Dopaminergic-induced changes in Mauthner cell excitability disrupt prepulse inhibition in the startle circuit of goldfish

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Medan V, Preuss T. Dopaminergic-induced changes in Mauthner cell excitability disrupt prepulse inhibition in the startle circuit of goldfish. J Neurophysiol 106: 3195–3204, 2011. First published September 28, 2011; doi:10.1152/jn.00644.2011.—Prepulse inhibition (PPI) is a widespread sensorimotor gating phenomenon characterized by a decrease in startle magnitude if a nonstartling stimulus is presented 20–1,000 ms before a startling stimulus. Dopaminergic agonists disrupt behavioral PPI in various animal models. This provides an important neuropharmacological link to schizophrenia patients that typically show PPI deficits at distinct (60 ms) prepulse-pulse intervals. Here, we study time-dependent effects of dopaminergic modulation in the goldfish Mauthner (M-cell) startle network, which shows PPI-like behavioral and physiological startle attenuations. The unique experimental accessibility of the M-cell system allows investigating the underlying cellular mechanism with physiological stimuli in vivo. Our results show that the dopaminergic agonist apomorphine (2 mg/kg body wt) reduced synaptic M-cell PPI by 23.6% (n = 18; P = 0.009) for prepulse-pulse intervals of 50 ms, whereas other intervals showed no reduction. Consistently, application of the dopamine antagonist haloperidol (0.4 mg/kg body wt) restored PPI to control level. Current ramp injections while recording M-cell membrane potential revealed that apomorphine acts through a postsynaptic, time-dependent mechanism by deactivating a M-cell membrane nonlinearity, effectively increasing input resistance close to threshold. This increase is most pronounced for prepulse-pulse intervals of 50 ms (47.9%, n = 8; P < 0.05) providing a time-dependent, cellular mechanism for dopaminergic disruption of PPI. These results provide, for the first time, direct evidence of dopaminergic modulation of PPI in the elementary startle circuit of vertebrates and reemphasize the potential of characterizing temporal aspects of PPI at the physiological level to understand its underlying mechanisms.

sensorimotor-gating; dopamine; cellular mechanisms; input resistance; time-specific neuromodulation

THE CENTRAL NERVOUS SYSTEM has to filter the overwhelming stream of information provided by the various sense organs while optimizing detection of behaviorally relevant information. One prominent filtering phenomenon is prepulse inhibition (PPI), which is thought of as an early sensory gate that reduces the sensory information of a secondary event (pulse) while a preceding event (prepulse) is being processed (Graham 1975; Hoffman and Ison 1980; Koch 1999). Experimentally, PPI is produced when a startling stimulus (pulse) is preceded within 20–1,000 ms by a subthreshold stimulus (prepulse) of the same or another modality (Graham 1975; Hoffman and Ison 1980; Koch 1999). The multimodal nature of PPI distinguishes it from unimodal synaptic modulations such as paired-pulse depression and short-term habituation (Aubert et al. 2006; Campeau and Davis 1995; Simons-Weidenmaier et al. 2006). The majority of PPI studies, however, including the present one, used auditory prepulse-pulse stimulus combinations.

PPI is typically associated with a sensory-evoked attenuation of the startle response in rats and mice or the eye-blink reflex in humans. Deficits in sensorimotor gating (PPI) are implicated in several cognitive and information-processing disorders (Braff et al. 1978, 2001b); for example, schizophrenia patients show reduced PPI for distinct prepulse/pulse intervals (~60 ms; Swerdlow et al. 2006). Although schizophrenia is a complex neurophysiological disorder likely modulated by many neurotransmitters, dopaminergic hyperactivity in schizophrenia was early postulated (Mansbach et al. 1988). Since then, disruption of auditory PPI by dopaminergic agonists became a powerful tool to investigate PPI deficits and its neuropharmacological basis (Geyer et al. 2001).

In mammals, the final common path for PPI lies within the elementary startle pathway, centered around a group of ~60 large reticulospinal neurons of the ventrocaudal pontine reticular formation (PnC) that integrate multimodal excitatory and inhibitory inputs to initiate the startle (Davis et al. 1982; Lingenhohl and Friauf 1994; Yeomans and Frankland 1995). However, the cellular mechanisms underlying PPI are far from clear, partly due to the difficulty of performing intracellular, in vivo recordings in the small and deep PnC nucleus. Moreover, the long and complex time course of PPI (20–1,000 ms) and its modulation by numerous neurotransmitters imply that this inhibition within the auditory startle circuit depends on several mechanisms activated serially and/or in parallel through forebrain and midbrain circuits (Bosch and Schmid 2008; Miller et al. 2010; Yeomans et al. 2006, 2010). This complexity has motivated the search for more accessible startle circuits such as the touch-reflex circuit of Tritonia (Frost et al. 2003) and the startle-escape circuit of fish (see below and Neumeister et al. 2008) where pre- and postsynaptic cellular mechanisms mediating PPI-type phenomena have been identified.

