International Journal of
Molecular Sciences

MDPI

Review

Molecular and Regulatory Mechanisms of Desensitization and Resensitization of $GABA_A$ Receptors with a Special Reference to Propofol/Barbiturate

Youngnam Kang [1,2,*], Mitsuru Saito [3] and Hiroki Toyoda [4,*]

1 Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Osaka 565-0871, Japan
2 Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul 110-749, Korea
3 Department of Oral Physiology, Graduate School of Medical and Dental Sciences, Kagoshima University, Sakuragaoka 8-35-1, Kagoshima 890-8544, Japan; mtrsaito@dent.kagoshima-u.ac.jp
4 Department of Neuroscience and Oral Physiology, Osaka University Graduate School of Dentistry, Osaka 565-0871, Japan
* Correspondence: kang@dent.osaka-u.ac.jp (Y.K.); toyoda@dent.osaka-u.ac.jp (H.T.)

Received: 25 November 2019; Accepted: 14 January 2020; Published: 15 January 2020

Abstract: It is known that desensitization of $GABA_A$ receptor ($GABA_AR$)-mediated currents is paradoxically correlated with the slowdown of their deactivation, i.e., resensitization. It has been shown that an upregulation of calcineurin enhances the desensitization of $GABA_AR$-mediated currents but paradoxically prolongs the decay phase of inhibitory postsynaptic currents/potentials without appreciable diminution of their amplitudes. The paradoxical correlation between desensitization and resensitization of $GABA_AR$-mediated currents can be more clearly seen in response to a prolonged application of GABA to allow more desensitization, instead of brief pulse used in previous studies. Indeed, hump-like $GABA_AR$ currents were produced after a strong desensitization at the offset of a prolonged puff application of GABA in pyramidal cells of the barrel cortex, in which calcineurin activity was enhanced by deleting phospholipase C-related catalytically inactive proteins to enhance the desensitization/resensitization of $GABA_AR$-mediated currents. Hump-like $GABA_AR$ currents were also evoked at the offset of propofol or barbiturate applications in hippocampal or sensory neurons, but not GABA applications. Propofol and barbiturate are useful to treat benzodiazepine/alcohol withdrawal syndrome, suggesting that regulatory mechanisms of desensitization/resensitization of $GABA_AR$-mediated currents are important in understanding benzodiazepine/alcohol withdrawal syndrome. In this review, we will discuss the molecular and regulatory mechanisms underlying the desensitization and resensitization of $GABA_AR$-mediated currents and their functional significances.

Keywords: $GABA_A$ receptor; desensitization; resensitization

1. Introduction

Ligand-gated channels open in response to the neurotransmitter binding but also close (desensitize) for long periods with the agonist still bound [1,2]. It is demonstrated that desensitization of $GABA_A$ receptor ($GABA_AR$)-mediated currents is paradoxically correlated with the slowdown of their deactivation, i.e., resensitization [3]. Desensitization tends to prolong inhibitory currents and keeps the transmitter in the bound state of $GABA_ARs$. The rate at which the receptors enter the desensitization state will affect the shape of inhibitory currents [4–6].

The desensitization of $GABA_AR$-mediated currents is modulated by various signal transductions. The PKA-mediated phosphorylation modulates the desensitization of $GABA_AR$-mediated currents in chick cortical neurons [7], rat sympathetic ganglion neurons [8], rat cerebellar granule neurons [9], and recombinant $GABA_ARs$ [10]. The PKC- and PKG-mediated phosphorylation decreases the fast component of desensitization in recombinant α1β1 $GABA_ARs$ [11] and rat cerebellar granule cells [9], respectively. CaMKII (Ca^{2+}/calmodulin-dependent protein kinase II) decreased the desensitization of $GABA_AR$-mediated currents in rat spinal dorsal horn neurons [12], while calcineurin enhanced the desensitization of $GABA_AR$-mediated currents in rat hippocampal neurons [13]. Calcineurin directly binds to the intracellular loop of the $GABA_AR$ γ2 subunit, thereby dephosphorylating the receptor [14]. Interestingly, it is reported that the desensitization of $GABA_AR$-mediated currents, which is caused by the enhanced calcineurin activity, paradoxically prolongs the decay phase of inhibitory postsynaptic currents/potentials without appreciable diminution of their amplitudes [4].

The paradoxical correlation between desensitization and resensitization of $GABA_AR$-mediated currents can be seen in response to a brief pulse in previous studies [3,4]. However, this relationship can be more clearly seen in response to a prolonged application of GABA for enough time to allow full desensitization. Indeed, hump-like $GABA_AR$ currents were produced after a strong desensitization at the offset of puff applications of GABA for 2 s in pyramidal cells of the barrel cortex in the phospholipase C-related catalytically inactive proteins (PRIP-1/2) double-knockout (PRIP-DKO) mice [15]. In these neurons, the increased calcineurin activity due to the potentiated Ca^{2+}-induced Ca^{2+} release (CICR) and store-operated Ca^{2+} entry (SOCE) enhances the desensitization of $GABA_AR$-mediated currents and subsequently causes resensitization of $GABA_AR$-mediated currents [15]. GABARAP ($GABA_AR$-associated protein) plays an important role in intracellular trafficking/clustering of $GABA_ARs$ [16,17] and the clustered $GABA_ARs$ display lower apparent affinity for GABA, faster deactivation, and slower desensitization [18]. The kinases and molecules involved in desensitization and resensitization (slowdown of deactivation) of $GABA_AR$-mediated currents are summarized in Table 1.

Table 1. Kinases and molecules involved in desensitization and slowdown of deactivation of $GABA_AR$-mediated currents.

Kinases/ Molecules	Neuron/Recombinant $GABA_ARs$	Effects	References
PKA	Chick cortical neurons	increases desensitization	[7]
	Rat sympathetic ganglion neurons	decreases peak amplitude and increases fast desensitization	[8]
	Rat cerebellar granule cells	decreases fast desensitization	[9]
	α1β1γ2S, α1β3γ2LS	increases desensitization and slows deactivation	[10]
PKC	α1β1	decreases fast desensitization	[11]
PKG	Rat cerebellar granule cells	decreases fast desensitization	[9]
CaMKII	Rat spinal dorsal horn neurons	decreases desensitization	[12]
Calcineurin	Rat hippocampal neurons	increases desensitization and slows deactivation	[4]
PRIP	Mouse cortical pyramidal neurons	PRIP deletion increases desensitization and generates hump-like currents through increased calcineurin activity	[15]
GABARAP	α1β2γ2L	promotes clustering of $GABA_ARs$, facilitates deactivation, and slows desensitization	[18]

