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RAPID REPORT | *Cellular and Molecular Properties of Neurons*

β-Adrenergic enhancement of neuronal excitability in the lateral amygdala is developmentally gated

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Fink AE, LeDoux JE. β-Adrenergic enhancement of neuronal excitability in the lateral amygdala is developmentally gated. *J Neurophysiol* 119: 1658–1664, 2018. First published January 17, 2018; doi:10.1152/jn.00853.2017.—Noradrenergic signaling in the amygdala is important for processing threats and other emotionally salient stimuli, and β-adrenergic receptor activation is known to enhance neuronal spiking in the lateral amygdala (LA) of juvenile animals. Nevertheless, intracellular recordings have not yet been conducted to determine the effect of β-adrenergic receptor activation on spike properties in the adult LA, despite the potential significance of developmental changes between adolescence and adulthood. Here we demonstrate that the β-adrenergic agonist isoproterenol (15 μM) enhances spike frequency in dorsal LA principal neurons of juvenile male C57BL/6 mice and fails to do so in strain- and sex-matched adults. Furthermore, we find that the age-dependent effect of isoproterenol on spike frequency is occluded by the $GABA_A$ receptor blocker picrotoxin (75 μM), suggesting that β-adrenergic receptors downregulate tonic inhibition specifically in juvenile animals. These findings indicate a significant shift during adolescence in the cellular mechanisms of β-adrenergic modulation in the amygdala.

NEW & NOTEWORTHY β-Adrenergic receptors (β-ARs) in amygdala are important in processing emotionally salient stimuli. Most cellular recordings have examined juvenile animals, while behavioral data are often obtained from adults. We replicate findings showing that β-ARs enhance spiking of principal cells in the lateral amygdala of juveniles, but we fail to find this in adults. These findings have notable scientific and clinical implications regarding the noradrenergic modulation of threat processing, alterations of which underlie fear and anxiety disorders.

β-adrenergic receptors; amygdala; developmental gating; isoproterenol; patch clamp; threat and fear

INTRODUCTION

Norepinephrine signaling through β-adrenergic receptors plays an important role in signaling arousal and motivational states in the mammalian brain. β-Adrenergic receptor activation has been shown to increase spike firing in the lateral (LA) and basal (BA) amygdala (Faber and Sah 2002; Huang et al. 1996; Tully et al. 2007), to enhance excitatory synaptic transmission (Faber et al. 2008; Ferry et al. 1997; Huang et al. 1996; Wang et al. 1999) and to activate molecular signaling cascades responsible for activation of protein kinases (Huang et al. 2000; Leonard et al. 2003). β-Adrenergic activation has also been found to permit or enhance the induction of long-term potentiation in the LA in vitro (Faber et al. 2008; Huang et al. 2000; Tully et al. 2007) and is an important mediator of aversive (threat) learning in rodents (Bush et al. 2010; Johansen et al. 2014) and emotionally salient learning in humans (Cahill et al. 1994; Hurlemann et al. 2010).

While most behavioral experiments take place in adult animals, previous recordings demonstrating noradrenergic enhancement of neuronal excitability (i.e., spiking properties) were obtained from acute slices of the LA and BA of rats ranging from 17 days to roughly 5–7 wk (100–200 g). These time points in rodents correspond best to developmental stages ranging from late childhood through adolescence (Sengupta 2013), but β-adrenergic modulation of neuronal excitability has not been studied in whole cell recordings from the adult amygdala. Here we use patch-clamp recordings in acute slices of the mouse amygdala to demonstrate that β-adrenergic activation enhances spike frequency only in principal cells of the juvenile LA and fails to do so in adult neurons.

MATERIALS AND METHODS

Animals. All animal use and care protocols were reviewed and approved by the University Animal Welfare Committee at New York University. Either adult (9–10 wk old) or juvenile [postnatal day (P)21–P28] male C57BL/6 mice were obtained from Charles River Laboratories (Wilmington, MA). In all experiments that compared adult and juvenile animals, both ages arrived concomitantly and recordings were conducted on subsequent days, with experimental order counterbalanced for animal age. Upon arrival, mice were singly housed in an animal facility on a 12:12-h light-dark cycle and received disposable (paper and cardboard) forms of enrichment in their cages.

Slice preparation. Animals were deeply anesthetized with halothane and then decapitated. The brain was immediately removed and placed in a beaker filled with ice-cold (4°C) slicing solution bubbled with 95% O_2-5% CO_2 and containing (in mM) 85 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgSO_4$, 25 glucose, 75 sucrose, and 0.5 ascorbic acid. After cooling briefly in the beaker, the brain was transferred to a chamber on an OTS-5000 tissue slicer (Electron Microscopy Sciences, Hatfield, PA), where we prepared 280-μm-thick coronal sections containing the LA. We transferred slices into a heated submerged chamber (32°C) containing the artificial cerebrospinal fluid (ACSF) used for recording, which contained (in mM) 125

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NaCl, 3.3 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1.2 $MgSO_4$, 0.01 D-serine, 15 glucose, and 0.5 ascorbic acid. Slices were kept in the heated chamber for 45–60 min of recovery and then were maintained at room temperature for at least 45 min until being used for recordings.

