Neurosteroid administration and withdrawal alter $GABA_A$ receptor kinetics in CA1 hippocampus of female rats

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Withdrawal from the GABA-modulatory steroid 3α-OH-5α-pregnan-20-one (3α,5α-THP) following exposure of female rats to the parent compound progesterone (P) produces a syndrome characterized by behavioural excitability in association with up-regulation of the α4 subunit of the $GABA_A$ receptor (GABAR) in the hippocampus. Similar changes are seen after 48 h exposure to its stereoisomer, 3α,5β-THP. Here, we further characterize the effects of P withdrawal on GABAR kinetics, using brief (1 ms) application of 5–10 mM GABA to outside-out patches from acutely isolated CA1 hippocampal pyramidal cells. Under control conditions, GABA-gated current deactivated biexponentially, with $\tau_{fast} = 12$–19 ms (45–60% of the current), and $\tau_{slow} = 80$–140 ms. P withdrawal resulted in marked acceleration of deactivation ($\tau_{fast} = 3$–7 ms and $\tau_{slow} = 30$–100 ms), as did 48 h exposure to 3α,5β-THP ($\tau_{fast} = 5$–8 ms; $\tau_{slow} = 40$–120 ms). When recombinant receptors were tested in HEK-293 cells, a similar acceleration in τ_{fast} was observed for $\alpha4\beta2\delta$ and $\alpha4\beta2\gamma2$ GABARs, compared to $\alpha1\beta2\gamma2$ and $\alpha5\beta2\gamma2$ receptors. In addition, τ_{slow} was also accelerated for $\alpha4\beta2\delta$ receptors, which are increased following steroid withdrawal. As predicted by the Jones-Westbrook model, this change was accompanied by reduced receptor desensitization as well as an acceleration of the rate of recovery from rapid desensitization. A theoretical analysis of the data suggested that steroid treatment leads to receptors with a greater stability of the bound, activatable state. This was achieved by altering multiple parameters, including desensitization and gating rates, within the model. These results suggest that fluctuations in endogenous steroids result in altered GABAR kinetics which may regulate neuronal excitability.

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The $GABA_A$ receptor (GABAR), as the primary mediator of fast inhibitory input in the CNS, is modulated by a wide array of exogenous compounds, including benzodiazepines (BDZs), barbiturates, alcohol (Hevers & Luddens, 1998), as well as endogenous steroids such as 3α-OH-5α-pregnan-20-one (or allopregnanolone; 3α,5α-THP) (Majewska *et al.* 1986) and its active isomer, pregnanolone (3α,5β-THP). Acutely applied, these steroids increase the duration of GABA-gated single channel openings (Twyman & Macdonald, 1992) leading to anxiolytic (Bitran *et al.* 1993) and anticonvulsant (Belelli *et al.* 1989; Frye, 1995) effects. Fluctuations in 3α,5α-THP during the human menstrual cycle, however, result in prolonged exposure times (Endicott *et al.* 1999) followed by abrupt declines ('withdrawal') (Rapkin *et al.* 1997).

In testing the consequences of these exposure conditions, animal studies from this laboratory have demonstrated a bimodal change in GABAR subunit expression in CA1 hippocampus in response to prolonged administration of 3α,5α[β]-THP or its parent compound, progesterone. An initial increase in α4 subunit expression is seen after 48–72 h of exposure, followed by recovery to control levels. A secondary increase in α4 expression then occurs 24 h after termination of steroid exposure ('withdrawal') (Smith *et al.* 1998*a*; Gulinello *et al.* 2001). Increases in hippocampal excitability as well as behavioural excitability (i.e. anxiety and seizure susceptibility) are tightly correlated with these increases in α4 expression (Smith *et al.* 1998*a*,*b*; Gulinello *et al.* 2001; Hsu & Smith, 2003). A similar bimodal pattern has been reported (Backstrom, 1976; Herzog *et al.* 1997; Endicott *et al.* 1999; Rapkin *et al.* 1997) for adverse mood and exacerbation of seizures across the menstrual cycle for women with premenstrual syndrome and catamenial epilepsy, respectively, when exacerbation of symptoms occurs both

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early and late in the luteal phase, suggesting a clinical correlation. Increased expression of the $\alpha 4$ subunit is also observed during the increased excitability observed after chronic exposure to or withdrawal from other GABA-modulatory drugs such as the BDZs (Holt *et al.* 1996; Follesa *et al.* 2001) and alcohol (Devaud *et al.* 1997; Mahmoudi *et al.* 1997).

Increased $\alpha 4$ expression following steroid exposure or withdrawal is also associated with a decrease in the decay time constant (τ) for GABA-gated current (Smith *et al.* 1998*a*; Smith & Gong, 2004). This change in kinetics, however, was determined using whole cell patch clamp recording, low agonist (EC_{20}) concentrations, as well as relatively slow agonist application and exposure times of 40–100 ms. This approach results in overlapping times for agonist binding, channel opening, deactivation and desensitization. Agonist binding and channel opening occur on a submillisecond time scale only when saturating concentrations of GABA are rapidly (100–300 μs) applied (Maconochie *et al.* 1994; Burkat *et al.* 2001). Similarly, τ for rapid desensitization is ~5–10 ms (Celentano & Wong, 1994; Jones & Westbrook, 1995; Haas & Macdonald, 1999; McClellan & Twyman, 1999; Celentano & Hawkes, 2004), which necessitates the use of brief (1–2 ms) exposure times to more accurately determine the time course of deactivation.

In order to distinguish between deactivation and desensitization, here we directly measure these parameters using brief (~1 ms) or prolonged (400 ms to 5 s) application of saturating concentrations of agonist rapidly applied to outside-out patches of membrane from acutely isolated hippocampal pyramidal cells. We also compare findings from native GABAR tested across these steroid administration protocols with results from recombinant receptors with known subunit composition expressed in HEK-293 cells. Our previous findings suggest that both $\alpha 4\beta\delta$ (Sundstrom-Poromaa *et al.* 2002) and $\alpha 4\beta\gamma 2$ (Hsu *et al.* 2003) GABARs are increased following 48 h steroid administration as well as following withdrawal from chronic steroid exposure. Several studies suggest that receptors composed of these subunit combinations display shorter mean open times, consistent with a faster deactivation, than GABARs normally expressed in CA1 hippocampus under control conditions (Saxena & Macdonald, 1994; Gingrich *et al.* 1995; Burgard *et al.* 1996; Fisher & Macdonald, 1997; Lavoie *et al.* 1997; Haas & Macdonald, 1999; Bianchi *et al.* 2001; Akk *et al.* 2004). Thus, these findings suggest that altered subunit composition may play a role in shaping receptor kinetics after steroid treatment/withdrawal. The results from the present study may be relevant to the alterations in CNS excitability and mood which have been reported across the menstrual cycle (Herzog *et al.* 1997; Endicott *et al.* 1999).

Methods

Experimental animals

Adult female Long-Evans rats (Charles River, 120–140 g) were housed in groups of three under a constant light : dark cycle (14 : 10 light : dark) and room temperature (21°C). Food and water were available for *ad libitum* consumption. Animals were killed by decapitation during the light phase of the cycle (approx. 11.00 h). Control rats were tested only on the day of dioestrus-1, a low hormone stage, as verified by microscopic evaluation of the vaginal lavage. In all cases, conditions of animal maintenance and use were in agreement with the SUNY Downstate animal welfare committee.

Steroid administration

Two distinct steroid administration protocols were studied.

Protocol I. P withdrawal. Animals were implanted subcutaneously in the dorsal flank with silastic implants containing crystalline progesterone (P) for a 3-week period (Moran & Smith, 1998). Effects of steroid withdrawal on GABAR function were studied by killing animals 24 h following removal of the implant ('P withdrawal'). Both placement and removal of the P implant were accomplished under halothane anaesthesia (2-bromo-2-chloro-1,1,1-trifluoro-ethane, 2% in oxygen).

Protocol II. Forty-eight hours of 3α,5β-THP. Other animals were injected with the GABA-modulatory steroid 3α,5β-THP (10 mg kg^{-1}, I.P.) for 3 days, beginning on dioestrus-1.

Both steroid administration protocols result in physiological concentrations of 3α,5α-THP or 3α,5β-THP in the hippocampus (Moran & Smith, 1998). Control animals were injected with vehicle and tested on dioestrus-1. At the conclusion of these steroid treatment protocols, animals were killed by decapitation, hippocampi were removed, and pyramidal cells acutely isolated from the CA1 region.

Recombinant receptors: transfection

Plasmids obtained from Drs S. Vicini (Georgetown University, Washington, DC, USA; rat $\alpha 1$, $\alpha 5$, $\beta 2$, $\gamma 2$) and P. Whiting (Merck, Sharp & Dohme, Essex, UK; human $\alpha 4$, $\alpha 5$, δ) were prepared using Qiagen Maxi- or Midi-prep kits. HEK-293 cells were maintained in medium (Dulbecco's modified Eagle's medium (DMEM) : Ham's F-12 1 : 1) supplemented with 10% fetal calf serum at 37°C in a humid 5% CO_2 atmosphere. Cells were transfected with various subunit combinations using Lipofectamine (Invitrogen) with the

following ratios: $\alpha1\beta2\gamma2$, 1 : 1 : 5 (based on findings from Boileau *et al.* 2002); $\alpha4\beta2\gamma2$, 10 : 1 : 1, $\alpha4\beta2\delta$, 10 : 1 : 10, and $\alpha5\beta2\gamma2$, 5 : 1 : 1. Currents were recorded from lifted cells 1–3 days later. Cells were also cotransfected with enhanced green fluorescent protein for visualization. Expression of $\alpha1\beta2\gamma2$ was distinguished from $\alpha1\beta2$ by a robust response to the benzodiazepine lorazepam and little or no response to zinc. Expression of $\alpha4\beta2$ yielded little or no GABA-gated current, with no response to lanthanum or RO15-4513, compared to $\alpha4\beta2\delta$ and $\alpha4\beta2\gamma2$ receptors, respectively.

