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Effects of Age on a New Animal Model of Tardive Dyskinesia

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BERGAMO, M., V.C. ABÍLIO, C.M.T. QUEIROZ, H.N. BARBOSA-JÚNIOR, L.R.A. ABDANUR AND R. FRUSSA-FILHO. *Effects of age on a new animal model of tardive dyskinesia*. NEUROBIOL AGING **18**(6) 623–629, 1997.—The effects of age were studied on a new animal model of tardive dyskinesia, i.e., the quantification of oral dyskinesia in rats repeatedly treated with reserpine. Adult and old rats received two injections of reserpine (0.5 or 1.0 mg/kg s.c.) or vehicle, separated by 48 h. One, 10, 25 and 40 days after the second injection of reserpine or vehicle, the animals were observed for quantification of the behavioral parameters of oral dyskinesia: tongue protrusion and vacuous chewing movement frequencies and duration of twitching of the facial musculature. Phenomenologically, control old rats and reserpine-treated adult animals showed very similar oral dyskinesia. When compared to control adult rats, the significant increase in tongue protrusion frequency induced by reserpine treatment was more persistent in the old rats than in the adult animals. Because it is well known that age increases the persistence of tardive dyskinesia, our data provide further support for the validation of reserpine-induced oral dyskinesia as an animal model of tardive dyskinesia. In addition, the possibility is raised that a common pathophysiological mechanism may underlie tardive dyskinesia and age- and reserpine-induced oral dyskinesia.

Oral dyskinesia Reserpine Rats Senility

TARDIVE dyskinesia is a syndrome characterized by repetitive involuntary movements, usually involving mouth, face, and tongue, and sometimes limb and trunk musculature. The syndrome is considered to be an adverse effect of prolonged administration of antipsychotic drugs. It usually persists for months after the neuroleptic has been stopped and may be irreversible (6,29).

In rats, abrupt withdrawal from long-term treatment with dopamine receptor blockers such as haloperidol (3,46), sulpiride (11), metoclopramide (10), and droperidol (12) not only enhanced general activity observed in an open-field but also the stereotyped behavior induced by the dopamine agonist apomorphine. This behavioral supersensitivity is thought to result from striatal D_2 receptor site proliferation in response to chronic dopamine receptor blockade (4,40), and has been proposed as a potential model for antipsychotic-induced tardive dyskinesia in humans (1,24).

Although the hypothesis of dopamine supersensitivity has dominated the conceptual approaches to studying tardive dyskinesia, some fundamental observations seem not to support this hypothesis (5,14,51). Perhaps one of the most important flaws is related to the effects of age on tardive dyskinesia/behavioral supersensitivity. In this regard, age is the single most frequently implicated risk factor for tardive dyskinesia, increasing both the risk of developing tardive dyskinesia and the severity and persistence of the condition (23,51). Conversely, old animals have a diminished capacity to develop both behavioral supersensitivity to the apomorphine-induced stereotyped behaviors and dopamine receptor up-regulation after chronic treatment with neuroleptics (13,31,37,48).

More recently, Neisewander et al. (34) have suggested that reserpine-induced oral dyskinesia may provide a new animal model of tardive dyskinesia. Indeed, rats treated with this monoamine depleting agent for at least 3 days develop orofacial dyskinesia characterized by twitching of the facial musculature, vacuous chewing movements, and tongue protrusions (34–36). This reserpine-induced oral dyskinesia has also other features that are consistent with tardive dyskinesia including persistence following termination of administration and dose-dependent blockade by a D_2-selective antagonist (35,36). Furthermore, although reserpine is not classified as a neuroleptic, it has been used as an antipsychotic agent and has been associated with the development of tardive dyskinesia (42,45). Finally, whereas reserpine-induced oral dyskinesia in rats is of insidious onset at low doses, the response develops very rapidly at high doses, a fact offering an outstanding methodological advantage over long-term neuroleptic administration (see 34).

However, it should be noted that although reserpine-induced oral dyskinesia may provide an efficient and practical model of tardive dyskinesia, it is not free of criticisms. For example, the

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above mentioned clinical reports of tardive dyskinesia following reserpine are exceedingly rare and are found only in very old literature. Indeed, the reliability of reserpine-induced dyskinesia in humans has been difficult to establish because reserpine is rarely prescribed alone at anti-psychotic doses. Another characteristic of reserpine-induced oral dyskinesia that is inconsistent with tardive dyskinesia is that the response develops too rapidly at high doses. Thus, further characterization of the model seems to be necessary.

