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Effects of risperidone, an atypical antipsychotic drug, on excitatory synaptic responses in the perforant path–dentate gyrus pathway in chronically prepared rabbits

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Abstract

The effects of an atypical antipsychotic drug, risperidone, were examined on excitatory synaptic responses in the dentate gyrus by single electrical stimulations to the perforant path and the induction of long-term potentiation (LTP) in this pathway in chronically prepared rabbits. Any of 0.5, 1.0 and 2.0 mg/kg doses of risperidone intraperitoneally injected had virtually no effect on the excitatory synaptic responses. However, these three doses of risperidone dose-dependently suppressed the LTP induction. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Risperidone; Perforant path–dentate gyrus pathway; Long-term potentiation

1. Introduction

5-HT and dopamine receptor antagonists, clozapine and risperidone, are atypical antipsychotics because of their effectiveness in treating polar and refractory symptoms in schizophrenia, i.e. negative symptoms such as abulia, autism and affective flattening, and their low potential to induce extrapyramidal side effects. In these respects, clozapine and risperidone are supposed to have a different pharmacological profile from typical antipsychotics such as haloperidol. We previously found that clozapine, 20 mg/kg intraperitoneally injected, potentiated excitatory synaptic responses elicited in the dentate gyrus by single electrical stimulations to the perforant path in chronically prepared rabbits and that the potentiation lasted for an observation period of at least 7 days. We called this phenomenon ''clozapine-induced potentiation'' (Kubota et al., 1996). Further, in a recent study, we found that a noncompetitive NMDA receptor antagonist, dizocilpine 1.0 mg/kg, completely prevented clozapine-induced potentiation (Kubota et al., 2000). This finding indicated that clozapine-induced potentiation is caused by NMDA receptor activation. On the other hand, it has been reported that such a potentiation was not induced by haloperidol (Arvanov et al., 1997; Jibiki et al., 1993; Krug et al., 1983). In particular, we have recently found that even haloperidol at low doses of 0.1 or 0.4 mg/kg injected intraperitoneally, as well as at a high dose, 0.8 mg/kg, in a previous study (Jibiki et al., 1993), exerted no such potentiation on the excitatory synaptic responses (Kubota et al., unpublished). However, haloperidol suppressed the induction of long-term potentiation (LTP) in the perforant path–dentate gyrus pathway in chronically prepared rabbits (Jibiki et al., 1993), whereas clozapine showed no inhibitory effect on the LTP induction (Kubota et al., 1996). Recently, a phenomenon similar to clozapine-induced potentiation has been reported by Arvanov et al. (1997) who found that the bath administration of clozapine potentiated NMDA-evoked responses in a concentration-dependent manner in the pyramidal cells of the medial prefrontal cortex in rat brain slices.

On the other hand, a role of a subtype of the glutamate receptors, the NMDA receptor, in the pathogenesis of schizophrenia has been recently suggested, as represented by so-called phencyclidine (PCP) psychosis, which signifies schizophrenia-like psychotic symptoms caused by intake of the noncompetitive NMDA receptor antagonist PCP) in humans (Olney and Farber, 1995).

It is well known that antipsychotic drugs, including clozapine, have only a low affinity for binding sites of glutamate receptors, whereas they show a high affinity for

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those of monoamine receptors such as the dopamine, 5-HT receptors and adrenoceptors (Arnt and Skarsfeldt, 1998). Therefore, it is unlikely that such a facilitating effect of clozapine on excitatory synaptic transmission results from a direct interaction with the glutamate receptors. Currently, the mechanisms underlying the extraordinary action of antipsychotics on excitatory or glutamatergic neurotransmission such as clozapine-induced potentiation are unknown.

Risperidone and clozapine share a pharmacological similarity in respect of their high affinity for 5-HT_{2A} receptor rather than dopamine D_2 receptors, although the both are different in detailed pharmacological profiles regarding their affinity for various subtypes of neurotransmitter receptors (Arnt and Skarsfeldt, 1998). In the present study, we investigated whether another type of atypical antipsychotic, risperidone, showed clozapine-like effects on the excitatory synaptic responses elicited in the dentate gyrus by single electrical stimulations to the perforant path and the LTP induction in this pathway.