Acute neuronal isolation

Pyramidal neurones were acutely dissociated as previously described (Smith *et al.* 1998*a*). Briefly, tissue was digested at 32°C for 50–60 min under 100% O_2 in Pipes-buffered saline containing (mM): NaCl 120, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, D-glucose 25, Pipes 20 and trypsin (type XI) or pronase (0.8 mg ml^{-1}), pH 7.0. Following a 1 h enzyme-free incubation at room temperature, tissue was dissociated by trituration in 1 ml of 20 mM Hepes-buffered DMEM which was replaced by recording medium following transfer to the recording chamber.

Electrophysiology

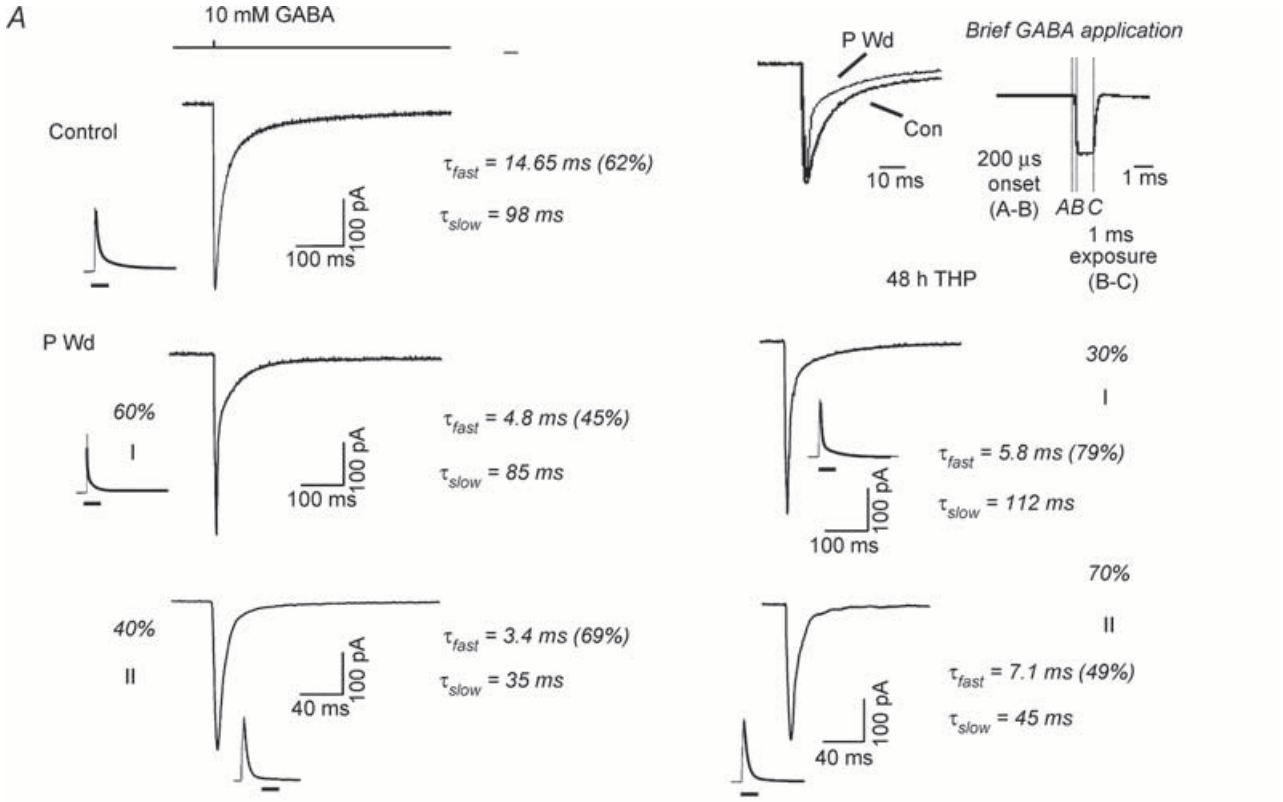
GABA-gated current was recorded at room temperature (20–25°C) at a holding potential of −50 mV in a bath containing (mM): NaCl 120, CsCl 5, $CaCl_2$ 2, $MgCl_2$ 1, Hepes 10 and glucose 25, pH 7.4, 320 mosmol (kg H_2O)$^{-1}$. Outside-out patches were pulled after a gigaseal was achieved using suction applied to 5–7 MΩ micropipettes (Sutter Instruments, filament-capillary tubes). The pipette solution contained (mM): *N*-methyl-D-glucamine chloride 120, Cs_4BAPTA 5 and Mg-ATP 5. The ATP regeneration system consisting of Tris phosphocreatine (20 mM) and creatine kinase was added to minimize GABA rundown.

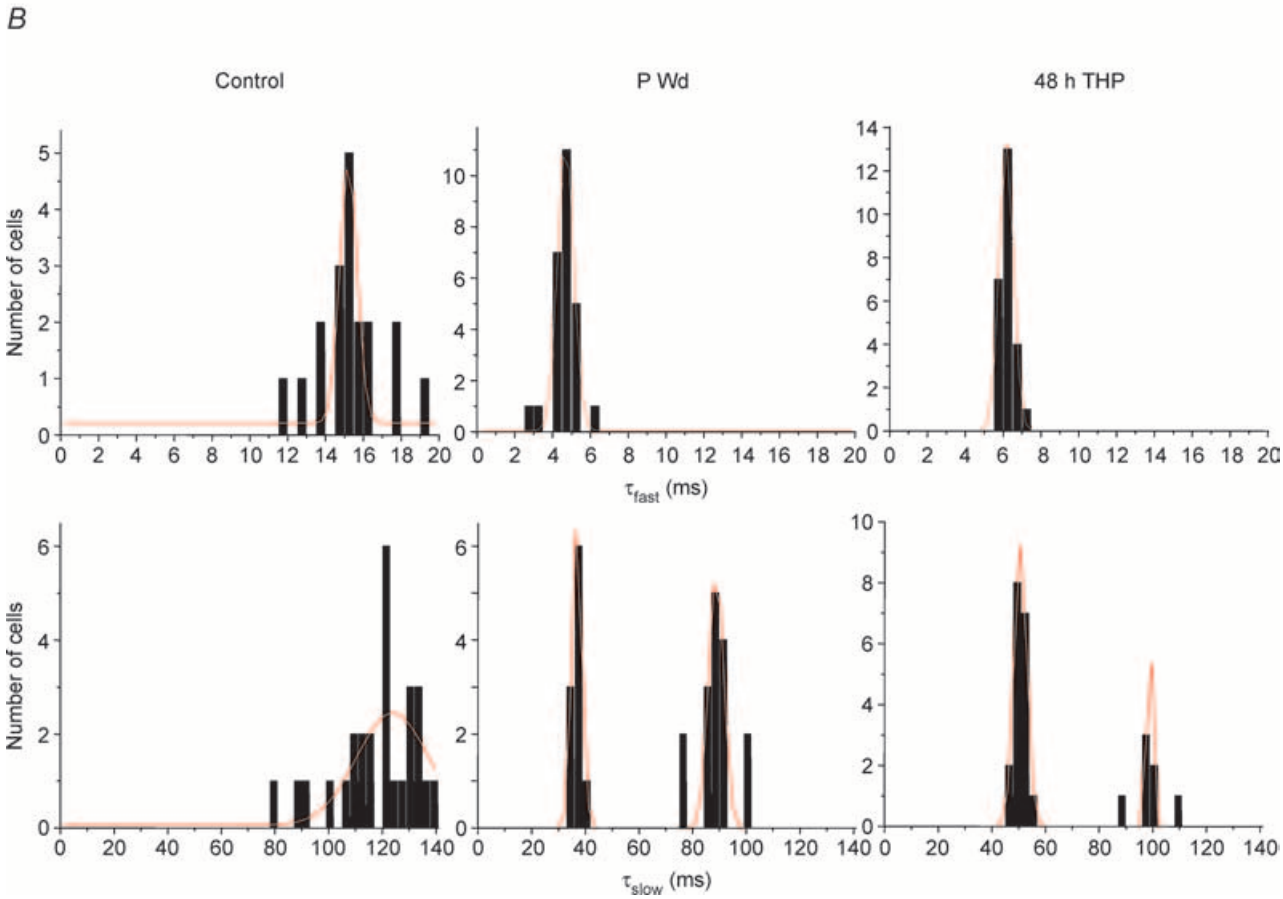
Currents were recorded using an Axopatch-1D amplifier (Axon Instruments, Union City, CA, USA) filtered at 2 kHz (four-pole Bessel filter) and acquired at an 8–10 kHz sampling frequency (pCLAMP 5.1, Axon Instruments). Ensemble averages of 6–10 responses per cell were used for determination of decay time constants (τ).