Whole cell current-clamp recordings. All recordings were conducted at 32 ± 0.5°C in the ACSF described above. If picrotoxin (PTX) was included in the solution, the concentration of divalent cations ($MgSO_4$ and $CaCl_2$) was increased to 2.5 mM. Slices were transferred to an Olympus BX51WIF upright microscope, with a ×10 air objective and a ×40 water immersion objective, where differential interference contrast with infrared illumination was used to visualize cells for patch-clamp recordings. For whole cell recordings, we used glass pipettes (4–7 MΩs) filled with an internal solution containing (in mM) 136 potassium gluconate, 4 $MgCl_2$, 1 EGTA, 0.1 $CaCl_2$, 10 HEPES, 4 Na-ATP, and 0.3 Na-GTP (pH = 7.3, osmolarity = 289–294 mosM). All membrane potentials are reported before correction for an estimated 14-mV liquid junction potential (LJP) estimated with the LJP calculator available in the pCLAMP software package. Cells were held at a potential of −58 mV (before correction for LJP). All salts, as well as PTX and isoproterenol bitartrate (ISO), were obtained from Sigma-Aldrich (St. Louis, MO).

Data acquisition and analysis. Data were acquired with pCLAMP software (Axon Instruments/Molecular Devices, Sunnyvale, CA). Recordings were conducted with an AxoClamp 2B amplifier, low-pass filtered at 10 kHz through a Brownlee model 440 filter (Palo Alto, CA), passed through a HumBug (Dagan, Minneapolis, MN) to remove 60-cycle noise and digitized through a Digidata 1440 (Molecular Devices) at 25 kHz.

Data were analyzed with a combination of the SPSS statistical package (IBM, Armonk, NY), GraphPad Prism (La Jolla, CA), and MATLAB software (MathWorks, Natick MA). Intrinsic neuronal properties were calculated with programs in MATLAB that extract membrane properties and spike parameters similarly to prior studies (Fink and O'Dell 2009). In our initial experiments (Figs. 1 and 2) we recorded intrinsic properties of principal excitatory cells in the dorsal portion of the LA by administering a series of six current steps (−100 to +400 pA in 100-pA increments) for a duration of 400 ms each, at an interval of 10 s. The set of current steps was repeated two or three times for each cell recorded, and intrinsic neuronal properties were averaged over the repetitions. In within-studies experiments (Fig. 3), ISO was perfused into the slice chamber for 15 min after a 5-min baseline recording. We continuously recorded spike properties during baseline and ISO washin with a series of 400-ms-long current steps ranging from −100 to +200 pA in 100-pA increments (1 current step delivered every 15 s).

Briefly, intrinsic properties were calculated as follows: Resting membrane potential (V_m) was measured in each cell before application of the holding current. Input resistance (R_{in}) was calculated from the maximal hyperpolarization obtained during the first 160 ms of the hyperpolarizing pulse, and %Sag (primarily measuring hyperpolarization-induced currents) was then calculated by dividing R_{in} by the average hyperpolarization during the last 100 ms of the hyperpolarizing current step. Action potential (AP) threshold was obtained from the rising portion of the first spike in a train, defined by the point where $dV/dt > 15$ mV/ms. Spike latency was measured by the time (in ms) from the start of the current step to the first spike. Spike frequency was calculated as f = 1/ISI (where ISI is the mean interspike interval during a given current step). The membrane time constant (τ_M) was approximated by fitting the single exponential equation $y = \exp(-x/\tau_M)$ to the initial 150 ms of the hyperpolarizing current pulse.

We used two-way ANOVAs (Drug × Age) to determine statistical significance on measures of V_m, AP threshold, R_{in}, %Sag, and τ_M in Figs. 1 and 2 after establishing that there was no significant difference in variance across groups. For measures of spike latency, number of spikes, and frequency, which were analyzed across increasing current steps, we conducted 2 × 2 repeated-measures ANOVAs (drug × current amplitude) to establish significance for each age group. Greenhouse-Geisser calculations were applied to correct for lack of sphericity in within-subjects comparisons. For the data in Fig. 3, we used a two-way repeated-measures ANOVA (Drug × Age) to determine statistical significance of the difference between baseline measures (averaged) and measures obtained after ISO application (10–15 min after application). All pairwise comparisons included a Bonferroni correction.

RESULTS

We conducted whole cell patch-clamp recordings in acute brain slices to test whether β-adrenergic modulation of LA cell excitability changes between a peripubertal time point and adulthood. To do this, we compared the intrinsic excitability of LA principal neurons from adult (63–70 days old) and juvenile (21–28 days old) C57BL/6 mice in slices bathed in plain ACSF or in ACSF containing the β-adrenergic receptor agonist ISO (15 μM). We used this concentration because it is a maximally activating concentration used in previous studies that examined the role of β-adrenergic receptor modulation of plasticity within the LA. We focused on the dorsal LA (LAd) because synaptic plasticity there has been explicitly linked to formation of aversive associative memories (Repa et al. 2001). In these experiments we measured R_{in}, %Sag in membrane potential in response to a 400-ms hyperpolarizing current, τ_M, V_m, AP threshold, and other spiking characteristics (latency, number of spikes, and frequency) during a series of 400-ms-long hyperpolarizing (−100 pA) and depolarizing (+100 to +400 pA) current steps (see MATERIALS AND METHODS).

