the mouse's skull at least 24 h prior to the first recording session (Muniak et al. 2012). Mice were anesthetized with isoflurane in an induction chamber and then placed in a stereotaxic apparatus with a bite bar and ear inserts to fix the head securely. Isoflurane anesthesia was maintained throughout the surgical procedure via a mask. After shaving the top of the head, we made an incision along the midline and reflected the skin laterally. We cemented a metal rod (the headpost) onto the skull using ultraviolet light-cured dental cement and inserted a tungsten ground electrode into the right cerebral cortex. Using stereotaxic measurements (4.9 to 5.8 mm caudally from bregma and 0.5 to 1.7 mm laterally from midline; Paxinos and Franklin 2001), we made a craniotomy (~1-mm square) above the left IC. We covered the hole with petroleum jelly or bone wax to prevent the brain from dehydrating, applied lidocaine and a triple antibiotic ointment (neomycin, polymyxin B, and bacitracin) to the exposed muscle, applied postsurgical analgesia (ketoprofen, 3–5 mg/kg), and returned the mouse to its home cage for recovery for at least 24 h. The petroleum jelly or bone wax was removed prior to each recording session and reapplied at the end.

Electrophysiological Recording and Drug Application

To record single units while applying dopamine, we used the "piggyback" electrode configuration, in which a single-barreled recording micropipette was glued to a five-barreled micropipette for microiontophoretic application of drugs (Havey and Caspary 1980; Mayko et al. 2012). We aimed our electrode into the center of the exposed IC, and all recordings were obtained at depths ranging from 500 to 2,000 μm. Thus, we presumed all recordings were from the central nucleus of the IC. The multi-barrel pipette was pulled and the tip broken to approximately 30 μm in diameter. The single-barrel pipette was pulled to a tip <1 μm in diameter. The single pipette was glued to the multi-barrel pipette such that it extended 10–25 μm beyond the multi-barrel pipette tips. The single pipette was filled with 1 M NaCl. One of the multi-barrel pipettes was filled with dopamine (drug channel), and a second was filled with 1 M NaCl (sum channel). The dopamine solution (Calbiochem, 500 mM, pH adjusted to approximately 3.5 with 1 M HCl) was prepared on the day of the experiment. Silver wires were inserted into the recording pipette, the drug pipette, and the sum pipette. The wires from the multi-barrel pipette were connected to a microiontophoresis current generator (model 650, David Kopf Instruments, Tujunga, CA) to control the retention and ejection currents. Dopamine was retained with negative current (−20 nA) and ejected

with positive current. In early experiments, the positive current was initially set at 20 nA and then gradually ramped up as high as 120 nA. In later experiments, a 90-nA ejection current was used because this current consistently affected neuronal responses. These current values are similar to those used in previous studies (Tierney et al. 2008).

Electrophysiological experiments were conducted in a sound-attenuating chamber. On experimental days, the animal was given the sedative acepromazine (2 mg/kg) and then was secured in a foam body mold with the head protruding. The headpost was attached to a custom-made stereotaxic apparatus (Muniak et al. 2012). If the animal showed signs of distress, the experiment was terminated. Experimental sessions lasted no more than 5 h, and we used each animal in one to three sessions.

Acoustic stimulation was computer controlled and included tone bursts (50–100 ms duration, 1 ms rise/fall time, 4/s), frequency modulations, and noise bursts. All stimuli were generated by computer and output through a high-speed, 16-bit digital-to-analog converter (Microstar Laboratories, Bellevue, WA, USA; 400,000 samples/s), fed to a programmable attenuator (Tucker Davis Technologies, Alachua, FL, USA; PA5), a power amplifier (Parasound), and to a freefield leaf tweeter speaker (Emit) set 10 cm from the mouse's ear contralateral to the IC from which we recorded. The speaker was calibrated using a 1/4-in. calibrated microphone (Bruel and Kjaer, Denmark; Model 4135) placed in the position normally occupied by the animal's ear. There was a smooth, gradual decrease in sound pressure level from 6 to 100 kHz of about 3 dB per 10 kHz. Distortion components in tonal stimuli were buried in the noise floor, at least 50 dB below the signal level, as measured by custom-designed software performing a fast Fourier transform of the digitized microphone signal.