Agonist application. The kinetics of GABA-gated current were tested using a brief application protocol to administer saturating concentrations of GABA (5–10 mM) to whole cells or excised outside-out patches for ∼1 ms (Lavoie *et al.* 1997). To this end, a double-barrelled theta tube (Sutter Instruments, 80–100 μm diameter tip) containing GABA and bath solution was positioned within 50–100 μm of the patch, such that the stream of control solution contacted the patch for 1–2 s periods which were interrupted by periodic brief (< 100–300 μs) transitions to the GABA stream (maintained at 2.5 ml h^{-1} to yield a forward flow velocity of 125 μm ms^{-1}). A computer generated pulse (pCLAMP 5.1, Axon Instruments) triggered the GABA application with a piezoelectric translator (Burleigh Instruments, LSS-3100). Following the recording, the patch was blown out, and the open tip potential recorded using solutions with a 5% difference in NaCl osmolarity to verify the approximate solution exchange time (see inset to Fig. 1*A*). Data from patches were analysed only for exposure times of < 2 ms duration and where the onset of agonist application times was < 300 μs.

Deactivation time constants were approximated as biexponential functions using nonlinear curve fitting routines with either Levenburg-Marquardt algorithms or the Simplex Minimization method depending on the level of background noise (Origin software, OriginLab Corp., Northampton, MA, USA). The formula $I = I_f e^{(-t/\tau_{fast})} + I_s e^{(-t/\tau_{slow})}$ was used, where I_f and I_s are the amplitudes of the fast and slow decay components, and τ_{fast} and τ_{slow} are their respective decay time constants. Goodness of fit was determined by minimizing the sum of the squares of deviations of the theoretical curve from the experimental points. Best fit was determined when this value was no longer improved by > 5%, with the sum of squared errors < 0.95. Averaged weighted values of τ were also determined for each case with the equation: $\tau_w = \tau_{fast}$(fractional amount of current) + τ_{slow}(fractional amount of current), in order to compare values of τ across steroid state. In some cases, total charge transfer was calculated by integrating the area under the curve (Origin software).

Desensitization. Desensitization in response to prolonged GABA exposure was studied by applying 5 mM GABA continuously for 400 ms or 5 s to excised outside-out patches using the piezoelectric-controlled theta tube to allow for rapid onset and offset of agonist. Desensitization was also studied using repetitive 1 ms applications of 5–10 mM GABA at different interpulse intervals to isolated pyramidal cells. As above, the open tip potential was used to verify times of agonist application and duration of exposure. The degree of desensitization following these various exposure periods was expressed as a percentage decrease from the initial GABA response. The time constant for desensitization ($\tau_{desensitization}$) was determined using similar non-linear curve fitting techniques as described above with two or three exponents. In this case, distinguishing between two- or three-exponential decay was accomplished using the *F* test (Prism statistical program, GraphPad Software Inc., San Diego, CA, USA), and best fit determined when $P < 0.05$ (Celentano & Wong, 1994). Deactivation following agonist removal was also evaluated as described above. As in the previous study, weighted averages of





τ (τ_w) were used for the purposes of statistical comparison between steroid-treatment groups.

The rate of recovery from fast desensitization was determined using paired 1 ms pulses of 10 mM GABA applied at interpulse intervals of 20, 70, 120, 240, 360, 500, 1000 and 2000 ms to outside-out patches (Jones & Westbrook, 1995; Bai *et al.* 1999). The amplitude of the second response was compared to that of the first (see Fig. 5) and adjusted for the baseline offset. Recovery was a biexponential function and time constants were calculated as described above.

Kinetic modelling

In order to investigate which microscopic parameters might produce the differences in macroscopic current observed experimentally, rate constants for agonist binding, desensitization and gating were estimated using a simplified version of the Jones-Westbrook model, which contained a single open state and two desensitized states (Fig. 6*A*). Biliganded binding was simplified as a single step to reduce the number of free parameters. Starting values for the rate constants for k_{on}, k_{off}, α and β were initially based on values derived from single channel studies published by other groups, which were used for models designed to simulate control (Model I, Bai *et al.* 1999; Jones & Westbrook, 1995; Mozrzymas *et al.* 2003; Shen *et al.* 2000), or steroid withdrawal conditions (Models III and IV, Akk *et al.* 2004; Fisher & Macdonald, 1997; Haas & Macdonald, 1999). Initial estimates of the forward (d_f, d_s) and reverse (r_f, r_s) rate constants (fast (f) and slow (s)) for desensitization were derived from values (time constants and extents of desensitization) obtained in the present study by solving the simultaneous equations:

$$d_f/(d_f + r_f) = \%\,\text{fast desensitization}$$

$$1/\tau_{fast} = (\alpha/(\alpha + \beta))d_f + r_f$$

as described in Celentano & Wong (1994). Forward and reverse rate constants for slow desensitization were determined by first estimating the fraction of current in the slow desensitized state under steady state conditions. Then, forward and reverse rate constants were derived using the equations:

$$d_s/r_s = F_{DS}/F_C$$

$$1/\tau_{slow} = F_C{}^* d_s + r_s$$

where F_{DS} is the fraction of receptors in the slow desensitized state, F_C is the fraction of receptors in the closed state under steady state conditions. $F_C{}^*$ is the fraction of current in the closed state at equilibrium with D_{fast}.

These values were used as starting estimates for the rate constants which were adjusted manually to best simulate the experimentally observed currents. Simulated current responses to 1 or 400 ms application of 10 mM GABA were generated using QUB software (Dr A. Auerbach, SUNY, Buffalo; Qin *et al.* 1997). Once optimal rate constants were obtained, the rate of recovery from fast desensitization was also simulated using a paired pulse protocol with locally written Q-matrix software (Celentano & Hawkes, 2004).

Statistical analysis

Differences between groups were assessed using Student's *t* test or ANOVA followed by the Tukey's *post hoc* analysis, for two or multiple groups, respectively. Differences were judged to be significant when $P < 0.05$. The Gaussian distribution of values for each group was determined using a Chi-square analysis (Origin).

Results

GABAR deactivation rate increases following steroid withdrawal

The deactivation of GABA-gated current was determined using brief application of GABA to outside-out patches. Agonist exposure times for the analysed currents varied between 1 and 1.4 ms (1.3 ± 0.28 ms, Control;

Figure 1. Progesterone withdrawal accelerates the deactivation of GABA-gated current
A, representative traces showing responses to brief (~1 ms) pulses of GABA (10 mM) recorded from outside-out patches of CA1 hippocampal pyramidal cells following progesterone withdrawal (P Wd), 48 h 3α,5β-THP (48 h THP) or sham conditions (Control). Each trace represents the average of 6–10 individual traces. (Fits are shown next to full traces.) The deactivation rate is best described as a biexponential decay, with a τ_{fast} in the range of 10–22 ms and a τ_{slow} of 80–145 ms for the control recordings. Following P withdrawal (P Wd), in Group I 60% of the current deactivated with a τ_{fast} of 3–6 ms (mean = 4.88 ± 0.61 ms), and a τ_{slow} of 80–120 ms (mean = 87.0 ± 12.0 ms). In Group II, 40% of the current recorded deactivated with a τ_{fast} of 3–7 ms, and a τ_{slow} of 30–40 ms. Forty-eight hours of treatment with 3α,5β-THP produced similar acceleration in deactivation times. Note that in both populations, τ_{fast} is significantly faster than control values, while in Group II τ_{slow} is also significantly faster than control. Average peak amplitude was unaffected by prior steroid treatment. The top trace indicates the open tip junctional current. (These results are representative of those recorded from 20 to 30 patches/group.) Inset: amplified traces illustrate an accelerated τ_{fast} following P Wd compared to control. Inset, representative open tip junction potential for a control recording. *B*, distribution of values for τ_{fast} and τ_{slow} for control (Con, left panels), progesterone withdrawal (P Wd, middle panels), and 48 h treatment with 3α,5β-THP (48 h THP, right panels). Values for τ_{slow} display a bimodal distribution for P Wd and 48 h THP conditions. All other distributions display a single mode.

Table 1. Deactivation time constants following brief GABA application

Group	Percentage of population	τ_{fast}	Percentage of total current (τ_{fast})	τ_{slow}	Weighted τ	n
Control	100	15.6 ± 3.8	55 ± 8	120.3 ± 12.6	70.1 ± 8.5	52
P Wd	60	4.88 ± 0.61*	50 ± 10	87.0 ± 12.0	32.5 ± 1.35*	30
	40	4.51 ± 0.52*	71 ± 9	36.87 ± 2.5*	13.8 ± 1.09*	20
Average					24.8 ± 1.25*	50
48 h THP	30	6.10 ± 0.78*	76 ± 8	98.2 ± 10.2	28.2 ± 2.10*	15
	70	6.50 ± 0.66*	66 ± 10	50.8 ± 3.2*	21.6 ± 1.52*	35
Average					23.6 ± 1.76*	50

Average decay time constants (in milliseconds) for the fast (τ_{fast}) and slow (τ_{slow}) components of deactivation (means ± S.E.M.), as well as the weighted values of τ. Values were assessed using brief application (~1 ms) of saturating concentrations of agonist (10 mM) to outside out patches of membrane from CA1 hippocampal pyramidal cells. Hippocampal tissue from female rats was isolated following withdrawal from 21 day progesterone treatment (P Wd) or 48 h treatment with the GABA-modulatory steroid 3α,5β-THP (48 h THP, 10 mg kg^{-1}, I.P., for 3 days). Significant decreases in τ_{fast} and the weighted τ were observed following both steroid protocols compared to control. Values for τ_{slow} exhibited a bimodal distribution following the steroid protocols (% of population indicated). (n = number of patches/group, $^*P < 0.05$ *versus* control).