Hump-like $GABA_AR$ currents after a strong desensitization were also seen at the offset of propofol applications at a high concentration (600 μM) in hippocampal pyramidal neurons [19], etomidate applications at a high concentration (1 mM) in rat spinal dorsal horn neurons [20], pentobarbital applications at high concentrations (1–3 mM) in frog sensory neurons [21,22], rat hippocampal neurons [23], and recombinant $GABA_ARs$ [24–29] or phenobarbital applications at a high concentration (10 mM) in rat hippocampal neurons [23], although these were not seen at the offset of GABA applications. Drugs that cause desensitization and resensitization of $GABA_AR$-mediated currents are summarized in Table 2. It is believed that the generation of hump-like currents may be caused by the removal of the blockade by anesthetic agents as partial antagonists [24], although their mechanisms remain unclear and the involvement of desensitization is not necessarily denied. Propofol and barbiturate are clinically used for treatment of benzodiazepine/alcohol withdrawal syndrome [30–32]. Considering that hump-like $GABA_AR$ currents that are seen after a strong desensitization or blockade were evoked at the offset of propofol or barbiturate applications, the regulatory mechanisms of desensitization/resensitization of $GABA_AR$-mediated currents might be important for understanding benzodiazepine/alcohol withdrawal syndrome. Here, we discuss the molecular and regulatory mechanisms underlying the desensitization and resensitization of $GABA_AR$-mediated currents in neurons of PRIP-DKO mice and their functional significances.

Table 2. Drugs that modulate GABA responses and directly activate $GABA_ARs$ at higher concentrations.

Drugs	Neurons/ Recombinant $GABA_ARs$	Effects	Refs.
Anesthetics			
Propofol	Mouse hippocampal neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations and directly elicits after-responses upon washout at high concentrations	[19]
Etomidate	Rat spinal dorsal horn neurons	slows deactivation of GABA responses at low concentrations while directly eliciting tail currents upon washout at high concentrations	[20]
Barbiturate			
Pentobarbital	Frog sensory neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations and directly elicits hump currents upon washout at high concentrations	[21,22]
	Rat hippocampal neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations and directly elicits rebound currents upon washout at high concentrations	[23]
	α1β2γ2L	directly elicits tail currents upon washout at high concentrations	[24,26]
	α1β3γ2L	slows deactivation and increases apparent desensitization of GABA responses at low concentrations and directly elicits rebound currents upon washout at high concentrations	[25]
	α1β2γ2S, α6β2γ2S	directly elicits hump currents upon washout at high concentrations	[27]
	β3	increases apparent desensitization of GABA responses and directly elicits rebound currents upon washout at high concentrations	[28]
	α1β3γ2L	directly elicits tail currents upon washout at high concentrations	[29]
Phenobarbital	Rat hippocampal neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations and directly elicits rebound currents upon washout at high concentrations	[23]

2. PRIP-1/2 are Involved in Desensitization and Resensitization of $GABA_AR$-Mediated Currents

PRIP-1/2 are involved in the membrane trafficking of $GABA_ARs$ and the regulation of intracellular Ca^{2+} stores [16,17]. Thus, it was investigated whether and how the deletion of PRIP-1/2 affects $GABA_AR$-mediated currents evoked by puff applications of GABA in layer III pyramidal cells of the barrel cortex. It was found that the deletion of PRIP-1/2 enhanced the desensitization of $GABA_AR$-mediated currents but paradoxically induced a hump-like tail-current at the offset of the GABA puff (Figure 1) [15]. Thus, it is likely that PRIP-1/2 are involved in the desensitization and resensitization of $GABA_AR$-mediated currents. Although similar tail-currents were observed following the removal of propofol [19], etomidate [20], pentobarbital [21–29], and phenobarbital [23], it was the first report on such hump-like tail-currents that were induced by GABA itself.

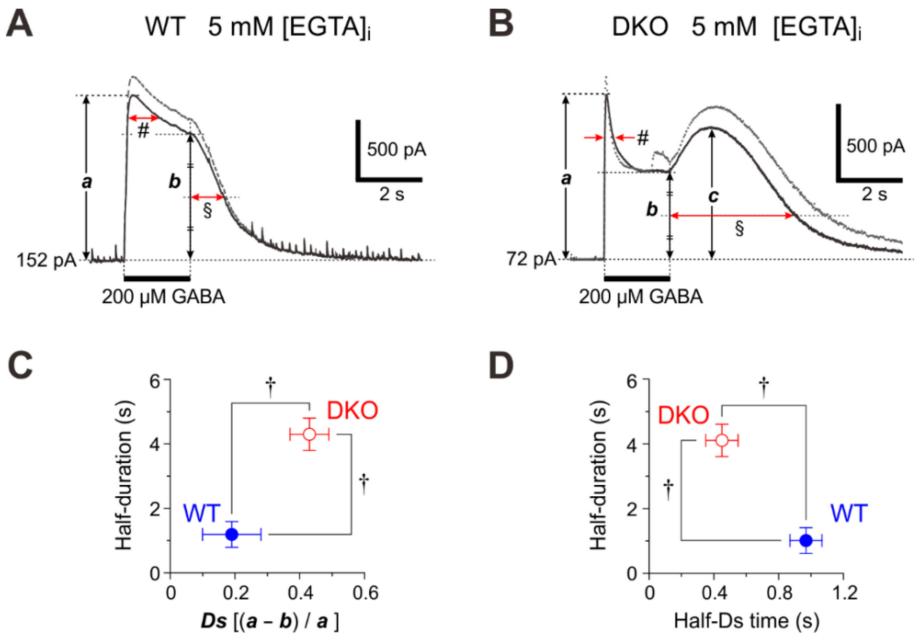

Figure 1. $GABA_AR$-mediated currents evoked by GABA puff applications in wild-type and PRIP-DKO pyramidal cells. (**A** and **B**) Sample traces of $GABA_AR$-mediated currents evoked at 0 mV in wild-type and PRIP-DKO pyramidal cells dialyzed with 5 mM EGTA, respectively, by puff application (4 and 6 psi) of GABA for 2 s. ***a***, ***b***, and ***c*** are the peak amplitude, the amplitude at the offset of the puff application, and the peak amplitude after the offset of the puff application, respectively. # and § are the durations at half amplitudes of desensitized component ([(a + b)/2]) and of tail-currents, respectively. (**C**) The relationship between the desensitization degree [Ds = (a – b)/a] of the $GABA_AR$-mediated currents and half-duration of the tail-current (§) induced by a puff with 4 psi. †: p <0.01. (D) The relationship between the half-desensitization time of the $GABA_AR$-mediated currents (#) and half-duration of the tail-current (§) induced by a puff with 4 psi. †: p <0.01. Adopted from [15].