Indeed, the auditory startle pathway is conserved in fish and mammals, involving a direct disynaptic pathway from the hair cell receptors to brain stem neurons that integrate multimodal inputs and activate downstream motor circuits with comparable latencies (10–15 ms; Korn and Faber 1996). Fish and mammals also show comparable temporal characteristics for effective prepulse/pulse interstimulus intervals (ISIs; 30–500 ms) during behavioral PPI of the auditory startle response (Braff et al. 1978; Burgess and Granato 2007; Mansbach and Geyer 1991; Neumeister et al. 2008).
Startle in fish is initiated by a pair of brain stem neurons, the Mauthner cells (M-cells), that, as the mammal’s PnC neurons, integrate multimodal information (Canfield 2003; Korn and Faber 2005; Preuss et al. 2006; Szabo et al. 2006). A single action potential (AP) in one of the M-cells is sufficient to activate contralateral motor execution networks for a fast body-bend (C-start escape) away from the startling stimulus (Eaton et al. 2001; Zottoli 1977). In other words, the M-cells are sensorimotor decision neurons that determine the likelihood, timing, and direction of startle escape in goldfish (Korn and Faber 2005; Preuss and Faber 2003; Preuss et al. 2006; Zottoli et al. 1999). The M-cell dendritic morphology is relatively simple: two primary dendrites, one lateral and one ventral, each extend ≥500 μm from the soma (Faber and Korn 1978; Korn and Faber 2005). Because of its size, morphology, and electrophysiological signature (an unusually large extracellular antidromic field potential), it is possible to record reliably from the M-cell and its dendrites in vivo (Furushpan and Furukawa 1962; Faber and Korn 1978; Korn and Faber 2005; Preuss et al. 2006; Szabo et al. 2006). Importantly, recent studies using physiological stimuli identified two distinct postsynaptic mechanisms that mediate PPI in the M-cell (Neumeister et al. 2008). In addition, dopamine agonists disrupt behavioral PPI in zebrafish, reproducing deficits induced in rodent models. (Burgess and Granato 2007).

The homology in auditory pathways and the similarities of dopaminergic modulation of PPI at the behavioral level among teleost and mammals along with the accessibility of the M-cell provided the rationale for the present study, which tested the possible modulation of M-cell PPI by dopamine and the underlying mechanism(s). Our results indicate that the dopaminergic agonist apomorphine disrupts PPI during a restricted time window by modulating nonlinear properties of the M-cell membrane.

MATERIALS AND METHODS

Animals. Adult goldfish (Carassius auratus), 7–10 cm in body length, were purchased from Billy Bland Fishery (Taylor, AR). They were acclimated to our holding system for ≥1 wk before experiments, allowing recovery from transportation and adjustment to laboratory conditions. Fish were housed in groups of 10–15 in 114-L holding tanks with recirculating conditioned water at 18°C under a 12:12-h light-dark cycle. Details for water conditioning have been described previously (Szabo et al. 2006). Water quality was monitored regularly and was the same for holding aquaria and recording chamber (pH 7.3 ± 0.1).

Electrophysiology. These experiments involved standard in vivo surgical and recording techniques as described previously (Preuss and Faber 2003). All surgical and experimental procedures were performed following protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Hunter College, City University of New York (CUNY) and are in accordance with all relevant guidelines and regulations. Goldfish were initially anesthetized by immersion in 10% tricaine (α-methylphenylalanine) placed at the position of the fish was used for sound calibration but was removed during actual recordings.