1.4 ± 0.3 ms, P Wd; 1.32 ± 0.2 ms, 48 h 3α,5β-THP, see inset to Fig. 1*A* for representative open tip potential). Agonist exposure times did not differ significantly between experimental and control groups.

Under control conditions, deactivation was best fitted as a biexponential equation, with an average $\tau_{fast} = 15.6 \pm 3.8$ ms (mean ± S.E.M.), which represented 55% of the current, and a $\tau_{slow} = 120.3 \pm 12.6$ ms (Fig. 1*A*, Table 1). Following withdrawal from P (Fig. 1*A*, Table 1, P Wd), the fast component of τ for GABA-gated current was significantly accelerated ($\tau_{fast} = 3$–7 ms, P Wd, $P < 0.05$) compared to corresponding control values, and a Gaussian fit of the data revealed a single peak (Fig. 1*B*). However, values for the slow component of deactivation were distributed bimodally ($r^2 = 0.90$, $P < 0.05$, Fig. 1*B*) following P withdrawal, which we have designated as separate groups. For one population, τ_{slow} was not significantly changed from control ($\tau_{slow} = 87.0 \pm 12.0$ ms, Group I, 60% of the population). However, for the second population, τ_{slow} was significantly accelerated compared to control values ($\tau_{slow} = 36.87 \pm 2.5$ ms, Group II, Fig. 1*A* and *B*). For these two populations, the distribution of current carried by the fast component was either unchanged (P Wd-I) or increased (P Wd-II) compared to control values (Fig. 1*A*).

Forty-eight hours of exposure to 3α,5β-THP resulted in altered kinetics similar to those observed following P withdrawal: τ_{fast} was consistently accelerated compared to control values ($\tau_{fast} = 5$–8 ms, 66–76% of the current, Fig. 1*A*, Table 1). As observed following P withdrawal, values for τ_{slow} displayed a bimodal distribution ($r^2 = 0.87$, $P < 0.05$, Fig. 1*B*): Group I, $\tau_{slow} = 98.2 \pm 10.2$ ms (30% of the population) and Group II, $\tau_{slow} = 50.8 \pm 3.2$ ms (Fig. 1*A* and *B*, and Table 1). The range of deactivation time constants obtained is within the range reported for native and recombinant receptor isoforms (Banks & Pearce, 2000; Jones & Westbrook, 1995; McClellan & Twyman, 1999), including the 30–50 ms values for τ_{slow} observed after steroid treatment and withdrawal. In addition, the 10–90% rise time was slightly accelerated when GABA responses were tested following either steroid exposure protocols (0.75–0.90 ms) compared to control (1.1–1.3 ms).

When the values of τ were converted to weighted values, both P withdrawal and 48 h 3α,5β-THP exposure resulted in a similar threefold faster rate of deactivation for GABA-gated current (Table 1) compared to the control value. Thus, these results demonstrate that both steroid treatment conditions significantly decrease τ for deactivation of GABA-gated current.

GABAR subunit composition alters deactivation kinetics

Because our previous findings have shown that a common outcome of the two steroid treatment protocols is to increase hippocampal expression of α4- and δ-containing GABARs (Smith *et al.* 1998*a*; Gulinello *et al.* 2001; Sundstrom-Poromaa *et al.* 2002), recombinant receptors were expressed in HEK-293 cells in order to compare deactivation rate as a function of subunit composition using whole cell mode and outside-out patches. As observed for native GABAR, all recombinant isoforms deactivated with a biexponential decay (Fig. 2*A* and *B*). Both α4-containing GABARs deactivated with an accelerated τ_{fast}, which was approximately 50% decreased compared to the $\alpha1\beta2\gamma2$ and $\alpha5\beta2\gamma2$ isoforms ($P < 0.05$). In addition, $\alpha4\beta2\delta$ GABAR deactivated with an accelerated τ_{slow} compared to the other isoforms ($P < 0.05$), while $\alpha4\beta2\gamma2$ deactivated with a τ_{slow} which was marginally slower than $\alpha1\beta2\gamma2$. In contrast, $\alpha5\beta2\gamma2$ GABAR deactivated with the slowest rate constants, displaying a τ_{slow} which was twofold greater than

$\alpha4\beta2\gamma2$ receptors ($P < 0.05$). These relative differences were similar in recordings from patches and whole cells, suggesting that the internal milieu is not required for the observed variations in kinetics associated with subunit composition. Values of τ_{fast} for all three subunit combinations were nearly identical in patches and whole cell recordings. However, values of τ_{slow} for $\alpha4\beta2\gamma2$ GABAR were more prolonged in whole cell recordings compared to patches. This may reflect different states of phosphorylation or other post-translational mechanisms (Jones & Westbrook, 1997), which have been shown to alter the slow component of deactivation.

When the area under the curve was integrated to produce a value for the total charge transfer, this value varied as predicted by the variations in deactivation τ. Total charge transfer was approximately twofold greater for $\alpha5\beta2\gamma2$ GABAR compared to $\alpha1\beta2\gamma2$ (1.25 ± 0.14, $\alpha5\beta2\gamma2$ *versus* 0.7 ± 0.08, $\alpha1\beta2\gamma2$, $P < 0.05$; all

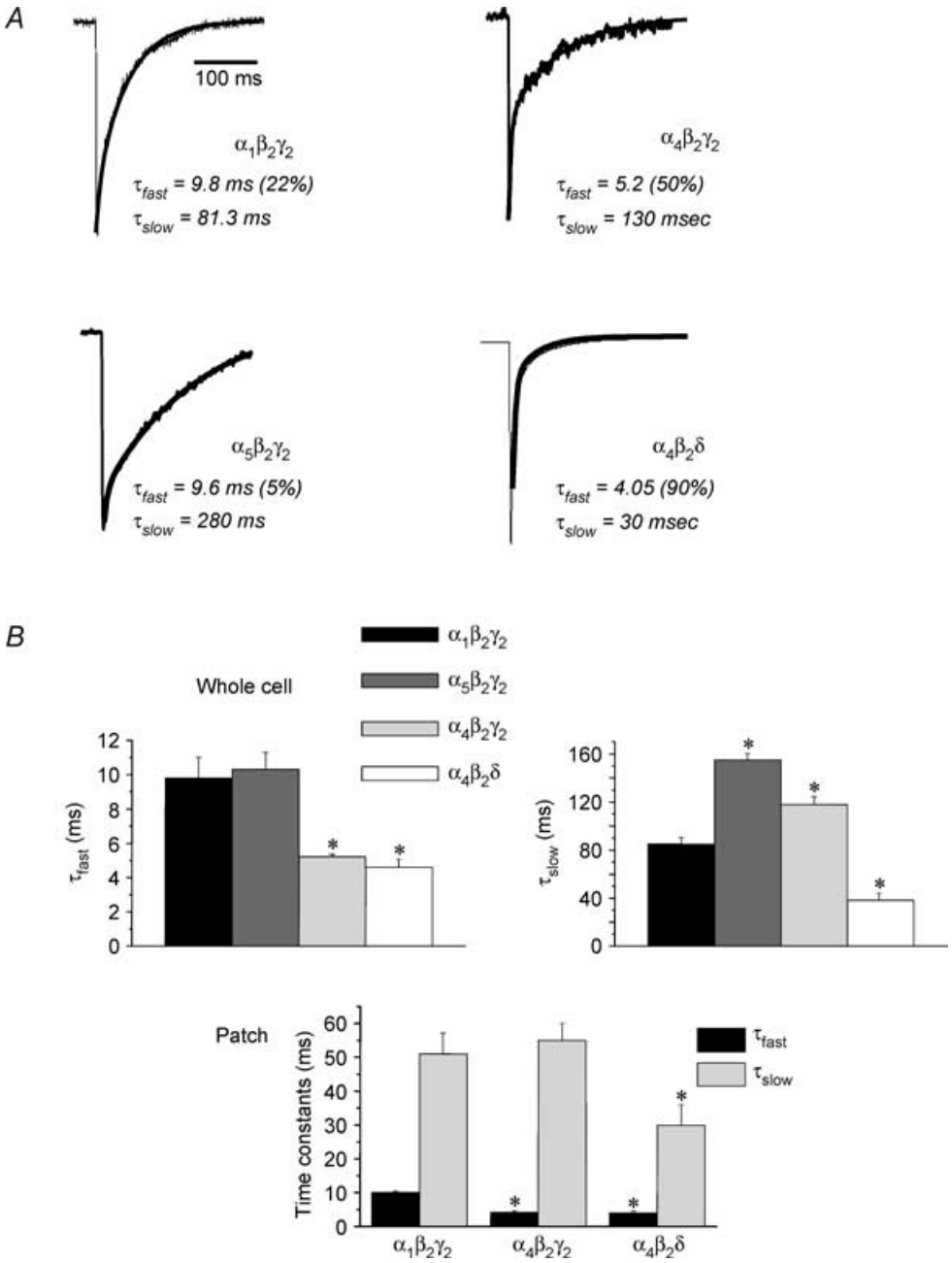


Figure 2. Kinetics of recombinant $GABA_A$ receptor isoforms
Representative current traces (*A*) and averaged values (*B*) illustrate the different kinetics exhibited by recombinant $\alpha1\beta2\gamma2$, $\alpha4\beta2\gamma2$, $\alpha5\beta2\gamma2$ or $\alpha4\beta2\delta$ GABAR recorded from HEK-293 cells using whole cell or outside-out patch recording techniques. Both $\alpha4$-containing GABAR isoforms deactivate with a faster τ_{fast} than $\alpha1$ or $\alpha5$-containing GABAR. (These results are averaged from 6–8 cells/group, $^*P < 0.05$ *versus* $\alpha1\beta2\gamma2$.)

values $\times 10^6$ per 500 pA). In contrast, both $\alpha4\beta2\gamma2$ and $\alpha4\beta2\delta$ GABARs resulted in values for total charge transfer which were significantly ($P < 0.05$) less than $\alpha1\beta2\gamma2$ (0.46 ± 0.04, $\alpha4\beta2\gamma2$; 0.265 ± 0.06, $\alpha4\beta2\delta$, $P < 0.05$).