Electrodes were advanced using a hydraulic micropositioner (David Kopf Instruments, Tujunga, CA) located outside the acoustic chamber. Extracellular action potentials were amplified (Dagan Corporation, Minneapolis, MN, USA), filtered (bandpass, 500–6,000 Hz; Krohn-Hite, Brockton, MA, USA), and passed through a spike enhancer (Fredrick Haer, Bowdoin, ME, USA) before being digitized (Microstar Laboratories, Bellevue, WA, USA; 10,000 samples/s). Neural waveforms were displayed and archived using custom-written C++ software. Waveforms, raster plots, peristimulus time histograms (PSTHs), and statistics were viewed online and stored for offline analysis.

We recorded single-unit responses to tones, noise, and/or frequency-modulated sweeps before, during, and after the application of dopamine. Because we were interested in the effects of dopamine and not concerned with neuronal selectivity in this study, the stimulus that evoked the best responses was used for all data collection conditions. We recorded responses to 50–300 presentations of the same sound and intensity combinations while dopamine was retained (control). We then applied dopamine and recorded responses to the same stimuli as in the control condition. We initially applied dopamine for up to 30 min. After we noted that effects were usually observed within 5 min, the application was reduced to 5–10 min. Recovery data were collected by retaining the dopamine. To ensure that effects were not due to current alone, we applied positive current through the barrel containing 1 M NaCl and observed no effects on neural responses. This is in accord with previous studies (Tierney et al. 2008).

Waveforms were analyzed offline using custom software written in IGOR Pro (Wavemetrics). Single-unit isolation was verified based on a signal-to-noise ratio of at least 4:1, consistent waveform, and interspike interval (ISI) greater than 1 ms. A bursting event was defined as a set of at least three spikes in a row with ISIs $\leq$3.0 ms. We calculated the response jitter as the standard deviation of the latency of the first spike after the onset of the acoustic stimulus. Unless otherwise noted, values presented are mean ± SEM. Unless otherwise stated, Student's t tests were used for statistical analyses using IGOR Pro software.

RESULTS

We recorded responses of 28 single units from the central nucleus of the IC to tones, frequency-modulated sweeps, and/or noise before, during, and when possible, after the application of dopamine.

Heterogeneous Effects of Dopamine on Response Rate

Dopamine had heterogeneous effects on the firing rate of IC neurons; some neurons showed an increase in firing, some showed a decrease, and some neurons were not affected by dopamine application. Figure 1 illustrates an example of each of these cases and the overall distribution of the effects of dopamine on our sample of IC neurons. In the first example, the responses of a neuron to 100-ms broadband noise bursts were reversibly increased by the application of dopamine (Fig. 1A). Dopamine caused the response to increase nearly twofold, from 15±0.5 to 26±1 spikes/s (T_{598}=−8.9, P=9.4E−18). Following cessation of dopamine application, the response returned to 17 ±0.5 spikes/s.

In other neurons, dopamine application reduced firing rate. The neuron shown in Figure 1B had a high spontaneous rate that was reduced by a best

frequency tone, and tone cessation resulted in a transient increase in firing rate. Application of dopamine reduced the neuron's response during sound playback from 20±0.3 spikes/s under control conditions to 11±0.3 spikes/s (T_{398}=16, P=7.7E–47). Within 5 min after dopamine application ended, the response recovered to 27±0.3 spikes/s.

The last example demonstrates that in some neurons, application of dopamine did not affect firing rate (Fig. 1C). After establishing the control response to a tone stimulus (40±0.7 spikes/s), dopamine was applied for approximately 15 min, and the firing did not change during this period (41±0.7 spikes/s, T_{238}= –0.11, P=0.91). The firing rate remained stable for 10 min after the end of dopamine application.

The majority of neurons tested (23/28) showed significant changes in firing rate following the iontophoretic application of dopamine (Fig. 1D). For each neuron, we plotted the mean spike rate during dopamine application normalized by the mean spike rate under control conditions. Fourteen of the 28 neurons showed a significant decrease in firing following dopamine application (ratio<0.9, P<0.05), whereas 9 of 28 showed an increase in firing rate (ratio>1.1, P<0.05). In the five neurons in which firing changed by 10 % or less, the changes were not significant (P>0.05).