2. Method

2.1. Animal model

Chronic experiments were carried out on 20 adult male rabbits weighing 2.5–3.5 kg. Each surgical procedure was conducted under intraperitoneal pentobarbital sodium anesthesia (20–30 mg/kg). A tungsten microelectrode for recordings (tip diameter: 1–2 μm, resistance: 1–5 kΩ) and a concentric stimulating electrode for laminar analysis (0.6 mm in diameter) were attached to a holder with the tips aligned 1 mm apart. The tungsten microelectrode was connected to a memory oscilloscope (Nihon Kohden: VC10, bandpath: 0.08–3000 Hz) through a preamplifier. After unilateral craniotomy, these electrodes were inserted from the pial surface at the P4 and L6 position on Ridge's map to the dentate gyrus, using an oil hydraulic microdrive (Narishige), with laminar analysis every 50 or 100 μm, as in the previous study (Jibiki et al., 1993; Kubota et al., 1994, 1996). Next, another concentric stimulating electrode was inserted from the pial surface at the P4 and L1 position to the perforant path ipsilateral to the dentate gyrus (Fig. 1) while observing the maximal responses elicited in the dentate gyrus by single shocks at a constant intensity delivered from the stimulating electrode. After a 10-day postsurgical recovery period, chronic experiments were performed as below. Animal care and use procedures were in accordance with approved protocols of the Animal Research Committee of the Kanazawa Medical University. In addition, risperidone was obtained from Janssen Pharmceutica (Belgium).

2.2. Experiment 1

In 5 of 20 rabbits, control experiments were performed to examine the magnitude of excitatory synaptic responses in the dentate gyrus. The threshold intensities of single shocks to the perforant path for inducing population spikes in the dentate gyrus were initially examined. The intensities just above the threshold were determined as those of single shocks to elicit control responses, which consisted of a small population spike with an amplitude of less than 0.5 mV preceded by the leading edge population EPSPs of a low positive wave, and the subsequent slow component (Kubota et al., 1994). Then, the baseline recording was performed for 30 min with single stimulus at a fixed intensity (monopolar square pulse of 0.2–0.5 ms duration, 400–800 μA, 30 s stimuli intervals). Next, vehicle solution [dimethylsulfoxide (0.5 ml)] was administered as a single injection intraperitoneally. Soon thereafter, single stimulus at a fixed intensity, the same as used for the control recording, was given to the perforant path for 60 min to observe the response changes in the dentate gyrus. Next, a tetanic stimulation for inducing LTP was delivered to the perforant path. It is well known that LTP is easily produced in the perforant path–dentate gyrus pathway, as shown in previous studies (Jibiki et al., 1993; Kubota et al., 1994, 1996). The tetanic stimulation was repeated three times at 3-min intervals. The stimulus parameters were monopolar square pulses of 0.2–0.4 ms duration, 200–600 μA, 60 Hz and 1 s in total duration. Soon thereafter, single shocks at the fixed intensity were delivered again for about 30 min to observe the response in the dentate gyrus.