GABAR desensitization – varying pulse frequency

Repetitive agonist application was used to study the effects of steroid treatment on GABAR desensitization. To this end, 1 ms pulses of GABA were applied at frequencies of 2, 8, 20 and 50 Hz (Fig. 3*A*). Under control conditions, a 15% desensitization was observed at frequencies as low as 2 Hz. As predicted, the degree of desensitization increased with increasing frequency of applied GABA pulses to a maximum level of 82.3 ± 15.0% desensitization at a 50 Hz GABA pulse frequency. Approximate values of τ for desensitization using this protocol were estimated as $\tau_{D1} = 12$–20 ms; $\tau_{D2} = 180$ ms. As seen for continuous agonist exposure (Table 2), desensitization in response to GABA application was markedly attenuated ($P < 0.05$) following P withdrawal for all frequencies, with significant desensitization beginning at 20 Hz frequencies of GABA application (Fig. 3*A*). Maximal desensitization (50 Hz) was 28.5 ± 5.2%, a value similar to that seen after 400 ms continuous agonist exposure (Table 2). The approximate τ for this desensitization process was 2200 ms, also similar to the τ for desensitization calculated after continuous agonist exposure (Table 2).

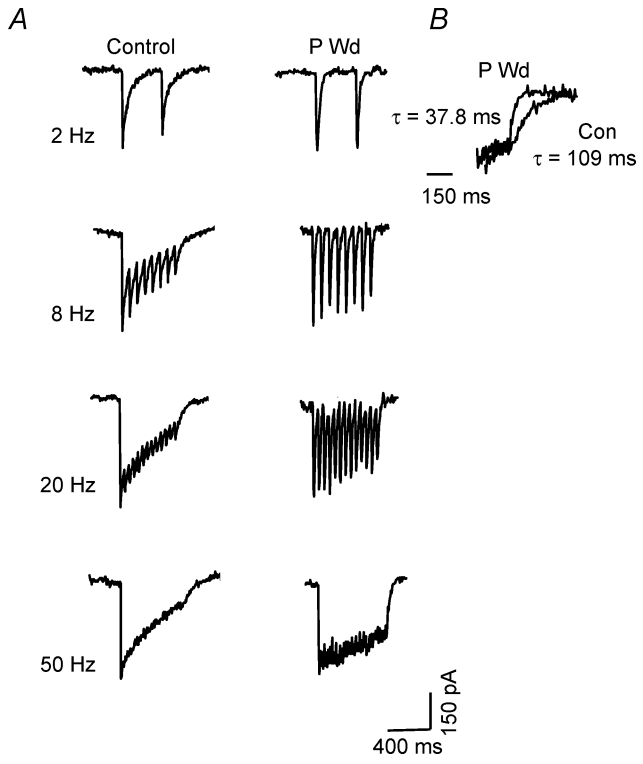


Figure 3. Desensitization in response to episodic agonist application is attenuated following progesterone withdrawal
A, representative traces illustrate responses of pyramidal cells to trains of 1 ms GABA (10 mM) pulses applied at frequencies of 2, 8, 20 or 50 Hz. Following progesterone withdrawal (P Wd), desensitization developed at higher agonist application frequencies than seen for control, first apparent at 8 Hz and reaching a maximum of 18% at 50 Hz application frequencies. In contrast, under control conditions, desensitization was apparent with 2 Hz GABA pulses (200 ms interpulse interval) and reached an 84% maximum desensitization at a 50 Hz application. *B*, deactivation following 50 Hz GABA application was ($P < 0.001$) faster following P withdrawal (38.2 ± 4.3 ms) compared to control (110.2 ± 5.6 ms). ($n = 12$–16 cells/group).

Using the 50 Hz GABA pulse application protocol, the rate of deactivation was also assessed following agonist washout (Fig. 3*B*). Deactivation following pulse agonist application was faster in ~50% of the cases tested ($\tau_w = 40.2 \pm 8.2$ ms, P Wd *versus* $\tau_w = 116 \pm 15.3$ ms, Control, $P < 0.05$).

Desensitization – prolonged agonist exposure

For this study, saturating concentrations of GABA were applied continuously for either 400 ms or 5 s with rapid onset and washout of agonist provided by the piezo-electric delivery system. Both desensitization and deactivation rate constants were determined.

Following 48 h 3α,5β-THP exposure, the extent of desensitization in patches from CA1 hippocampal pyramidal cells exposed to 5 mM GABA for 5 s was only 36% compared with 93% in control patches ($P < 0.001$, Fig. 4, Table 2). In addition, the rate of desensitization was also significantly slower after steroid treatment compared to control (Fig. 4, Table 2). This comparison is more easily made with the weighted time constants: $\tau_w = 2550 \pm 265$ ms, 48 h 3α,5β-THP *versus* 1148 ± 272 ms, Control ($P < 0.01$). When the individual exponential components were evaluated, desensitization was best fitted as a three-exponential decay for control patches, as reported by others (Celentano & Wong, 1994; Jones & Westbrook, 1995; Haas & Macdonald, 1999; Celentano & Hawkes, 2004), while 48 h 3α,5β-THP treatment resulted in desensitization kinetics best fitted as a two-exponential decay. Both τ_{fast} and $\tau_{slow,1}$ were significantly faster for control *versus* steroid treatment conditions (Table 2), with the faster values of τ representing a greater fraction of the desensitizing current for control traces. However, a smaller percentage of the cells recorded (15%) following steroid treatment exhibited a faster rate of desensitization (not shown), $\tau_w = 620$ ms, than the observed control traces. Deactivation, following washout of agonist after a 5 s application, also reflected a bimodal distribution following steroid treatment, with values either faster (20–27 ms) or not significantly different (150–200 ms) from control (80–170 ms), similar to the bimodal distribution of τ_{slow}.

Desensitization in response to continuous application of GABA for 400 ms was also attenuated following

Table 2. Desensitization time constants

Group	Control	P Wd	Control	48 h THP
Duration of GABA application	400 ms	400 ms	5 s	5 s
τ_{fast}	31.1 ± 4.05	97.6 ± 17.1*	18.5 ± 4.2	63.2 ± 8.5*
% of total desensitization	80 ± 11	5 ± 2	34 ± 5	18 ± 4
$\tau_{slow,1}$	308.6 ± 44.1	2888 ± 274*	205.1 ± 31.2	3100 ± 280*
% of total desensitization	18 ± 2	95 ± 1	14 ± 2	82 ± 11
$\tau_{slow,2}$			2156 ± 236	
% of total desensitization			52 ± 7	
Weighted τ	87.3 ± 21.1	2546 ± 433*	1148 ± 272	2550 ± 265*
% Desensitization	86.1 ± 7.8	28.5 ± 3.4*	92.6 ± 5.6	36.2 ± 2.3*

Average values of τ (means ± S.E.M. in milliseconds) calculated for multiexponential desensitization kinetics and the combined weighted τ. Values were assessed following prolonged application of 5 mM GABA (400 ms, left or 5 s, right) to outside-out patches using a theta tube. Progesterone withdrawal (P Wd) and 48 h treatment with 3α,5β-THP (48 h THP) both resulted in a slower rate of desensitization best fitted as a biexponential function compared to control, where a three-exponential fit was optimal. In addition, the extent of desensitization compared to the peak current (% desensitization) was attenuated following steroid treatment. (n = 20–25 excised patches/group, *$P < 0.05$ *versus* control values).

P withdrawal (Table 2): $\tau_w = 2545.6 \pm 433$ ms, compared to control ($\tau_w = 87.3 \pm 21.1$ ms), with the desensitization τ calculated independently of the slowest component ($\tau_{slow,2}$). This shorter exposure time is sufficient to compare the fastest two components of desensitization across control and experimental groups. In this case, the difference in decay time constants observed across steroid treatment groups is highly significant ($P < 0.001$), suggesting that the greatest difference in decay times for desensitization is observed during the initial period of desensitization for P withdrawal *versus* control. After P withdrawal, the extent of desensitization was markedly attenuated to about 29% of the maximal GABA-gated current, compared to an 86% desensitization observed under control conditions (Table 2), similar to results obtained with repetitive agonist application (Fig. 3*A*). Deactivation following removal of agonist after sustained administration was also faster after P withdrawal ($\tau_w = 27$ *versus* 79 ms, control, $P < 0.05$), as predicted by deactivation kinetics following brief exposure (Fig. 1*A*, Table 1). Thus, both steroid administration protocols decreased the rate and extent of desensitization compared to control conditions.