3. $[Ca^{2+}]_i$ Dependence of Desensitization and Resensitization of $GABA_AR$-Mediated Currents and Their Abolishment by a Calcineurin Inhibitor

It is well known that the desensitization of $GABA_AR$-mediated currents is accelerated by increases in $[Ca^{2+}]_i$ [33,34]. As expected, it was clearly demonstrated that both the acceleration of desensitization of $GABA_AR$-mediated currents and the generation of the hump-like tail-currents were caused by increases in $[Ca^{2+}]_i$ [15]. Consistent with the idea that desensitization is mechanistically related to the deactivation of $GABA_AR$-mediated currents [3], the progress of desensitization of $GABA_AR$-mediated currents was invariably accompanied by the enhancement of the hump-like tail-currents [15]. These results suggested that the deletion of PRIP-1/2 results in an enhancement of the desensitization and resensitization of $GABA_AR$-mediated currents through increases in $[Ca^{2+}]_i$. The involvement of CICR and the following SOCE in both the desensitization of $GABA_AR$-mediated

currents and the generation of the hump-like tail-currents in PRIP-DKO pyramidal cells was also demonstrated by an intracellular application of ruthenium red [15].

It has been demonstrated that a calcineurin inhibitor, cyclosporin A-cyclophilin A complex, suppressed the desensitization of $GABA_A$R-mediated currents in acutely dissociated hippocampal neurons [13]. It has also been reported that the inhibition of calcineurin increased the rate of GABA unbinding from $GABA_A$Rs [4]. Consistent with these previous studies, the bath application of a calcineurin inhibitor, fenvalerate, alleviated the desensitization of $GABA_A$R-mediated currents and markedly decreased the hump-like tail-currents [15]. Thus, it is likely that the hump-like tail-currents in PRIP-DKO pyramidal cells were generated as a result of an acceleration of desensitization of $GABA_A$R-mediated currents coupled with a slowdown of the GABA unbinding, which was mediated by Ca^{2+}-dependent activation of calcineurin. Furthermore, Ca^{2+} imaging revealed that CICR and the following SOCE were more potent in PRIP-DKO pyramidal cells than in wild-type pyramidal cells [15]. Taken together, these results strongly suggest that the enhancement of desensitization and resensitization of $GABA_A$R-mediated currents in PRIP-DKO pyramidal cells was largely mediated by the upregulation of Ca^{2+}-dependent activity of calcineurin due to the potentiation of CICR followed by SOCE.

4. Deletion of PRIP-1/2 Prolongs eIPSCs in Layer II/III Pyramidal Cells

The differences in the kinetic properties of $GABA_A$R-mediated currents between pyramidal cells of wild-type and PRIP-DKO mice should be reflected in the difference in inhibitory postsynaptic responses. Then, it was investigated how inhibitory postsynaptic responses reflect the changes in the kinetic properties of the $GABA_A$R-mediated currents in layer III pyramidal cells of the PRIP-DKO barrel cortex.

It was found that the deletion of PRIP-1/2 resulted in the prolongation of the decay phase of inhibitory postsynaptic currents/potentials (IPSCs/IPSPs) in layer II/III pyramidal cells evoked by stimulation of layer III (Figure 2), leaving the overall features of miniature IPSCs unchanged [35]. These observations suggest that the prolongation of inhibitory synaptic actions is likely to result from an enhancement of desensitization followed by an enhanced resensitization of $GABA_A$R-mediated currents. It has been reported that the PRIP-DKO mice exhibited a reduced expression of synaptic $GABA_A$Rs containing γ2 subunits by 40% in hippocampal neurons [36] and by 18% in cerebellar granule cells [37] as a consequence of the lack of binding between PRIP-1/2 and $GABA_A$R-associated protein [38]. The mean peak amplitudes of the IPSCs and IPSPs in the PRIP-DKO pyramidal cells were not significantly different from those in the wild-type pyramidal cells. In any case, the amplitude of eIPSPs would not be increased by deletion of PRIP-1/2 [35]. Then, an increase in duration instead of amplitude of eIPSPs is likely to be caused in PRIP-DKO mice.

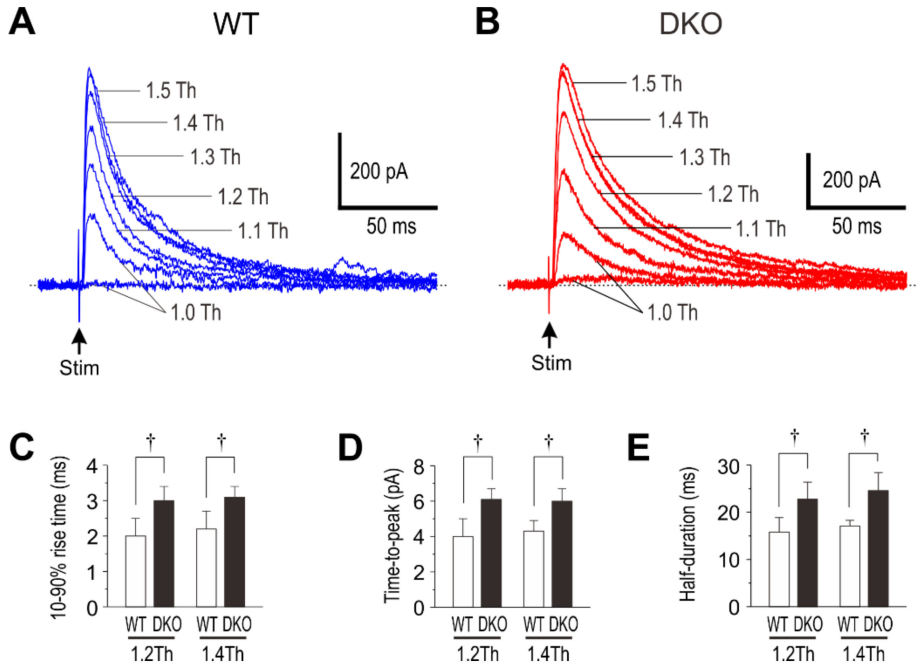

Figure 2. Evoked IPSCs (eIPSCs) in wild-type and PRIP-DKO pyramidal cells. (**A** and **B**) Superimposed sample traces of IPSCs evoked by stimulation with 1.0–1.5 times threshold (1.0–1.5 Th) in wild-type (**A**) and PRIP-DKO pyramidal cells (**B**). (**C**) The mean 10%–90% rise times of IPSCs evoked by stimulation with 1.2 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7) and those evoked by stimulation with 1.4 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7). †: p <0.01. (**D**) The mean times-to-peak of IPSCs evoked by stimulation with 1.2 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7) and those evoked by stimulation with 1.4 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7). †: p <0.01. (**E**) The mean half-durations of IPSCs evoked by stimulation with 1.2 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7) and those evoked by stimulation with 1.4 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7). †: p <0.01. Adopted from [35].

