Lateral Orbitofrontal Cortical Modulation on the Medial Prefrontal Cortex-Amygdala Pathway: Differential Regulation of Intra-Amygdala GABA\textsubscript{A} and GABA\textsubscript{B} Receptors

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Abstract

Background: The basolateral complex of the amygdala receives inputs from neocortical areas, including the medial prefrontal cortex and lateral orbitofrontal cortex. Earlier studies have shown that lateral orbitofrontal cortex activation exerts an inhibitory gating on medial prefrontal cortex-amygdala information flow. Here we examined the individual role of GABA\textsubscript{A} and GABA\textsubscript{B} receptors in this process.

Methods: In vivo extracellular single-unit recordings were done in anesthetized rats. We searched amygdala neurons that fire in response to medial prefrontal cortex activation, tested lateral orbitofrontal cortex gating at different delays (lateral orbitofrontal cortex-medial prefrontal cortex delays: 25, 50, 100, 250, 500, and 1000 milliseconds), and examined differential contribution of GABA\textsubscript{A} and GABA\textsubscript{B} receptors with iontophoresis.

Results: Relative to baseline, lateral orbitofrontal cortex stimulation exerted an inhibitory modulatory gating on the medial prefrontal cortex-amygdala pathway and was effective up to a long delay of 500 milliseconds (long-delay latencies at 100, 250, and 500 milliseconds). Moreover, blockade of intra-amygdala GABA\textsubscript{A} receptors with bicuculline abolished the lateral orbitofrontal cortex inhibitory gating at both short- (25 milliseconds) and long-delay (100 milliseconds) intervals, while blockade of GABA\textsubscript{B} receptors with saclofen reversed the inhibitory gating at long delay (100 milliseconds) only. Among the majority of the neurons examined (8 of 9), inactivation of either GABA\textsubscript{A} or GABA\textsubscript{B} receptors during baseline did not change evoked probability per se, suggesting that local feed-forward inhibitory mechanism is pathway specific.

Conclusions: Our results suggest that the effect of lateral orbitofrontal cortex inhibitory modulatory gating was effective up to 500 milliseconds and that intra-amygdala GABA\textsubscript{A} and GABA\textsubscript{B} receptors differentially modulate the short- and long-delay lateral orbitofrontal cortex inhibitory gating on the medial prefrontal cortex-amygdala pathway.

Keywords: in vivo electrophysiology, prefrontal cortex, amygdala

Introduction

The amygdala serves as the key emotion center in the brain (LeDoux, 2000; Phelps and LeDoux, 2005; Roozenendaal and McGaugh, 2011). The lateral nucleus (LA) and the basolateral nucleus (BLA) of the amygdala are traditionally viewed as the sensory interface (LeDoux et al., 1990) where inputs from cortical and subcortical areas converge (Maren, 1999; Orsini and Maren, 2012).
Significance Statement

Earlier study suggested that activation of the lateral orbitofrontal cortex (LOFC) decreased amygdala neurons responsive to stimulation of medial prefrontal cortex (mPFC) through intra-amygdala feed-forward inhibition. Here we further examined the differential role of GABA <sub>A</sub> and GABA <sub>B</sub> receptors in this process. We extended the findings that LOFC inhibitory modulatory gating was effective up to a long delay of 500 milliseconds (compared with an earlier study of 100 milliseconds). Mechanisms of intra-amygdala GABA receptors were examined at LOFC-mPFC stimuli delays of 25 and 100 milliseconds. Evidence suggested that GABA <sub>A</sub> receptors dominated in the inhibitory gating process, while GABA <sub>B</sub> receptors were critical for long delay (100 milliseconds) only. Our results provided detailed mechanisms of this inhibitory modulatory information processing.

The emotional contingencies are potently modulated by neocortical afferents from the prelimbic (PL) and the infralimbic (IL) divisions of the medial prefrontal cortex (mPFC) (Sotres-Bayon and Quirk, 2010) and generally require the integration of spatial and contextual information from the hippocampus (Canteras and Swanson, 1992; Pitkanen et al., 2000; Quirk and Mueller, 2008; Orsini et al., 2011). Input from the mPFC, for example, is critical for proper fear regulation after extinction (Quirk and Mueller, 2008; Sierra-Mercado et al., 2011; Milad and Quirk, 2012).