Recovery from fast desensitization

According to the Jones-Westbrook GABAR model (1995) an observed change in the τ_{slow} of deactivation could be a result of a change in the rate of recovery from fast desensitization. Therefore, we assessed this parameter in outside-out patches by measuring the current response to the second of two paired 1 ms pulses of 5–10 mM GABA delivered at varying interpulse intervals (Fig. 5*A*).

Using this protocol, the rate of recovery from fast desensitization was significantly accelerated following P withdrawal or 48 h 3α,5β-THP exposure (Fig. 5*A* and *B*) compared to control. The time course of recovery was best described by a biexponential function, as has been reported previously (Jones & Westbrook, 1995). Values of both τ_{fast} and τ_{slow} were significantly reduced following the steroid treatment protocols compared to control ($\tau_{fast} = 13.4 \pm 2.0$ ms, control, *versus* $\tau_{fast} = 5.6 \pm 0.67$ ms, P Wd and $\tau_{fast} = 6.0 \pm 0.41$ ms, 48 h 3α,5β-THP, $P < 0.05$; $\tau_{slow} = 210 \pm 31.3$ ms, control, *versus* $\tau_{slow} = 54 \pm 4.8$ ms, P Wd and $\tau_{slow} = 75 \pm 6.2$ ms, 48 h 3α,5β-THP, $P < 0.05$). In addition, a greater fraction of the current was carried by the fast component following the steroid protocols (78–80%, P Wd and 48 h 3α,5β-THP, *versus* 21%, control).

Kinetic modelling

In order to explore the microscopic parameters which might predict the observed change in macroscopic kinetics obtained following steroid treatment, a biliganded model of the GABAR was employed, based on Jones & Westbrook (1995) and Celentano & Wong (1994), but simplified to reduce the number of free parameters. Briefly, the model included the predominant biliganded state with a single open state as well as fast and slow desensitized states. In order to reduce the number of free parameters, the binding of two molecules of GABA was reduced to a single binding step. Rate constants were approximated from values reported in a number of studies (Jones & Westbrook, 1995; Fisher & Macdonald, 1997; Bai *et al.* 1999; Haas & Macdonald, 1999; Shen *et al.* 2000; Mozrzymas *et al.* 2003) and combined with those approximated here from the desensitization studies. Computer simulations of macroscopic current in response to a 1 ms pulse of agonist (Fig. 6*A*) resulted in a deactivation similar to the control traces (Fig. 1*A*) with a biexponential decay: $\tau_{fast} = 10$ ms; $\tau_{slow} = 80$ ms (Model 1, Fig. 6*A*). Desensitization in response to a 400 ms pulse of agonist resulted in a 75% desensitization,

with a $\tau_{fast} = 9$ ms; $\tau_{slow} = 379$ ms, approximating the control values reported in Fig. 4. Deactivation following desensitization was identical to τ_{slow}, 80 ms.

A number of alterations in the rate constants were tested to determine which would predict a deactivation and desensitization time course similar to that observed following steroid exposure. The Jones-Westbrook model predicts that a faster recovery from the fast desensitized state, as observed following steroid treatment, would accelerate the slow component of deactivation. Amending the control model to incorporate an increased rate of recovery from D_{fast}, r_f (Model II, Fig. 6*A*) indeed yielded a faster τ_{slow} of deactivation, but was insufficient to model the steroid data. This current appeared to exhibit a monoexponential decay, because τ_{fast} was equal to τ_{slow} under these conditions. In contrast, for the biological data, τ_{fast} was consistently faster than τ_{slow}. Because, we have shown increased expression of $\alpha 4\beta\delta$ GABAR following P withdrawal (Sundstrom-Poromaa *et al.* 2002), we also incorporated a decrease in α and an increase in β, to approximate values derived from single channel recordings of δ-containing GABAR (Fisher & Macdonald, 1997; Haas & Macdonald, 1999; Akk *et al.* 2004). This combination of changes to the model yielded a current response to a 1 ms application of agonist which closely replicated current deactivation following steroid treatment, with acceleration in both τ_{fast} and τ_{slow} (4.7 ms and 36 ms, respectively, Fig. 6*A*). Use of such a model also effectively reflected current desensitization where the extent (36% *versus* 75%, respectively) and rate of desensitization were reduced compared to control (Fig. 6*A*). In the absence of a significant fast desensitized state, prolongation of τ_{slow} could be accomplished with incorporation of a slower k_{off} (Model IV, Fig. 6*A*), consistent with the findings of Chang & Weiss (1999), and reflecting the second population of currents recorded following steroid treatment ($\tau_{fast} = 4$ ms, $\tau_{slow} = 90$ ms). Values for the 10–90% rise time using these simulations closely corresponded to those obtained with patch recordings (0.8–0.9 ms, Models III and IV *versus* 1.2 ms, Model I).

Models I and III were also tested for their ability to simulate the rate of recovery from fast desensitization for control and steroid withdrawal conditions, respectively (Fig. 6*B*). These simulations approximated the actual data in their relative rates of recovery, estimated as biexponential decays: Model I produced values of τ_{fast} and τ_{slow} for recovery which were markedly slower than calculated for Model III. Values for the 80% recovery time estimated for Models I and III (approximately 220 and 30 ms, respectively) were close to values estimated from the data for control and P withdrawal conditions (approximately 270 and 30 ms, respectively). Thus, these models were successful in approximating deactivation, desensitization and recovery from fast desensitization under control and steroid withdrawal conditions. Therefore, the models most successful in simulating steroid withdrawal kinetics incorporated both an accelerated rate of recovery from fast desensitization, as well as shorter mean open times consistent with increased expression of δ-containing GABAR.

Discussion

The results from this study indicate that steroid withdrawal alters the kinetics of GABA-gated current in pyramidal cells of CA1 hippocampus, producing faster deactivation and slower desensitization. Similar changes in kinetics

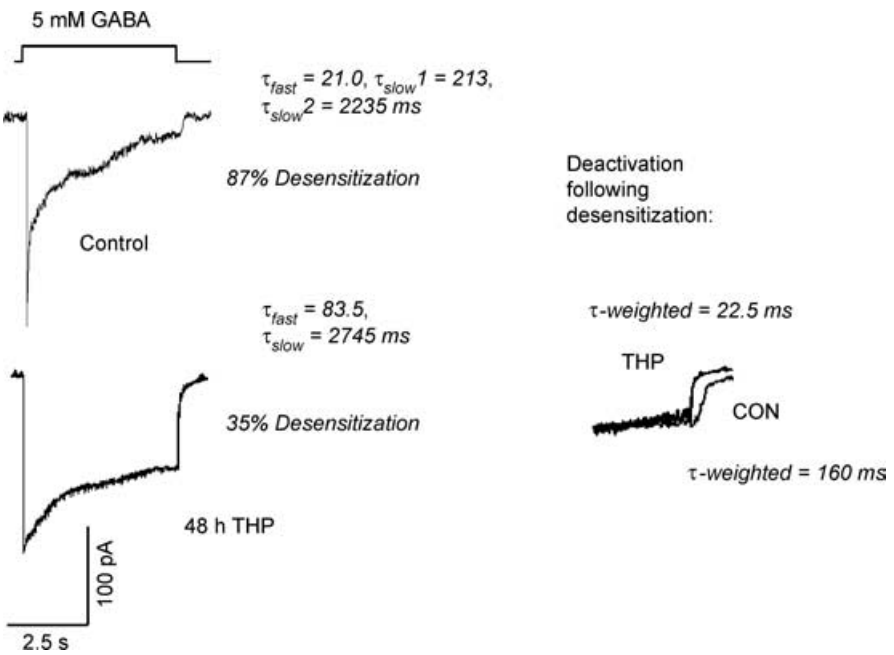


Figure 4. Desensitization in response to prolonged agonist exposure is attenuated following steroid treatment
Representative traces illustrate significant attenuation in both the rate and degree of desensitization of GABA response following 48 h treatment with 3α,5β-THP (48 h THP). Desensitization kinetics (τ_{fast}, τ_{slow}) were determined for 5 s exposure to GABA (5 mM) using outside-out patches of membrane from acutely isolated CA1 hippocampal pyramidal cells following steroid treatment. Inset: deactivation following this prolonged exposure period was also significantly ($P < 0.01$) faster following steroid pretreatment (THP, average weighted $\tau = 20.45 \pm 9.2$ ms) compared to control (CON, average weighted $\tau = 148.11 \pm 18.8$ ms). ($n = 20$–25 patches/group).

were observed following 48 h exposure to 3α,5β-THP. We suggest that the decrease in deactivation τ, which would decrease the total charge transfer for inhibitory current, may contribute to the neuronal excitability which characterizes steroid withdrawal (Smith *et al.* 1998*a*; Hsu & Smith, 2003), and is similar to the withdrawal hyperexcitability of other GABA-modulatory compounds (Xie & Tietz, 1991; Kang *et al.* 1996).