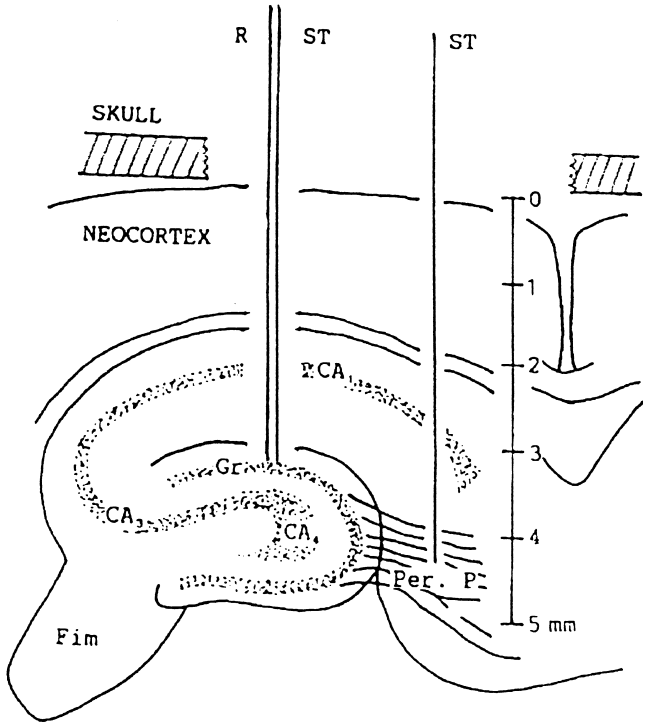

Fig. 1. Experimental schema of rabbit brain. CA1, CA3 and CA4 show hippocampal regions, respectively. Fim, fimbria; Gr, granular cell layer; Per. P, perforant path; R, a tungsten recording electrode; ST, stimulating electrode.

2.3. Experiment 2

In 15 rabbits, experiments were performed to examine whether changes in the excitatory synaptic responses and LTP were induced after risperidone administration. The baseline recording was initially performed like in Experiment 1, after which risperidone dissolved in dimethylsulfoxide (0.5 ml) was administered as a single injection intraperitoneally. The doses of risperidone were 0.5, 1.0 and 2.0 mg/kg, using five rabbits for each group. Soon after the risperidone injection, single shocks at the fixed intensity were again given to the perforant path for 60 min to observe the response changes like in Experiment 1. Next, LTP-inducing tetanic stimulation was delivered to the perforant path like in Experiment 1. Soon thereafter, single shocks at the fixed intensity were delivered again for about 30 min.

2.4. Data analysis

In each experiment, four sets of responses were averaged using a DAT 1100 (Nihon Kohden) and recorded with an X–Y recorder. To analyze the response changes, the amplitude of the population spike and the slope of the population EPSP were measured like in previous studies (Jibiki et al., 1993; Kubota et al., 1994, 1996). Both the population spike amplitudes and the EPSP slopes in the 60 responses averaged during the whole experimental time of 120 min were first analyzed by repeated-measure analysis of variance (ANOVA) to examine whether there were significant differences between the four groups, i.e. the control group of Experiment 1 and 0.5, 1.0 and 2.0 mg/kg dose groups of risperidfone of Experiment 2. Then, the respective values in the 15 responses averaged over 30 min in each of the four sessions, i.e. baseline, after vehicle or risperidone injection, the first half and latter half in the observation period of 60 min after risperidone injection, and after tetanus were analyzed by one-way ANOVA followed by Scheffe's multiple comparison to examine whether they differed significantly between the four sessions in each group and whether they differed significantly between the four groups in each session.

3. Results

3.1. Experiment 1

In all five rabbits in Experiment 1, the baseline responses were virtually unaltered during the baseline recordings. After subsequent vehicle injection, the responses showed no changes either during the 60 min observation period after the administration of vehicle with regard to both the population spikes and the EPSP slopes. Next, the responses were markedly potentiated soon after the tetanic stimulations, with regard to population spikes and EPSP slope, showing the induction of LTP (Fig. 2; control and as to real responses to single shocks, refer to Fig. 1 in Kubota et al., 1994, p. 172).

One-way ANOVA showed significant differences among the four sessions for both the population spike amplitudes and EPSP slopes [main time course effect, $F(3,16)=8.718$, $P=.001$ in population spike and $F(3,16)=5.889$, $P=.006$ in EPSP]. The subsequent Scheffe's multiple comparison showed no significant differences between baseline and the first half after vehicle injection ($P=.999$ in population spike, $P=.999$ in EPSP) and the latter half after vehicle injection ($P=.999$ in population spike, $P=.999$ in EPSP), whereas they showed significant differences between baseline and after tetanus ($P=.006$ in population spike, $P=.029$ in EPSP) with regard to both the population spike amplitudes and the EPSP slopes.

3.2. Experiment 2

In the risperidone 0.5 mg/kg dose group, the responses were virtually unaltered during the baseline recordings and during the 60 min observation period after risperidone administration. After tetanic stimulation, the responses to single shocks looked potentiated as compared with the previous responses for both population spike amplitudes and EPSP slopes, seemingly showing the induction of LTP in all five rabbits (Fig. 2; risperidone 0.5 mg/kg).