While we observed a β-adrenergic receptor-mediated enhancement of LAd cell spiking in the juvenile animals, this effect was surprisingly absent in adult neurons (Fig. 1, *C* and *D*). Specifically, in the juvenile animals, a given current pulse in the presence of ISO was able to elicit more spikes [$F(1,36) = 4.931$, $P = 0.033$], and a higher spike frequency [$F(1,36) = 5.293$, $P = 0.027$], than equivalent current pulses delivered to cells in control solution. In contrast, ISO had no effect on spike number [$F(1,31) = 0.613$, $P = 0.439$] or frequency [$F(1,31) = 0.673$, $P = 0.418$] in adult slices. We found no interactions between ISO application and amplitude of the current injection at either age (see Table 1) and no effect of ISO on spike latency (Fig. 1*E*) in adult [$F(1,31) = 1.405$, $P = 0.245$] or juvenile [$F(1,36) = 1.221$, $P = 0.277$] mice. In two-way comparisons (see Table 2 for statistics), we found no significant effect of age or ISO application on V_m (Fig. 1*F*) but we did observe a significant effect of ISO on AP threshold (Fig. 1*G*), with no main effect of animal age or interaction. We also observed a significant difference in R_{in} (Fig. 1*H*) between adult and juvenile LA neurons, with no main effect of ISO or interaction between age and drug application. Differences in %Sag (Fig. 1*I*) and τ_M (Fig. 1*J*) approached but did not reach significance in comparisons between juvenile and adult animals. Notably, we observed no difference in basal neuronal spike number [$F(1,33) = 1.227$, $P = 0.276$], spike frequency [$F(1,33) = 1.559$, $P = 0.221$], or spike latency [$F(1,33) = 0.777$, $P = 0.384$] between the adult and juvenile LAd cells (2-way repeated-measures ANOVA), in agreement with previous findings indicating that basal spiking properties of neurons in the LA approach maturity at P21–P28 (Ehrlich et al. 2012).

We next repeated these experiments in the presence of the $GABA_A$ receptor antagonist PTX (75 μM), to determine

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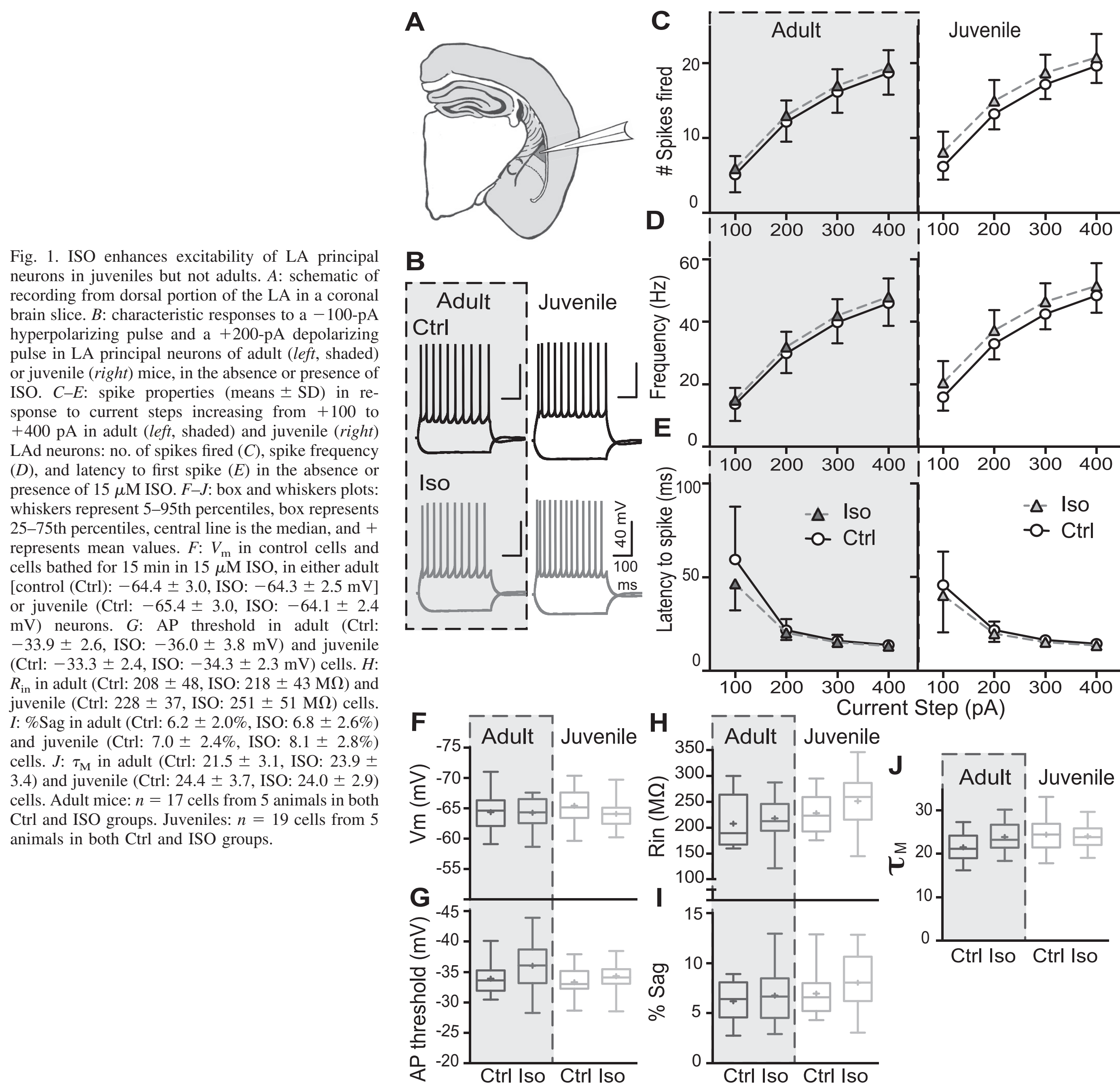