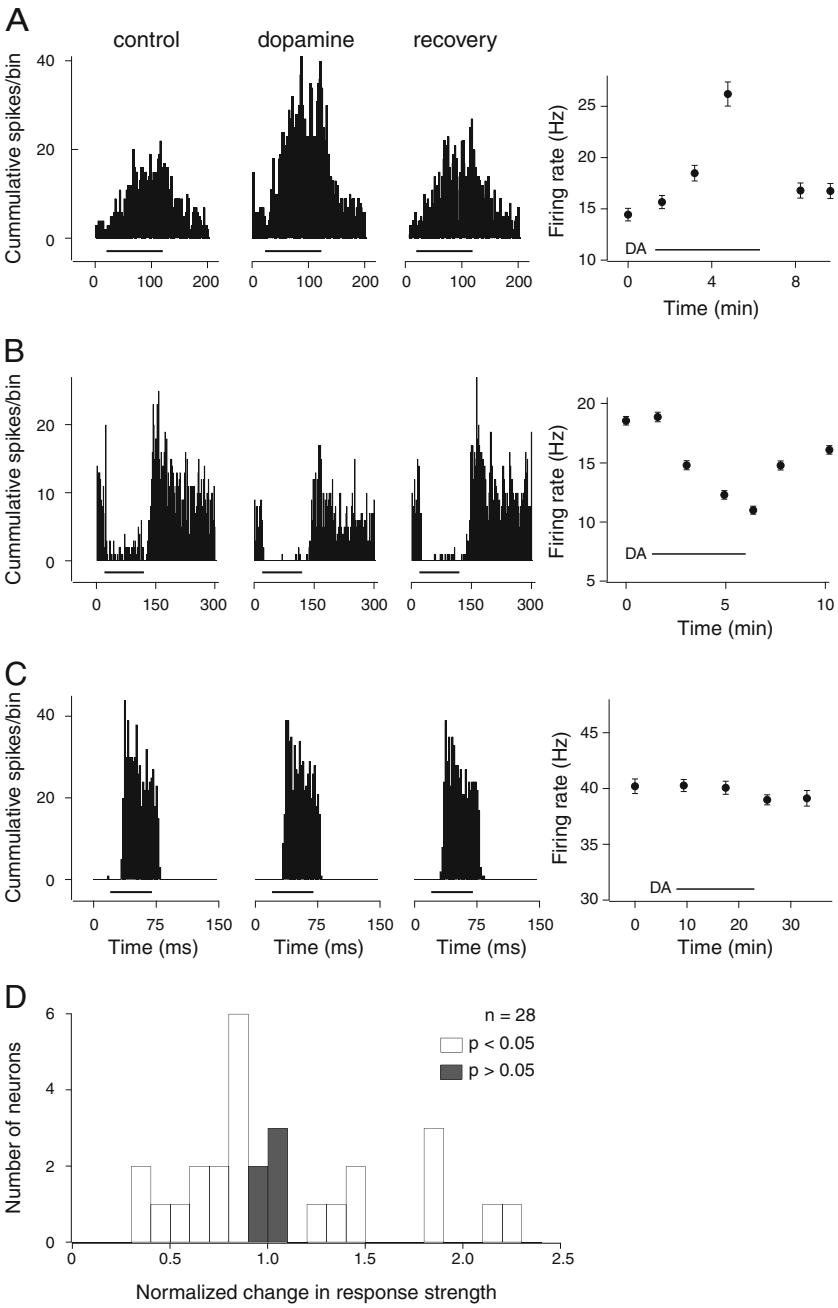

FIG. 1. Dopamine has heterogeneous effects on IC neurons. **A** Example neuron in which dopamine application increased the firing rate. The first three panels are PSTHs of that neuron's responses to 300 presentations of a 100-ms 24-dB sound pressure level (SPL) broadband noise stimulus (*horizontal bar*) during control, dopamine application, and recovery periods. The fourth panel represents firing rate throughout the recording period. Each point represents the average firing rate to 300 stimulus presentations. The *bar* labeled DA represents time of dopamine application. **B** Example neuron that decreased its firing rate during dopamine application. Same conventions as in **A** except that the stimulus was a 100-ms 65-dB SPL 18-kHz tone (*horizontal bar*) that was presented 200 times at each time point. **C** Example neuron that showed no change in its firing rate during dopamine application. Same conventions as in **A** except that the stimulus was a 100-ms 25-dB SPL 9-kHz tone (*horizontal bar*) that was presented 120 times. **D** Normalized change in response rate with dopamine application for the sample of 28 neurons. The mean number of spikes/trial during dopamine application was divided by the mean number of spikes/trial before dopamine. The majority of neurons showed a significant change in firing rate with dopamine application. *Open bars* represent neurons showing a significant effect of dopamine. *Filled bars* represent neurons that did not show a significant effect of dopamine. Values less than 1.0 represent decreases in response strength, and values greater than 1.0 represent increases in response strength.

