Guanfacine’s mechanism of action in treating prefrontal cortical disorders: Successful translation across species

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ABSTRACT

The selective norepinephrine (NE) α2A-adrenoceptor (α2A-AR) agonist, guanfacine (Intuniv™), is FDA-approved for treating Attention Deficit Hyperactivity Disorder (ADHD) based on research in animals, a translational success story. Guanfacine is also widely used off-label in additional mental disorders that involve impaired functioning of the prefrontal cortex (PFC), including stress-related disorders such as substance abuse, schizotypic cognitive deficits, and traumatic brain injury. The PFC subserves high order cognitive and executive functions including working memory, abstract reasoning, insight and judgment, and top-down control of attention, action and emotion. These abilities arise from PFC microcircuits with extensive recurrent excitation through NMDAR synapses. There is powerful modulation of these synapses, where cAMP-PKA opening of nearby potassium (K⁺) channels can rapidly and dynamically alter synaptic strength to coordinate arousal state with cognitive state, e.g. to take PFC “offline” during uncontrollable stress. A variety of evidence shows that guanfacine acts within the PFC via post-synaptic α2A-AR on dendritic spines to inhibit cAMP-PKA-K⁺ channel signaling, thus strengthening network connectivity, enhancing PFC neuronal firing, and improving PFC cognitive functions. Although guanfacine’s beneficial effects are present in rodent, they are especially evident in primates, where the PFC greatly expands and differentiates. In addition to therapeutic actions in PFC, stress-related disorders may also benefit from additional α2-AR actions, such as weakening plasticity in the amygdala, reducing NE release, and anti-inflammatory actions by deactivating microglia. Altogether, these NE α2-AR actions optimize top-down control by PFC networks, which may explain guanfacine’s benefits in a variety of mental disorders.

1. Introduction

The norepinephrine (NE) α2A-adrenoceptor (α2A-AR) agonist, guanfacine, was approved by the FDA for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in 2009 under the brand name, Intuniv™, one of the rare success stories where basic neuroscience research in animals has successfully translated to human patients. The beneficial effects of α2-AR agonists for higher cognitive function were first discovered in aged monkeys (Arnsten, Cai, & Goldman-Rakic, 1988; Arnsten & Goldman-Rakic, 1985), who naturally develop cognitive impairments on tasks dependent on the prefrontal cortex (PFC), a newly evolved brain region that subserves working memory, abstract reasoning, and the top down regulation of attention, action and emotion (Szczepanski & Knight, 2014). Although α2-ARs are classically considered as presynaptic receptors, early research determined that the beneficial effects of α2-AR agonists on cognition arose from postsynaptic receptor actions in the PFC (Arnsten & Goldman-Rakic, 1985; Cai, Ma, Xu, & Hu, 1993), with a pharmacological profile consistent with the α2A-AR subtype (Arnsten et al., 1988; Arnsten & Leslie, 1991), a finding later confirmed in genetically altered mice (Franowicz et al., 2002). Subsequent research determined the cellular basis for guanfacine’s beneficial actions, strengthening network connections in PFC through intracellular signaling events in dendritic spines (Wang et al., 2007). Guanfacine is now in widespread clinical use, not only in ADHD, but in additional disorders associated with impaired PFC function. The following review describes guanfacine’s mechanism of action in PFC, enhancing the network connections needed for healthy cognitive experience and top-down control.

It is noteworthy that many of the discoveries made in this field were by women neuroscientists. As this recognition may provide inspiration to young women and girls wanting to become scientists, many of their names will be emphasized in this review.
2. The cognitive functions of PFC and their dysfunction in mental disorders

2.1. Cognitive functions of the PFC

The newly evolved PFC subserves many of our highest order cognitive functions, representing, evaluating and manipulating information in the absence of sensory stimulation, and using that information for goal-directed behavior (Fuster, 2008). In general, the PFC in primates is topographically organized, with circuits in the dorsal and lateral PFC (dLPFC) representing information from the external world (e.g. through vision and audition), and the ventral and medial subregions representing the internal world (e.g. taste and smell, pain, reward and punishment) (Ongür & Price, 2000). The ability to generate mental representations underlies a range of cognitive operations, including working memory, high-order decision-making, insight and judgment, and the top-down regulation of attention, action and emotion (summarized in Fig. 1A). Many of the highest order abilities, i.e. meta-cognitive abilities (‘thinking about thinking’, ‘remembering to remember’, and insight and judgment), reside in the frontal pole, which expands greatly in human vs. nonhuman primates (Tsujimoto, Genovesio, & Wise, 2011). The human PFC also shows evidence of laterality, with the left hemisphere specialized for language production, and the right hemisphere especially important for behavioral inhibition, i.e. inhibiting inappropriate responses.

2.2. Mental disorders involving the PFC

PFC functions are impaired in a host of mental disorders (summarized in Fig. 1B). Some of these disorders arise from developmental insults and are evident early in life. For example, ADHD is associated with slowed (Shaw et al., 2007) or impaired (Shaw et al., 2009) development of the right lateral PFC, the PFC region needed for inhibiting inappropriate actions and attention to distractors (Aron, Robbins, & Poldrack, 2004). Autism spectrum disorders can afflict a variety of circuits with a range of severity, but share in common alterations in the circuits mediating social cognition, many of which reside in PFC (Shamay-Tsoory & Aharon-Peretz, 2007). Mood disorders such as depression are associated with changes in the medial PFC, including overactivation of the cingulate circuits that process the suffering aspects of pain (Mayberg et al., 2005; Opler, Opler, & Arnsten, 2016). In contrast, Blumberg has shown that these circuits are underactive in the manic phase of bipolar disorder, as are the areas affecting impulse control and insight (Blumberg et al., 2003, 1999), helping to explain why these disorders can sometimes look so similar in children. Schizophrenia is associated with accelerated gray matter loss in PFC (Cannon, Chung, He, Sun, Jacobson, van Erp, & Consortium, 2014), including loss of dendrites and spines in the dLPFC (Glantz & Lewis, 2000). Indeed, hypofrontality during working memory correlates with symptoms of thought disorder in patients with schizophrenia (Perlstein, Carter, Noll, & Cohen, 2001), a symptom that is worsened by stress exposure (Docherty, Evans, Sledge, Seibyl, & Krystal, 1994). As described below, stress exposure weakens PFC function, and severe stressors that cause PTSD can cause atrophy of PFC (Kühn & Gallinat, 2013; Meng & iang, Jin, Liu, Zhao, Wang, Gong, 2016). Stress exposure is also often a cause of substance abuse, which in turn further weakens PFC abilities, creating a vicious cycle (Sinha, 2007). Finally, aging causes weakening and removal of dLPFC synapses on spines (Morrison & Baxter, 2012), a process which is worsened by Alzheimer’s Disease (AD), where tau pathology often begins in the medial and ventral areas and moves to dLPFC as the disease progresses, correlating with the rise in dementia (Bussière et al., 2003). If you are interested in learning more about the role of the PFC in mental disorders, the effects of stress on the PFC, or the neurobiology of AD, please access the following educational YouTube videos at the Yale School of Medicine YouTube channel:

The PFC and mental disorders: https://www.youtube.com/watch?v=DEtnoiKGDwI
The PFC and stress: https://www.youtube.com/watch?v=TsQUeNuvlDY
The neurobiology of Alzheimer’s Disease: https://youtu.be/wa3dWrszEFQ

3. The neuronal basis of higher cognition in PFC

Much of our understanding of the cellular neurobiology of dLPFC function is due to the pioneering research of Patricia Goldman-Rakic, whose anatomical and physiological studies uncovered the cellular basis for working memory and abstract thought (Goldman-Rakic, 1995). Her early lesion studies determined the subregion of the dLPFC surrounding the principal sulcus that was essential for visuospatial working memory. Physiological recordings from this area, in concert with neuroanatomical mapping, revealed how dLPFC microcircuits, connected with multiple cortical and subcortical networks, can generate the mental representations needed for thoughtful behavior.
3.1. Cortical networks

Goldman-Rakic’s anatomical tracing studies (Goldman-Rakic, 1987; Selemon & Goldman-Rakic, 1988), along with the elegant work of many other women neuroscientists (e.g. Ann Graybiel, Lynn Selemon, Helen Barbas, Suzanne Haber), revealed the extensive PFC connections with both cortical and subcortical brain regions. These studies have shown that PFC circuits allow the top-down regulation of sensory association cortices e.g. for control of attention (Barbas et al., 2005), and of subcortical structures such as the amygdala for control of emotion (Ghashghaei, Hilgetag, & Barbas, 2007), and with the basal ganglia, such as the projections to the caudate and subthalamic nucleus, e.g. for the inhibition of inappropriate responses (Graybiel, 1991; Haber, 2016; Haynes & Haber, 2013; Selemon & Goldman-Rakic, 1985).