Acoustic stimuli. A function generator (33210A; Agilent, Santa Clara, CA) connected to a subwoofer (SA-WN250; Sony) located 30 cm to the side of the recording chamber produced sound pips (single cycle sine waves) of 200 Hz to stimulate M-cell orthodromically. Sound transmission through the recording chamber wall limited the maximum underwater sound intensity to 147 dB relative (re) to 1 μPa in water, which is essentially subthreshold for evoking behavioral startle (Neumeister et al. 2008). However, this limitation does not impair studying the inhibitory PPI effects on synaptic responses and membrane properties, since prepulse stimuli are subthreshold by definition and prepulse intensities were similar to those used successfully in behavioral PPI in goldfish (Neumeister et al. 2008). Sound pips were recorded with a microphone positioned 10 cm over the fish head and stored online together with intracellular recordings. A hydrophone (SQ10; Sensor Technology, Collingwood, Ontario, Canada) placed at the position of the fish was used for sound calibration but was removed during actual recordings.

Pharmacology. The dopaminergic agonist apomorphine (in saline, 10% ascorbic acid; Sigma) was superfused on the brain surface (0.27 mM, n = 7) or injected intramuscularly (2 mg/kg body wt, n = 13). Our results indicated that both routes of administration produced the same effects on PPI in the M-cell system (F1,15 = 0.92), therefore data were combined and analyzed as a single group. The dopaminergic agonist haloperidol (0.6 mg/kg body wt; Sigma) was injected intramuscularly in the caudal part of the fish body. Injection volumes never exceeded 200 μl.

Experiments and data analysis. In the M-cell, physiological PPI can be observed as an amplitude reduction of a sound-evoked test postsynaptic potential (pulse, PSPPulse) when preceded by a conditioning sound stimulus (prepulse, PSPprepulse) presented at distinct ISIs (Neumeister et al. 2008). PSPs recorded in the M-cell in response to a sound pip have a first, brief, largely excitatory component followed by a longer period (>5 ms) wherein excitation is rapidly balanced by feedforward inhibition (Preuss and Faber 2003). Therefore, postsynaptic potentials (PSP) peak amplitude measurements were performed within the initial 5-ms time window following sound onset (Fig. 1A, dashed lines). When instead of peak amplitude, PSP area (within the same 5-ms time window) was considered for measurements, results were qualitatively the same (data not shown).

The PPI effect was then calculated as 100 − (PSPPulse / PSPprepulse) × 100, therefore higher percentages reflect stronger PPI. The influence of dopaminergic modulation on the time course and magnitude of PPI was determined at four different ISIs ranging from 20 to 500 ms and analyzed offline with IGOR Pro (WaveMetrics, Lake Oswego, OR) and MATLAB (The MathWorks, Natick, MA). PSP peak amplitude measurements were done on individual responses. Averages of 5–10 responses were used for statistical analysis.
prepulse sound stimulus remained for (pulse) recording. Note a residual membrane depolarization evoked by the effects (see MATERIALS AND METHODS).

Followed by a similar series of ramp stimuli preceded by a prepulse series of ramp stimuli every 4 s for 2–3 min to assess baseline between the electrodes. A typical experiment involved applying a compensation circuit built into the amplifier eliminated cross talk.

M-cell membrane properties (e.g., input resistance and threshold current) following a prepulse were determined by injecting a positive current ramp (0–200 nA/20 ms; Wavetek 39, Norwich, United Kingdom) into the M-cell via an intrasomatic electrode while simultaneously recording membrane voltage with a second intracellular electrode located ~100 μm from the soma (see diagram in Fig. 4A). A compensation circuit built into the amplifier eliminated cross talk between the electrodes. A typical experiment involved applying a series of ramp stimuli every 4 s for 2–3 min to assess baseline properties or possible indications for habituation or sensitization, followed by a similar series of ramp stimuli preceded by a prepulse stimulus at different ISIs.

Only one M-cell was recorded and analyzed in each animal. Resting membrane potential (RMP) was monitored throughout the experiment, and experiments where its value changed ≥10% during the recording session were not included in the analysis. Previous studies, however, have shown that RMP is not affected by PPI (Neumeister et al. 2008). Finally, experiments where PPI levels for 50-ms ISI were below 10% were excluded as indicative of an unhealthy preparation (typical M-cell PPI levels for 50-ms ISI are ~23%; Neumeister et al. 2008).

KaleidaGraph (Synergy Software, Reading, PA) or MATLAB were used for statistical analysis. One- or two-way repeated-measures ANOVA followed by post hoc comparisons (Dunnett or Tukey methods) was used to describe differences among ISI and drug treatment. Two-tailed Student’s t-test was used for pairwise comparisons. A critical significance level of α = 0.05 was used throughout the study. Results are reported as means ± SE.