One-way ANOVA showed significant differences among the four sessions for the population spike amplitudes but showed no significant differences for the EPSP slopes [main time course effects, $F(3,16)=3.922$, $P=.0282$ in population spike and $F(3,16)=2.546$, $P=.092$ in EPSP]. So, the subsequent Scheffe's multiple comparison was carried out only on the population spike amplitudes. It showed no significant differences between baseline and the first half after risperidone injection ($P=.999$ in population spike) or between baseline and the latter half after risperidone injection ($P=.979$ in population spike). Moreover, unexpectedly, the multiple comparison showed no significant differences also between baseline and after tetanus for the population spike amplitudes ($P=.066$ in population spike), indicating that the potentiation of responses to single shocks after tetanus was a show and that risperidone suppressed the induction of LTP on the whole.

In the risperidone 1.0 mg/kg dose group, the responses were unaltered throughout the experimental time, i.e. during the baseline recordings, after risperidone injection and tetanic stimulation (Fig. 2; risperidone 1.0 mg/kg), clearly indicating that risperidone suppressed the induction of LTP.

One-way ANOVA showed no significant differences among the four sessions [main time course effect, $F(3,16)=1.771$, $P=.193$ in population spike, $F(3,16)=1.358$, $P=.291$ in EPSP] with regard to both the population spike amplitudes or EPSP slopes.

Also, in the risperidone 2.0 mg/kg dose group, the responses were virtually unaltered throughout the experimental time, i.e. during the baseline recordings, after risper-