Dopamine may affect responses of IC neurons in different ways. For example, if spontaneous activity is generated only intrinsically within IC neurons and dopamine affects only synaptic transmission, we would expect dopamine to affect evoked activity. Alternatively, if dopamine acts on intrinsic neuronal excitability or if spontaneous activity is synaptically driven, we would expect dopamine to alter spontaneous and evoked activity in the same manner. Therefore, we compared the effects of dopamine on spontaneous and evoked firing.

In general, dopamine altered sound-evoked and spontaneous firing rate in the same manner. Figure 2A shows spontaneous and sound-evoked firing of the neuron shown in Figure 1A. Under control conditions, spontaneous rate was 4.7±1.0 spikes/s and significantly increased during dopamine application to a peak value of 9.3±1.7 spikes/s (T_{598}=−2.4, P=0.016; Fig. 2A*2*). Similarly, the sound-evoked spike rate significantly increased from 20±1.0 spikes/s under control conditions to a peak of 39±1.9 spikes/s during dopamine application (T_{598}=−8.7, P=4.1E−17; Fig. 2A*3*). Thus, dopamine altered the spontaneous and sound-evoked activity in the same manner.

A similar trend was found in the example neuron shown in Figure 2B. Under control conditions, spontaneous activity was 25±1.8 spikes/s and then significantly decreased to a minimum of 12±1.5 spikes/s during dopamine application (T_{398}=5.3, P=2.3E−7). While the tone was presented, the spike rate decreased from 3.5±0.4 spikes/s under control conditions to 0.36±0.1 during application of dopamine (T_{398}=7.5, P=5.6E−13). This neuron also had an offset response (Fig. 2B*4*), and the spike rate during the offset response decreased from 58±1.9 spikes/s under control conditions to 37±1.7 spikes/s during application of dopamine (T_{398}=8.2, P=2.4E−15). During each measurement period (spontaneous activity, tone evoked, offset response), the spike rates decreased following application of dopamine (Fig. 2B*2–4*). Indeed, once dopamine was applied, the tone eliminated nearly all firing (Fig. 2B*3*).

We compared the effect of dopamine on spontaneous and sound-evoked firing in the 17/28 neurons that had sufficient spontaneous activity for analysis. For each neuron, we plotted the normalized dopamine-evoked change in spontaneous firing rate versus the normalized dopamine-evoked change in evoked firing rate. Although the magnitude of the effect of dopamine was often different on spontaneous activity compared to sound-evoked activity, there was a significant correlation between the change in spontaneous firing and the change in sound-evoked firing (Fig. 2C, R^2=0.68, P<0.001). These results are consistent with the hypotheses that dopamine modulates overall excitability of the postsynaptic membrane or that dopamine modulates synapses that contribute to both spontaneous and sound-evoked activity.

Heterogeneous Effects of Dopamine on First Spike Latency and Jitter

We measured the first spike latency and jitter before and during dopamine application in 25/28 neurons (one neuron had only spontaneous activity, and in two neurons, sound completely suppressed firing). As with firing rate, the effects of dopamine on spike timing were diverse. In some neurons, the first spike latency and jitter increased following dopamine application (Fig. 3A). In response to a best frequency tone, the example neuron illustrated had a mean first spike latency of 39 ms under control conditions, and this significantly increased to 52 ms following dopamine application (T_{394}=−10.6, P=2.0E−23). The range of latencies was 27.1–70.5 ms in the control and 27.5–105 ms with dopamine application. Jitter also significantly increased with application of dopamine from 7.7 to 16 ms (Levene's test using median, $F_{199,\ 195}$=51, P=4.0E−12). These changes were accompanied by a decrease in the sound-evoked response rate. Under control conditions, the average spike rate was 58±1.4 spikes/s, and this decreased to 33±1.1 spikes/s during application of dopamine. In other neurons, first spike latency and jitter decreased following dopamine application (Fig. 3B). Under control conditions, the example neuron illustrated had a mean first spike latency of 22 ms in response to a downward FM sweep. Following dopamine application, the latency significantly decreased to 18 ms (T_{321}=12, P=9.6E−29). The range of latencies was 16.5–31.5 ms in the control condition and 13.2–32.4 ms with dopamine application. Jitter also significantly decreased from 3.1 ms in the control condition to 2.0 with dopamine application (Levene's test using median, $F_{131,\ 190}$=25, P=1.1E−6). In this example, dopamine also increased the sound-evoked spike rate from 26±1.5 to 57±1.8 spikes/s.