RESULTS

Effect of prepulse/pulse ISI on PPI. Sound-evoked PSPs recorded in the M-cell proximal dendrite (~100 μm from soma) are characterized by a short-latency (~1.5-ms) fast-rising membrane depolarization with multiple peaks (Fig. 1A, no prepulse). These PSPs are composed of a fast-decaying electrical excitation superimposed on a slow-decaying chemical component (Szabo et al. 2006). The sound-evoked slow component of the PSP remains visible as a small membrane depolarization for ≥50 ms (Fig. 1A, 20- and 50-ms ISI, arrowheads). To study the time course of the synaptic PPI, we compared PSPs in response to sound pips (200 Hz, 145 dB re 1 μPa in water) without and with a preceding sound pip (prepulse) of similar strength at different ISIs (20, 50, 150, and 500 ms). The results indicate a large reduction of the PSP_pulse for shorter lead times (Fig. 1A) that becomes progressively weaker at longer ISIs. The quantification of this effect shows a significant negative relationship between ISI duration and PPI magnitude (Fig. 1B; F3,57 = 104.05, P < 0.001, n = 20).

Time-dependent dopaminergic neuromodulation of physiological M-cell PPI. We first asked whether the dopaminergic agonist apomorphine (0.27 mM in superfusion experiments or 2 mg/kg im in systemic application experiments; see MATERIALS AND METHODS for details) has any non-PPI-related effect on the synaptic response. Indeed, apomorphine significantly reduced the sound-evoked M-cell PSP by 14.68 ± 4.54% (Fig. 2A; paired t-test: t = 2.77, P = 0.01, n = 18). In contrast, within the PPI paradigm, apomorphine had an opposite effect on the synaptic response. Namely, the drug increased the PSP_pulse following a prepulse compared with controls (Fig. 2B, gray vs. black trace), i.e., it functionally decreased PPI levels in the M-cell. However, this disruption of PPI only occurred for one distinct prepulse/pulse ISI interval (Fig. 2C). A two-way repeated-measures ANOVA with ISI interval and drug effect as factors showed an interaction between the two (F3,51 = 3.05, P = 0.037, n = 18) indicating that apomorphine effect was dependent on the ISI. Post hoc tests revealed a significant reduction in PPI by apomorphine only for 50-ms ISI: from 20.3 ± 2.0 to 15.5 ± 1.8% (Tukey test, q = 3.81, P = 0.009, n = 18). No significant differences were found for shorter (20-ms) or longer (150- and 500-ms) ISIs (Fig. 2C), implying a time-dependent action of apomorphine on PPI. Note that synaptic PPI levels were calculated for each experimental trial by comparing two pulse PSPs, one with and one without a prepulse; i.e., each trial comprised its own control. Accordingly, any shift in the synaptic response will have little impact on the calculated PPI levels. It is conceivable that, at least for 500-ms ISIs, the lack of drug action was masked by a PPI floor effect, however, the lack of an apomorphine effect at 150-ms ISI when PPI was still substantial argues against this interpretation.

To describe the pharmacokinetics of apomorphine in our preparation, we assessed its effect on PPI for 50-ms ISI at four distinct postinjection time windows; namely, between 0 and 10, 11 and 40, 41 and 70, and 70 and 120 min. The results indicate a maximal and stable drug effect between 11 and 70 min, which started to diminish afterward (Fig. 2D). Accordingly, we used PPI measures within the 11- to 70-min period for data analysis.
In a subset of the above experiments \((n = 5)\), we injected haloperidol (0.4 mg/kg) 60–70 min (mean: 68 ± 9 min) after systemic application of apomorphine to test whether this D2 dopaminergic agonist could reverse the apomorphine disruption of PPI (Fig. 3A). We found no overall effect of the drug alone or a significant interaction between drugs or ISI (drug, \(F_{2,24} = 4.23, P = 0.06\); interaction, \(F_{6,24} = 1.02, P = 0.43\)). However, when focusing our analysis on 50-ms ISI, we found that haloperidol reversed the effects of apomorphine to control levels (Fig. 3A; \(F_{2,8} = 4.91, P = 0.04\) followed by Tukey comparisons: haloperidol vs. apomorphine, \(q = 4.25, P = 0.04\); haloperidol vs. control, \(q = 1.03, P = 0.75\)). When applied alone, the same dose of haloperidol (0.4 mg/kg) had no effect on PPI (control vs. haloperidol for 50-ms ISI: 22.2 ± 4.9 vs. 21.7 ± 6.7%; \(F_{1,9} = 0.47, n = 4\); Fig. 3B) or on PSP amplitude in response to single pips (means ± SE; PSP amplitude of control vs. haloperidol: 3.9 ± 0.7 vs. 3.8 ± 0.5 mV, \(n = 4\)).