In the following experiment, the effects of age on reserpine-induced oral dyskinesia were investigated. In short, the objectives of the present study were: 1) to determine whether reserpine-induced oral dyskinesia would be increased in old rats, in contrast to conventional behavioral models of tardive dyskinesia, and 2) to further characterize the mechanisms underlying the well known relationship between age and oral dyskinesia.

METHOD

Drugs

Reserpine (methyl reserpate 3,4,5-trimethoxycinnamic acid ester: Sigma Chemical Co. St. Louis, MO) was dissolved in glacial acetic acid and then diluted to the correct concentration with distilled water. Vehicle consisted of the same amount of acetic acid and water as in the reserpine solution. Both solutions were injected subcutaneously (s.c.) at a volume of 1 mL/kg.

Animals and Drug Administration

Healthy adult (4 months of age, weighing approximately 350 g) and old (20–24 months of age, weighing approximately 400 g) male Wistar EPM-1 rats, born and raised under our laboratory conditions, were used. The animals were housed under conditions of controlled temperature (22–23°C) and lighting (12 h light:12 h dark, lights on 6:30 a.m.). Food and water were available ad libitum throughout the experiment. The rats were divided into 5 groups of 8–10 animals each. Adult animals (A) received two injections of vehicle (V) or 1.0 mg/kg reserpine (R 1.0), respectively. Old rats (O) received two injections of vehicle, 0.5 mg/kg reserpine (R 0.5) or 1.0 mg/kg reserpine, respectively. Because it is well known that drug effects can be increased by age due to a decrease in overall metabolic capacity of the liver, old animals were also treated with this lower dose of reserpine (0.5 mg/kg). Thus, the five groups of animals were as follows: AV, AR 1.0, OV, OR 0.5, and OR 1.0. In both adult and old animals, the second injection of vehicle or reserpine was administered 48 h after the first one.

In order to avoid severe weight loss due to reserpine administration, during the first 5 days after the first drug administration, experimental and control animals were also given access to ground wet mash.

Behavioral Testing

On each test day, the rats were placed individually in observation cages (16 × 30 × 19 cm). To quantify the occurrence of oral dyskinesia, hand operated counters were employed to score tongue protrusion and vacuous chewing frequencies and stopwatches were employed to score duration (total seconds) of twitching of the facial musculature. In the present study, vacuous chewing movements are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion, vacuous chewing movements, or twitching of the facial musculature occurred during a period of grooming, they were not taken into account. Mirrors were placed under the floor and behind the backwall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for 15 min. Animals were observed for oral dyskinesia 1, 10, 25, and 40 days after the second injection of reserpine or vehicle. The observations were made by five observers who were blinded to the five animal groups. As far as possible, the same number of animals in each group was observed by each observer. It is worth pointing out that the observation criterions were not subjective; an excellent inter-observer agreement was found in previous pilot experiments (Pearson's correlation was r = 0.98, 0.97, and 0.98 for tongue protrusion frequency, vacuous chewing frequency, and duration of facial twitching, respectively).

Statistical Analysis

Bartlett's test (21) was performed and it was concluded that the three behavioral parameters of reserpine-induced oral dyskinesia were non-parametric. Thus, the data were analyzed by Kruskal-Wallis analysis of variance followed by the two-tailed Mann-Whitney *U* test (44). A probability of $p < 0.05$ was considered to show significant differences for all comparisons made.

RESULTS

The median number of tongue protrusions on days 1, 10, 25, and 40 after reserpine or vehicle treatment is shown in Figure 1. Kruskal-Wallis analysis of variance revealed significant differences in all testing sessions ($H = 15.4$, $p < 0.01$; $H = 18.2$, $p < 0.01$; $H = 10.4$, $p < 0.05$; and $H = 11.0$, $p < 0.05$, for 1, 10, 25, and 40 days post-injection, respectively). Although rats of the OV group showed an increased frequency of tongue protrusions in the four observation sessions when compared to animals of the AV group, this enhancement only reached statistical significance at 25 days post-injection. Animals of the AR 1.0 group exhibited an increase in tongue protrusion relative to rats of the AV group at 1, 10, and 25 days post-injection, but not 40 days after reserpine or vehicle treatment. On the other hand, tongue protrusion frequency for animals of both the OR 0.5 and OR 1.0 groups was significantly higher than that of the AV group in all testing sessions. In addition, at 10 days post-injection, rats of the OR 1.0 group showed an increased tongue protrusion frequency even when compared to animals of the OV group.