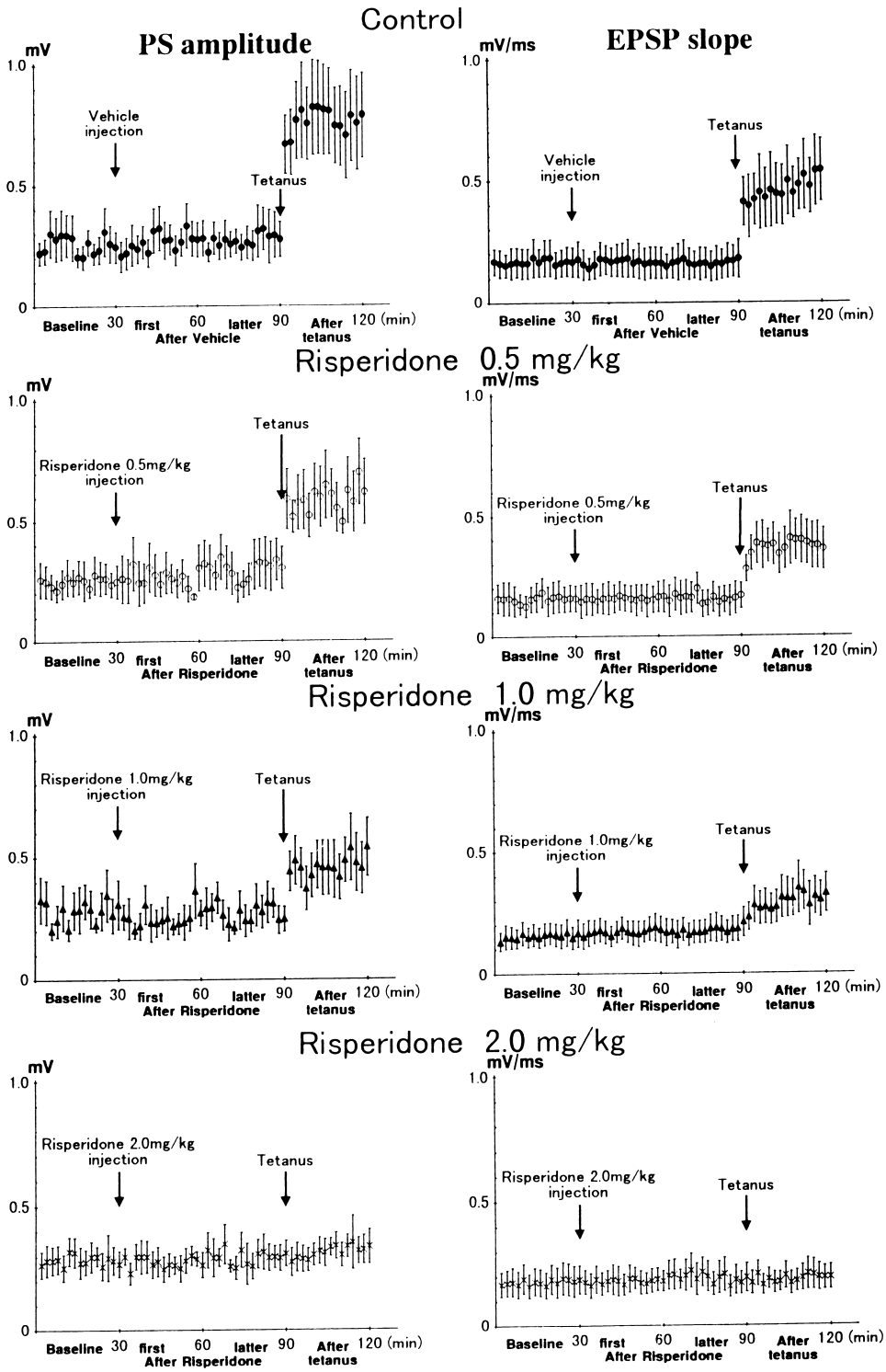

Fig. 2. Serial changes of mean and S.E. values of the population spike (PS) amplitudes and EPSP slopes in the 60 averaged responses, i.e. 240 real responses during 120 min in four sessions, namely baseline, the first half and latter half of the observation period of 60 min after vehicle or risperidone and after tetanus in each experimental group consisting of five rabbits.

idone injection and tetanic stimulation (Figs. 2 and 3; risperidone 2.0 mg/kg).

One-way ANOVA showed no significant differences among the four sessions [main time course effect, $F(3,16)=0.078$, $P=.970$ in population spike, $F(3,16)=0.022$, $P=.995$ in EPSP] with regard to both the population spike amplitudes or EPSP slopes.

In addition, repeated-measure ANOVA showed significant differences among the four groups with regard to both the population spike amplitude and EPSP slopes in the 60

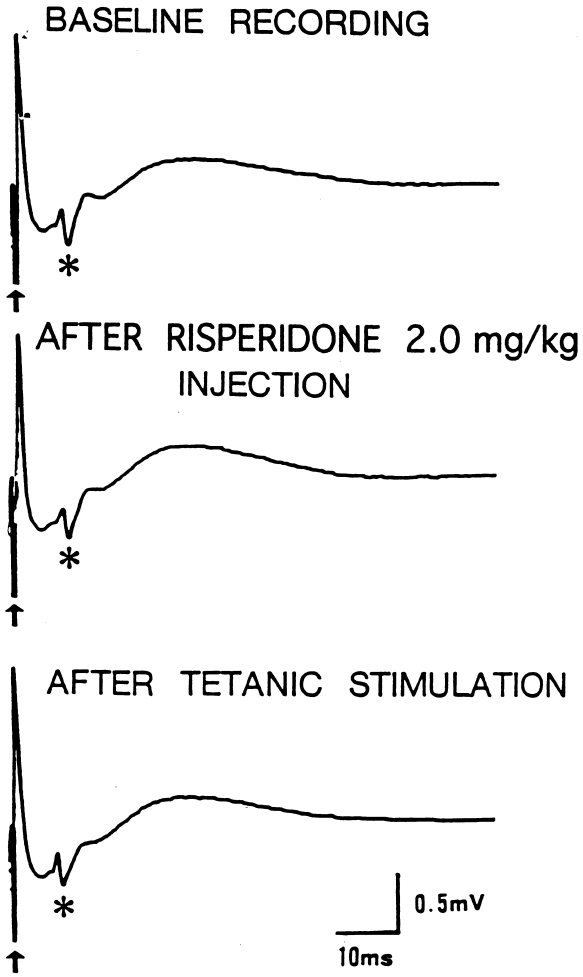

Fig. 3. Typical averaged responses evoked in the dentate gyrus by single shocks at fixed intensity to the perforant path in each session in a single rabbit in experiment of risperidone 2.0 mg/kg ip injection. Arrow: the single shock; asterisk: population spike.