3.2. Delay cell physiology

The early work of Fuster found neurons in the dlPFC that were able to maintain firing across the delay period in a working memory task, e.g. for 18 sec (Fuster & Alexander, 1971). Goldman-Rakic and colleagues (Funahashi, Bruce, & Goldman-Rakic, 1989) built on this original discovery using an oculomotor visuospatial working memory task (Fig. 2A). They found diPFC “Delay cells” can represent a position in visual space across the delay period when there is no sensory stimulation through spatially-tuned, persistent firing for their preferred spatial direction, but not for other, nonpreferred locations (Fig. 2B). These Delay cells can maintain firing even through distractions (e.g. the work of Jacqueline Gottlieb (Suzuki & Gottlieb, 2013)). The ability to generate mental representations is fundamental to abstract reasoning and top down control, e.g. inhibiting inappropriate behavior (Funahashi, Chafee, & Goldman-Rakic, 1993), and regulation of attention (Buschman & Miller, 2007).

3.3. Layer III microcircuits

Goldman-Rakic also discovered the diPFC microcircuits that generate mental representations, concentrated in deep layer III of the diPFC (Fig. 2C), a layer that expands greatly in primates. With Mary Kritzer, she showed that deep layer III contains the extensive horizontal connections needed for extensive recurrent excitation (Kritzer & Goldman-
Rakic, 1995). She posited that the persistent firing across the delay period arose from pyramidal cells with shared “preferred directions”, exciting each other through glutamatergic synapses on spines (Fig. 2C-D). Thus, one needs a large number of strong, excitatory connections to maintain the firing across a long delay period. Conversely, she showed evidence that the spatial tuning was refined by lateral inhibition from GABAergic interneurons, whereby pyramidal cells could inhibit their neighbors with dissimilar preferred directions to enhance “signal to noise” (Fig. 2C). This working model was confirmed by extraordinary in vitro studies by Gonzalez-Burgos, identifying the neurons involved in recurrent excitation and lateral inhibition (González-Burgos, Barrionuevo, & Lewis, 2000; González-Burgos, Krimer, Povysheva, Barrionuevo, & Lewis, 2005).

Fig. 3. cAMP-PKA actions in classic circuits vs. dlPFC. (A) In classic circuits, cAMP-PKA signaling in spines enhances plasticity, e.g. via activation of CREB, and PDE4 inhibition improves learning and memory. (B) cAMP-PKA signaling also has classic actions at pre-synaptic sites, e.g. enhancing glutamate release, as occurs in monkey primary visual cortex V1. C. In layer III dlPFC spines, cAMP-PKA signaling opens nearby K⁺ channels (e.g. KCNQ2) to reduce firing. Thus, PDE4 inhibition can be harmful in these circuits.

4. Unique molecular regulation of layer PFC microcircuits

Layer III dlPFC microcircuits that generate mental representations have unique molecular needs for both neurotransmission and neuromodulation that allow them to sustain long periods of neuronal firing without bottom-up sensory stimulation (Arnsten, 2015; Arnsten, Wang, & Paspalas, 2012). However, these same molecular signaling requirements confer vulnerability to dysfunction when not properly regulated due to genetic and/or environmental insults.

4.1. Unique neurotransmission-

Classic glutamate neurotransmission depends on stimulation of
AMPAR to depolarize the membrane, to eject the magnesium (Mg$^{2+}$) block from the NMDAR pore, and permit NMDAR transmission. Neurons in primary visual cortex (V1) of the rhesus monkey exhibit classic neurotransmission, relying heavily on AMPAR neurotransmission, with only a minor NMDAR component (Yang et al., 2018). The rapid kinetics of AMPAR are appropriate for these circuits, whose function is to rapidly and accurately encode the appearance/disappearance of visual events in the environment. In contrast, the work of Min Wang and colleagues (Wang et al., 2013) have shown that Delay cells in the dlPFC have remarkably little reliance on AMPAR, and instead rely heavily on NMDAR, including NMDAR with NR2B subunits, that close slowly and transfer large amounts of calcium (Ca$^{2+}$) into the spine (Fig. 2D). This experimental finding was predicted by computational models (Wang, 1999). Wang went on to show that the permissive role of AMPAR in classic synapses is instead played by acetylcholine in dlPFC Delay cells. Her work with Yang Yang showed that cholinergic stimulation of nic-α7R, which are localized within the glutamate PSD in layer III dlPFC, are permissive for NMDAR stimulation (Fig. 2D; (Yang et al., 2013)). Nic-α7R are particularly effective in fluxing Ca$^{2+}$ (Fucile, 2004), which may help to sustain membrane depolarization needed for continuous firing. A more recent study by Wang and Galvin showed that muscarinic M1R also permits NMDAR Delay cell firing, possible exciting the synaptic membrane via closure of synaptic KCNQ “m” channels, and/or by increasing internal Ca$^{2+}$ release via Gq-IP3 signaling (Galvin et al., 2020). As acetylcholine is released during waking but not deep sleep, this unusual form of synaptic transmission may explain why dlPFC microcircuits can communicate during waking to allow conscious mental activity, but not during deep sleep when we are unconscious.

4.2. Unique neuromodulation

Layer III dlPFC microcircuits also have different neuromodulatory needs than typical circuits, where neuromodulators further coordinate cognitive state with arousal state, including rapidly disconnecting dlPFC circuits during uncontrollable stress (Arnsten, 2015; Arnsten...
In classic circuits, cAMP signaling strengthens synaptic actions, e.g. by enhancing plasticity in hippocampal circuits, including activation of transcriptional events for very long-term changes in synaptic strength (Fig. 3A; (Abel et al., 1997; MacKenzie & Houssay, 2000)), or by presynaptic facilitation of glutamate release, as occurs in V1 where cAMP signaling increases neuronal firing (Fig. 3B; (Yang et al., 2018)). Thus, in hippocampus, phosphodiesterases such as PDE4A impair memory consolidation, and PDE4 inhibitors improve long-term memory storage (eg (Havekes et al., 2016; Wimmer, Blackwell, & Abel, 2020)).

In contrast to these classic circuits, the working memory circuits of the dlPFC are modulated very differently, consistent with their role in long-term memory storage (eg (Havekes et al., 2016; Wimmer, Blackwell, & Abel, 2020)). Early studies with Jane Taylor showed that activation of cAMP-PKA signaling in rat mPFC impaired working memory performance (Taylor, Birnbaum, Ubriani, & Arnsten, 1999), opposite to its catalytic effects on long-term memory consolidation in hippocampus and amygdala. Ultrastructural analysis of the primate layer III dlPFC by Paspalas and colleagues showed a constellation of cAMP-related proteins near the Ca²⁺-containing spine apparatus (the extension of the smooth endoplasmic reticulum into the spine), near HCN and KCNQ channels, providing structural evidence for feedforward Ca²⁺-cAMP-K⁺ channel signaling in layer III dlPFC spines (Carlton et al., 2014; Galvin et al., 2020; Paspalas, Wang, & Arnsten, 2013; Wang et al., 2007). Thus, rapid changes in camp-Ca²⁺-K⁺ channel signaling can produce dynamic changes in synaptic strength to coordinate environmental events and arousal with cognitive state, a process termed Dynamic Network Connectivity (Arnsten et al., 2012). Under optimal conditions (alert, safe, interested), moderate levels of NE release engage high affinity α2A-AR that inhibit cAMP-K⁺ channel signaling to strengthen synaptic connectivity (Fig. 4A; (Wang et al., 2007)). Similar effects have now been seen with post-synaptic mGluR3 signaling (Liu et al., 2018), a receptor consistently linked to risk of schizophrenia (Saini et al., 2017), and more recently, human intelligence (Zink et al., 2020). Thus, strong layer III recurrent connections are important for strong cognition. However, under conditions of uncontrollable stress, there are high levels of NE and DA release in the PFC (Finlay, Zigmond, & Abercrombie, 1995; Murphy, Arnsten, Goldman-Rakic, & Roth, 1996), which engage α1-AR and D1R to drive Ca²⁺-PKC and cAMP-PKA signaling, rapidly opening nearby K⁺ channels to weaken PFC network connectivity and impair working memory and top-down control (Fig. 4B; (Arnsten et al., 2019; Birnbaum et al., 2004; Gamo et al., 2015). Thus, feedforward Ca²⁺-PKC-cAMP-PKA-K⁺ signaling can very quickly take PFC “offline”.