Fig. 1. ISO enhances excitability of LA principal neurons in juveniles but not adults. *A*: schematic of recording from dorsal portion of the LA in a coronal brain slice. *B*: characteristic responses to a −100-pA hyperpolarizing pulse and a +200-pA depolarizing pulse in LA principal neurons of adult (*left*, shaded) or juvenile (*right*) mice, in the absence or presence of ISO. *C–E*: spike properties (means ± SD) in response to current steps increasing from +100 to +400 pA in adult (*left*, shaded) and juvenile (*right*) LAd neurons: no. of spikes fired (*C*), spike frequency (*D*), and latency to first spike (*E*) in the absence or presence of 15 μM ISO. *F–J*: box and whiskers plots: whiskers represent 5–95th percentiles, box represents 25–75th percentiles, central line is the median, and + represents mean values. *F*: V_m in control cells and cells bathed for 15 min in 15 μM ISO, in either adult [control (Ctrl): −64.4 ± 3.0, ISO: −64.3 ± 2.5 mV] or juvenile (Ctrl: −65.4 ± 3.0, ISO: −64.1 ± 2.4 mV) neurons. *G*: AP threshold in adult (Ctrl: −33.9 ± 2.6, ISO: −36.0 ± 3.8 mV) and juvenile (Ctrl: −33.3 ± 2.4, ISO: −34.3 ± 2.3 mV) cells. *H*: R_{in} in adult (Ctrl: 208 ± 48, ISO: 218 ± 43 MΩ) and juvenile (Ctrl: 228 ± 37, ISO: 251 ± 51 MΩ) cells. *I*: %Sag in adult (Ctrl: 6.2 ± 2.0%, ISO: 6.8 ± 2.6%) and juvenile (Ctrl: 7.0 ± 2.4%, ISO: 8.1 ± 2.8%) cells. *J*: τ_M in adult (Ctrl: 21.5 ± 3.1, ISO: 23.9 ± 3.4) and juvenile (Ctrl: 24.4 ± 3.7, ISO: 24.0 ± 2.9) cells. Adult mice: n = 17 cells from 5 animals in both Ctrl and ISO groups. Juveniles: n = 19 cells from 5 animals in both Ctrl and ISO groups.

whether intact inhibition could be masking excitability changes in adult animals and whether the effects observed in juvenile animals required intact $GABA_A$-mediated inhibition. In the presence of PTX, the enhancement of excitability by ISO was abolished in young animals; we observed no difference in spike latency [$F(1,27) = 0.047$, $P = 0.829$], the number of spikes fired [$F(1,27) = 0.903$, $P = 0.350$], or spike frequency [$F(1,27) = 0.971$, $P = 0.333$] between cells bathed in ISO or in control ACSF (Fig. 2, *F–H*, *right*). In the adult animals, we still observed no ISO-induced change in spike latency [$F(1,22) = 0.765$, $P = 0.391$], number of spikes fired [$F(1,22) = 0.138$, $P = 0.714$], or firing frequency [$F(1,22) = 0.204$, $P = 0.656$] in the presence of PTX (Fig. 2, *F–H*, *left*). In 2 × 2 comparisons, we found no effects of age or drug on V_m or AP threshold in these experiments (Fig. 2, *A* and *B*, respectively), although we did find an interaction of Age × Drug on AP threshold (see Table 2 for 2 × 2 statistics). We observed once again an effect of age on R_{in}, with no ISO effect or interaction (Fig. 2*C*). In the presence of PTX, we additionally found an effect of age on %Sag (Fig. 2*D*) and τ_M (Fig. 2*E*), suggesting that the absence of inhibition exaggerated differences in membrane properties between ages. Taken together, these findings suggest that the effects of ISO on spiking properties in juvenile LA cells found under control conditions are likely due to changes in tonic inhibition.

Finally, as shown in Fig. 3, we confirmed our findings via wash-in of ISO in adult and juvenile brain slices containing the