The amygdala is also recruited by diverse high-level behaviors, such as its interaction with the orbitofrontal cortex (OFC) (Orsini et al., 2015a). OFC and the amygdala are heavily interconnected (Aggleton et al., 1980). The more medially situated ventral orbital (VO) and ventrolateral orbital (VLO) areas provide inputs to autonomic output areas of the amygdala, including the medial and central nuclei, while the more laterally situated lateral orbital (LO) area and the adjacent ventral agranular insular (AI; including the ventral and dorsal subdivisions) area project heavily to the sensory input area of the LA and BLA (McDonald et al., 1996). Functionally, the OFC-amygdala pathway, especially the lateral OFC (LOF; LO and AI) (Lapatina et al., 2015), is critical for the development of cue-outcome contingencies (Schoenbaum and Roesch, 2005; Lucantoni et al., 2015; Sharpe and Schoenbaum, 2016). For example, reversal learning (shifting between different stimulus-reward association) is facilitated by the OFC (Schoenbaum et al., 2007; Ghods-Sharif et al., 2008).

The LA/BLA as the hub receives convergent inputs from the mPFC and OFC (McDonald et al., 1996; Vertes, 2004; Rempel-Clower, 2007), suggesting its potential role in processing emotionally relevant actions based on cue-outcome contingencies. Physiologically, it has been reported that OFC activation exerts an inhibitory modulatory gating on the mPFC-amygdala pathway through intra-amygdala feed-forward inhibition (Chang and Grace, 2016). However, there are critical questions that await further study, such as the individual role of GABA <sub>A</sub> and GABA <sub>B</sub> receptors in this process, and whether there is a general lift from the inhibitory tone under blockade of intra-amygdala GABA receptors. GABA <sub>A</sub> receptors are ionotropic receptors, whereas GABA <sub>B</sub> receptors are metabotropic receptors. They have different kinetics, and GABA <sub>A</sub> receptors mediate the long-lasting inhibitory effect in vitro (Perez-Garcia et al., 2006) and in vivo (Li et al., 1996). In this study, we used combined techniques of extracellular single unit recordings and iontophoretic administration of either GABA <sub>A</sub> or GABA <sub>B</sub> antagonist into the amygdala in anesthetized rats to examine these questions.

Methods

Subjects

Male Sprague-Dawley rats (250–400 g; BioLASCO) were housed for at least 5 days upon arrival in groups (maximum of 3) in a temperature- (22 ± 1°C) and humidity- (60% – 70%) controlled facility on a 12-h-light/-dark cycle (7:00 AM to 7:00 PM) with food and water available ad libitum. Animals were handled according to protocols approved by the Institutional Animal Care and Use Committees of both National Tsing Hua University and National Chiao Tung University.

Surgery

All recordings were performed on anesthetized rats (Rosenkranz et al., 2003; Buffalari and Grace, 2007). Rats were anesthetized with 8% chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting Co.); core body temperature was maintained at 37°C by a temperature-controlled heating pad (CWE Inc.). Incisions were then made in the scalp to expose the skull. Supplemental doses of chloral hydrate were administered as needed throughout the entire recording session.

Electrically Evoked Responses of mPFC-Amygdala Pathway

For electrical stimulation, a burr hole was drilled into the skull overlying the mPFC (from bregma: anteroposterior [AP], +3.5 mm; mediolateral [ML], +0.6 mm; dorsoventral [DV], -5.0 mm) for the placement of the electrode. The stimulation electrode targeted the IL subdivision of mPFC. However, current spread to adjacent PI subdivision could not be ruled out; hence, the stimulation site is identified as mPFC. A bipolar concentric electrode (FHC) was lowered into the target, and stimulation was delivered using a dual-output stimulator (S88; Grass Instruments) at an intensity of 1.0 mA and duration of 0.25 milliseconds at 0.5 Hz in search of evoked responses in the amygdala, focused on the LA and BLA nuclei (Belujon et al., 2014).