**Effects of apomorphine on M-cell membrane properties.** The M-cell resting potential is close to the chloride reversal potential. Thus inhibition in the M-cell is of the shunting type, i.e., not accompanied by a frank potential change but rather by a change in membrane conductance (i.e., input resistance). This type of inhibition is powerful since it is thought to have a divisive rather than a subtractive effect on the synaptic response (Koch and Segev 2000).

To analyze inhibition we injected current ramps in the M-cell soma (150–200 nA, 20 ms) with one electrode while recording membrane voltage in the proximal dendrite with a second intracellular electrode (Fig. 4A; see MATERIALS AND METHODS for details). This method allowed us to analyze pre-pulse-induced postsynaptic changes in M-cell membrane properties (e.g., input resistance, threshold voltage, and nonlinearity) in a standardized way over the full dynamic range of membrane depolarization (Neumeister et al. 2008). The ramp experiments are thus a tool to reveal potential voltage-dependent effects of dopamine close to threshold, which may not be fully activated by subthreshold PSPs. The example voltage-current (V-I) plots in Fig. 4B show that in control conditions (i.e., without a prepulse), the M-cell membrane behaves linearly during the initial depolarization phase but becomes increasingly nonlinear for depolarization >5 mV (Fig. 4B, black trace). Functionally, this nonlinearity can be understood as a dynamic increase in input resistance, which will boost the postsynaptic response as the M-cell approaches threshold (Faber and Korn 1986). However, when a sound prepulse is presented before the current injection, the nonlinearity is absent (Fig. 4B, gray trace), i.e., a prepulse stimulus effectively impedes AP generation by linearizing the M-cell membrane (Neumeister et al. 2008). To quantify this effect, we measured the slopes of linear fits to two distinct regions of averaged \((n = 10\) traces) V-I plots, which functionally represent the input resistance during early and late phases of membrane depolarization. Specifically, slope region one was defined as the initial 2-ms time window after ramp onset, whereas slope region two encompassed a 2-ms time window that ended 1 ms before AP threshold (Fig. 4B, dashed lines). The results of 15 experiments indicate that for 20- and 50-ms ISI, an auditory prepulse significantly decreased the M-cell input resistance during the early (slope 1) and late (slope 2) phases of membrane depolarization (ANOVA followed by Dunnett comparisons, slope 1: \(F_{4,56} = 4.98, P = 0.002\), no prepulse vs. 20-ms ISI and no prepulse vs. 50-ms ISI, \(P < 0.05\); slope 2: \(F_{4,56} = 4.21, P = 0.005\), no prepulse vs. 20-ms ISI and no prepulse vs. 50-ms ISI, \(P < 0.05\); Fig. 4, C and D). Longer ISIs also evoked sizable
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**Fig. 3. Effects of the dopamine antagonist haloperidol on PPI. A: mean PPI (± SE) levels before (black) and after (light gray) apomorphine application followed by a subsequent injection of haloperidol (dark gray). Haloperidol significantly increased PPI for 50-ms ISI, restoring it to control levels (APcontrol /A Ptest × 100) evoked at different ISIs (range 2–500 ms) after activation of the feedforward inhibitory network by a sound stimulus (Fig. 6A, inset; Faber and Korn 1989; Preuss and Faber 2003; Weiss et al. 2008). This silent or shunting inhibition activates glycine and GABA gated Cl− channels that shunt incoming excitatory synaptic currents out from the M-cell (Faber and Korn 1988). The time course and magnitude of this inhibition can be quantified as a fractional reduction of a test AP (defined as 100 − APtest /A Pcontrol × 100) evoked at different ISIs (range 2–500 ms) after activation of the feedforward inhibitory network by a sound stimulus (Fig. 6A, inset; Faber and Korn 1982). For statistical analysis, we calculated the root mean square values of the fractional shunt over the entire range of ISIs. Figure 6B illustrates that feedforward inhibition reaches its maximum within 10–12 ms and then declines rapidly. Apomorphine produced an overall nonsignificant decrease in inhibition (paired t-test, P = 0.332; n = 9) and also did not change its time course (Fig. 6B, red trace). Moreover, the lack of an apomorphine effect on the feedforward inhibition at 50-ms ISI suggests that this presynaptic circuit is not responsible for the time-dependent effect on PPI by apomorphine described above.