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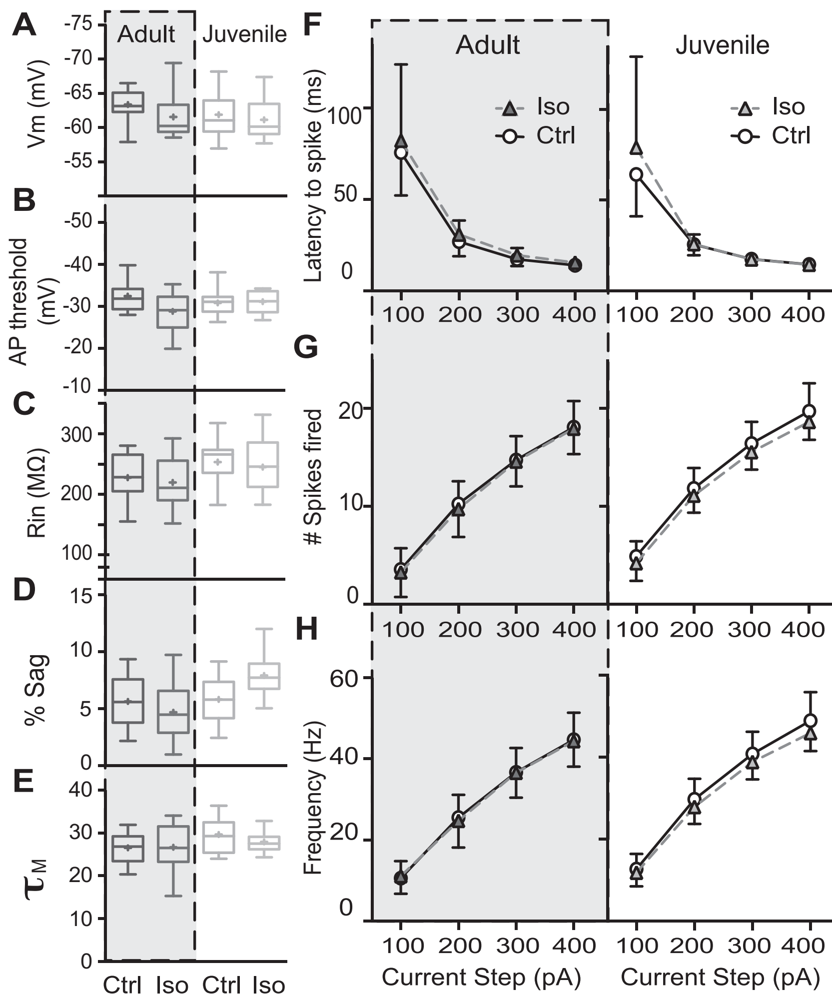

Fig. 2. β-Adrenergic enhancement of excitability in the juvenile LA is occluded by picrotoxin application. *A–E*: box and whiskers plots of membrane properties in adult and juvenile cells bathed in 75 μM picrotoxin, in the presence or absence of 15 μM ISO: whiskers represent 5–95th percentiles, box represents 25–75th percentiles, central line is the median, and + represents the mean. *A*: V_m in adult (Ctrl: −63.4 ± 2.2, ISO: −61.6 ± 3.1 mV) and juvenile (Ctrl: −61.9 ± 3.2, ISO: −61.1 ± 2.9 mV) LAd neurons. *B*: AP threshold in adult (Ctrl: −32.4 ± 3.6, ISO: −28.8 ± 4.4 mV) and juvenile (Ctrl: −30.8 ± 2.9, ISO: −31.1 ± 2.6 mV) neurons. *C*: R_{in} in adult (Ctrl: 228 ± 38, ISO: 220 ± 43 MΩ) and juvenile (Ctrl: 253 ± 36, ISO: 245 ± 44 MΩ) neurons. *D*: %Sag in adult (Ctrl: 5.6 ± 2.2%, ISO: 4.7 ± 2.5%) and juvenile (Ctrl: 5.8 ± 1.9%, ISO: 7.9 ± 2.0%) neurons. *E*: τ_M in adult (Ctrl: 26.5 ± 3.5, ISO: 26.7 ± 5.4) and juvenile (Ctrl: 30.0 ± 4.1, ISO: 28.0 ± 2.2) LAd neurons. *F–H*: values in point plots represent mean ± SD spike properties in response to current steps increasing from +100 to +400 pA: latency to first spike (*F*), no. of spikes fired (*G*), and spike frequency (*H*) at increasing current injection amplitudes in the absence or presence of ISO. Adult values are shown on *left* (shaded) and juvenile values on *right*. Adults: n = 14 cells from 4 animals in both Ctrl and ISO groups. Juveniles: n = 15 cells from 4 animals in both Ctrl and ISO groups.