5. A Possible Kinetic Mechanism Underlying the Generation of the Hump-Like Tail-Currents and the Prolongation of eIPSCs

To understand the kinetic mechanisms underlying the generation of the hump-like tail-currents and the prolongation of eIPSCs, these currents were simulated using a previously proposed model [3] (Figure 3). It was examined whether the possible increase in the fast desensitization rate (d_2) and the possible decrease in the unbinding rate (k_{off}) can lead to a generation of the hump-like tail-current at the offset of the GABA puff.

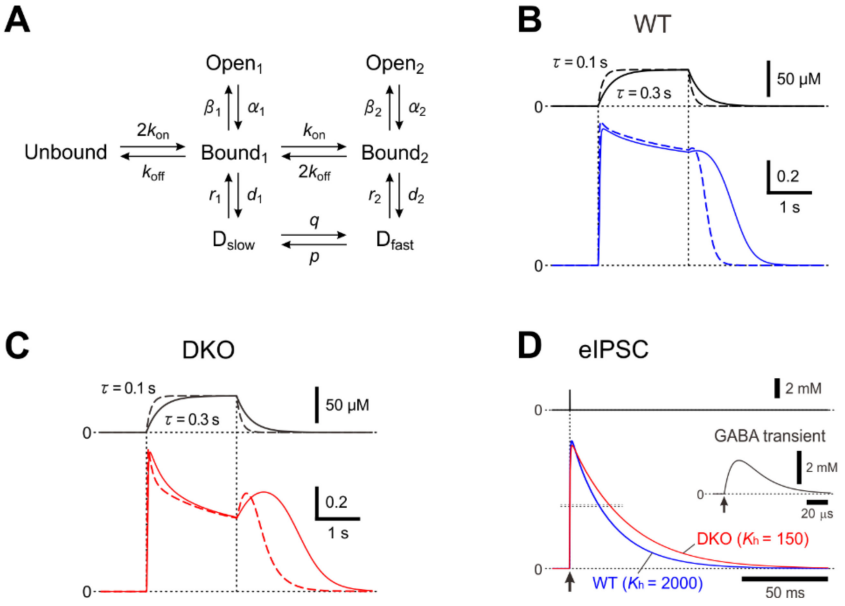

Figure 3. A kinetic model for a hump-like tail-current. (**A**) A kinetic model of $GABA_A$Rs representing mono- and double-liganded states, each providing access to open and desensitized states. (**B** and **C**) Top; Presumed [GABA] changes created by puff application of GABA with a rectangular pressure pulse through a puff pipette containing 200 μM GABA in the extracellular medium was assumed to be diluted 4 times and the onset and offset of the puff application were assumed to be attenuated with a time constant ranging between 0.1 and 0.3 s. Bottom; superimposed traces of the simulated $GABA_A$R-mediated currents under the condition that the attenuation time constant is 0.3 and 0.1 s (solid and interrupted traces, respectively) in simulated wild-type (**B**) and PRIP-DKO (**C**) pyramidal cells. The rate constants were as follows (in s^{-1}): k_{on} = 15 μM^{-1}, β_2 = 2500, α_2 = 142, r_2 = 50, β_1 = 200, α_1 = 1100, r_1 = 0.35, d_1 = 6, $q = 1 \times 10^{-8}$ μM^{-1}, and p = 1. The values of k_{off} in WT and PRIP-DKO $GABA_A$Rs were 90 and 30 s^{-1}, respectively. The value of d_{max} in WT and PRIP-DKO $GABA_A$Rs was 3600. The values of k_h in WT and PRIP-DKO $GABA_A$Rs were 2000 and 200, respectively. (**D**) Superimposed traces of a simulated wild-type and PRIP-DKO eIPSC induced by a GABA transient shown on an expanded time scale (inset) with a small maximum conductance. The rate constants were as follows (in s^{-1}): k_{on} = 20 μM^{-1}, β_2 = 2500, α_2 = 195, r_2 = 55, β_1 = 100, α_1 = 600, r_1 = 0.35, d_1 = 11, $q = 1 \times 10^{-8}$ μM^{-1}, p = 0, and d_{max} = 3100. The values of k_{off} in WT and PRIP-DKO $GABA_A$Rs were 550 and 410 s^{-1}, respectively. The value of d_{max} in WT and PRIP-DKO $GABA_A$Rs was 310. The values of k_h in WT and PRIP-DKO $GABA_A$Rs were 2000 and 150, respectively. Adopted from [15] and [35].

It is known that GABA binding affinity was much larger in the desensitized $GABA_A$Rs compared to the non-desensitized $GABA_A$Rs and the binding affinity of the desensitized $GABA_A$Rs increased depending on the concentration of the pre-applied GABA as was the case with the degree of desensitization of $GABA_A$R-mediated currents [39]. Then, when the probability of being in the desensitized state (D_{fast}) for $GABA_A$Rs was increased by increasing GABA concentration ([GABA]) or during the 2 s puff application of GABA, D_{fast} would be further recruited, leaving $Open_2$ unchanged. Thus, it is reasonable to assume that the d_2, but not β_2, increase in a manner dependent on [GABA] [15,39]. Because $Bound_2$, which is bifurcated into $Open_2$ and D_{fast}, increases in a manner dependent on [GABA], the idea was incorporated in this model by defining d_2 as follows;

$$d_2 = \frac{d_{max}}{1 + \left(\frac{K_h}{[GABA]}\right)^n}$$

where d_{max} is the maximum desensitization rate, K_h is the [GABA] that yields the half maximum desensitization rate, and n is the Hill coefficient [15]. It was assumed that calcineurin increased d_2

by increasing its [GABA] dependency through a reduction of k_h, and the d_2 and k_{off} were changed between the simulated wild-type and PRIP-DKO pyramidal cells. These changes were comparable to those caused by the activation of calcineurin reported previously [4,13].