**DISCUSSION**

Here, we used intracellular in vivo recordings to elucidate the cellular mechanisms by which dopamine regulates PPI in the auditory startle network of the goldfish. The two main results discussed below are that 1) apomorphine disrupts PPI predominantly within a distinct prepulse/pulse time window (50 ms) by 2) a deactivation of a M-cell membrane nonlinearity that effectively increases input resistance. This demonstrates, for the first time, a direct dopaminergic influence on sensorimotor gating of the vertebrate auditory startle pathway by a postsynaptic, time-dependent mechanism.

**Time-dependent PPI effects of a dopamine on the M-cell synaptic response.** Intracellular M-cell recordings in response to sounds revealed a dopaminergic-mediated disruption of PPI. This finding complements previous behavior studies in zebrafish showing disruptive effects of apomorphine on behave-
ioral PPI (Burgess and Granato 2007) but also provides a comparative link to the auditory startle response of mammals where dopamine agonists causes similar PPI deficits (Geyer et al. 2001; Swerdlow et al. 2008). Importantly, our results showed that apomorphine did not produce a general decrease in M-cell PPI but acted predominately at a prepulse/pulse interval of 50 ms because it reduced PPI by ~25% compared with controls. This reduction in synaptic PPI (produced by a time-dependent increase in input resistance; see below) has significant functional consequences for the all-or-none expression of

![Fig. 4. Postsynaptic effects of a sound prepulse on the M-cell membrane. A: M-cell membrane response (solid gray trace) to a current ramp injection (150 nA, 20 ms, dashed gray) with a sound stimulus (147 dB, 200 Hz, dashed black) preceding the current ramp by 50 ms. Black circle: action potential (AP) firing threshold. Inset: schematic showing the M-cell and its direct excitatory 8th nerve input to the lateral dendrite. A current-injecting electrode (I) is placed in the soma of the M-cell, and voltage (V_m) is recorded in the proximal dendrite. B: voltage-current (V-I) plots covering the subthreshold portion of M-cell depolarization during a current ramp experiment with (gray) or without (black) a prepulse. Note: without a prepulse, the M-cell membrane shows initially a linear V-I relationship that becomes increasingly nonlinear for depolarization above 4–5 mV. A sound prepulse attenuates the nonlinear behavior of the membrane (gray trace). The hypothetical linear response is shown in dotted line. Slopes 1 and 2 represent the 2 regions of the V-I plots used to quantify M-cell input resistance (i.e., V-I slope) during a ramp experiment. Means (± SE) for slope 1 (C) and slope 2 (D) for no prepulse controls (open bars) and for indicated prepulse/ramp ISIs are shown. Slopes 1 and 2 were significantly decreased at 20- and 50-ms ISI (*P < 0.05; n = 15). RMP, resting membrane potential.

![Fig. 5. Effect of apomorphine on M-cell input resistance during PPI. A: sample V-I plots following a prepulse (50-ms ISI) before (black) and after (gray) apomorphine. Apomorphine increases M-cell input resistance especially for slope 2, i.e., for M-cell depolarization nearing threshold. B and C: plots of the relative (Rel.) change in slopes 2 and 1 (means ± SE) before and after apomorphine (apomorphine slope1,2/control slope1,2) for indicated ISIs and no prepulse controls. Apomorphine significantly increased slope 2 at 50-ms ISI (*P < 0.05). D: mean ± SE of ratios between slope 2 and 1 with or without a prepulse and before and after apomorphine injection were calculated to get a measure of the prepulse linearization effect (n = 9). A ratio of 1 indicates a linear membrane response, whereas ratios >1 reveal the nonlinear behavior of the M-cell. Means (control vs. apomorphine) were compared using t-tests, *P = 0.026.
Haloperidol reversed the apomorphine-induced decrease in PPI, although not having an effect by itself, reproducing behavioral findings in fish and rodents (Burgess and Granato 2007; Schall et al. 1999; Swerdlow et al. 1986). We applied haloperidol subsequently to apomorphine, and therefore we cannot rule out that some of the PPI recovery we find after haloperidol is due to apomorphine washout.