LAd (in control ACSF). This within-subjects comparison allowed us to observe whether there were time-dependent effects during drug application, as the slow perfusion system results in a gradual increase in concentration over 2–4 min. In these experiments we delivered an increasing series of current pulses, from −100 pA to +200 pA, once every minute. After a 5-min baseline recording, we applied 15 μM ISO for a period of 15 min. Wash-in of 15 μM ISO significantly increased spikes fired [$F(1,29)$ = 16, P = 0.0004] and frequency [$F(1,29)$ = 14, P = 0.0009] in response to +100-pA current injection, with no main effect of age [$F(1,29)$ = 0.9, P = 0.3509 and $F(1,29)$ = 1.3, P = 0.2612, respectively] or interaction [$F(1,29)$ = 2.4, P = 0.1301 and $F(1,29)$ = 2.7, P = 0.1080, respectively]. Paired comparisons (Fig. 3*D*) revealed a significant increase in spike number [$t(29)$ = 4.0, P = 0.0008] and frequency [$t(29)$ = 3.9, P = 0.0012] in juvenile LAd neurons at 10–15 min (averaged) after washin of ISO but no effect on spike number [$t(29)$ = 1.7, P = 0.1910] or frequency [$t(29)$ = 1.4, P = 0.3251] in adult LAd neurons in response to +100-pA current injections. In contrast, ISO application did not lead to a significant increase in spike number [$t(29)$ = 1.4, P = 0.3281] or frequency [$t(29)$ = 1.1, P = 0.0.5570] elicited by +200-pA current injections in juvenile cells or spike number or frequency in adult cells [$t(29)$ = 0.28, $P \geq$ 0.9999 and $t(29)$ = 0.76, P = 0.9087, respectively]. Wash-in of ISO decreased spike latency in response to +100-pA [$F(1,29)$ = 28, P < 0.0001] and +200-pA [$F(1,29)$ = 64, P < 0.0001] current injections, with significant pre-post differences in both juvenile [$t(29)$ = 3.8, P = 0.0014 at 100 pA and $t(29)$ = 6.3, P < 0.0001 at 200 pA] and adult [t(29) = 3.8, P = 0.0016 at 100 pA and $t(29)$ = 5.1, P < 0.0001 at 200 pA] animals. Finally, in agreement with our earlier findings, we found that ISO application led to a decrease in AP threshold [$F(1,29)$ = 21, P < 0.0001] with no main effect of age [$F(1,29)$ = 1.4, P = 0.2432] or interaction [$F(1,29)$ = 0.30, P = 0.5867]. Paired comparisons revealed a pre-post effect of ISO on AP threshold for both juvenile [$t(29)$ = 2.9, P = 0.0138] and adult [$t(29)$ = 3.6, P = 0.0025] animals.

As our earlier experiments suggested that the developmental differences in the effects of ISO are dependent on tonic GABAergic signaling, we also analyzed these data for changes in both R_{in} and τ_M over the course of ISO application. Surprisingly, we found no effect of ISO on R_{in} or τ_M in adult or juvenile cells (Fig. 3*I*). We did, however, document an acute depolarization in both adult [$t(29)$ = 3.8, P = 0.0013] and juvenile [$t(29)$ = 5.8, P < 0.0001] cells immediately after ISO bath application (Fig. 3*H*). Because we then injected current to maintain cells at −58 mV throughout each recording, we were not able to determine whether this depolarization was maintained in an age-dependent manner.

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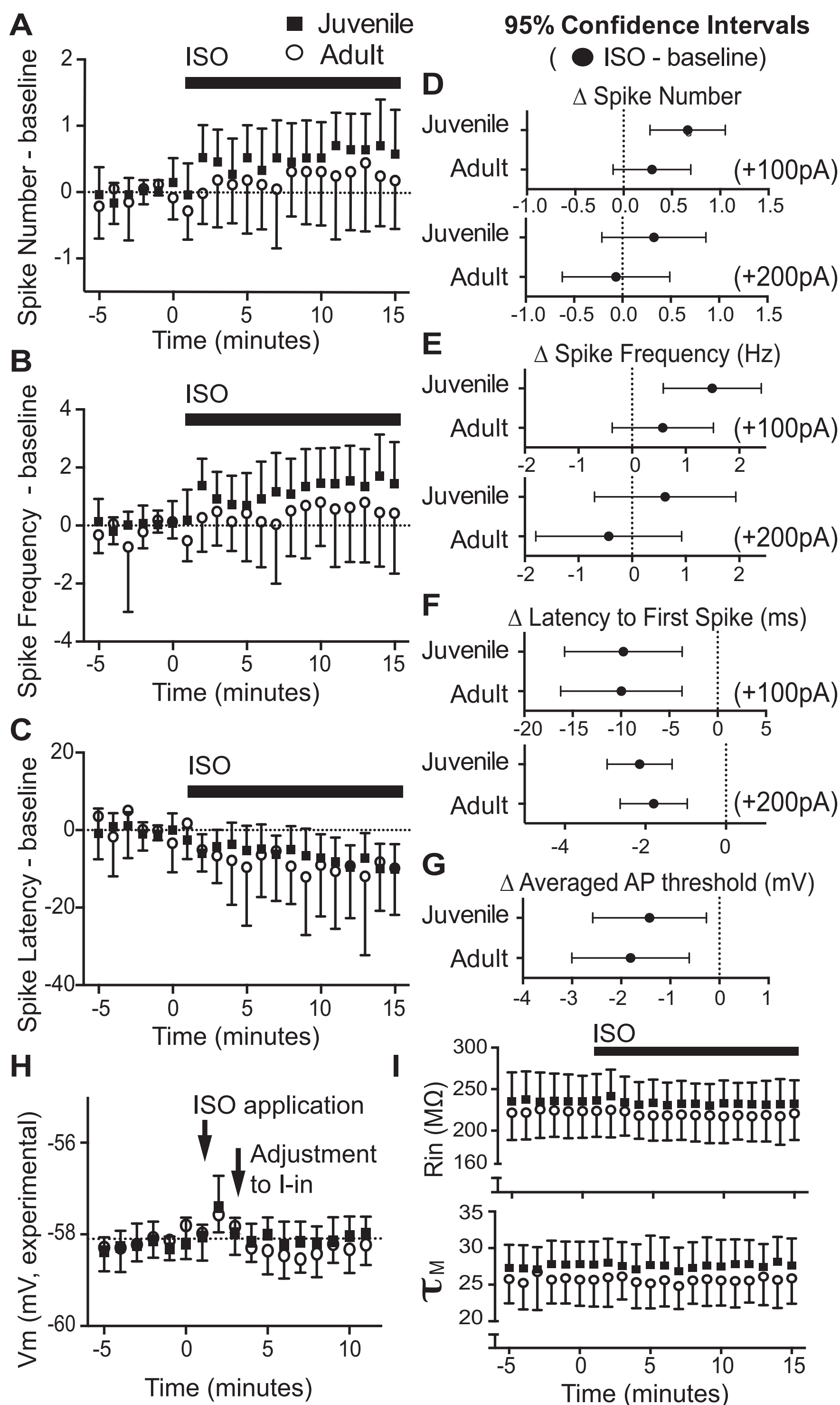