The amygdala responds to NE in an opposite manner to the mPFC, thus producing a chemical “flip-flop” switch to determine which structure is positioned to orchestrate the brain’s response. The engagement of α2A-AR by moderate levels of NE release under safe conditions weakens the amygdala (DeBock et al., 2003), while high levels of catecholamine release during stress strengthen its emotional responses through α1-AR and β-AR (Debiec & LeDoux, 2006; Ferry, Roozenaal, & McLaugh, 1999). Thus, under nonstress conditions of basal NE release, high affinity α2A-AR strengthen the top-down orchestration by the PFC and weaken the amygdala, while under conditions of stress, high levels of NE release engage low affinity α1-AR and β-AR to weaken the PFC and strengthen the amygdala. This rapid switch may be beneficial under some conditions, e.g. being cut off on the highway, but can be devastating when dealing with a stressor that requires PFC abstract reasoning, e.g. an invisible virus such as COVID-19.

4.3. The effects of chronic stress exposure

With chronic stress exposure, there is architectural loss of mPFC spines and dendrites (Moda-Sava et al., 2019; Radley et al., 2006) that correlates with impaired PFC cognitive functioning (Hains et al., 2009; Hains, Yabe, & Arnsten, 2015; Liston et al., 2006). In contrast, the dendrites of amygdala neurons actually expand with chronic stress exposure (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002), as do the dendrites of the subset of neurons in PFC that excite the amygdala (Shansky, Hamo, Hof, McEwen, & Morrison, 2009), while cortical-cortical neurons in mPFC lose their dendrites (Shansky et al., 2009). In this way, the switch from thoughtful to reactive network connectivity is modulated by cortical changes. Reduced PFC gray matter and weaker functional connectivity with chronic has been seen in humans as well, (Ansell, Rando, Tuit, Guarnaccia, & Sinha, 2012; Liston, McEwen, & Casey, 2009). These changes can be reversed in both rats and humans with extended periods of nonstress, at least in young subjects (Bloss et al., 2011; Liston et al., 2009). The molecular basis for spine loss is particularly interesting, given its relevance to many mental disorders. Research in rats by Avis Hains has shown that inhibition of PKC or PKA signaling can prevent the loss of mPFC dendritic spines and working memory abilities during chronic stress exposure (Hains et al., 2009, 2015), suggesting that the sustained weakening of synaptic connectivity via K⁺ channel opening contributes to spine loss. These data also have immediate relevance to treatment of mental disorders, as agents such as lithium and atypical antipsychotics reduce PKC signaling (Manji & Lenox, 1999), and daily guanfacine administration prevented spine loss and working memory deficits (Hains et al., 2015), which may help to explain its utility in treating stress-related disorders.

Evidence suggests that physiological stressors such as traumatic brain injury (Kobori, Clifton, & Dash, 2006; Kobori, Hu, & Dash, 2011; Kobori, Moore, & Dash, 2015), and hypoxia (Rauser, Sahu, Kumar, & Panjwani, 2013) have similar physiological actions as psychological stress within the PFC. These physical stressors also induce catecholamine release in mPFC, and activate the same intracellular signaling events (e.g. activation of cAMP-PKA signaling), in association with dendritic spine loss and working memory impairment. Thus, multiple starting points can lead to the same phenotype of impaired PFC structure and function.

It is noteworthy that there are marked sex differences in the stress response, where female animals and humans have a greater stress response (Shansky et al., 2004) and humans (Qin et al., 2012), which may help to explain the greater prevalence of PTSD and depression in women, e.g. as shown by Weissman and Mazer (Breslau, Chilcoat, Kessler, Peterson, & Lucia, 1999; Maciejewski, Prigerson, & Mazer, 2001; Weissman et al., 1996). Research in rodents has begun to illuminate the biological factors that underlie these sex differences. Rita Valentino has found that the extended amygdala produces greater activation of the NE locus coeruleus in females (Bangasser & Valentino, 2012), while Rebecca Shansky has shown a range of sex-specific differences in the stress response (Rincón-Cortés, Herman, Lupien, Maguire, & Shansky, 2019), including exaggerated dendritic changes in the PFC of estrogen-exposed females (Shansky et al., 2010). Estrogen also reduces the expression of COMT (Tunbridge, 2010), one of the catalytic enzymes that breaks down catecholamines, which would thus exacerbate the stress response in women. Recent data also indicate that the increased risk of stress-induced depression in females is associated with long non-coding RNAs that regulate CREB signaling in the PFC (Issler et al., 2020). As discussed below, these factors may help to explain why guanfacine is particularly helpful in treating women with stress-related disorders such as substance abuse.

5. Guanfacine’s mechanism of action in PFC

A variety of evidence indicates that guanfacine improves PFC function by mimicking NE’s beneficial actions at post-synaptic α2A-AR
on dlPFC spines, strengthening PFC network connectivity via inhibition of cAMP-PKA-K⁺ signaling (Fig. 5A). This mechanism of action has been clarified by decades of research in animals and humans.

5.1. Actions at post-synaptic receptors of the α2A-AR subtype

Early studies discovered that α2-AR agonists markedly improved working memory performance in young monkeys with experimental catecholamine depletion in the dlPFC, with effects consistent with actions at post-synaptic receptors in the dlPFC (Arnsten & Goldman-Rakic, 1985). α2-AR agonists also improved working memory performance in aged rhesus monkeys with naturally occurring catecholamine depletion (Arnsten et al., 1988; Arnsten & Goldman-Rakic, 1985; Arnsten & Leslie, 1991; Rama, Linnankoski, Tanila, Pertovaara, & Carlson, 1996). It is noteworthy that cAMP signaling becomes dysregulated in the aging PFC due to loss of PDE4 expression (Carlisle et al., 2014), and that regulation through α2A-AR is especially helpful in restoring PFC neuronal firing (Wang et al., 2011). Thus, the use of aged animals likely contributed to the prominence of this initial discovery. Research with visiting Chinese scientist, Jing-Xia Cai, showed that the α2A-AR-selective agonist, guanfacine, was the most potent agent with the least side effects, suggesting that the beneficial effects arose from what is now called the α2A-AR subtype (Arnsten et al., 1988). This hypothesis was later confirmed in mice with genetic mutations to the α2A-AR (Franowicz et al., 2002). Dr. Cai returned to China and taught generations of scientists about the PFC, thus establishing a global collaboration that continues to this day.

5.2. Actions directly in the PFC

At least some of guanfacine’s beneficial actions on cognition result from actions within the PFC. For example, direct infusions of guanfacine into the lateral PFC improved performance of working memory and associative learning tasks in monkeys (Mao, Arnsten, & Li, 1999;
Guanfacine infusions into the aged rat mPFC also improve working memory, and are reversed by local activation of cAMP-PKA signaling (Ramos, Stark, Verduzco, van Dyck, & Arnsten, 2006). Conversely, infusion of the α2-AR antagonist, yohimbine, into the monkey dlPFC impaired working memory (Li & Mei, 1994), impulse control (Ma, Qi, Peng, & Li, 2003) and regulation of locomotor activity (Ma, Arnsten, & Li, 2005), producing an “ADHD-like” phenotype.

5.3. Actions at the cellular level, inhibiting cAMP opening of K⁺ channels on spines

Guanfacine and yohimbine also produce markedly opposing effects on the neuronal firing of dlPFC Delay cells in monkeys performing the ODR working memory task. Local iontophoresis of yohimbine directly onto dlPFC neurons markedly reduced their firing (Li, Mao, Wang, & Mei, 1999; Wang et al., 2007), while iontophoresis of guanfacine enhanced neuronal firing (Fig. 5C; Wang et al., 2007)). It is noteworthy that there could be ceiling effects; i.e. neurons with strong Delay firing under basal conditions showed less effect of guanfacine, which may help to explain why guanfacine has minimal effects in healthy subjects with strong cognition. The physiological data also showed that α2A-AR stimulation enhances Delay cell firing by inhibiting cAMP-PKA opening of HCN channels. The enhancing effects of guanfacine were reversed by co-activation of cAMP-PKA signaling, while the loss of firing with yohimbine was reversed by HCN channel blockade (Wang et al., 2007).

ImmunoeM demonstrated the physical substrate for these physiological interactions, showing that HCN channel co-localized with α2A-AR on dlPFC dendritic spines, e.g. on the spine head (Fig. 5B) and in the spine neck. Later studies showed that KCNQ2 channels, which are opened by PKA signaling, are also localized on spines near the synapse and the spine apparatus (Arnsten et al., 2019). Thus, the constellation of signaling molecules needed to dynamically alter network strength are all concentrated in layer III dlPFC dendritic spines, supporting the model proposed in Fig. 5. Computational models are also consistent with the hypothesis (Duggins, Stewart, Choo, & Eliasmith, 2017; Hassani et al., 2017). Thus, support comes from a range of approaches and perspectives.