Application of dopamine produced a significant change in first spike latency in 11/25 neurons (Fig. 3C, P<0.05). In 7/11 neurons, latency increased, and in 4/11 neurons, latency decreased. Dopamine application also produced a significant change in jitter in 9/25 neurons (Fig. 3D, Levene's test using median rather than mean, P<0.05). In six of nine neurons, jitter increased, and in three of nine neurons, jitter deceased.

There was a strong correlation between change in latency and change in jitter with application of dopamine as would be expected if dopamine modulated membrane excitability (Fig. 3E, R^2=0.92). Eight neurons showed a significant change in both latency and jitter, three neurons showed a significant change

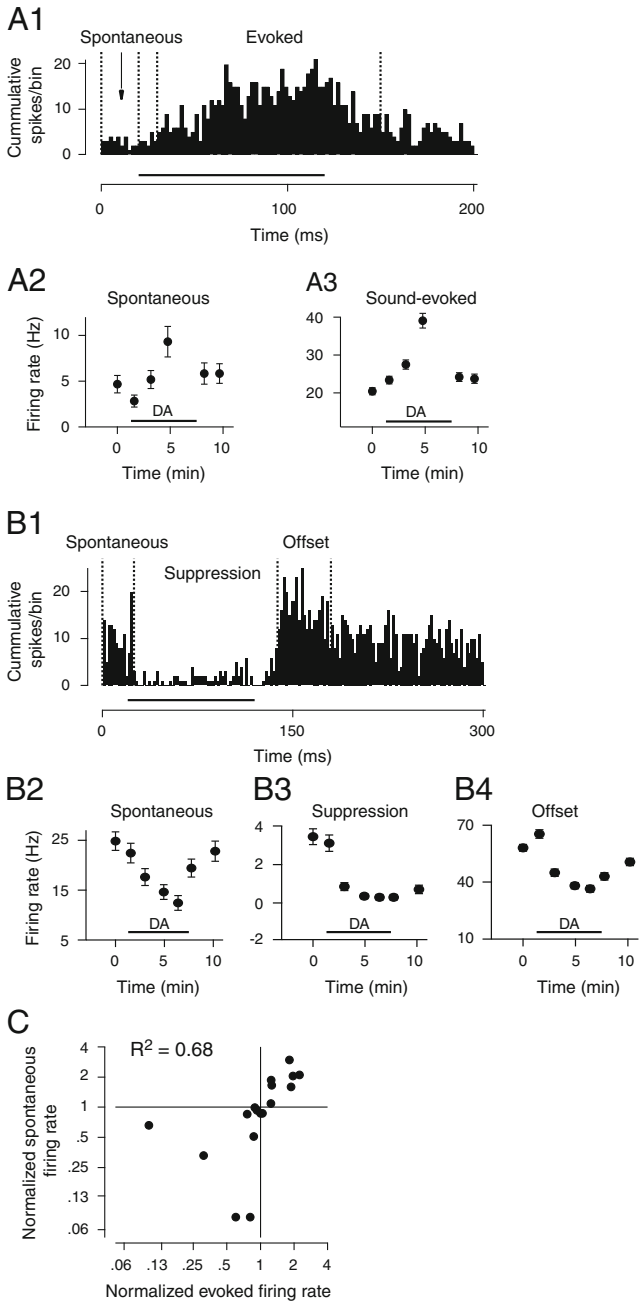

FIG. 2. Dopamine modulates spontaneous activity and sound-evoked firing similarly. **A** An example neuron that showed increased spontaneous and evoked firing rates with the application of dopamine. Same neuron as in Figure 1A. **A***1* PSTH showing time points where spontaneous and evoked firing rates were calculated. **A***2–3* Time courses of spontaneous and evoked firing rate throughout the recording period. Each point represents the average firing rate to 300 stimulus presentations. **B** An example neuron that showed decreased spontaneous and evoked firing rates with the application of dopamine. Same neuron as in Figure 1B. **B***1* PSTH showing time points where spontaneous, suppressed, and offset firing rates were calculated. **B***2–4* Time courses of spontaneous, evoked, and offset firing rates throughout the recording period. Each point represents the average firing rate to 200 stimulus presentations. **C** Scatter plot of normalized change in spontaneous firing rate versus normalized change in evoked firing rate in response to dopamine shows that dopamine affected spontaneous and evoked firing in the same direction.

in latency only, and one neuron showed a significant change in jitter only.