For recording, burr holes were drilled into the skull, and the dura was removed in an area overlying the LA/BLA (from bregma: AP, -2.8 mm; ML, +5.0 mm; DV, -6.5 ~ -9.0 mm). Single- (Exp 1; 2-mm outer diameter Omegadot filament glass; World Precision Instruments) or 5-barrel microelectrodes (Exp 2; ASI Instruments) were constructed using a vertical microelectrode puller (PE-22; Narishige), and the tip was broken back under microscopic control. The recording barrel of the microelectrode was filled with 2% Pontamine sky blue in 2 M NaCl with in situ impedance of 4 ~ 8 MΩ (measured at 1 kHz) for electrophysiological recordings. The microelectrode was slowly lowered into the LA/BLA using an oil hydraulic microdrive (MO-10; Narishige) in search of neurons responsive to mPFC stimulation. Once a responsive single unit was identified, stimulation current was adjusted to determine a baseline (BL) evoked spike response probability of ~50% (of 50 stimulation trials). In some cases, multiple tracks were searched with at least 0.2 mm apart (AP and/or ML) between tracks.

LOFC Long-Delay Gating

For LOFC gating, another bipolar concentric electrode was lowered into the IOFC (relative to bregma: AP +3.5 mm; ML +3.0 mm;DV, -6.0 mm) and water available ad libitum. Animals were handled according to protocols approved by the Institutional Animal Care and Use Committees of both National Tsing Hua University and National Chiao Tung University.
on 26 July 2018

Intra-LA/BLA Iontophoretic Application of Drug

For iontophoretic application of drug, 5-barrel microelectrodes were used. Other than the central recording barrel, 2 of the outer barrels were filled with GABA, antagonist bicuculline methiodide (5 mM, pH 4.5) and the other 2 with GABA, antagonist saclofen (20 mM, pH 4.5). Antagonists were dissolved in 100 mM NaCl (Stutzmann and LeDoux, 1999) and were held with (+) retaining current at 10 nA until ejection with (+) iontophoretic current at 40 nA during testing. Drugs were applied continuously over the testing period (Exp 2; during BLs and 2 gating delays), with one-half of the neurons tested with bicuculline first and the other one-half with saclofen first.

Data Acquisition

Signals from the recording electrode were amplified by a headstage before being fed into an amplifier (1000 gain, 100-10k Hz bandpass; Model 1800, A-M Systems), then into an audio monitor (Model AM3300; A-M Systems), and displayed on an oscilloscope (Tektronix) for real-time monitoring. Data were collected using a data acquisition board interface, monitored on-line, and analyzed off-line using the computer software Powerlab (AD instruments).

Neuronal spikes were with a signal-to-noise ratio >3. We included only single units with response onset latencies <30 milliseconds (presumably monosynaptic) for further analyses. Onset latency was measured from the start of stimulation artifact to the initial rising phase of the evoked action potential that crossed the threshold set in the computer software. These LA/BLA neurons showed little shift in latency when increasing the stimulus intensity, yet they showed some range (generally < 5 milliseconds) in latency distribution (“jitter”), ruling out antidromic activation. All of the neurons reported in this study were putative projection neurons in that they exhibited very low spontaneous firing rates (<0.5 Hz) and long duration action potential waveforms (>2.5 milliseconds); the duration of the action was quantified as the time from the initial deflection from baseline to the return to baseline) as determined previously (Rosenkranz and Grace, 1999).

Histology

One or 2 neurons were recorded from a single track of search. At the conclusion of each experiment, the microelectrode was replaced to the depth of the neuron recorded and the location verified via electrophoretic ejection (BAB-501; Kation Scientific) of Pontamine sky blue dye into the recording site for 30 minutes (~20 μA constant current). To verify the placement of the stimulation electrode, a 10-second pulse at 100 μA was administered. Rats were then killed by an overdose of anesthetic (chloral hydrate, additional 400 mg/kg, i.p.). All rats were decapitated, their brains removed, fixed for at least 2 days (8% paraformaldehyde in 0.2 M phosphate buffer solution [PBS]), and cryoprotected (25% sucrose in 0.1 M PBS) until saturated. Brains were sectioned (60-μm coronal sections), mounted onto gelatin-chrome alum-coated slides, and stained with a combination of neutral red and cresyl violet for histochemical verification of the stimulating and recording sites.