One possible mechanism for the decrease in τ_{fast} produced by steroid administration and withdrawal is via α4-containing GABARs, which we have shown are increased by these steroid protocols (Smith *et al.* 1998*a*; Gulinello *et al.* 2001). Results from the present study demonstrate that recombinant $\alpha4\beta2\delta$ and $\alpha4\beta2\gamma2$ GABARs deactivate with an accelerated τ_{fast}, compared to $\alpha1\beta2\gamma2$ and $\alpha5\beta2\gamma2$, which are normally highly expressed in CA1 hippocampal pyramidal cells (Wisden *et al.* 1992; Nusser *et al.* 1996; Crestani *et al.* 2002; Liang *et al.* 2004). We have previously shown that 48 h treatment with 3α,5β-THP also results in miniature inhibitory postsynaptic currents (mIPSCs) which deactivate with an accelerated τ_{fast} (Hsu *et al.* 2003). In this study, τ_{fast} was prolonged when α4 expression was suppressed with *in vivo* antisense treatment, suggesting that increased expression of α4-containing GABAR resulted in this change in kinetics (Hsu *et al.* 2003). Other conditions, such as ethanol withdrawal, which increase hippocampal α4 expression also produce acceleration in the τ_{fast} of mIPSCs (Cagetti *et al.* 2003). In a separate study, suppression of α4 expression prevented the faster decay of GABA-gated current recorded following P withdrawal, which was assessed using slower agonist exposure times (Smith *et al.* 1998*a*). Taken together, these findings support the idea that increases in α4 expression underlie the faster deactivation τ observed in the present study after steroid exposure and withdrawal.

A number of reports have demonstrated that $\alpha2\beta\gamma2$ (Lavoie *et al.* 1997), $\alpha3\beta\gamma2$ (Gingrich *et al.* 1995) and $\alpha6\beta\gamma2$ (Tia *et al.* 1996) GABARs deactivate more slowly than $\alpha1\beta2\gamma2$. Taken together with the present data, these findings suggest that α4-containing GABARs may exhibit the fastest deactivation rates of the diverse population of GABAR subunit combinations which have been evaluated to date.

Unlike τ_{fast}, values for τ_{slow}, assessed in the present study, displayed a bimodal distribution following steroid treatment. This bimodal pattern could be a result of two different GABAR isoforms with altered kinetics or result from state-dependent changes in a single isoform. The first possibility is more likely, as the $\alpha4\beta\delta$ and $\alpha4\beta\gamma2$ receptors, which are increased by these steroid administration protocols (Smith *et al.* 1998*a*; Sundstrom-Poromaa *et al.* 2002), were shown in the present study to deactivate with slow components similar to the two modes distinguished after steroid treatment. Alternatively, recent reports have identified modal gating patterns in ligand-gated receptors such as NMDA and acetylcholine receptors (Naranjo & Brehm, 1993; Popescu & Auerbach, 2003), which result from transitions of the fully liganded receptor to open and closed states. In these studies, modal gating at the single channel level produced alterations in decay of synaptic

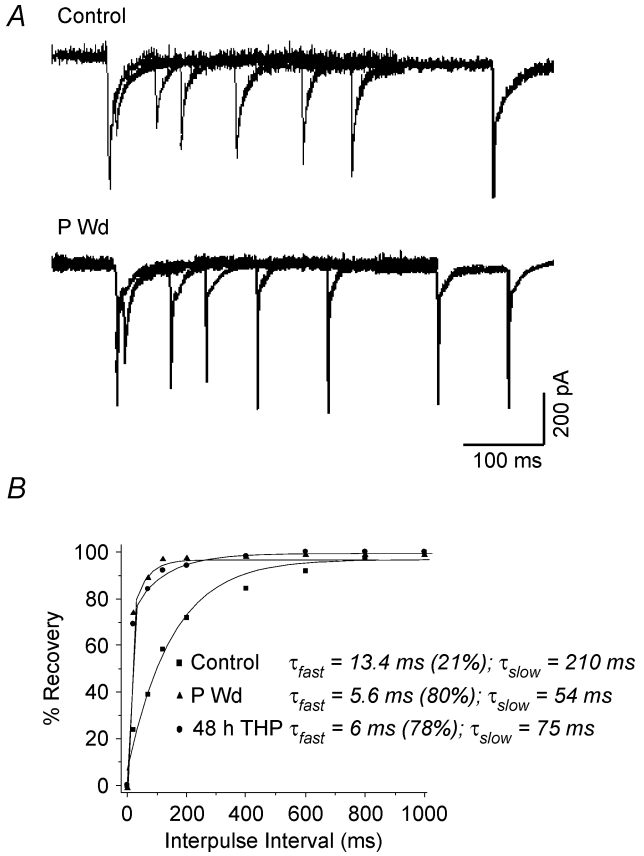
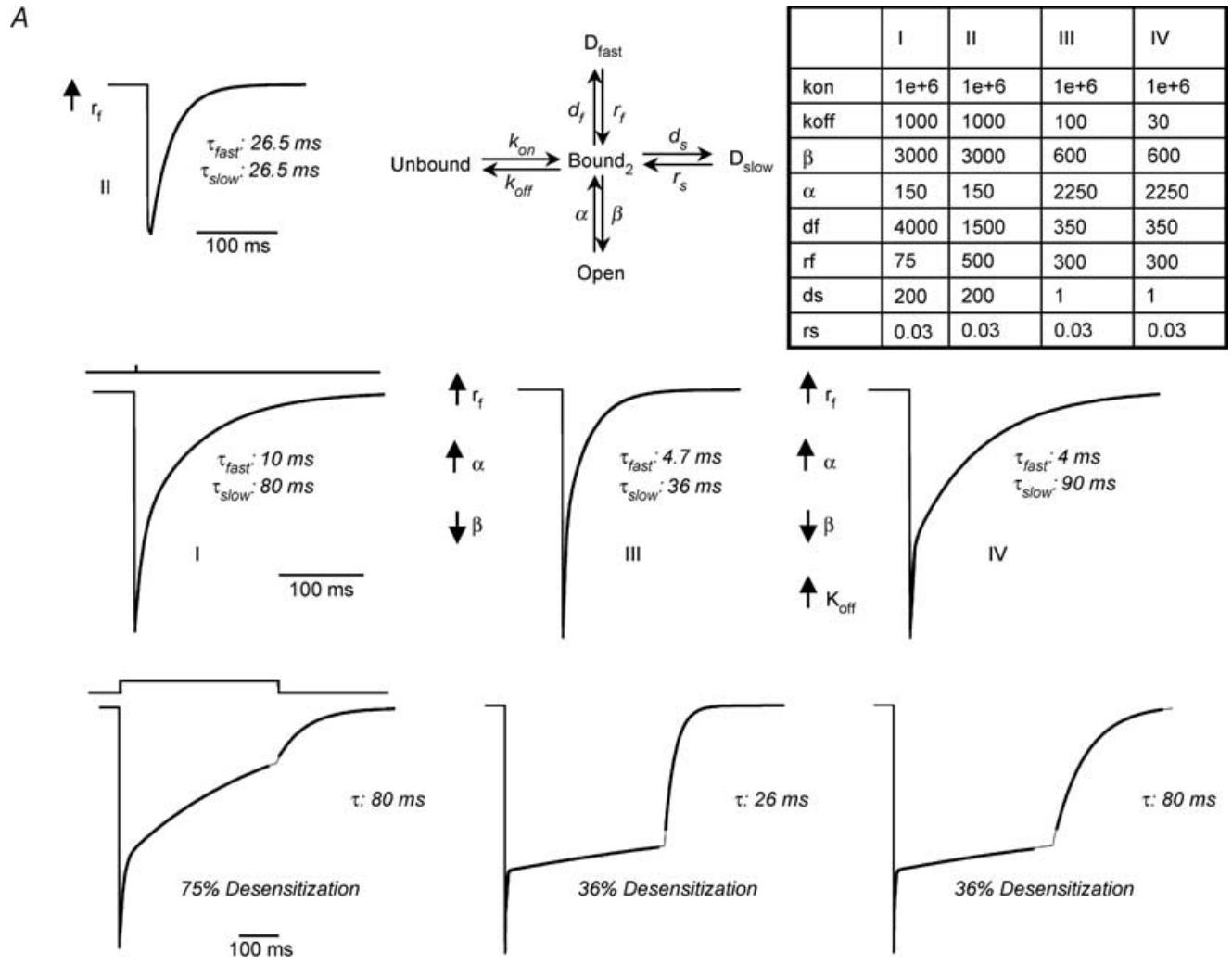
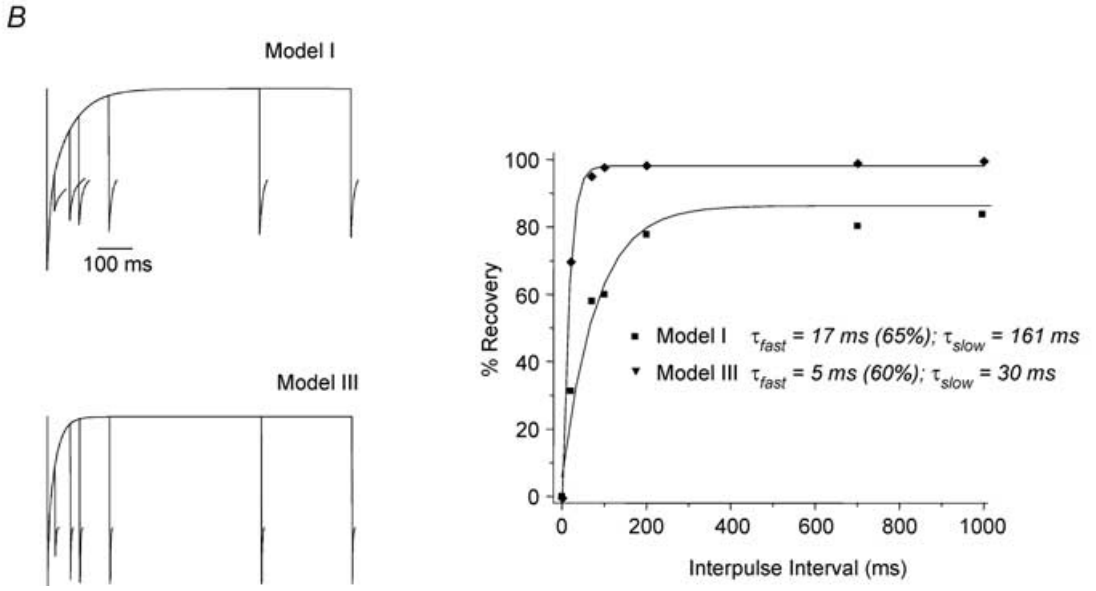