responses averaged during the whole experimental time of 120 min [main time effect, $F(59,944)=34.111$, $P<.001$ in population spike and $F(59,944)=48.729$, $P<.001$ in EPSP; Group × Time course, $F(177,944)=7.220$, $P<.001$ in population spike and $F(177,944)=8.947$, $P<.001$ in EPSP]. Then, one-way ANOVA showed significant differences among the four groups with regard to both the population spike amplitude and EPSP slopes in the 15 responses averaged during each 30 min only in the last session, i.e. after tetanus [main group effect, $F(3,16)=4.825$, $P=.014$ in population spike and $F(3,16)=3.840$, $P=.030$ in EPSP in after tetanus]. The subsequent Scheffe's multiple comparison showed significant differences between the control and risperidone 2.0 mg/kg dose groups in the last session ($P=.021$ in population spike and $P=.037$ in EPSP, respectively), suggesting that the suppression of LTP induction was striking in risperidone 2.0 mg/kg dose groups. However, there were no significant differences between control and risperidone 0.5 and 1.0 mg/kg dose groups and between risperidone 2.0 mg/kg group and risperidone 0.5 and 1.0 mg/kg dose groups with regard to either the population spike amplitudes or EPSP slopes (Fig. 2; comparison between control and risperidone 0.5, 1.0 and 2.0 mg/kg).

4. Discussion

In the present study, we found that risperidone showed no effects on excitatory synaptic responses elicited in the dentate gyrus by single electrical stimulations and suppressed the induction of LTP. These results indicate that the effects of risperidone are different from those of the other atypical antipsychotic, clozapine, and are rather similar to those of a typical antipsychotic drug, haloperidol, in view of our past studies, in which clozapine markedly potentiated the excitatory synaptic responses, and contrastingly, haloperidol had no such effect but blocked the LTP induction (Jibiki et al., 1993; Kubota et al., 1996).

Studies with in vivo intracerebral microdialysis have shown that the acute and systemic administration of clozapine at more than 20 mg/kg dose-dependently produced an increase in extracellular concentrations of glutamate in the medial prefrontal cortex of freely moving rats, whereas haloperidol induced virtually no increase (Daly and Moghaddam, 1993; Yamamoto et al., 1994). Also, risperidone may induce no increase in the extracellular glutamate concentrations. It has been reported that clozapine activates the noradrenergic system because plasma catecholamines and their metabolites increase in the acute and chronic administration of clozapine (Green et al., 1993). Further, it has been known that noradrenaline facilitates the ordinary synaptic transmission in the perforant path–dentate gyrus pathway and increases over a prolonged period in the postsynaptic population spikes and EPSPs elicited in the neurons in the dentate gyrus (Stanton and Sarvay, 1987). Risperidone may not have such an action activating the noradrenergic system, although we do not know any past studies available about it. As another possibility, clozapine have higher affinity for D_1 and D_4 receptors than does risperidone. In particular, it has been reported that D_4 receptors relates to GABAergic inhibition in the cerebral cortex, hippocampus and other brain regions and that the blockade of D_4 receptors causes GABAergic disinhibition (Mrzljak et al., 1996). Risperidone may have no potency to induce such D_4 receptor-related GABAergic disinhibitions on account of the lower affinity for D_4 receptors.

In the present study, risperidone dose-dependently suppressed LTP. This suppression may be caused by risperidone-induced inactivation of calmodulin in common with haloperidol (Jibiki et al., 1993).

Clinically, clozapine is known to be associated with a higher prevalence of seizures than traditional neuroleptics and risperidone (Haller and Binder, 1990). This complication may be related to clozapine-induced potentiation. Further, it has been reported that risperidone has less

advantage for negative and positive symptoms and is associated with a higher prevalence of extrapyramidal side effects as compared with clozapine (Breier et al., 1999). In view of these differences between risperidone and clozapine, the two as atypical antipsychotics do not always share common pharmacological actions, with this also helping to explain the differences noted between them here.

In conclusion, risperidone had little effect on excitatory synaptic responses in the dentate gyrus by single electrical stimulations to the perforant path but prevented the induction of LTP in this pathway in a dose-dependent manner. These results indicate that the effects of risperidone are different from those of the other atypical antipsychotic drug, clozapine, but are rather similar to those of a typical antipsychotic drug, haloperidol.

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