There was a significant inverse correlation between the change in onset latency and the change in evoked firing (Fig. 3F, R^2=0.54, P=0.007). In the seven neurons in which dopamine application increased the latency, overall evoked firing decreased. In the four neurons in which dopamine decreased the

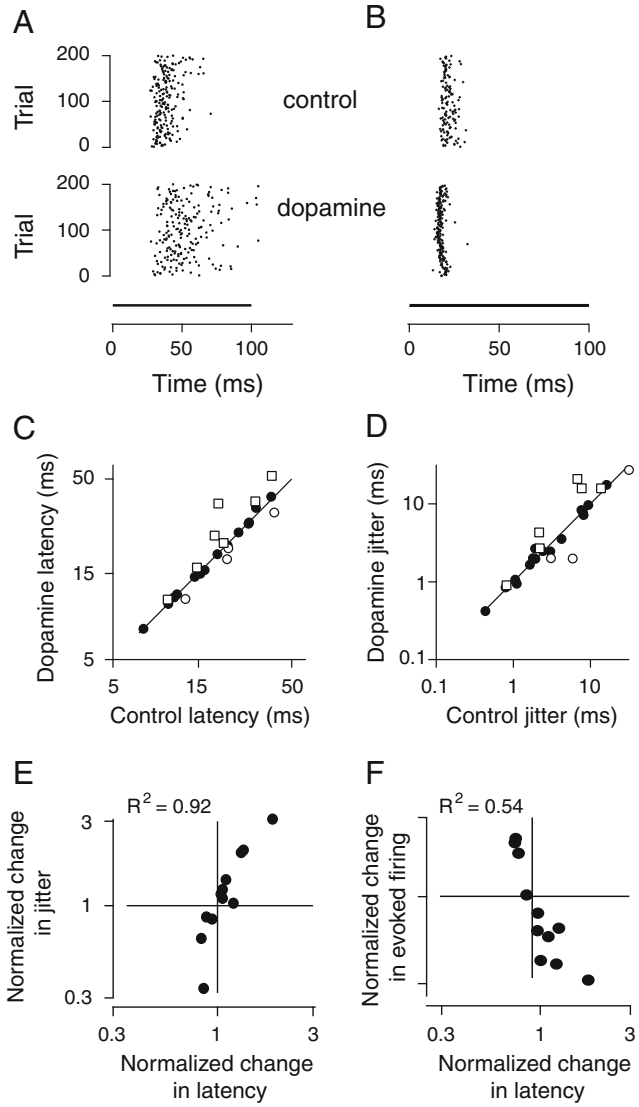

FIG. 3. Dopamine affects first spike latency and jitter. **A** Raster plots of an example neuron showing that dopamine application could increase first spike latency. Stimulus was a 50-dB SPL 23-kHz tone. **B** Raster plots of an example neuron showing that dopamine application could decrease first spike latency. Stimulus was a 70-dB SPL downward FM sweep with center frequency of 15 kHz and bandwidth of 6 kHz. The *bar* below the traces indicates acoustic stimulus. Only the first spike is shown for each trial. **C** Scatter plot illustrating mean first spike latency in the presence of dopamine versus that under control conditions. In some neurons, dopamine increased mean first spike latency (*open squares*; $P<0.05$); in other neurons, dopamine decreased mean latency (*open circles*; $P<0.05$). The remaining neurons showed no significant effect of dopamine on latency (*filled circles*; $P>0.05$). **D** Scatter plot illustrating the standard deviation of first spike latency (jitter) during dopamine application plotted against the jitter under control conditions. Same symbols as in **C**. **E** Scatter plot of the normalized effect of dopamine on jitter plotted against the normalized effect of dopamine on latency. Changes in jitter and latency were highly correlated. **F** Scatter plot of the normalized effect of dopamine on the mean number of sound-evoked spikes per trial plotted against the normalized effect of dopamine on mean first spike latency. The change in mean evoked firing was inversely correlated with the change in latency.

latency, evoked firing increased (although, in one of these neurons the increase in evoked firing was not significant). This inverse relationship is consistent with the dopaminergic effect on spike timing being due to modulation of overall neuronal excitability.

Effects of dopamine on bursting activity

Finally, we observed that dopamine modulated the probability of burst firing in a small number of neurons. Only 7/28 neurons exhibited enough burst firing for analysis, but in three of those seven, dopamine modulated burst probability. As illustrated in Figure 4A, application of dopamine altered the auditory response to include burst firing. Burst firing largely disappeared after dopamine was retained (Fig. 4A, recovery). In this neuron, dopamine significantly increased the burst probability from 0.1 to 5 % ($T_{598}=-7.2$, $P=2.0E-12$). In a second example (Fig. 4B), dopamine significantly decreased burst probability from 5 to 1 % ($T_{398}=4.3$, $P=$

2.2E–5). In two neurons, dopamine increased burst probability, and in one neuron, dopamine decreased burst probability. Although the sample size is small, these observations suggest that dopamine affects bursting in a heterogeneous manner.