5.4. Guanfacine protects PFC neurons from psychological and physiological stress

Evidence from both in vivo and in vitro studies show that guanfacine can protect PFC circuits from stress exposure, including preventing atrophy due to chronic stress exposure. This has been seen for both psychological stressors such as restraint stress in rodents (Hains et al., 2015), as well as for physiological stressors such as reduced oxygen (Kauser et al., 2013). As both psychological and physiological stressors drive feedforward calcium-cAMP-PKA-K⁺ signaling and impair working memory, guanfacine’s ability to inhibit these actions may preserve network connectivity and PFC function (schematically shown in Fig. 6A).

The work of Shari Birnbaum showed that pretreatment with guanfacine protects working memory from acute stress exposure in rats (Birnbaum, Podell, & Arnsten, 2000). Interestingly, guanfacine was more effective than clonidine (Birnbaum et al., 2000), suggesting that these benefits are not primarily due to reducing NE release, as clonidine is more potent than guanfacine in this regard. With chronic stress exposure, dendritic spines are lost from layer II/III pyramidal cells in rat mPFC (Fig. 6B-C; Hains et al., 2009, 2015). The work of Avis Hains showed that daily treatment with guanfacine prevented stress-induced loss of spines (Fig. 6C), and preserved working memory performance (Hains et al., 2015). These data may have relevance to guanfacine’s therapeutic clinical effects in stress-related disorders.

Guanfacine also protects mPFC dendritic spines, neurons and working memory performance from the physiological stress of hypoxia (Kauser et al., 2013). Guanfacine’s prevention of neuronal loss was associated with increased BDNF and decreased expression of caspase 3 (Kauser, Sahu, & Panjwani, 2016), which may be induced by calcium overload of mitochondria with high levels of cAMP-calcium signaling (Lencseova & Krizmanova, 2012). These in vivo data are consistent with in vitro experiments showing that guanfacine enhances the maturation of dendritic spines in PFC neuronal cultures (Ren, Liu, & Li, 2011).

In addition to direct actions on dendritic spines, guanfacine’s protective effects may involve anti-inflammatory actions through α2A-AR on glia (Gyoneva & Traynelis, 2013). As schematized in Fig. 6A, α2A-AR are expressed on activated microglia, where their engagement deactivates microglial activity (Gyoneva & Traynelis, 2013). Thus, guanfacine may reduce microglial phagocytosis of spines. The anti-inflammatory effects of α2A-AR agonists are well-established, and contribute to their widespread use as co-anesthetics in surgery, where they reduce the incidence of emergence delirium (Zhang et al., 2020). These anti-inflammatory effects may also contribute to guanfacine’s protections from hypoxia in animal models (Kauser et al., 2013, 2016), and from encephalitis in humans (Singh-Curry, Malhotra, Farmer, & Husain, 2011). Thus, guanfacine’s engagement of α2A-AR in PFC may have coordinated actions, both strengthening connections on spines and reducing local phagocytic actions by glia.

6. Guanfacine improves PFC-dependent cognition across species

Systemic administration of guanfacine improves PFC cognitive functions in rodents, monkeys and humans, a rare instance where a mechanism can be bridged across species. As the PFC expands greatly in primates (Wise, 2008), these actions are particularly important in these higher species, and may be why α2A-AR agonist pro-cognitive effects were initially discovered in monkeys. For example, rodents do not have a dlPFC or frontal pole, and even the medial aspects of PFC have re-localized actions at the α2A-AR subtype (Franowicz et al., 2002). With chronic stress exposure, dendritic spines are lost from layer II/III pyramidal cells in rat mPFC (Fig. 6B-C; Hains et al., 2009, 2015). Emerging data also indicate primate-unique cell types, and profound differences in transcriptomic signatures between rodents and primates (Krienen et al., 2019). Thus, translational research for cognitive disorders should try to use primates whenever possible.

6.1. Mice

Systemic administration of guanfacine significantly improves working memory performance in wild-type mice performing the delayed alternation task (Fig. 7A; Franowicz et al., 2002). Importantly, guanfacine does not improve mice with a genetic mutation of the α2A-AR where the receptor is no longer able to bind agonists, confirming actions at the α2A-AR subtype (Franowicz et al., 2002).

6.2. Rats

Guanfacine also improves working memory performance on the delayed alternation task in rats. Working memory performance was improved by systemic administration of guanfacine in stressed rats (Birnbaum et al., 2000), and by direct infusion into the mPFC of aged rats, with benefits reversed by the cAMP-PKA activator, Sp-cAMPS (Fig. 7B; Ramos et al., 2006)). Systemic administration of guanfacine also improves attentional performance in a rat model of ADHD, Spon-taneously Hypertensive rats (Kawaura, Karasawa, Chaki, & Hikichi, 2014; Savgolden, 2006), that have altered catecholamines in mPFC (Russell, 2002).

6.3. Monkeys

Systemic administration of guanfacine in rhesus macaques improves a variety of cognitive functions dependent on the PFC. Guanfacine administration to aged monkeys improves working memory (Arnsten et al., 1988) and reduces disruption by distractors (Fig. 7C; Arnsten &
Contant, 1992; O’Neill, Fitten, Siembieda, Ortiz, & Halgren, 2000)), a key function of the dlPFC (Suzuki & Gottlieb, 2013). A SPECT imaging study in monkeys showed that improved working memory performance was accompanied by greater activation of the dlPFC (Avery, Franowicz, Studholme, van Dyck, & Arnsten, 2000). Guanfacine also improves the ability of a young monkey to wait for a larger reward (Kim, Bobeica, Gamo, Arnsten, & Lee, 2012), a measure of impulse control, which in children is often assessed using the “marshmallow test”. As described above, these abilities depend on the right inferior PFC in humans. Guanfacine also improves functions dependent on the ventral PFC. Systemic guanfacine administration in aged monkeys enhances performance of an object reversal task that requires flexible responding to reward (Steere & Arnsten, 1995), a task that Elizabeth Murray’s lab at NIMH has shown depends on more ventral PFC circuits (Rudebeck, Saunders, Lundgren, & Murray, 2017). Either systemic or direct infusion of guanfacine into the ventral PFC improved the performance of monkeys performing a paired associates learning task (Wang, Ji, & Li, 2004; Wang, Tang, et al., 2004).

6.4. Healthy humans

Low, systemic guanfacine doses have been shown to improve performance of PFC cognitive tasks in young, healthy human subjects, with small but significant enhancing effects on working memory, paired associates learning and planning (Jakala, Riekkinen, et al., 1999; Jakala, Sirvio, et al., 1999). Guanfacine has also been shown to activate dlPFC in human functional imaging studies (Clerkin et al., 2009; Swartz, McDonald, Patel, & Torgersen, 2008). However, it can be challenging to improve cognition in healthy subjects (Muller et al., 2005), possibly related to ceiling effects on neuronal firing, as described above. Thus, most studies of guanfacine in human subjects have focused on patients with PFC disorders who would benefit most from its therapeutic actions.

7. Guanfacine treats PFC cognitive disorders in humans

A wide variety of disorders that involve PFC dysfunction are

Fig. 6. Guanfacine counteracts the effects of chronic stress in PFC. (A) A schematic illustration showing how stress increases, while guanfacine inhibits, the feed-forward Ca\(^{2+}\)-cAMP-PKA-K\(^+\) actions that weaken PFC network connectivity, neuronal firing and function. α2A-AR are also expressed on activated microglia, where α2A-AR stimulation deactivates microglia and thus has anti-inflammatory actions. (B) Example of a layer II/III mPFC pyramidal cell distal dendrite from a control vs. chronic stressed rat, showing the reduced spine density in the stressed PFC. Scale bar indicates 25 μm. Adapted from (Hains et al., 2009) with permission. (C) Chronic stress (Str) exposure reduces PFC spine density in vehicle-treated rats, but not in those receiving daily guanfacine treatment. Con = control; Str = chronic stress; Veh = vehicle; Gfc = guanfacine. Adapted from (Hains et al., 2015) with permission.
Guanfacine is currently approved for treating ADHD in the USA, Canada, Europe and Japan, the latter in adults (Iwanami, Saito, Fujiwara, Okutsu, & Ichikawa, 2020) as well as children. Historically, the α2-AR agonist, clonidine, was tested in ADHD, where it was thought that the powerful sedative effects of this nonselective agonist were helping to make patients less active (Hunt, Capper, & O'Connell, 1990). It is now known that the therapeutic effects are independent of, and indeed in spite of, these sedative side effects, with the development of guanfacine’s use in ADHD based on understanding its prefrontal enhancement in monkeys (Arnsten & Contant, 1992; Arnsten, Steere, & Hunt, 1996), and the recognition that ADHD is a PFC disorder.