In this simulation, the onset and offset of the 2 s puff application of GABA were assumed to be attenuated with a time constant raging between 0.1 and 0.3 s. In the simulated wild-type pyramidal cell, $GABA_A$R-mediated currents were induced without a hump-like tail-current in response to 2 s GABA puff at 50 μM [15]. In contrast, in the simulated PRIP-DKO pyramidal cell, $GABA_A$R-mediated currents displayed a prominent desensitization and were followed by a prominent hump-like tail-current [15]. Thus, a slowdown of k_{off} and an acceleration of d_2 resulted in a generation of a hump-like tail-current. Following a sharp decrease in [GABA] at the offset of GABA puff, a sharp decrease in d_2 to a level smaller than the fast de-desensitization (i.e., resensitization) rate constant (r_2) occurred to subsequently induce a hump-like tail-current. Indeed, decreases in the decay time constant at the offset of GABA puff pulse from 0.3 to 0.1 sec decreased the half-duration of the hump-like tail-current, leaving its amplitude almost unchanged [15]. Only PRIP-DKO pyramidal cells, but not wild-type pyramidal cells, displayed hump-like tail-currents in response to the same GABA puff that may have decayed slowly. These observations clearly indicate that the generation of the hump-like tail-current reflects kinetic differences between $GABA_A$R-mediated currents in wild-type and PRIP-DKO pyramidal cells. Taken together, it can be concluded that a higher calcineurin activity in PRIP-DKO layer III pyramidal cells might have caused a slowdown of k_{off} and an acceleration of d_2 through the modulation of its GABA concentration dependency, leading to a generation of hump-like tail-currents in PRIP-DKO pyramidal cells.

Because there were no significant differences in the single-channel current and the number of $GABA_A$Rs between eIPSCs in PRIP-DKO and wild-type pyramidal cells [35], it can be investigated whether the increase in d_2 and the decrease in k_{off} can also lead to the prolongation of eIPSCs. Simulated IPSCs in PRIP-DKO and the wild-type pyramidal cells that have half-durations similar to those obtained in the real experiments [35] revealed that a prolongation of eIPSCs/eIPSPs in PRIP-DKO pyramidal cells results from resensitization of $GABA_A$R-mediated currents, which is brought about by an acceleration of d_2 through the modulation of its [GABA] dependency together with a slowdown of k_{off}. The finding of a negative skewness coefficient in PRIP-DKO eIPSCs obtained by the nonstationary variance analysis [35] is consistent with the occurrence of de-desensitization (resensitization) of $GABA_A$R-mediated currents during the decay phase of PRIP-DKO eIPSCs.

Based on the experimental and simulation studies, the regulatory mechanisms of $GABA_A$Rs are schematically depicted (Figure 4).

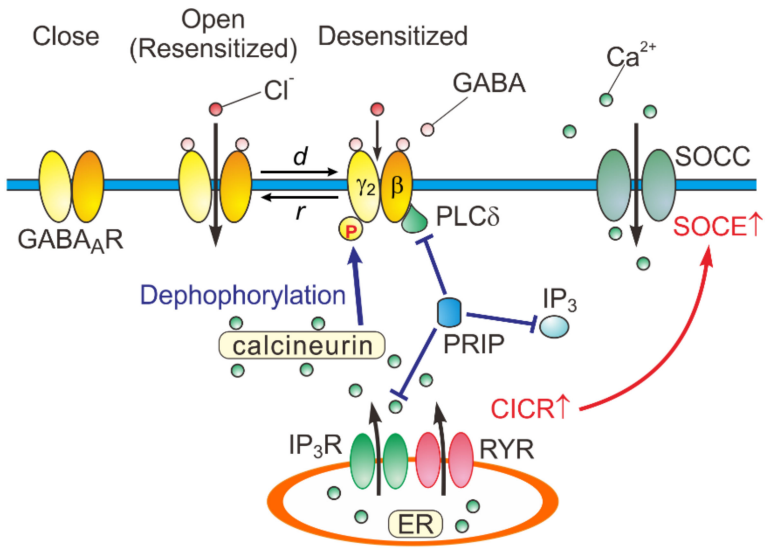

Figure 4. Close, open (resensitized), and desensitized states of $GABA_AR$s. When GABA binds to $GABA_AR$s, the receptors open the pore and consequently increase the permeability of the ion pore to Cl^-. In response to a prolonged application of GABA, $GABA_AR$s are desensitized (*d*) by increased calcineurin activity due to potentiated Ca^{2+}-induced Ca^{2+} release (CICR) followed by store-operated Ca^{2+} entry (SOCE) [15]. $GABA_AR$s are resensitized through de-desensitization (*r*) at the offset of the GABA puff. PRIP outcompetes the PLCδ in binding to $GABA_AR$ β subunits [40]. *d*: desensitization, *r*: resensitization, RYR: ryanodine receptor, SOCC: store-operated Ca^{2+} channel, IP_3R: inositol trisphosphate receptor.

6. Physiological Significance of Desensitization and Resensitization of $GABA_AR$-Mediated Currents

A single whisker deflection elicits an excitation in a subset of layer IV neurons within a single barrel-related column [41], which subsequently causes an excitation in layer II/III in the same column and then spreads horizontally into neighboring columns [42,43]. The spatio-temporal profile of the excitation spread in layer II/III evoked by stimulation of layer IV was narrower and faster in the barrel cortex of the PRIP-DKO mice compared to the wild-type mice [35].

Such a horizontal excitation spread in layer II/III seems to be strictly controlled by $GABA_AR$-mediated lateral inhibition [42,44,45]. Indeed, bicuculline application abolished such a difference in the spatio-temporal profile of the excitation spread in layer II/III between the two genotypes [35]. It is reported that the PRIP-DKO mice exhibited a greater decrease in performance in the rotarod test [36], which is commonly used to assess the sensorimotor integration [46]. Then, the enhanced phasic inhibition caused by the PRIP-1/2 deletion would suppress the inter-columnar integration in the barrel cortex, consequently decreasing spatial recognition. Further studies are required to clarify the roles of PRIP-1/2 in sensorimotor processing in the barrel cortex.

7. Clinical Significance of Desensitization and Resensitization of $GABA_AR$-Mediated Currents

Central nervous system depressants slow brain activity, making them useful for treating anxiety, panic, and sleep disorders. Alcohol and benzodiazepine are useful to mitigate anxiety through enhancing $GABA_AR$-mediated inhibition. However, alcohol and benzodiazepine are known as abused drugs. Alcohol or benzodiazepine withdrawal syndrome appears following a reduction in alcohol or benzodiazepine use after a period of excessive use [47–50]. The alcohol or benzodiazepine withdrawal symptoms typically include anxiety, sweating, hand tremor, and sleep disturbance. The underlying mechanisms involve neuronal adaptations, which are revealed as decreased GABAergic responses [51] and enhancement of NMDA responses [52–55]. Although the exact mechanism for the reduced responsiveness of $GABA_AR$s remains uncertain, changes in surface $GABA_AR$ protein level and subunit composition, changes in turnover, recycling, and production rates, degree of phosphorylation, and decreased coupling mechanisms between GABA and alcohol/benzodiazepine sites are thought