Statistics

All data are represented as the mean ± SEM and were submitted to repeated measures ANOVA. “Delay” served as the within-subject factor in Exp 1, while both “Delay” and “Drug” were within-subject factors in Exp 2. Post hoc comparisons using Fisher’s LSD test were performed for ANOVAs that achieved a significance of P < .05. All statistics were calculated using SPSS (IBM) or SigmaStat (Systat Software Inc.).

Results

Exp 1: IOFC Stimulation Exerted a Long-Delay Inhibitory Gating on mPFC-LA/BLA Evoked Responses

Recently, it was reported that inhibitory gating of the IOFC on the mPFC-amygdala pathway was effective at a wide range of IOFC-mPFC delay intervals up to 100 milliseconds (Chang and Grace, 2016). In this experiment, we extended this finding by electrically engaging the activity of the IOFC with delay latencies up to 1000 milliseconds (IOFC-mPFC delays: 25, 50, 100, 250, 500, and 1000 milliseconds). A total of 11 LA/BLA neurons responsive to mPFC stimulation were recorded from 5 rats. Consistent with the earlier report, the majority of mPFC-evoked response (9 of 11 neurons) was attenuated by IOFC prepulse (Figure 1, black circles), among which 3 of 9 received convergent inputs from both the mPFC and the IOFC.

Figure 1. The placements of (A) all the stimulation electrodes in the medial prefrontal cortex (mPFC) (PL and IL) and the lateral orbitofrontal cortex (IOFC) (LO and Alv) and (B) the distribution of all the neurons recorded (+3.72 and -3.36; anterior-posterior [AP] distance [mm] to bregma) in Exp 1 and 2. White arrowhead, dye mark of an exemplary recording site. Alv, ventral agranular insula; BLA, basolateral nucleus of the amygdala; IL, infralimbic cortex; LA, lateral nucleus of the amygdala; LO, lateral orbital cortex; PL, prelimbic cortex.
lOFC prepulse exerted a long-delay inhibitory gating on the mPFC-LA/BLA-evoked response (Figure 2). The gating effect was robust at shorter intervals and gradually lost the modulation at longer latencies. There was a significant main effect of Delay \(F(7,56) = 4.020, P = .001\). Compared with BL, evoked probability was significantly decreased at delay latencies up to 500 milliseconds (25, 50, 100, 250, and 500 milliseconds; “a”, \(P < .05\)), with no statistical difference at 1000 milliseconds or post BL. When compared with post BL controls, evoked probability was significantly lower at delay latencies of 25, 50, and 100 milliseconds (“b”, \(P < .05\)). Because there was no statistical difference between BL and post BL, the long-delay inhibitory modulation was unlikely due to the accumulation of GABA.

Exp 2: lOFC Inhibitory Gating on mPFC-LA/BLA Pathway Is Differentially Regulated by Intra-Amygdala GABA\(_A\) and GABA\(_B\) Receptors

From an earlier study (Chang and Grace, 2016), total blockade of intra-amygdala GABA receptors reversed the lOFC inhibitory gating on the mPFC-amygdala information flow. In this experiment, we specifically examined the differential regulation of GABA\(_A\) and GABA\(_B\) receptors in this process. We chose to verify the effect at delay latencies of 25 milliseconds (short) and 100 milliseconds (long), which had significant decreased evoked probability compared with both BL and post BL in Exp 1. A total of 9 LA/BLA neurons (from 6 rats) responsive to mPFC stimulation was recorded in this experiment; all were inhibitory gated by lOFC prepulse (Figure 1, white circles). To avoid the potential confounding results due to residual drug effects, we counterbalanced the test order so that 5 neurons were examined with GABA\(_A\) antagonist (bicuculline) first followed by GABA\(_B\) antagonist (saclofen), while the other 4 with the reversed sequence. One of the 9 neurons displayed dramatic increase in evoked responses (>3 SEM from mean) at BLs when we applied either GABA\(_A\) or GABA\(_B\) antagonist and was thus singled out from analyses.