Research by Judy Rapoport, Chief of the Child Psychiatry Branch at NIMH, working with Philip Shaw, demonstrated that ADHD is associated with altered development of the right inferior PFC (Shaw et al., 2009). Functional imaging studies had also highlighted dysfunction of this PFC region in ADHD e.g. studies by Casey (Cassey et al., 1997) and by Rubia (Rubia, 2011) showed underactivity and/or reduced functional connectivity of the right inferior PFC as children with ADHD performed tasks requiring top-down control of behavior and attention.

Guanfacine has been shown to relieve cardinal symptoms of ADHD in children and adolescents, including improved impulse control and better regulation of attention, in early, small trials (Hunt, Arnsten, & Asbell, 1995; Scailth et al., 2001), and later in multiple, large Phase III studies in the USA, Europe and Japan (Biederman et al., 2008; Hervas et al., 2014; Sallee, McCough, Wigal, Donahue, & Lyn, Biederman, Group., 2009). An early, small study of adults with ADHD showed that guanfacine could improve performance of the Stroop interference task, a task that challenges the inhibitory functions of the PFC (Taylor & Russo, 2001), and a large Phase 3 Trial showed success in adults (Iwanami et al., 2020). Guanfacine is often used as adjunct to stimulant medication, as the combination optimizes the therapeutic actions while diminishing side effects (McCracken et al., 2016). It is also used to treat patients with co-morbid ADHD and Tourette’s syndrome, where stimulant medication can aggravate the appearance of tics (Scailth et al., 2001).

### 7.2. Use in other PFC disorders

“Off label” use of guanfacine in additional PFC disorders is widespread, but often with only limited empirical demonstrations of efficacy, as there is little corporate interest in funding large trials now that Intuniv is generically available. Interestingly, many of the disorders that are helped by guanfacine treatment have psychological or physiological stress as an etiologic factor. The following is a brief review.

#### 7.2.1. Oppositional Defiant/Conduct Disorders

These disorders of inappropriate aggression are often found in...
children and adolescents who have suffered abuse. Functional imaging studies show that children with conduct disorder exhibit underactivity of more ventral PFC regions, consistent with these areas being important centers for the regulation of emotion (Rubia, 2011). Guanfacine is helpful in treating oppositional symptoms (Connor et al., 2010; Findling, McBurnett, White, & Youcha, 2014), consistent with it improving ventral PFC functions in monkeys.

### 7.2.2. Autism-spectrum disorders

The diagnosis of autism now represents a range of disorders of varying severity. Evidence from case reports and clinical trials indicate that guanfacine can be helpful in boosting top down control to reduce oppositional, self-injurious and repetitive behaviors in children on the autism spectrum, including children with severe (e.g. nonverbal) cognitive impairment (Coleman, Adams, Anderson, & Frye, 2019; Propper, 2018). A placebo-controlled study showed that guanfacine could be helpful for reducing oppositional and repetitive behaviors, although social behaviors are usually not improved (Politte et al., 2018).

### 7.2.3. Psychological trauma and abuse

Guanfacine has been especially useful in treating traumatized or abused children, who often have PFC dysfunction reflected in symptoms of impaired self-control. An open label study found that guanfacine reduced symptoms of re-experiencing the trauma, avoidance of others, and hyperarousal (Arnsten, Raskind, Taylor, & Connor, 2015; Connor, Grasso, Slivinsky, Pearson, & Banga, 2013). Guanfacine’s use in adults with PTSD has been mixed. Although guanfacine has not shown efficacy in a placebo-controlled trial of veterans with longstanding PTSD (Davis et al., 2008), it may be effective in patients in earlier stages of the disorder with less severe PFC atrophy. Positive effects with guanfacine are in concert with animal studies showing that it can protect PFC connections from psychological stress exposure.

### 7.2.4. Traumatic brain injury, stroke, and infection

Studies in rats have shown that TBI to the posterior cortex increases PKA signaling in the PFC (Kobori et al., 2015), as well as reducing PFC spine density and impairing working memory (Zhao et al., 2018). Thus, guanfacine treatment may be helpful to normalize intracellular signaling and promote synaptic strength. Studies of humans with TBI support this view (McAllister, Flashman, Sparling, & Saykin, 2004; McAllister et al., 2011), including evidence of improved working memory and enhanced fMRI BOLD activation of PFC (McAllister et al., 2011). Guanfacine has also been shown to enhance cognition and attention in patients with impairments due to encephalitis (Singh-Curry et al., 2011), or strokes, as long as the PFC itself remains sufficiently intact to provide a therapeutic target for drug actions (Malhotra, Parton, Greenwood, & Hasain, 2006).

### 7.2.5. Aging

Given guanfacine’s benefit in aging monkeys, one would also predict it may be helpful in aged human subjects who, like aged monkeys, have naturally-occurring PFC cognitive deficits. A recent trial of low dose guanfacine vs. placebo in elderly humans (> 75 years) did not show significant improvement (Barcelos et al., 2018). As dendritic spines appear to lose their resilience in very old brains (Bloss et al., 2011), it may be that guanfacine treatment would need to begin at a younger age (e.g. late middle age) when the therapeutic substrate remains.

### 7.2.6. Substance abuse

A large number of studies indicate that guanfacine may be efficacious in treating substance abuse disorders, by strengthening PFC top-down control to resist cravings and impulsive consumption, and reducing the stress responses that often underlie drug intake. A group of women scientists at the Yale Stress Center (Rajita Sinha, with colleagues Sherry McKee, Helen Fox and Verica Milivojvic) have shown that guanfacine can reduce stress-induced PFC dysfunction and decrease substance abuse in subjects struggling with addictions to nicotine or cocaine. McKee et al. (2015) found that guanfacine reduced stress-induced smoking in the lab, and helped people quit smoking at

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**Fig. 8.** Mental disorders where treatment with guanfacine is either approved for treatment, is used off-label, or is under experimental investigation.
home. The extended release formulation was more effective than an equivalent dose of immediate release guanfacine (Verplaetse et al., 2019). Fox et al. showed that guanfacine could improve PFC functions (inhibitory control, Stroop interference) and reduce drug cravings in cocaine addicts (Fox, Sofuoglu, & Sinha, 2015; Fox et al., 2012). Guanfacine was particularly helpful in women (Milivojevic, Fox, Jayaram-Lindstrom, Hermes, & Sinha, 2017), consistent with the greater stress response in females (see above), and stress as a leading cause of substance abuse in women (Mckee, Maciejewski, Falba, & Mazure, 2003).

Recent studies suggest that guanfacine may also be useful in addiction to cannabis. Guanfacine treatment was shown to reduce the cognitive impairment induced by THC (Mathai et al., 2018), and new data suggest that it may be helpful in treating cannabis addiction (Dakwar et al., 2020), although there are mixed results regarding its effects on withdrawal symptoms (Haney et al., 2019; Holst et al., 2019). As cannabis use in adolescents is a risk factor for subsequent schizophrenia (Hasan et al., 2020), guanfacine treatment may also be protective in the prodrome to mental illness in high risk adolescents who are cannabis users.

7.2.7. Schizophrenia spectrum disorders

An early study suggested that guanfacine may be helpful in patients with schizophrenia who are still in early, but not later stages of the disease (Friedman et al., 2001). Studies of patients with schizophrenia are complicated by the fact that many atypical antipsychotics have complex pharmacological profiles including α2A-AR-blocking properties. However, a case report by Bardoloi (2020) suggests that guanfacine may be especially helpful in young adults with schizophrenia and a childhood history of abuse, and that it can improve insight, and thus have global benefits (Bardoloi, 2018).

Patients diagnosed with schizotypal personality disorder have cognitive deficits similar to patients with schizophrenia, including thought disorder symptoms such as ideas of reference, odd beliefs and magical thinking, and thus can be helpful in observing the cognitive effects of potential treatments without drug interactions. Recent studies indicate that symptoms of schizotypy have a linear relationship to severe childhood trauma, and are associated with worse PFC cognitive functioning (Velikonja et al., 2019). The work of Margaret McClure has shown that guanfacine can improve working memory (McClure, Barch, Romero, Harvey, & Siever, 2007) and significantly enhance the beneficial effects of cognitive remediation (McClure et al., 2019) in schizotypal patients. The latter is particularly interesting, as a combined approach of boosting PFC physiology while engaged in cognitive training may optimize success.