to be involved in the reduced responsiveness [56–59]. It has recently been demonstrated that the benzodiazepine diazepam caused downregulation of GABAergic inhibition through the phospholipase C (PLCδ)/Ca^{2+}/calcineurin signaling pathway [40]. The study showed that overexpression of PRIP-1 suppressed diazepam-dependent activation of PLCδ and diazepam-dependent downregulation of $GABA_A$Rs in HEK293 cells [40], indicating that PRIP-1 acts as an inhibitor by outcompeting the PLCδ binding to $GABA_A$Rs. Because intracellular Ca^{2+} and calcineurin activity are increased in PRIP-DKO mice [15], these findings suggest that the diazepam-induced long-term downregulation of GABAergic inhibition is mediated by the PLCδ/Ca^{2+}/calcineurin signaling pathway. Nevertheless, it is also true that calcineurin causes resensitization of $GABA_A$R-mediated currents by facilitating their desensitization [4,15]. Given the apparently contradictory behaviors of $GABA_A$R-mediated currents by calcineurin activation, the two different behaviors of $GABA_A$R-mediated currents may depend on whether calcineurin activation occurs before or after activation of $GABA_A$Rs.

As for the treatment of benzodiazepine/alcohol withdrawal syndrome, propofol and barbiturate which enhance $GABA_A$R-mediated inhibition are useful. Indeed, it was demonstrated that propofol and barbiturates (pentobarbital and phenobarbital) were effective for the treatment of alcohol withdrawal syndrome [30,32] and barbiturate (pentobarbital) was effective for the treatment of benzodiazepine withdrawal syndrome [60]. However, it remains unclear how propofol and barbiturate ameliorate reduced GABA responsiveness in patients with benzodiazepine/alcohol withdrawal syndrome. Although the concentrations of propofol and barbiturates that generated the hump-like current are very high [19,21,22] compared to the dose used for treatment of the withdrawal syndrome [30,32], the generation of hump-like $GABA_A$R currents itself may suggest the occurrence of resensitization of $GABA_A$R-mediated currents. Indeed, the desensitization and deactivation of $GABA_A$R-mediated currents are facilitated and slowed, respectively, by propofol/barbiturate at much lower concentrations [19,22]. Then, propofol and barbiturate may improve the reduced GABA responsiveness through the resensitization of $GABA_A$R-mediated currents. Therefore, the regulatory mechanisms of desensitization/resensitization of $GABA_A$R-mediated currents are important to better understand benzodiazepine/alcohol withdrawal syndrome and to develop the treatment method.

Author Contributions: Y.K. and H.T. conceptualized, drafted, reviewed, and revised the manuscript. M.S. performed a simulation study. All authors have read and agreed to the published version of the manuscript

Funding: This research was funded by Japan Society for the Promotion of Science (17K07055 to Y.K. and 17K08538 to H.T.).

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CICR	Ca^{2+}-induced Ca^{2+} release
DKO	double-knockout
$GABA_A$R	$GABA_A$ receptor
GABARAP	$GABA_A$R-associated protein
IPSC	inhibitory postsynaptic current
IPSP	inhibitory postsynaptic potential
NMDA	N-methyl-D-aspartate
PLC	phospholipase C
PRIP	phospholipase C-related catalytically inactive protein
RYR	ryanodine receptor
SOCC	store-operated Ca^{2+} channel
SOCE	store-operated Ca^{2+} entry

References

1. Jones, M.V.; Westbrook, G.L. The impact of receptor desensitization on fast synaptic transmission. *Trends Neurosci.* **1996**, *19*, 96–101. [CrossRef]
2. Keramidas, A.; Lynch, J.W. An outline of desensitization in pentameric ligand-gated ion channel receptors. *Cell Mol. Life Sci.* **2013**, *70*, 1241–1253. [CrossRef] [PubMed]
3. Jones, M.V.; Westbrook, G.L. Desensitized states prolong $GABA_A$ channel responses to brief agonist pulses. *Neuron* **1995**, *15*, 181–191. [CrossRef]
4. Jones, M.V.; Westbrook, G.L. Shaping of IPSCs by endogenous calcineurin activity. *J. Neurosci.* **1997**, *17*, 7626–7633. [CrossRef] [PubMed]
5. Overstreet, L.S.; Jones, M.V.; Westbrook, G.L. Slow desensitization regulates the availability of synaptic $GABA_A$ receptors. *J. Neurosci.* **2000**, *20*, 7914–7921. [CrossRef] [PubMed]
6. Qazi, S.; Caberlin, M.; Nigam, N. Mechanism of psychoactive drug action in the brain: Simulation modeling of GABAA receptor interactions at non-equilibrium conditions. *Curr. Pharm. Des.* **2007**, *13*, 1437–1455. [CrossRef] [PubMed]
7. Tehrani, M.H.; Hablitz, J.J.; Barnes, E.M., Jr. cAMP increases the rate of $GABA_A$ receptor desensitization in chick cortical neurons. *Synapse* **1989**, *4*, 126–131. [CrossRef]
8. Moss, S.J.; Smart, T.G.; Blackstone, C.D.; Huganir, R.L. Functional modulation of $GABA_A$ receptors by cAMP-dependent protein phosphorylation. *Science* **1992**, *257*, 661–665. [CrossRef]
9. Robello, M.; Amico, C.; Cupello, A. Evidence of two populations of $GABA_A$ receptors in cerebellar granule cells in culture: Different desensitization kinetics, pharmacology, serine/threonine kinase sensitivity, and localization. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 603–608. [CrossRef]
10. Hinkle, D.J.; Macdonald, R.L. Beta subunit phosphorylation selectively increases fast desensitization and prolongs deactivation of $\alpha_1\beta_1\gamma_{2L}$ and $\alpha_1\beta_3\gamma_{2L}$ $GABA_A$ receptor currents. *J. Neurosci.* **2003**, *23*, 11698–11710. [CrossRef]
11. Krishek, B.J.; Xie, X.; Blackstone, C.; Huganir, R.L.; Moss, S.J.; Smart, T.G. Regulation of $GABA_A$ receptor function by protein kinase C phosphorylation. *Neuron* **1994**, *12*, 1081–1095. [CrossRef]
12. Wang, R.A.; Cheng, G.; Kolaj, M.; Randic, M. Alpha-subunit of calcium/calmodulin-dependent protein kinase II enhances gamma-aminobutyric acid and inhibitory synaptic responses of rat neurons in vitro. *J. Neurophysiol.* **1995**, *73*, 2099–2106. [CrossRef] [PubMed]
13. Martina, M.; Mozrzymas, J.W.; Boddeke, H.W.; Cherubini, E. The calcineurin inhibitor cyclosporin A-cyclophilin A complex reduces desensitization of $GABA_A$-mediated responses in acutely dissociated rat hippocampal neurons. *Neurosci. Lett.* **1996**, *215*, 95–98. [CrossRef]
14. Muir, J.; Arancibia-Carcamo, I.L.; MacAskill, A.F.; Smith, K.R.; Griffin, L.D.; Kittler, J.T. NMDA receptors regulate $GABA_A$ receptor lateral mobility and clustering at inhibitory synapses through serine 327 on the 2 subunit. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 16679–16684. [CrossRef] [PubMed]
15. Toyoda, H.; Saito, M.; Sato, H.; Tanaka, T.; Ogawa, T.; Yatani, H.; Kanematsu, T.; Hirata, M.; Kang, Y. Deletion of phospholipase C-related inactive protein-1/2 enhances desensitization and resensitization of $GABA_A$ receptors in pyramidal cells of the barrel cortex. *Pflugers Arch.* **2015**, *467*, 267–284. [CrossRef]
16. Jacob, T.C.; Moss, S.J.; Jurd, R. $GABA_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat. Rev. Neurosci.* **2008**, *9*, 331–343. [CrossRef]
17. Luscher, B.; Fuchs, T.; Kilpatrick, C.L. $GABA_A$ receptor trafficking-mediated plasticity of inhibitory synapses. *Neuron* **2011**, *70*, 385–409. [CrossRef]
18. Chen, L.; Wang, H.; Vicini, S.; Olsen, R.W. The γ-aminobutyric acid type A ($GABA_A$) receptor-associated protein (GABARAP) promotes $GABA_A$ receptor clustering and modulates the channel kinetics. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 11557–11562. [CrossRef]
19. Orser, B.A.; Wang, L.Y.; Pennefather, P.S.; MacDonald, J.F. Propofol modulates activation and desensitization of $GABA_A$ receptors in cultured murine hippocampal neurons. *J. Neurosci.* **1994**, *14*, 7747–7760. [CrossRef]
20. Zhang, Z.X.; Lu, H.; Dong, X.P.; Liu, J.; Xu, T.L. Kinetics of etomidate actions on $GABA_A$ receptors in the rat spinal dorsal horn neurons. *Brain Res.* **2002**, *953*, 93–100. [CrossRef]
21. Akaike, N.; Hattori, K.; Inomata, N.; Oomura, Y. γ-Aminobutyric-acid- and pentobarbitone-gated chloride currents in internally perfused frog sensory neurones. *J. Physiol.* **1985**, *360*, 367–386. [CrossRef] [PubMed]