Among the majority of the neurons (8 of 9) examined in this experiment (Figure 3), blockade of either GABA\(_A\) or GABA\(_B\) receptor did not change the BL evoked probability, but had differential regulation on lOFC inhibitory gating at the 2 delay intervals tested. There was a significant interaction between Drug and Delay \(F(4,28) = 7.839, P < .001\). Post hoc comparisons suggested that there was no statistical difference among BLs regardless of drug administration. Comparing to respective BLs, lOFC inhibitory gating was abolished at both short and long delays under GABA\(_A\) antagonist, while the inhibitory gating was reversed at long delay under GABA\(_B\) antagonist (all \(P > .05\)).

There was one neuron that displayed a unique response with robust increase in evoked spikes at BL when we applied either GABA\(_A\) or GABA\(_B\) antagonist (Figure 4). At the basal condition, lOFC exerted an inhibitory gating only at short delay (25 milliseconds). Blockade of GABA\(_A\) receptor not only dramatically increased the evoked response at BL but also abolished the lOFC short-delay gating. Interestingly, blockade of GABA\(_A\) receptor had a general upward shift in evoked responses but retained the short-delay inhibitory gating.

Lastly, we managed to reexamine the BL in 6 of the 9 neurons. There was no significant difference between pre- (49.3 ± 3.6%) and post- (50.3 ± 7.6%) drug administration \([\text{paired}-t(5) = 0.207, P = .84}\].

Discussion

In this study, the combined techniques of in vivo electrophysiology and iontophoretic administration of drugs were used in anesthetized rats. lOFC activation exerted a long-delay inhibitory gating on the mPFC-amygdala pathway effective up to 500 milliseconds. Our results suggested that under basal condition, there was no direct inhibitory tone from lOFC onto the mPFC-amygdala pathway in the majority of neurons recorded. However, there may be tonic inhibition from other sources, or activation of mPFC itself recruited feed-forward inhibition (Figure 5A). When lOFC was brought online, lOFC exerted an

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**Figure 2.** (A) Electrophysiological recording of an amygdala neuron responsive to medial prefrontal cortex (mPFC) stimulation (left) was decreased with 100-millisecond lateral orbitofrontal cortex (lOFC) prepulse (right). n/50 = evoked spikes of 50 trials. Gray and black traces were superimposed on 50 recorded trials, with one black trace highlighted to demonstrate the evoked spike. Arrows, electrical stimulation artifacts from mPFC and lOFC stimulation. (B) lOFC activation exerted a long-delay inhibitory gating on the mPFC-amygdala pathway \((P < .05; a, \text{relative to baseline [BL]; } b, \text{relative to post BL})\). Other abbreviations, refer to Figure 1.
inhibitory gating on the mPFC-amygdala pathway, which was differentially modulated by GABA_{A} and GABA_{B} receptors in that blockade of GABA_{A} receptors abolished both short- and long-delay gating, while blockade of GABA_{B} receptors reversed long-delay gating only (Figure 5B).

Compared with the medially located areas of the OFC (VO and VLO) that provided inputs to the automatic output areas of the amygdala, lOFC had widespread terminals in the sensory-related interface of LA and BLA but less densely to the central nucleus, with particularly strong connections with the BLA (Rempel-Clower, 2007). This is consistent with our recordings (Figure 1B, bottom panel) that we targeted neurons responsive to lOFC modulation in the LA/BLA area, and the majority distributed in the BLA. Although we did not compare the potential contrast of lOFC onto LA or BLA, there are anatomical and functional differences between the 2 subdivisions (Orsini and Maren, 2012; Toyote et al., 2015). Conservatively, the results in the present study were concluded from recordings in BLA in majority (neurons in BLA or at the boarder of LA/BLA combined, n = 7 and 8 in Exp 1 and 2, respectively). lOFC exerted a long-delay inhibitory gating on the mPFC-amygdala pathway effective up to inter-stimulus latency of 500 milliseconds (Figure 2, compared with BL "a"). The variance in evoked probability started to increase with delay latencies longer than 100 milliseconds (delays at 250, 500, and 1000 milliseconds) potentially due to the kinetics of the neurotransmitters and receptors involved. Conservatively, the inhibitory gating was effective up to 100 milliseconds (Figure 2, compared with BL "a" and post BL "b"), consistent with the earlier study (Chang and Grace, 2016). Another potential mechanism that we cannot entirely rule out is that the inhibitory modulation engaged a more complicated multi-synaptic pathway that involved at least one feed-forward inhibitory connection. The latter may also explain the increased variance for longer delays >100 milliseconds.