Fig. 3. Wash-in application of ISO leads to enhanced spiking in neurons of the juvenile, but not the adult, LA. *A–C*: spiking characteristics plotted during 15-min washin of 15 μM ISO. All values represent mean ± SD difference (Δ) from average baseline values. *A*: Δ spike numbers elicited in response to +100-pA current pulses in adult and juvenile animals. *B*: Δ spike frequency in response to +100-pA current pulses. *C*: Δ spike latency in response to +100-pA current pulses. *D–G*: difference scores with 95% confidence intervals obtained from paired comparisons between baseline values and values obtained at 15 min after ISO application. *D*: ISO-dependent shift in spike number in juvenile and adult animals at +100 pA (mean shift = +0.66 spikes in juveniles and +0.29 spikes in adults) and +200 pA (mean shift = +0.33 spikes in juveniles and −0.07 spikes in adults). *E*: ISO-dependent shift in spike frequency in juvenile and adult animals at +100 pA (mean shift = +1.5 Hz in juveniles and +0.57 Hz in adults) and +200 pA (mean shift = +0.62 Hz in juveniles and −0.44 Hz in adults). *F*: ISO-dependent shift in latency to first spike in juvenile and adult animals at +100 pA (mean shift = −9.8 ms in juveniles and −10 ms in adults) and +200 pA (mean shift = −2.1 ms in juveniles and −1.8 ms in adults). *G*: ISO-dependent shifts in AP threshold (mean shift = −1.4 mV in juveniles and −1.8 mV in adults). *H*: time course of V_m displaying acute ISO-induced depolarization (mean shift = +0.84 mV in juveniles and +0.57 mV in adults) and return to holding potential by increased negative current injection. I-in, injected current. *I*, *top*: R_{in} values before and 15 min after ISO application in adult cells (224 ± 31 vs. 219 ± 32 MΩ) and in juvenile cells (236 ± 33 vs. 232 ± 27 MΩ). *Bottom*: τ_M values before and 15 min after ISO application in adult cells (25.8 ± 3.7 vs. 25.7 ± 3.4) and in juvenile cells (27.5 ± 3.0 vs. 27.8 ± 3.2 MΩ). Values shown are means ± SD. Adult mice: *n* = 15 cells from 7 animals. Juvenile mice: *n* = 16 cells from 7 animals.

DISCUSSION

Our results demonstrate that β-adrenergic modulation does not enhance AP number or frequency in LAd cells of adult mice. Instead, β-adrenergic receptor activation only alters these measures of excitability in the LAd of juvenile mice, in a $GABA_A$ receptor-dependent manner. While we speculate that the change in spiking observed in juvenile neurons is due to downregulation of tonic inhibition, future experiments will be required to compare β-adrenergic effects on inhibitory neuronal activity in the adult and juvenile LA. Bath application of ISO resulted in a rapid depolarization of both juvenile and adult neurons that could have resulted from either disinhibition or intrinsic variables. The fact that we injected current to maintain cells at −58 mV limits interpretation of these findings by obscuring any lasting age difference in membrane potential as well as anticipated changes in R_{in} over time. The effects of ISO-induced changes in $GABA_A$-mediated signaling could also be minimal near the Cl^- equilibrium potential, resulting

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Table 1. *Additional F-statistics for Figs. 1 and 2 [interaction (ISO × current)]*

	Juvenile		Adult	
	F(DFn, DFd)	*P* value	*F*(DFn, DFd)	*P* value
Fig. 1: intrinsic neuronal properties in regular ACSF				
Latency	*F*(1.0,36.3) = 0.63	0.434	*F*(1.0,31.4) = 1.97	0.171
No. of spikes	*F*(1.4,49.2) = 0.77	0.424	*F*(1.3,39.1) = 0.04	0.889
Frequency	*F*(1.3,45.4) = 0.47	0.541	*F*(1.2,37.3) = 0.05	0.869
Fig. 2: *intrinsic neuronal properties in picrotoxin*				
Latency	*F*(1.0,27.5) = 0.22	0.65	*F*(1.0,22.3) = 0.12	0.738
No. of spikes	*F*(1.2,32.3) = 0.43	0.554	*F*(1.3,28.2) = 0.07	0.847
Frequency	*F*(1.2,32.1) = 1.06	0.325	*F*(1.2,26.0) = 0.25	0.620

only in significant effects near AP threshold. Prior in vivo single-unit recordings have produced complex evidence for inhibitory modulation, indicating that locus coeruleus activation or β-adrenergic receptor activation has the potential to either enhance or suppress basolateral neuron firing (Buffalari and Grace 2007, 2009; Chen and Sara 2007) in rats approaching adult age. This is thought to be the result of simultaneous modulation of excitatory synaptic transmission, local inhibitory transmission, network properties, and intrinsic membrane properties.