DISCUSSION

Our main finding is that dopamine modulates neural activity in the mouse IC in a heterogeneous manner. Dopamine could increase or decrease spontaneous or sound-evoked firing rate, first spike latency, spike jitter, and/or probability of burst firing. Overall, our findings indicate that dopamine modulates auditory processing in the IC.

Potential Mechanisms Underlying the Effects of Dopamine

Because the IC expresses predominantly D2-like dopamine receptors (Wamsley et al. 1989; Weiner et al. 1991), we hypothesize that these receptors underlie the effects we observed. Activation of D2 receptors in other brain regions can have diverse actions, including altering synaptic strength and intrinsic neuronal excitability (Trantham-Davidson et al. 2004; Surmeier et al. 2011). All of the heterogeneous effects of dopamine we observed in the IC could be explained by mechanisms that have been described in other brain areas.

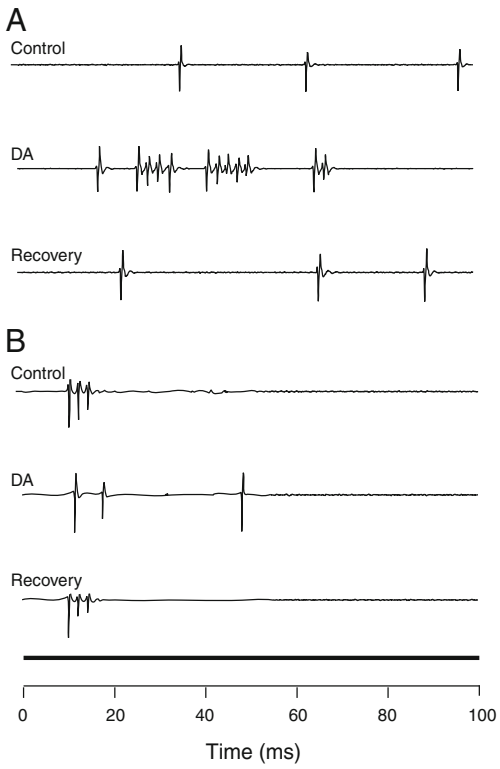

FIG. 4. Dopamine can modulate burst firing. **A** Voltage traces from individual trials from an example neuron showing single spikes during control and recovery (dopamine retained) and an increase in burst firing during dopamine application. The *horizontal bar* indicates time of the 24-dB SPL broadband noise stimulus. **B** Voltage traces from individual trials from an example neuron showing bursting during control and recovery (dopamine retained) and a decrease in burst firing during dopamine application. The *horizontal bar* indicates time of the 25-dB SPL 16-kHz (best frequency) tone.

Dopamine is known to alter synaptic properties. For example, in striatal medium spiny neurons, D2 receptor activation reduces corticostriatal glutamatergic synaptic transmission (Calabresi et al. 1993; Bamford et al. 2004; Higley and Sabatini 2010). A similar reduction of glutamatergic synaptic transmission could explain the decrease in both spontaneous and sound-evoked activity observed in some IC neurons, assuming that the spontaneous activity is synaptically driven. In addition, iontophoretic application of dopamine could spread and increase neural activity in the IC by reducing excitatory inputs to nearby inhibitory interneurons. Activation of D2 receptors is known to reduce GABA release in the ventral tegmental area (Michaeli and Yaka 2010). Thus, dopamine could increase IC neuronal firing by reducing the strength of extrinsic or intrinsic inhibitory synaptic inputs to the IC. Because the IC receives inhibitory inputs from multiple brainstem nuclei (Adams and Mugnaini 1984; Saint Marie et al. 1989; Gonzalez-Hernandez et al. 1996; Kulesza et al. 2003), the heterogeneous actions of dopamine in the IC could result from differential actions of dopamine on distinct sources of inhibition.

Dopamine can also alter intrinsic properties of neurons (Nicola et al. 2000), altering both spontaneous and evoked activity. Indeed, dopamine-induced modulation of any of a number of ion-channel types could account for the effects we observed. For example, D2 receptor activation can increase transient (A type) potassium currents (Perez et al. 2006) or modulate the hyperpolarization-activated cation current (I_h) (Vandecasteele et al. 2008). Alteration of either of these currents could reduce overall neuronal firing. Because both of these currents are expressed in a subset of IC neurons (Sivaramakrishnan and Oliver 2001; Koch and Grothe 2003), their modulation by dopamine could contribute to the reduced firing that we observed in some IC neurons.