GABA_{A} and GABA_{B} receptors mediated intra-amygdala information processing of emotional learning and memory, as well as synaptic plasticity (Watanabe et al., 1995; Li et al., 1996; Bissiere et al., 2003; Ehrlich et al., 2009; Pan et al., 2009). GABA_{B} receptors are metabotropic, and the action is known to last a long time.

Figure 3. (A) Electrophysiological recording of an amygdala neuron that is responsive to medial prefrontal cortex (mPFC) stimulation that exhibited a decrease in evoked responses following lateral orbitofrontal cortex (lOFC) 25- or 100-millisecond prepulse. The decrease (both 25 and 100 milliseconds) was reversed upon local administration of GABA_{A} antagonist, bicuculline, while the long-delay gating (100-millisecond prepulse) was also blocked by local administration of GABA_{B} antagonist, saclofen. n/50 = evoked spikes of 50 trials. Gray and black traces were superimposed on 50 recorded trials, with one black trace highlighted to demonstrate the evoked spike. (B) Compared with respective baselines (BLs), evoked probability was significantly decreased with lOFC prepulse (25 and 100 milliseconds; both P < .05) and was reversed under the influence of GABA_{A} antagonist at both delays and under GABA_{B} antagonist at 100-millisecond long-delay. There was no statistical difference among BLs. Abbreviations, refer to Figures 1 and 2.
An earlier study on the auditory thalamo-amygdala pathway suggested that using paired-pulse stimulation, both short (<30 milliseconds) and longer (>50 milliseconds) latency inhibitory processes were revealed, while the former was eliminated by GABA_A and the latter blocked by GABA_B receptor antagonist, respectively (Li et al., 1996). Here, we reported that blockade of GABA_A receptors (Figure 3, middle panels) abolished both the short (25 milliseconds) and long-delay (100 milliseconds) gating, while blockade of GABA_B receptors (Figure 3, lower panels) only reversed the long-delay (100 milliseconds) gating. The effect we observed cannot be simply explained by “current injection” alone because of the differential influence of drugs during BL and the 2 gating latencies (25 and 100 milliseconds) tested. We are also aware of evidence from in vitro systems that bicuculline acted at Ca^{2+}-activated potassium channels (Seutin et al., 1997; Debarbieux et al., 1998; Khawaled et al., 1999). The nonspecific effect has not been explored in in vivo systems, and the iontophoretic current intensity used in this study was carefully chosen and standard for the in vivo approach based on earlier literatures (Li et al., 1996, 2002; Stutzmann and LeDoux, 1999; Gervasoni et al., 2000; Staak and Pape, 2001; Jia et al., 2004; Urbain et al., 2004; Windels and Kiyatkin, 2004; Sardo et al., 2009; Malmierca et al., 2012). Our results suggested that with functional ionotropic GABA_A receptors (blockade of the GABA_B receptors with saclofen), the short-delay inhibitory gating was preserved. However, functional metabotropic GABA_B receptors per se (blockade of GABA_A receptors with bicuculline) were not sufficient to support the normal inhibitory gating as in the basal conditions. Thus, GABA_A receptors are critical for long-delay (100 milliseconds) inhibitory gating in our study, and our results also

Figure 4. One amygdala neuron that displayed a unique response with a robust increase in evoked spikes at baselines (BLs) when either GABA_A or GABA_B antagonist was applied. n/50 = evoked spikes of 50 trials. Gray and black traces were superimposed on 50 recorded trials, with one black trace highlighted to demonstrate the evoked spike. Abbreviations, refer to Figures 1 and 2.
support a dominant role of GABA_\textsubscript{A} receptors for gating within 100 milliseconds.