7.3. Future potential uses

The research described above suggests that guanfacine may be particularly helpful during the schizophrenia prodrome, protecting PFC circuits from the psychological stressors and substance abuse (e.g. cannabis) that are such large risk factors for conversion to schizophrenia. As the descent into schizophrenic illness is accompanied by accelerated gray matter loss and signs of inflammation (Mongan, Ramesar, Föcking, Cannon, & Cotter, 2019), guanfacine’s anti-inflammatory actions may be particularly helpful. The anti-inflammatory effects of guanfacine may also be useful for treating the long-term neural consequences of COVID-19, where a subset of patients report sustained mental problems (Heneka, Golenbock, Latz, Morgan, & Brown, 2020; Pinna et al., 2020).

In summary, guanfacine strengthens connectivity and enhances the firing of the newly evolved PFC circuits that underlie higher cognitive function. As these circuits expand in humans, and are subject to dysfunction in multiple mental disorders, guanfacine may be helpful in a range of cognitive disorders that benefit from improving PFC function. However, some substrate for guanfacine’s beneficial actions must remain, i.e. the presence of α2A-AR—expressing PFC dentritic spines. Thus, treatment early in the course of the disorder may be essential for beneficial guanfacine actions.

References

Abel, T., Nguyen, P. V., Barad, M., Deuel, T. A., Kandel, E. R., & Bourjouis, R. (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell, 88, 615–626.

Amell, E. B., Rando, K., Tait, K., Guarnaccia, J., & Sinha, K. (2012). Cumulative adversity and smaller gray matter volume in medial prefrontal, anterior cingulate, and insula regions. Biological Psychiatry, 72, 57–64.

Arnsten, A. F. (2015). Stress weakens prefrontal networks: Molecular insults to higher cognition. Nature Neuroscience, 18, 1756–1765.

Arnsten, A. F. T., Cai, J. X., & Goldman-Rakic, P. S. (1988). The α2-agonist adenosine guanfacine improves memory in aged monkeys without sedative or hypotensive side effects. Journal of Neuroscience, 8, 4287–4298.

Arnsten, A. F. T., & Contant, T. A. (1992). Alpha-2 adrenergic agonists decrease tractability in aged monkeys performing a delayed response task. Psychopharmacology (Berl), 108, 159–169.

Arnsten, A. F. T., & Goldman-Rakic, P. S. (1985). Alpha-2 adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. Science, 230, 1273–1276.

Arnsten, A. F. T., Jin, L. E., Gamo, N. J., Ramos, B., Papasalas, C. D., Morozov, Y., ... El Hassar, L. (2019). Role of NCGN channelopathies in stress-induced deficit of working memory. Neurako, 11, Article 100187.

Arnsten, A. F. T., & Leslie, F. M. (1991). Behavioral and receptor binding analysis of the α2-adrenergic agonist, UK-14304 (5-bromo-6-[2-imidazoline-2-y1] quinoline): Evidence for cognitive enhancement at an α2-adrenergic subtype. Biological Pharmacology, 30, 1279–1289.

Arnsten, A. F. T., Raskind, M., Taylor, F. B., & Connor, D. F. (2015). The effects of stress exposure on prefrontal cortex: Translating basic research into successful treatments for Post-Traumatic Stress Disorder. Neurobiology of Stress, 1, 89–99.

Arnsten, A. F. T., Steere, J. C., & Hunt, R. D. (1996). The contribution of α2- nora- drnergic mechanisms to prefrontal cortical cognitive function: Potential significance to Attention Deficit Hyperactivity Disorder. Archives of General Psychiatry, 53, 448–455.

Arnsten, A. F. T., Wang, M., & Papasalas, C. D. (2012). Neuromodulation of thought: Flexibilities and vulnerabilities in prefrontal cortical network synapses. Neuron, 76(1), 223–239.

Aron, A. R., Robbins, T. W., & Poldrack, R. A. (2004). Inhibition and the right inferior frontal cortex. Trends in Cognitive Sciences, 8, 170–177.

Avery, R. A., Franovick, J. S., Studholme, C., van Dyck, C. H., & Arnsten, A. F. T. (2000). The alpha-2-adrenoceptor agonist, guanfacine, increases regional cerebral blood flow in dorsolateral prefrontal cortex of monkeys performing a spatial working memory task. Neuropsychopharmacology, 23, 240–249.

Bangasser, D. A., & Valentino, R. J. (2012). Sex differences in molecular and cellular substrates of stress. Cellular and Molecular Neurobiology, 32, 709–723.

Barb, H., Medalla, M., Alade, O., Suski, J., Zikopoulos, B., & Lera, P. (2005). Relationship of prefrontal connections to inhibitory systems in superior temporal areas in the rhesus monkey. Cerebral Cortex, 15(9), 1356–1370.

Barcelos, N. M., Van Ness, P. H., Wagner, A. F., MacAvoy, M. G., Mecca, A. P., Anderson, G. M., ... van Dyck, C. H. (2018). Guanfacine treatment for prefrontal cortical dysfunction in older participants: A randomized clinical trial. Neurobiology of Aging, 70, 117–124.

Bardoloi, P. (2018). Treating executive dysfunction to improve insight in psychiatric disorders. Archives Neurology Neu Disorders, 1, 27–30.

Bardoloi, P. S. (2020). Enhancement of Function of the Prefrontal Cortex to Improve Symptoms of Schizophrenia. Acta Scientfica Neurology 3, 19-23. doi: https://actascientific.com/ASNE/pdf/ASNE-03-0185.pdf.

Bardey, J., Melsom, B. D., Patel, A., McBurnett, K., Konow, J., Lyne, A., ... Group, S. S. (2008). A randomized, double-blind, placebo-controlled study of guanfacine extended release in children and adolescents with attention-deficit/hyperactivity disorder. Pediatrics, 121, e73–84.

Birnbaum, S. B., Yuan, P., Wang, M., Vijayraghavan, S., Bloom, A., Davis, D., ... Arnsten, A. F. T. (2004). Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. Science, 306, 882–884.

Birnbaum, S. G., Podell, D. M., & Arnsten, A. F. T. (2000). Noadrenergic alpha-2 receptor agonists reverse working memory deficits induced by the anxiogenic drug, FG7142, in rats. Pharmacology, Biochemistry and Behavior, 67, 397–403.

Bliss, E. B., Janssen, W. G., Ohm, D. T., Yuki, F. J., Wadsworth, S., Saudri, K. M., ... Morrison, J. H. (2011). Evidence for reduced experience-dependent dendritic spine plasticity in the aging prefrontal cortex. Journal of Neuroscience, 31, 7831–7839.

Blumberg, H. P., Leung, H. C., Skudlarski, P., Lasacic, C. M., Fredericks, A. C., Harris, B. C., ... Peterson, B. S. (2013). A functional magnetic resonance imaging study of bipolar disorder: State- and trait-related dysfunction in ventral prefrontal cortices. Archives of General Psychiatry, 60, 601–609.

Blumberg, H. P., Stern, E., Ricketts, S., Martinez, D., de Asis, J., White, T., ... Silbersweig, D. A. (1999). Rostral and orbital prefrontal cortical dysfunction in the manic state of bipolar disorder. American Journal of Psychiatry, 156(12), 1986–1988.

Breslau, N., Chilcoat, H. D., Kessler, R. C., Peterson, E. L., & Lucia, V. C. (1999). Vulnerability to assaultive violence: Further specification of the sex difference in post-traumatic stress disorder. Psychological Medicine, 29, 813–821.
Buschman, T. J., & Miller, E. K. (2007). Top-down versus bottom-up control of attention in the prefrontal and posterior parietal cortices. Science, 315, 1860–1862.

Bustí, T., Giannakopoulos, P., Borsus, C., Perl, D. P., Morrison, J. H., & Hof, P. R. (2005). Progressive degeneration of synaptophysin-containing neurelament protein-enriched pyramidal neurons predicts cognitive unresponsiveness in Alzheimer’s disease. Stereologic analysis of prefrontal cortex area 9. The Journal of Comparative Neurology, 463, 301–302.

Cai, J. X., Ma, Y., Xu, L., & Hu, X. (2019). Receptor impairments spatial working memory performance in monkeys: Reversal by the alpha 2-adrenergic agonist clonidine. Brain Research, 1614, 191–196.

Cannon, T. D., Chung, Y., He, G., Sun, D., Jacobson, A., van Erp, T. G., & Consortium, a. t. N. A. P. L. S. (2014). Progressive reduction in cortical thickness as psychosis develops: A multivariate longitudinal neuroimaging study of youth at elevated clinical risk. Biological Psychiatry 77, 147–157.