Depending on which ion channels are expressed and the precise nature of the inputs, I_h reduction can also increase excitability (Koch and Grothe 2003; Khurana et al. 2011). In addition, D2 receptor activation can decrease two types of potassium current, thereby increasing overall neuronal firing. Specifically, D2 receptor activation can reduce the apamin-sensitive, calcium-activated potassium current and the dendrotoxin-sensitive potassium current

(Ramanathan et al. 2008; Govindaiah et al. 2010). Because both of these potassium currents are believed to be present in IC neurons (Sivaramakrishnan and Oliver 2001; Rosenberger et al. 2003; Tan et al. 2007; Xie et al. 2008; Gittelman et al. 2012), their reduction due to activation of D2 receptors could explain the increase in firing that we observed in some IC neurons.

The diverse effects of dopamine receptor activation on neuronal firing rate described above could also underlie the observed changes in temporal firing properties of IC neurons, including bursting. For example, modulation of I_h could either increase or decrease bursting (Tobin and Calabrese 2005; Vandecasteele et al. 2008; Orio et al. 2012). Detailed intracellular analysis of the effects of dopamine on intrinsic properties of IC neurons will be essential to determine the specific mechanisms underlying the heterogeneous effects of dopamine that we observed.

Functional Relevance of Dopamine Modulation in the IC

Considering that the IC contains both tyrosine hydroxylase-positive terminals and D2-like dopamine receptors (Wamsley et al. 1989; Weiner et al. 1991; Tong et al. 2005), it is likely that endogenous dopamine modulates neural activity in the IC. In addition, acute dopamine injection into the IC reduces prepulse inhibition of the acoustic startle reflex (Satake et al. 2012), indicating that dopamine modulation in the IC has functional relevance. Moreover, midbrain dopamine neurons in songbirds show selective responses to playback of the bird's own song (Gale and Perkel 2010), and dopamine can directly modulate auditory responses in the songbird striatum (Leblois et al. 2010). Because IC neurons in mice display selective responses to specific subsets of vocalizations (Portfors et al. 2009; Holmstrom et al. 2010; Mayko et al. 2012) and are modulated by dopamine (as shown here), it is plausible that modulation of IC responses by endogenous dopamine could shape perceptual detection or discrimination of vocal communication sounds.

Understanding how dopamine modulates auditory responses in the IC is likely to be an integral part of understanding and interpreting results from studies that use prepulse inhibition (PPI) as a behavioral endpoint. This simple modulation of a reflex is a tool in basic research and in translationally relevant animal models of numerous neuropsychiatric disorders, most notably schizophrenia (Braff et al. 1978; Swerdlow et al. 2008). Using sound as the prepulse stimulus, PPI has been used to illuminate the genetic substrates of gap detection, transient sound detection, and auditory spatial acuity (Allen and Ison 2010, 2012; Ison and Allen 2012) and is being developed as a screen for tinnitus (Turner et al. 2006; Lobarinas et al. 2013).

Although the IC is not needed for acoustic startle, lesion studies strongly suggest that it is required when using sound as the prepulse in PPI (Li et al. 1998). The findings that apomorphine injected directly into the IC reduces PPI and that this effect is blocked by the D2-antagonist haloperidol indicate that D2-like receptors modulate the auditory responses of IC neurons that contribute to the PPI circuit (Satake et al. 2012).

Finally, it is clear that responses in the IC are modulated by attention- (Rinne et al. 2008) and reward-based learning (Metzger et al. 2006). Our finding that dopamine modulates neural response properties in the IC provides further evidence that dopamine may contribute to such forms of behavior-related modulation of sensory processing.

Based on work in other systems, we would expect that endogenous dopamine levels increase in response to novel or especially salient cues, stimuli that predict reward or in a courtship context (Schultz et al. 1997; Phillips et al. 2003; Aragona et al. 2003; Charlier et al. 2005; Sasaki et al. 2006; Schultz 2010; Flagel et al. 2011). Thus, we speculate that during such times, it would be advantageous for the auditory system to modulate its neuronal properties to selectively enhance processing of relevant stimuli and reduce sensitivity to other inputs. Dopamine actions in the IC may function to help modulate auditory processing in a context-dependent manner.

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