22. Akaike, N.; Maruyama, T.; Tokutomi, N. Kinetic properties of the pentobarbitone-gated chloride current in frog sensory neurones. *J. Physiol.* **1987**, *394*, 85–98. [CrossRef] [PubMed]
23. Rho, J.M.; Donevan, S.D.; Rogawski, M.A. Direct activation of $GABA_A$ receptors by barbiturates in cultured rat hippocampal neurons. *J. Physiol.* **1996**, *497*, 509–522. [CrossRef] [PubMed]
24. Gingrich, K.J.; Burkat, P.M.; Roberts, W.A. Pentobarbital produces activation and block of $\alpha_1\beta_2\gamma_2$S $GABA_A$ receptors in rapidly perfused whole cells and membrane patches: Divergent results can be explained by pharmacokinetics. *J. Gen. Physiol.* **2009**, *133*, 171–188. [CrossRef] [PubMed]
25. Feng, H.J.; Bianchi, M.T.; Macdonald, R.L. Pentobarbital differentially modulates $\alpha1\beta3\delta$ and $\alpha1\beta3\gamma2$L $GABA_A$ receptor currents. *Mol. Pharmacol.* **2004**, *66*, 988–1003. [CrossRef]
26. Krampfl, K.; Wolfes, H.; Dengler, R.; Bufler, J. Kinetic analysis of the agonistic and blocking properties of pentobarbital on recombinant rat $\alpha1\beta2\gamma2$S $GABA_A$ receptor channels. *Eur. J. Pharmacol.* **2002**, *435*, 1–8. [CrossRef]
27. Thompson, S.A.; Whiting, P.J.; Wafford, K.A. Barbiturate interactions at the human $GABA_A$ receptor: Dependence on receptor subunit combination. *Br. J. Pharmacol.* **1996**, *117*, 521–527. [CrossRef]
28. Wooltorton, J.R.; Moss, S.J.; Smart, T.G. Pharmacological and physiological characterization of murine homomeric $\beta3$ $GABA_A$ receptors. *Eur. J. Neurosci.* **1997**, *9*, 2225–2235. [CrossRef]
29. Ziemba, A.M.; Forman, S.A. Correction for inhibition leads to an allosteric co-agonist model for pentobarbital modulation and activation of $\alpha1\beta3\gamma2$L $GABA_A$ receptors. *PLoS ONE* **2016**, *11*, e0154031. [CrossRef]
30. Brotherton, A.L.; Hamilton, E.P.; Kloss, H.G.; Hammond, D.A. Propofol for treatment of refractory alcohol withdrawal syndrome: A review of the literature. *Pharmacotherapy* **2016**, *36*, 433–442. [CrossRef]
31. MacKinnon, G.L.; Parker, W.A. Benzodiazepine withdrawal syndrome: A literature review and evaluation. *Am. J. Drug Alcohol Abuse* **1982**, *9*, 19–33. [CrossRef] [PubMed]
32. Martin, K.; Katz, A. The role of barbiturates for alcohol withdrawal syndrome. *Psychosomatics* **2016**, *57*, 341–347. [CrossRef] [PubMed]
33. Inoue, M.; Oomura, Y.; Yakushiji, T.; Akaike, N. Intracellular calcium ions decrease the affinity of the GABA receptor. *Nature* **1986**, *324*, 156–158. [CrossRef] [PubMed]
34. Mozrzymas, J.W.; Cherubini, E. Changes in intracellular calcium concentration affect desensitization of $GABA_A$ receptors in acutely dissociated P2–P6 rat hippocampal neurons. *J. Neurophysiol.* **1998**, *79*, 1321–1328. [CrossRef]
35. Toyoda, H.; Saito, M.; Sato, H.; Kawano, T.; Kawakami, S.; Yatani, H.; Kanematsu, T.; Hirata, M.; Kang, Y. Enhanced lateral inhibition in the barrel cortex by deletion of phospholipase C-related catalytically inactive protein-1/2 in mice. *Pflugers Arch.* **2015**, *467*, 1445–1456. [CrossRef]
36. Mizokami, A.; Tanaka, H.; Ishibashi, H.; Umebayashi, H.; Fukami, K.; Takenawa, T.; Nakayama, K.I.; Yokoyama, T.; Nabekura, J.; Kanematsu, T.; et al. $GABA_A$ receptor subunit alteration-dependent diazepam insensitivity in the cerebellum of phospholipase C-related inactive protein knockout mice. *J. Neurochem.* **2010**, *114*, 302–310. [CrossRef]
37. Mizokami, A.; Kanematsu, T.; Ishibashi, H.; Yamaguchi, T.; Tanida, I.; Takenaka, K.; Nakayama, K.I.; Fukami, K.; Takenawa, T.; Kominami, E.; et al. Phospholipase C-related inactive protein is involved in trafficking of $\gamma2$ subunit-containing $GABA_A$ receptors to the cell surface. *J. Neurosci.* **2007**, *27*, 1692–1701. [CrossRef]
38. Kanematsu, T.; Jang, I.S.; Yamaguchi, T.; Nagahama, H.; Yoshimura, K.; Hidaka, K.; Matsuda, M.; Takeuchi, H.; Misumi, Y.; Nakayama, K.; et al. Role of the PLC-related, catalytically inactive protein p130 in $GABA_A$ receptor function. *EMBO J.* **2002**, *21*, 1004–1011. [CrossRef]
39. Chang, Y.; Ghansah, E.; Chen, Y.; Ye, J.; Weiss, D.S. Desensitization mechanism of GABA receptors revealed by single oocyte binding and receptor function. *J. Neurosci.* **2002**, *22*, 7982–7990. [CrossRef]
40. Nicholson, M.W.; Sweeney, A.; Pekle, E.; Alam, S.; Ali, A.B.; Duchen, M.; Jovanovic, J.N. Diazepam-induced loss of inhibitory synapses mediated by PLCδ/Ca^{2+}/calcineurin signalling downstream of $GABA_A$ receptors. *Mol. Psychiatry* **2018**, *23*, 1851–1867. [CrossRef]
41. Armstrong-James, M.; Fox, K.; Das-Gupta, A. Flow of excitation within rat barrel cortex on striking a single vibrissa. *J. Neurophysiol.* **1992**, *68*, 1345–1358. [CrossRef] [PubMed]
42. Petersen, C.C.; Sakmann, B. Functionally independent columns of rat somatosensory barrel cortex revealed with voltage-sensitive dye imaging. *J. Neurosci.* **2001**, *21*, 8435–8446. [CrossRef] [PubMed]