We found that both M-cell PSPs and M-cell input resistance (see below) show consistent time-dependent effects of apomorphine during PPI. On the other hand, under our experimental conditions, we likely activated the receptors tonically. How can we reconcile the apparent time-dependent dopaminergic effects during a presumably tonic activation (time independent) of dopamine receptors? Indeed, apomorphine has a tonic effect (PPI-independent) on the M-cell input resistance evident in a nonsignificant but still noticeable increase in slope 1 (Fig. 5B). However, when the membrane is further depolarized, a different PPI-dependent mechanism is engaged. This independent mechanism peaks ~50 ms and then declines (Fig. 5C, slope 2).

Interestingly, studies in humans also emphasize the importance of time-dependent PPI deficits. Namely, schizophrenia patients show PPI deficits of the auditory eye-blink reflex only at ~60-ms prepulse/pulse ISIs (Braff et al. 1978, 2001a; Ludewig and Vollenweider 2002; Swerdlow et al. 2006). In addition, some of the symptoms of the disorder have been attributed to abnormal activation of dopamine (D2) receptors (Geyer et al. 2001). Indeed, the use of dopamine agonists to disrupt sensorimotor gating (PPI) in various animal models represents an important experimental link between neuroscience and clinical research in schizophrenia patients (Braff et al. 1978, 2001a; Ludewig and Vollenweider 2002; Swerdlow et al. 2006). We believe our results strengthen this link significantly by mechanistically tying dopamine directly to time-dependent PPI deficits.

However, can startle and PPI in mammals and fish be compared directly? In fish, startle is an all-or-none response triggered by a single AP in the M-cell, whereas in mammals, startle is a graded response produced by the population response of neurons in the PnC nucleus (Lingenhohl and Friauf 1994; Yeomans et al. 2006). These differences may affect the time course of PPI, and thus the aforementioned timing concurrence should be interpreted with some caution. On the other hand, sound-evoked PSPs and physiological PPI recorded in individual PnC neurons and the M-cell show a striking resemblance. For example, M-cells and PnC neurons receive similar direct input from auditory afferences indicated by short-latency synaptic responses (<3 ms) for abrupt stimuli. They also share a high firing threshold and show a similar sensitivity during paired-pulse stimulation (PPI) with respect to ISI (Lingenhohl and Friauf 1994; Neumeister et al. 2008). Finally, the axons of both, M-cell and PnC neurons establish output synapses with numerous motoneurons along the spinal cord for a massive motor (startle) response, which further underlines their analog function (Fetcho and Faber 1988; Lingenhohl and Friauf 1994).

Postsynaptic effects of dopamine on the M-cell membrane properties. As noted, the M-cell is a high-threshold neuron due to its low input resistance (90–120 kΩ; Fukami et al. 1965 and this study) and a relatively low RMP (~75–~80 mV; Faber and Korn 1986 and this study). In combination with fast onset of feedforward inhibition (Fig. 6), this ensures that only intense and abrupt signals lead to a startle (Zottoli 1977). Moreover, one putative mechanism that also aids a selective response to
intense stimuli is a membrane nonlinearity that dynamically increases the input resistance once the M-cell is depolarized by about 5–6 mV above RMP (slope in Fig. 5D). This nonlinearity will provide a selective boost for large evoked PSP as they propagate toward the axon hillock (Faber and Korn 1986) and significantly influence AP generation (Neumeister et al. 2008).

Our results indicate that a prepulse stimulus reduced this nonlinearity (i.e., the M-cell membrane showed a quasilinear $V-I$ relationship; Figs. 4D and 5D). This provides a potent postsynaptic mechanism for PPI since it effectively decreases input resistance when the cell approaches threshold. After application of apomorphine, however, the prepulse-mediated membrane linearization was no longer observed. Importantly, this drug effect was predominantly observed for 50-ms ISI providing a mechanism for the time-dependent disruption of M-cell PPI observed (Fig. 5C and D). In addition, the apparent influences of dopamine on the dendritic cable (filtering) properties extends, we believe, the significance of these findings beyond the PPI paradigm since it underlines the role of single neurons in information processing.

Work by Faber and Korn (1986) identified an inward potassium rectifier as the likely source for the above described membrane nonlinearity. Thus, although the present study provides no direct evidence, our results are compatible with a dopaminergic modulation of potassium channels. Indeed, dopamine modulates potassium inward rectifiers in medium spiny neurons of the nucleus accumbens (Perez et al. 2006; Podda et al. 2010) and in fast spiking interneurons and pyramidal neurons of the prefrontal cortex (Gorelova et al. 2002; Witkowski et al. 2008). Future experiments using specific dopaminergic antagonists and/or inward rectifier $K^+$ channel blockers will test the hypothesis that such a regulation is present in the M-cell.