Carle, B. C., Nairn, A. C., Wang, M., Yang, Y., Jin, L. E., Simen, A. A., ... Paspals, C. D. (2016). CAMP-RIA phosphorylation of tau confers resistance for degeneration in aging association cortex. Proceedings of the National Academy of Sciences of the U.S.A. 110, 5036–5041.

Casey, B. J., Castellanos, F. X., Giedd, J. N., Marsh, W. L., Hamburger, S. D., Schubert, A. B., Rapoport, J. L. (1997). Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. Journal of the American Academy of Child and Adolescent Psychiatry, 36, 374–383.

Clarkin, S. M., Schulz, K. R., Palperin, J. M., Newcorn, J. H., Ivanov, I., Tang, C. Y., & Fan, J. (2009). Guanfacine potentiates the activation of prefrontal cortex evoked by warning signals. Biological Psychiatry, 66, 307–312.

Coleman, D. M., Adams, J. B., Anderson, A. L., & Frye, R. E. (2019). Rating of the effec-
tiveness of 26 psychiatric and seizure medications for autism spectrum disorder: Results of a national survey. Journal of Child and Adolescent Psychopharmacology Feb ahead of print.

Connor, D. F., Findling, R. L., Collins, S. H., Sallee, F., López, F. A., Lyne, A., & Tremblay, G. (2010). Effects of guanfacine extended release on oppositional symptoms in children aged 6–12 years with attention-deficit/hyperactivity disorder and oppositional symptoms: A randomized, double-blind, placebo-controlled trial. CNS Drugs, 24, 755–768.

Connor, D. F., Grasso, D. J., Slivinsky, M. D., Pearson, G. S., & Banga, A. (2013). An open-
labeled study of guanfacine extended release for trau-ma stress-related symptoms in children and adolescents. Journal of Child and Adolescent Psychopharmacology, 23, 244–251.

Dekwar, E., Mahony, A., Choi, C. J., Pavlica, M., Brooks, D., Martini, J. P., & Levin, F. (2020). Guanfacine extended-release for cannabis use disorder: A pilot feasibility trial. American Journal of Drug and Alcohol Abuse, 46, 44–48.

Davis, L. W., Ward, C., Rasmuason, A., Newell, J. M., Frazier, E., & Southwick, S. M. (2008). A placebo-controlled trial of guanfacine for the treatment of posttraumatic stress disorder in veterans. Psychopharmacology Bulletin, 41(1), 8–18.

Debiec, J., & Le Doux, J. E. (2006). Noradrenergic signaling in the amygdala contributes to the reconsolidation of fear memory: Treatment implications for PTSD. Annals of the New York Academy of Sciences, 1071, 521–524.

DeBock, F., Kurz, J., Azad, S. C., Parsons, C. G., Hafplmeier, G., Zieglgansberger, W., & Rammes, G. (2003). Alpha2-adrenoreceptor activation inhibits LTP and LTD in the basolateral amygdala: Involvement of Gi/o-mediated modulation of Ca2++ channels and involvement of K+ channels in LTD. European Journal of Neuroscience, 17, 1411–1424.

Docherty, N. M., Evans, I. M., Slade, W. H., Seibyl, J. P., & Krystal, J. H. (1994). Affective reactivity of language in schizophrenia. The Journal of Nervous and Mental Disease, 182, 123–131.

Duggins, P., Stewart, T. C., Choo, X., & Eliaslam, C. (2017). The effects of guanfacine and phenylephrine on a spiking neuron model of working memory. Topics in Cognitive Neuroscience, 28, 15291–15302.

Huber, S. N. (2016). Corticostriatal circuitry. Dialogues Clinical Neuroscience, 18, 7–21.

Hains, A. B., Vu, M. A., Maciejewski, P. K., van Dyck, C. H., Gottron, M., & Arnsten, A. F. T. (2009). Inhibition of protein kinase C signaling protects prefrontal cortex dendritic spines and cognition from the effects of chronic stress. Proceedings of the National Academy of Sciences of the USA, 106(42), 17957–17962.

Hains, A. B., Yabe, Y., & Arnsten, A. F. T. (2015). Chronic stimulation of alpha2A-adrenoreceptors with guanfacine protects rodent prefrontal cortex dendritic spines and cognition from the effects of chronic stress. Neurobiology of Stress, 2, 1–12.

Hayes, M., Cooper, Z. D., Bedi, G., Herrmann, E., Comer, S. D., Reed, S. C., Levin, F. R. (2019). Guanfacine decreases symptoms of cannabis withdrawal in daily cannabis smokers. Addiction Biology, 24, 707–726.

Hazan, A., van Keller, R., Friend, G. M., Hall, W., Schneider, M., Koethe, D., ... Hoch, E. (2020). Cannabis use and psychosis: A review of reviews. European Archives of Psychiatry and Clinical Neuroscience, 270, 403–412.

Hassani, S. A., Oemisch, M., Balcarras, M., Westendorf, S., Ardil, S., van der Meer, M. A., & Welschendorf, T. (2017). A computational psychiatry approach identifies how alpha- 2A noradrenergic agonist guanfacine affects feature-based reinforcement learning in the macaque. Scientific Reports, 7, 40606.

Havekes, R., Park, A. J., Tolentino, R. E., Bruneinberg, V. M., Tudor, J. C., Lee, Y., ... Abel, T. (2004). Compartmentalized PDE4A5 Signaling Impairs Hippocampal Synchrony Plasticity and Long-Term Memory. Journal of Neuroscience, 36, 8936–8946.

Haynes, W. I., & Haber, S. N. (2013). The organization of prefrontal-subthalamic inputs in primates provides an anatomical substrate for both functional specificity and integration: Implications for Basal Ganglia models and deep brain stimulation. Journal of Neuroscience, 33, 4804–4816.

Heneka, M. T., Golenbock, D. L., Inagaki, M., Saito, K., Fujiwara, M., Okutsu, D., & Ichikawa, H. (2020). Efficacy and safety of guanfacine and modafinil in attention-deficit/hyperactivity disorder in adults. Current Drug Therapy, 19, 19m12979.

Hoffman, J. M., Zigmond, M. J., & Abercrombie, E. D. (1995). Increased dopamine and norepinephrine release in prefrontal cortex following transcranial magnetic stimulation. Experimental Brain Research, 104, 403–412.

Iwanami, A., Saito, K., Fujiwara, M., Okutsu, D., & Ichikawa, H. (2020). Efficacy and safety of guanfacine and modafinil in attention-deficit/hyperactivity disorder in adults. Current Drug Therapy, 19, 19m12979.

Holst, M., Mathis, D. S., Patel, M. M., Rodgman, C., Keller, J., Hussain, M. Z., ... Verrico, C. D. (2019). Use of Guanfacine for Cannabis Use Disorder and Related Problems. American Journal of Addictions, 28, 619–628.

Hunt, R. D., Arnsten, A. F. T., & Asbell, M. D. (1995). An open trial of guanfacine in the treatment of attention deficit hyperactivity disorder. Journal of the American Academy of Child and Adolescent Psychiatry, 34, 50–54.

Hunt, R. D., Capper, L., & O’Connor, P. (1999). Clonidine in child and adolescent psy-
chiatry. Journal of Child and Adolescent Psychiatry, 1, 87–102.

Istler, O., van der Zee, Y. Y., Ramakrishnan, A., Wang, J., Tan, C., Loh, Y. E., ... Nestler, E. J. (2020). Sex-Specific Role for the Long Non-coding RNA LINCO0473 in Depression. Cell, 106, 912–926.

Iwaynami, A., Saito, K., Fujisawa, M., Okutsu, D., & Ichikawa, H. (2020). Efficacy and safety of guanfacine and modafinil in attention-deficit/hyperactivity disorder in adults: A randomized, double-blind, placebo-controlled study. Journal of Clinical Psychiatry, 81, 10m12979.

Jakab, P., Riekkien, M., Sirvio, J., Koivisto, E., Kejonen, K., Vanhanen, M., & Riekkinen, P. J. (1999). Guanfacine, but not clonidine, improves planning and working memory performance in humans. Neuropsychopharmacology, 20, 460–470.
Liston, C., McEwen, B. S., & Casey, B. J. (2009). Psychosocial stress reversibly disrupts memory. Proceedings of the National Academy of Sciences of the USA, 106, 259–264.

McEwen, B. S. (2006). Stress-induced alterations in prefrontal cortical dendritic spine morphology predict selective impairments in perceptual attention shifting. Neuron, 50, 895–903.