43. Sato, H.; Shimanuki, Y.; Saito, M.; Toyoda, H.; Nokubi, T.; Maeda, Y.; Yamamoto, T.; Kang, Y. Differential columnar processing in local circuits of barrel and insular cortices. *J. Neurosci.* **2008**, *28*, 3076–3089. [CrossRef] [PubMed]
44. Laaris, N.; Carlson, G.C.; Keller, A. Thalamic-evoked synaptic interactions in barrel cortex revealed by optical imaging. *J. Neurosci.* **2000**, *20*, 1529–1537. [CrossRef]
45. Laaris, N.; Keller, A. Functional independence of layer IV barrels. *J. Neurophysiol.* **2002**, *87*, 1028–1034. [CrossRef]
46. Anstrom, K.K.; Schallert, T.; Woodlee, M.T.; Shattuck, A.; Roberts, D.C. Repetitive vibrissae-elicited forelimb placing before and immediately after unilateral 6-hydroxydopamine improves outcome in a model of Parkinson's disease. *Behav. Brain Res.* **2007**, *179*, 183–191. [CrossRef]
47. Bayard, M.; McIntyre, J.; Hill, K.R.; Woodside, J., Jr. Alcohol withdrawal syndrome. *Am. Fam. Physician* **2004**, *69*, 1443–1450.
48. McKeon, A.; Frye, M.A.; Delanty, N. The alcohol withdrawal syndrome. *J. Neurol. Neurosurg. Psychiatry* **2008**, *79*, 854–862. [CrossRef]
49. Onyett, S.R. The benzodiazepine withdrawal syndrome and its management. *J. R. Coll. Gen. Pract.* **1989**, *39*, 160–163.
50. Petursson, H. The benzodiazepine withdrawal syndrome. *Addiction* **1994**, *89*, 1455–1459. [CrossRef]
51. Allison, C.; Pratt, J.A. Neuroadaptive processes in GABAergic and glutamatergic systems in benzodiazepine dependence. *Pharmacol. Ther.* **2003**, *98*, 171–195. [CrossRef]
52. Chandler, L.J.; Newsom, H.; Sumners, C.; Crews, F. Chronic ethanol exposure potentiates NMDA excitotoxicity in cerebral cortical neurons. *J. Neurochem.* **1993**, *60*, 1578–1581. [CrossRef] [PubMed]
53. Grant, K.A.; Valverius, P.; Hudspith, M.; Tabakoff, B. Ethanol withdrawal seizures and the NMDA receptor complex. *Eur. J. Pharmacol.* **1990**, *176*, 289–296. [CrossRef]
54. Koff, J.M.; Pritchard, G.A.; Greenblatt, D.J.; Miller, L.G. The NMDA receptor competitive antagonist CPP modulates benzodiazepine tolerance and discontinuation. *Pharmacology* **1997**, *55*, 217–227. [CrossRef]
55. Tsuda, M.; Shimizu, N.; Yajima, Y.; Suzuki, T.; Misawa, M. Hypersusceptibility to DMCM-induced seizures during diazepam withdrawal in mice: Evidence for upregulation of NMDA receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **1998**, *357*, 309–315. [CrossRef]
56. Olsen, R.W.; Liang, J.; Cagetti, E.; Spigelman, I. Plasticity of $GABA_A$ receptors in brains of rats treated with chronic intermittent ethanol. *Neurochem. Res.* **2005**, *30*, 1579–1588. [CrossRef]
57. Olsen, R.W.; Spigelman, I. GABAA Receptor Plasticity in Alcohol Withdrawal. In *Jasper's Basic Mechanisms of the Epilepsies*, 4th ed.; Noebels, J.L., Avoli, M., Rogawski, M.A., Olsen, R.W., Delgado-Escueta, A.V., Eds.; National Center for Biotechnology Information: Bethesda, MD, USA, 2012.
58. Tan, K.R.; Rudolph, U.; Luscher, C. Hooked on benzodiazepines: $GABA_A$ receptor subtypes and addiction. *Trends Neurosci.* **2011**, *34*, 188–197. [CrossRef]
59. Wafford, K.A. $GABA_A$ receptor subtypes: Any clues to the mechanism of benzodiazepine dependence? *Curr. Opin. Pharmacol.* **2005**, *5*, 47–52. [CrossRef]
60. Preskorn, S.H.; Denner, L.J. Benzodiazepines and withdrawal psychosis. Report of three cases. *JAMA* **1977**, *237*, 36–38. [CrossRef]