Presynaptic effects of apomorphine in the M-cell startle network? Apomorphine had also tonic effects on the M-cell outside of the PPI paradigm. Specifically, the drug diminished sound-evoked PSP in the M-cell to single sound pips (i.e., during pulse-only stimulation; Fig. 2A). On the other hand, the $V-I$ plots of the ramp experiments without a prepulse indicated a slight nonsignificant increase in steady-state M-cell input resistance after apomorphine (no sound; Fig. 5B, slope $I$). The latter, however, should rather increase the synaptic response. These seemingly inconsistent results can in principle be explained by separate pre- and postsynaptic effects of dopamine. Possible presynaptic mechanisms include a dopamine-dependent decrease of excitatory input and/or an increase in feedforward inhibition mediated by presynaptic interneurons (PHP neurons: Faber et al. 1989). We did not observe, however, significant changes in feedforward inhibition following apomorphine (Fig. 6B), leaving a decrease of afferent input as the likely presynaptic source for the PSP reduction. On the other hand, recent work has shown that dopaminergic activation of postsynaptic $D_{1/5}$ receptors mediated by endocannabinoid release increases PKA activity in the M-cell leading to a potentiation of electrical and glutamatergic synaptic transmission in response to electrical stimulation of the auditory nerve (Caçopé et al. 2007; Pereda et al. 1992, 1994). The discrepancy with our results (i.e., to the observed decrease in the synaptic response) is likely due to a difference in the stimulation protocols. Electrical stimulation of the auditory nerve fires all afferences synchronously, activating both the electrical and chemical components of the synaptic response. In contrast, we used a physiological (and weaker) stimulus that only recruited a fraction of the afferences activating primarily the electrical component of the synapse, as most of the chemical contacts are silent in normal conditions (Lin and Faber 1988; Szabo et al. 2006). As dopamine enhancement of the synaptic response is most prominent in the chemical component (Pereda et al. 1994), this increase may have been masked by subsequent inhibition in our experiments. Alternatively, apomorphine acting through different postsynaptic receptors (e.g., type $D_2$ instead of $D_{1/5}$) may attenuate the synaptic response by affecting the chemical synapses and/or the hemichannels of the electrical synapses.

PPI is mediated by multiple mechanisms. In fish, behavioral PPI can last for $\geq 500$ ms (Burgess and Granato 2007; Hoffman and Ison 1980; Neumeister et al. 2008) and is mediated by at least two postsynaptic mechanisms acting at distinct ISI intervals (Neumeister et al. 2008). These as well as the present results are consistent with the notion that the long-lasting inhibitory effects of PPI are mediated by multiple mechanisms with distinct time dependencies. Similarly, recent work in rats shows that stimulation of the pedunculopontine nucleus in the brain stem inhibits startle-mediating PnC neurons at two distinct time windows. Specifically, although direct cholinergic projections acting through presynaptic muscarinic receptors are responsible for the 1,000-ms interval, a different postsynaptic receptor seems responsible for the 300-ms time window (Bosch and Schmid 2008). Moreover, patch-clamp recordings showed that PnC neurons are also affected by combined activation of GABA$_A$ and GABA$_B$ receptors to maintain a fast-onset (30 ms) and long-lasting (100–500 ms) postsynaptic inhibition, respectively (Yeomans et al. 2010).

Interestingly, GABA also mediates synaptic inhibition in the M-cell lateral dendrite (Diamond and Huxley 1968; Hatta et al. 2001; Lee et al. 1993; Triller et al. 1993), although its role in teleost PPI still has to be assessed. These similarities not only suggest conserved auditory startle and PPI pathways in vertebrates (see above), but also provide evidence that similar pre- and postsynaptic mechanisms for PPI act in teleost fish and mammals.

Since Braff and coworkers (1978) found deficiencies in PPI in schizophrenia patients, PPI has become an important research tool to study the neurobiology and pharmacology of sensorimotor-gating deficits of this disease (Geyer et al. 2001; Swardlow et al. 2008). Defining the cellular mechanisms of PPI, including its complex time course and the locus of neurotransmitters action, represents a key step toward a better understanding of this phenomenon.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

Author contributions: V.M. and T.P. conception and design of research; V.M. performed experiments; V.M. and T.P. analyzed data; V.M. and T.P. interpreted results of experiments; V.M. prepared figures; V.M. and T.P. drafted manuscript; V.M. and T.P. edited and revised manuscript; V.M. and T.P. approved final version of manuscript.

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