Our whole cell experiments complement these in vivo findings by specifying the changes in spiking properties that contribute to the effects of β-adrenergic receptor activation at two different ages. These data have wide-ranging implications. First, our findings suggest that the importance of β-adrenergic receptor signaling for aversive learning in adult rodents (Bush et al. 2010; Johansen et al. 2014) is largely due to activation of signaling molecules involved in bolstering synaptic plasticity (Huang et al. 2000) or AP initiation, rather than generalized effects on spike number or frequency. Second, our findings indicate that while LAd neurons exhibit largely mature cellular properties at this peripubertal time point (Ehrlich et al. 2012), neuromodulatory signaling can have strikingly different effects on spike properties.

These findings might relate to differences in amygdala function, attachment, and aversive learning observed across development in rodents and humans (Gao et al. 2010; Landers and Sullivan 2012; Pattwell et al. 2013; Poulos et al. 2014). Specifically, β-adrenergic modulation of cellular excitability in juvenile animals could play an important organizational role in peripubertal sensitivity to stress. Our findings indicate that both adult and juvenile animals responded to ISO application with a decreased AP threshold and latency, which could increase the probability of coincidence detection and the induction of input-specific plasticity, as well as stimulus-specific aversive learning. In contrast, the enhanced overall spike number and frequency induced by ISO in juvenile animals represents a more generalized form of plasticity.

Generalized plasticity has multiple interpretations: one is a potential cellular mechanism of fear/threat generalization (Ghosh and Chattarji 2015), which could increase vulnerability to conditions such as posttraumatic stress disorder. Alternatively, more generalized plasticity could result in a greater capacity to alter or erase threat-related memories. A period of more robust extinction learning corresponds to the presence of immature perineuronal nets up to the upper limit of our juvenile range (28 days; Gogolla et al. 2009). Perineuronal nets, moreover, interact with parvalbumin-expressing interneuron terminals (Pizzorusso et al. 2002), providing a potential mechanism for inhibitory modulation in such extinction mechanisms. These results raise the intriguing possibility that microstructural variables shape neuromodulatory capacity, and mutability of threat-related memories, across development. Developmental changes in neuromodulation represent an especially critical clinical consideration given uncertainty over the use of psychiatric medications such as serotonin and norepinephrine reuptake inhibitors within child and adolescent populations (Miller et al. 2014; Tsapakis et al. 2008).

As this is a brief study that examines for the first time differential effects of β-adrenergic receptor activation on juvenile and adult neurons, a number of open questions remain. First of all, future studies might examine concentration-dependent effects of β-adrenergic receptor agonists or of other adrenergic receptor agonists. Further experiments might also continue to delineate effects of adrenergic receptor agonists and antagonists on inhibitory synaptic transmission across development. Finally, future work should establish whether developmental changes in β-adrenergic modulation within the amygdala are consistent across rodent strains and sexes and across species.

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Table 2. *Additional F-statistics for Figs. 1 and 2 (two-way ANOVA)*

	AP Threshold		V_m		R_{in}		τ_M		%Sag	
	F value (df)	*P* value	*F* value (df)	*P* value	*F* value (df)	*P* value	*F* value (df)	*P* value	*F* value (df)	*P* value
Fig. 1: intrinsic neuronal properties in regular ACSF										
ISO	**F(1,68) = 5.6**	**0.0203**	*F*(1,68) = 1.2	0.2721	*F*(1,68) = 2.5	0.1198	*F*(1,68) = 1.6	0.2099	*F*(1,68) = 2.0	0.1608
Age	*F*(1,68) = 3.0	0.0866	*F*(1,68) = 0.47	0.4937	**F(1,68) = 6.4**	**0.0136**	*F*(1,68) = 3.9	0.0515	*F*(1,68) = 3.1	0.0820
Interaction	F(1,68) = 0.72	0.3985	*F*(1,68) = 0.91	0.3447	F(1,68) = 0.37	0.5434	*F*(1,68) = 3.1	0.0831	*F*(1,68) = 0.20	0.6573
Fig. 2: intrinsic neuronal properties in picrotoxin										
ISO	*F*(1,54) = 3.4	0.0689	*F*(1,46) = 1.1	0.3090	*F*(1,54) = 0.58	0.4496	*F*(1,54) = 0.61	0.4389	*F*(1,54) = 1.0	0.3118
Age	*F*(1,54) = 0.14	0.7083	*F*(1,46) = 0.76	0.3880	**F(1,54) = 5.8**	**0.0190**	**F(1,54) = 4.5**	**0.0386**	**F(1,54) = 9.1**	**0.0038**
Interaction	**F(1,54) = 4.7**	**0.0341**	*F*(1,46) = 1.1	0.2944	*F*(1,54) = 0.00038	0.9845	*F*(1,54) = 0.83	0.3676	**F(1,54) = 7.3**	**0.0090**

Significant values are in bold.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.E.F. and J.E.L. conceived and designed research; A.E.F. performed experiments; A.E.F. analyzed data; A.E.F. and J.E.L. interpreted results of experiments; A.E.F. prepared figures; A.E.F. drafted manuscript; A.E.F. and J.E.L. edited and revised manuscript; A.E.F. and J.E.L. approved final version of manuscript.

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