Morgan, D., Ramesar, M., Ficking, M., Cannon, M., & Cotter, D. (2019). Role of inflammation in the pathogenesis of schizophrenia: A review of the evidence, proposed mechanisms and implications for treatment. Early Interv Psychiatry, 31, (epub ahead of print).

Morrison, J. H., & Baxter, M. G. (2012). The age-related cortical synapse: Hallmarks and implications for cognitive decline. Nature Reviews Neuroscience, 13, 240–250.

Opler, L. A., Opler, M. G., & Arnsten, A. T. (2016). Ameliorating treatment-refractory depression with intranasal ketamine: Potential NMDA receptor actions in the pain circuitry representing mental anguish. CNS Spectrums, 21, 12–22.

Perlstein, W. M., Carter, C. S., O’Reilly, R. E., Noll, D. C., & Cohen, J. D. (2001). Relation of prefrontal cortex activity to executive function during go/no-go performance. The Journal of Neuroscience, 21, 206–219.
Desirability versus Availability of Outcomes. *Neuron*, 95, 1208–1220.

Russell, V. A. (2002). Hypodopaminergic and hypernoradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder—the spontaneously hypertensive rat. *Behavioural Brain Research*, 130, 191–196.

Sagvolden, T. (2006). The alpha-2A adrenoceptor agonist guanfacine improves sustained attention and reduces overactivity and impulsiveness in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioural Brain Function*, 2, 41.

Saini, S. M., Mancuso, S. G., Mostad, M. S., Liu, C., Pantelis, C., Everill, I. P., & Bouman, C. A. (2017). Meta-analysis supports GWAS-implicated link between GRM3 and schizophrenia risk. *Translationl Psychiatry*, 7, Article e1196.

Sallee, F. R., McGough, J. J., Wigal, T., Donahue, J., Lyn, E. A., Biederman, J., & GROUP, S. S. (2009). Guanfacine Extended Release in Children and Adolescents With Attention-Deficit/Hyperactivity Disorder: A Placebo-Controlled Trial. *Journal of the American Academy of Child and Adolescent Psychiatry*, 48, 155-165.

Sculli, L., Chappell, P. B., Kim, Y. S., Schulte, R. T., Katsovich, L., Shepherd, E., ... Leckman, J. F. (2001). A placebo-controlled study of guanfacine in the treatment of children with tic disorders and attention deficit hyperactivity disorder. *American Journal of Psychiatry*, 158, 1067–1074.

Selemon, L. D., & Goldman-Rakic, P. S. (1985). Longitudinal topography and inter-digitation of corticostriatal projections in the rhesus monkey. *Journal of Neuroscience*, 5, 776–794.

Selemon, L. D., & Goldman-Rakic, P. S. (1988). Common cortical and subcortical targets of the dorsolateral prefrontal and posterior parietal cortices in the rhesus monkey: Evidence for a distributed neural network subserving spatially guided behavior. *Journal of Neuroscience*, 8(11), 4049–4068.

Shamay-Tsoory, S. G., & Aharon-Peretz, J. (2007). Dissociable prefrontal networks for cognitive and affective theory of mind: A lesion study. *Neuropsychologia*, 45, 3054–3067.

Shansky, R. M., Glavis-Bloom, C., Lerman, D., McRae, P., Benson, C., Miller, K., ... Arnsten, A. F. T. (2004). Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. *Molecular Psychiatry*, 9, 531–538.

Shansky, R. M., Hamo, C., Hof, P. R., Lou, W., McEwen, B. S., & Morrison, J. H. (2010). Estrogen promotes stress sensitivity in a prefrontal cortex-amgydala pathway. *Cerebral Cortex*, 20, 2560–2567.

Shansky, R. M., Hamo, C., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2009). Stress-induced dendritic remodeling in the prefrontal cortex is circuit specific. *Cerebral Cortex*, 106(42), 17957–17962.

Shaw, P., Eckstrand, K., Sharp, W., Blumenshal, J., Jerch, J. P., Greenstein, D., ... Rapoport, J. L. (2007). Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proceedings of the National Academy of Sciences of the USA*, 104, 19649–19654.

Shaw, P., Lalonde, F. M., Lepage, C., Rabin, C., Eckstrand, K., Sharp, W., ... Peters-Rodriguez, M. M. (2019). Severe childhood trauma and clinical and neuro-cognitive features in schizophrenia spectrum disorder. *Acta Psychiatrica Scand.*, 140, 50–64.

Verplante, T. L., Roberts, W., Moore, K. E., Pelteir, M. R., Oberleitner, L. M., & McKee, S. A. (2019). Pharmacokinetics and pharmacodynamics of immediate-release versus extended-release guanfacine in adult daily smokers. *Journal of Clinical Psychopharmacology*, 39, 124–128.

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience*, 22(15), 6810–6818.

Wallis, C. U., Cardinal, R. N., Alexander, L., Roberts, A. C., & Clarke, H. F. (2017). Opposing roles of primate areas 25 and 32 and their putative rodent homologs in the regulation of negative emotion. *Proceedings of the National Academy of Sciences of the United States of America*, 114, E4075–E4084.

Wang, M., Gamo, N. J., Yang, Y., Jin, L. E., Wang, X. J., Laubach, M., ... Arnsten, A. F. T. (2011). Neuronal basis of age-related working memory decline. *Nature*, 476, 210–213. https://doi.org/10.1038/nature10483.

Wang, M., J., J. Z., & Li, B. M. (2004). The alpha(2A)-adrenergic agonist guanfacine improves visuosomotor associative learning in monkeys. *Neuropsychopharmacology*, 29, 96–92.

Wang, M., Ramos, B., Paspalas, C., Shu, Y., Simen, A., Duque, A., ... Arnsten, A. F. T. (2007). Alpha2A-adrenoceptor stimulation strengthens working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. *Cell*, 129, 397–410.

Wang, M., Tang, Z. X., & Li, B. M. (2004). Enhanced visuosomotor associative learning following stimulation of alpha 2A-adrenoceptors in the ventral prefrontal cortex in monkeys. *Brain Research*, 1024, 176–182.

Wang, M., Yang, Y., Wang, C. J., Gamo, N. J., Jin, L. E., Mazer, J. A., ... Arnsten, A. F. T. (2013). NMDA receptors subserve working memory persistent neuronal firing in dorsolateral prefrontal cortex. *Neuron*, 77(4), 736–749.

Wang, X. J. (1999). Synaptic basis of cortical persistent activity: The importance of NMDA receptors to working memory. *Journal of Neuroscience*, 19, 9587–9603.

Weisman, M. M., Bland, R. C., Canino, G. J., Faravelli, C., Greenwald, S., Hwu, H. G., ... Yeh, E. K. (1996). Cross-national epidemiology of major depression and bipolar disorder. *JAMA*, 276, 293–299.

Wimmer, M. E., Blackwell, J. M., & Abel, T. (2005). Rollipram treatment during consolidation ameliorates long-term object location memory in aged male mice. *Neurobiology of Learning and Memory*, 87, 107–116.

Wise, S. P. (2008). Forward frontal fields: Phylogeny and fundamental function. *Trends in Neurosciences*, 31, 599–608.

Yang, S. T., Wang, M., Paspalas, C. P., Crimins, J. L., Altman, M. T., Mazer, J. A., ... Arnsten, A. F. T. (2018). Core differences in synaptic signaling between primary visual and dorsolateral prefrontal cortex. *Journal of Neuroscience*, 38(28), 5999–6008.

Yang, S. T., Wang, M., Paspalas, C. D., Jin, L. E., Picciotto, M. R., Arnsten, A. F. T., & Wang, M. (2013). Nicotinic α7 receptors enhance NMDA cognitive circuits in dorsolateral prefrontal cortex. *Proceedings of the National Academy of Sciences of the USA*, 110(29), 12078–12083.

Zhang, W., Wang, T. J., Wang, G., Yang, M., Zhou, Y., & Yuan, Y. (2020). Effects of dezmedetomidine on postoperative delirium and expression of IL-1β, IL-6, and TNF-α in elderly patients after hip fracture operation. *Frontiers in Pharmacology*, 11, 678.

Zhao, J., Huyun, J., Hylin, M. J., O'Malley, J. J., Perez, A., Moore, A. N., & Dash, P. F. (2018). Mild traumatic brain injury reduces spine density of projection neurons in the medial prefrontal cortex and impairs extinction of contextual fear memory. *Journal of Neurotrauma*, 35, 149–156.

Zink, C., Barker, P., Sawa, A., Weinberger, D., Wang, A., Quillian, H., ... Bigos, K. (2020). Masenine mutation in FOLHI1 is associated with decreased NAAG levels and impaired working memory circuitry and cognition. *American Journal of Psychiatry in press.*