Figure 5. Recovery from fast desensitization is accelerated following progesterone withdrawal
A, superimposed currents gated by paired 1 ms pulses of 10 mM GABA, at varying interpulse intervals (20– 2000 ms) are depicted for control, progesterone withdrawal (P Wd) or 48 h treatment with 3α,5β-THP (48 h THP). Following both steroid protocols, the extent of fast desensitization was reduced, and the rate of recovery from this desensitized state was accelerated compared to control as determined by the amplitude of the second GABA response relative to the first. *B*, the percentage recovery of current amplitude for the second GABA response relative to the first represents recovery from the fast desensitized state (D_{fast}), and is plotted as a function of the interpulse interval for averaged datapoints from both groups. Percentage recovery was calculated as $((Amp_{test} - Onset_{test})/(Amp_{init} - Onset_{test})) \times 100$, where Amp_{init} is the amplitude of the initial GABA response, Amp_{test} is the amplitude of the second (test) GABA response, and $Onset_{test}$ is the value of the current at the onset of the second response. In all cases, the amplitude of the initial response was normalized to its maximal value during the experiment to account for variability in current. Each point represents the average from 8 to 10 different patches from 5 to 6 animals. The rate of recovery was best fitted by a biexponential equation, which was markedly faster following the steroid treatment protocols compared to control ($P < 0.05$). ($n = 8$–10 samples per point).



	I	II	III	IV
kon	1e+6	1e+6	1e+6	1e+6
koff	1000	1000	100	30
β	3000	3000	600	600
α	150	150	2250	2250
df	4000	1500	350	350
rf	75	500	300	300
ds	200	200	1	1
rs	0.03	0.03	0.03	0.03



current comparable to the bimodal distribution reported here. Modal gating cannot be ruled out in the present study, where it could result from spontaneous thermodynamic changes in a single GABAR isoform or post-translational mechanisms.

The GABAR kinetics recorded after steroid treatment and withdrawal are similar to those reported for δ-containing GABARs which deactivate more quickly and exhibit much less desensitization (Haas & Macdonald, 1999; Bianchi *et al.* 2001; Brown *et al.* 2002) than most commonly expressed GABAR subtypes in CA1 hippocampus (Gingrich *et al.* 1995; Burgard *et al.* 1996; Lavoie *et al.* 1997; Haas & Macdonald, 1999; Bianchi *et al.* 2001), although variations in $\alpha\beta\gamma2$ kinetics have been reported (Boileau *et al.* 2003). δ-Containing GABARs also desensitize with a two-exponential decay (Haas & Macdonald, 1999; Bianchi *et al.* 2001) similar to the desensitization kinetics we report here following steroid treatment. This contrasts with the three-exponential decay (Celentano & Wong, 1994; Haas & Macdonald, 1999; Celentano & Hawkes, 2004) reported for desensitization of native hippocampal GABA-gated currents and recombinant $\alpha1\beta\gamma2$ GABAR. These similarities between our kinetic findings following steroid treatment/withdrawal and those exhibited by $\alpha4\beta\delta$ and $\alpha4\beta\gamma2$ GABARs suggest that these receptors may mediate the faster deactivation and slower desensitization observed following steroid exposure and withdrawal.

In determining the microscopic rate constants which might change in order to produce the macroscopic changes observed after steroid treatment, we implemented a receptor model. Although multiple models for GABAR binding and gating have been proposed (Jones & Westbrook, 1995; Lavoie *et al.* 1997; Bianchi *et al.* 2001; Burkat *et al.* 2001; Weiss & Magleby, 2001; Chang *et al.* 2002; Mozrzymas *et al.* 2003; Celentano & Hawkes, 2004), the model of Jones & Westbrook (1995) is useful in relating deactivation and desensitization rates. Increases in the fast desensitized state of the receptor prolong deactivation, by delaying unbinding and subsequent relaxation of the channel. Both steroids and anaesthetics can prolong deactivation by delaying recovery from this fast desensitized state (Zhu & Vicini, 1997; Bai *et al.* 1999; Banks & Pearce, 1999; Shen *et al.* 2000). In the present study, the accelerated rate of recovery from D_{fast} after the steroid protocols reduced τ_{slow} in the model, but required additional reductions in β, to reflect the decreased mean open time and reduced open probability for δ-containing GABAR, to accurately simulate the data. In contrast to τ_{fast}, the bimodal population of values for τ_{slow} may be reflected by differences in k_{off}, in agreement with recent studies (Chang & Weiss, 1999). These changes would stabilize the bound, activatable state of the receptor, which may then yield a receptor which is highly modifiable, as demonstrated for δ-containing GABAR, which have increased sensitivity to ethanol (Sundstrom-Poromaa *et al.* 2002; Wallner *et al.* 2003) and neurosteroids (Bianchi *et al.* 2002; Stell *et al.* 2003).

Consistent with the modelling results, single channel recording experiments have established a mean open time for $\alpha4\beta2\gamma2$ GABAR (Akk *et al.* 2004) which is approximately one-half of that reported for $\alpha1\beta\gamma2$ receptors (Fisher & Macdonald, 1997). δ-Containing GABARs, including $\alpha4\beta2\delta$, have a yet lower open probability than $\alpha4\beta2\gamma2$ (Akk *et al.* 2004) and lack the longest open channel time that is reported for $\alpha1\beta\gamma2$, resulting in a mean open time only one-third that established for $\alpha1\beta\gamma2$ (Fisher & Macdonald, 1997; Haas & Macdonald, 1999; Bianchi *et al.* 2001; Bianchi *et al.* 2002).

The currents recorded in the present study may largely represent currents gated by extrasynaptic receptors. In fact, $\alpha4\beta\delta$ GABARs, which are increased by steroid treatment/withdrawal, are extrasynaptic or perisynaptic (Wei *et al.* 2003). Under conditions of increased activity from GABAergic afferents, spillover (Wei *et al.* 2003) from adjacent synapses may then access the peri-synaptic receptor population, to act as a resistive

Figure 6. Computer simulation of GABAR gating following steroid treatment
A simplified biliganded model (*A*, upper part, centre), based on Jones & Westbrook (1995) and Celentano & Wong (1994) was modified to simulate the data from this study. It includes one open state and two desensitized states, with rate constants (table, upper right) derived from single channel data, modified from other models and approximated from desensitization data from the present study. *A*, Model I results in simulated current with deactivation and desensitization kinetics similar to that from control hippocampal pyramidal cell patches. Incorporation of a more rapid rate of recovery from the fast desensitized state ($\uparrow r_f$) markedly accelerated τ_{slow} (Model II, inset), consistent with the Jones-Westbrook model, but failed to modify τ_{fast}. Additionally, incorporation of $\uparrow\alpha$ and $\downarrow\beta$ to replicate single channel properties of δ-containing GABAR (Model III) replicated one subpopulation of currents following steroid treatment, with acceleration in both τ_{fast} and τ_{slow}. The second population of currents recorded following steroid treatment (faster τ_{fast} only) was simulated by additionally incorporating a $\downarrow k_{off}$ (Model IV). Bottom panel, simulations resulted in markedly different rates and extent of desensitization, comparable to those obtained from control (75% desensitization) and steroid-treated animals (36% desensitization). *B*, rate of recovery from fast desensitization using a paired pulse protocol. Models I and III simulate the relative differences between control and P Wd data, respectively. Left, simulated traces. (The current response to the second agonist application is truncated.) Right, percentage recovery from the fast desensitized state, estimated as a biexponential decay.

shunt to decrease excitability (Brickley *et al.* 2001; Bai *et al.* 2001; Hamann *et al.* 2002; Nusser & Mody, 2002; Wei *et al.* 2003; Yeung *et al.* 2003). In the absence of a change in the total GABAR population, as suggested by similar peak current amplitudes following steroid treatment/withdrawal (Smith *et al.* 1998*a*), steroid-induced increases in these extrasynaptic GABARs which deactivate quickly, would result in less inhibition during transient spill-over events (Wei *et al.* 2003) than would slower deactivating GABARs (such as $\alpha5\beta\gamma2$) found under control conditions (Crestani *et al.* 2002; Caraiscos *et al.* 2004). However, following steroid withdrawal, more prolonged activation of GABARs at sites distant from the synapse would increase inhibition due to their relative lack of desensitization. Thus, the effect of altered GABAR kinetics observed after steroid withdrawal may depend upon the ambient activity of inhibitory afferents to individual pyramidal cells which would determine the time course of transmitter exposure.

Conclusions

In conclusion, the results from the present study suggest that 48 h exposure to and withdrawal from the GABA-modulatory steroid $3\alpha,5\alpha/\beta$-THP produces GABARs which deactivate quickly, due at least in part to a decrease in the fast desensitized state. The resulting decrease in inhibition may serve as a mechanism for alterations in mood, susceptibility to seizures and CNS activation associated with fluctuations in endogenous steroids across the menstrual cycle.

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