Application of GABA_\textsubscript{A} or GABA_\textsubscript{B} receptor antagonist did not change the BL evoked probability in the majority of the neurons examined (Figure 3; 8 of 9). This result is consistent with an earlier report that IOFC did not actively exert an inhibitory tone on the mPFC-amygdala pathway during basal condition (Chang and Grace, 2016), and, moreover, our data further suggest that there was no apparent inhibitory tone from other sources to these amygdala neurons responsive to mPFC stimulation. We are aware of the fact that the effective distance of iontophoresis is very local and confined. When combined with the small tip diameter (approximately 5 µm total) compared with the neuron size (40–50 µm), it is not likely that the drug diffused even to the entire extent of the neuron. However, the iontophoretic current (40 nA) chosen was on the high end based on earlier studies (Rosenkranz and Grace, 1999; Stutzmann and LeDoux, 1999; Buffalari and Grace, 2007; Lipski and Grace, 2013; Chang and Grace, 2015), and the fact that drug effect was observed during gating (25 and/or 100 milliseconds), especially the total blockade of GABA_\textsubscript{A} function with bicuculline, supported the null results.

Under basal condition, IOFC did not actively exert inhibitory tone on the mPFC-amygdala pathway. Although minor, there may be tonic inhibition from other sources, or activation of the mPFC itself may recruit feed-forward inhibition. (B) Electrical activation of the IOFC exerted an inhibitory gating of the mPFC-amygdala pathway, which was differentially modulated by GABA_\textsubscript{A} and GABA_\textsubscript{B} receptors in that blockade of GABA_\textsubscript{A} receptors reversed both short- and long-delay gating, while blockade of GABA_\textsubscript{B} receptors reversed long-delay gating only. GLU, glutamate. Other abbreviations, refer to Figure 1.

Figure 5. One potential model that may account for the lateral orbitofrontal cortex (IOFC) modulation of the medial prefrontal cortex (mPFC)-amygdala pathway (A) Under basal condition, IOFC did not actively exert inhibitory tone on the mPFC-amygdala pathway. Although minor, there may be tonic inhibition from other sources, or activation of the mPFC itself may recruit feed-forward inhibition. (B) Electrical activation of the IOFC exerted an inhibitory gating of the mPFC-amygdala pathway, which was differentially modulated by GABA_\textsubscript{A} and GABA_\textsubscript{B} receptors in that blockade of GABA_\textsubscript{A} receptors reversed both short- and long-delay gating, while blockade of GABA_\textsubscript{B} receptors reversed long-delay gating only. GLU, glutamate. Other abbreviations, refer to Figure 1.

feed-forward inhibitory modulation is specific to inputs from mPFC and IOFC or serves as a general modulatory process. At the functional level, what could benefit from this inhibitory modulation? IOFC-amygdala integrity is critical for the development and update of cue-outcome contingencies (Ghods-Sharifi et al., 2008; Sharpe and Schoenbaum, 2016), while the mPFC-amygdala pathway is critical for processing of emotional reactions (Sotres-Bayon and Quirk, 2010). Several lines of research proposed that individuals constantly faced the challenge of decision-making that they have to weigh the consequences of multiple options before selecting the most beneficial (Orsini et al., 2015b), and sometimes the choice of a highly valuable option may be accompanied by a risk of adverse consequences. In laboratory settings, risk-taking behavior can be assessed in rats using a task that incorporates both rewards and risks by delivery of a mild footshock (Simon and Setlow, 2012). Animals make decisions and switch between conflicting behaviors of reward seeking (IOFC-amygdala dependent) and freezing (mPFC-amygdala dependent) in scenarios where the animal suppressed the tendency of freezing to attend the on-going task. Our physiology data provided a potential mechanism that engaging the IOFC suppressed the mPFC-amygdala information flow. According to the circuitry interaction we reported, we expect optogenetically engaging the mPFC-amygdala pathway during risk decision making with mild footshock would shift the behavior curve toward less risky decisions to avoid punishment, which awaits future validation.

Acknowledgments

We thank the animal care team at the Laboratory Animal Center of National Chiao Tung University for their support in animal housing.

This work was supported by the Ministry of Science and Technology (Taiwan) (MOST 104-2320-B-007-004- to C.-h.C.).

Statement of Interest

None.

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