Figure 2 shows the effects of the age on vacuous chewing frequency. Kruskal-Wallis analysis of variance revealed significant differences in all observation sessions ($H = 19.5$, $p < 0.001$; $H = 19.9$, $p < 0.001$; $H = 21.4$, $p < 0.001$; and $H = 11.7$, $p < 0.05$, for 1, 10, 25, and 40 days post-injection, respectively). Animals of the OV, AR 1.0, OR 0.5, and OR 1.0 groups exhibited a significantly higher vacuous chewing frequency than that of the AV group in all testing sessions. The only exceptions were the value of the OV group at 10 days post-injection and the value of the AR 1.0 group at 40 days post-injection. At 10 days post-treatment, rats of the OR 1.0 group also showed an increase in vacuous chewing frequency when compared to the OV group.

The duration of twitching of the facial musculature on days 1, 10, 25, and 40 after reserpine or vehicle treatment is shown in Figure 3. Once again, Kruskal-Wallis analysis of variance revealed significant differences in all testing sessions ($H = 19.9$, $p < 0.001$; $H = 16.9$, $p < 0.01$; $H = 15.9$, $p < 0.01$; and $H = 16.1$, $p < 0.01$, for 1, 10, 25, and 40 days post-injection, respectively). In all observation sessions, animals of the OV, OR 0.5, and OR 1.0 groups exhibited a significant increase in the duration of twitching of the facial musculature relative to the rats of the AV group. However, when compared to animals of the AV group, rats of the AR 1.0 group showed increased duration of this behavioral parameter only at 1 and 10 days post-injection. In addition, the duration of twitching shown by the AR 1.0 group was significantly

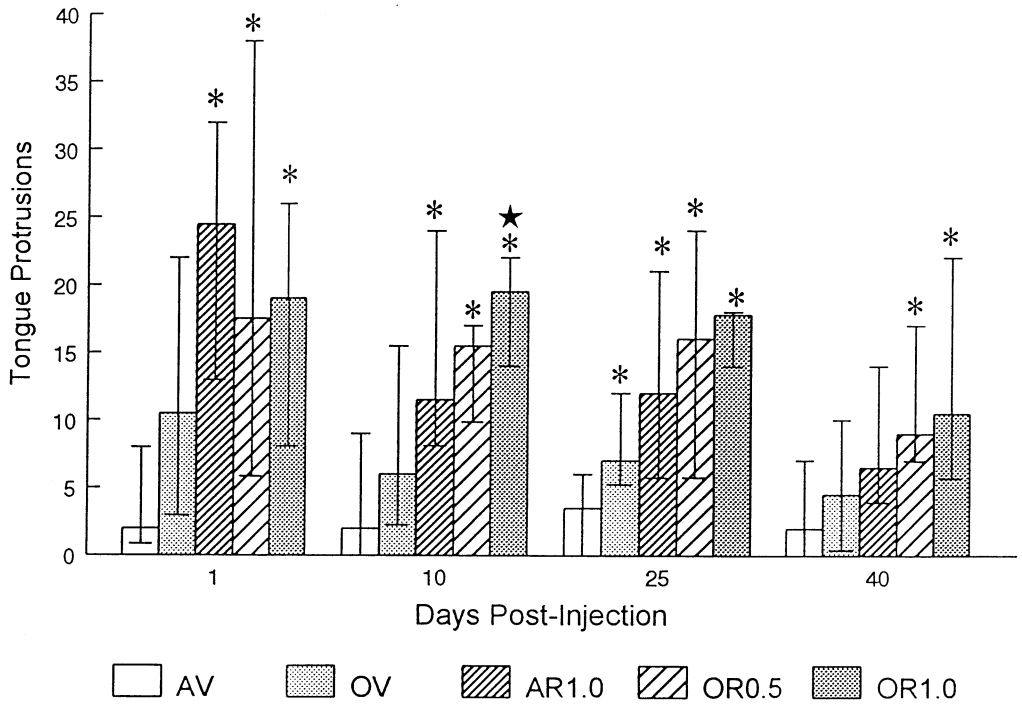

FIG. 1. Median and interquartile range of tongue protrusion frequency exhibited by adult (*A*) or old (*O*) rats treated with vehicle (*V*), 0.5 mg/kg reserpine (*R0.5*) or 1.0 mg/kg reserpine (*R1.0*) and observed 1, 10, 25, and 40 days afterwards (sessions 1, 2, 3, and 4, respectively).
* $p < 0.05$ compared to AV animals; ★ $p < 0.05$ compared to OV rats. Kruskal-Wallis analysis of variance and two-tailed Mann-Whitney *U* test.

lower that those of the OV and OR 1.0 group at 40 days post-injection.

DISCUSSION

The major findings of the present investigation were that: 1) adult rats treated with reserpine developed an orofacial dyskinesia characterized by tongue protrusion, vacuous chewing movement, and twitching of the facial musculature, 2) phenomenologically, control old rats and reserpine-treated adult animals showed a very similar oral dyskinesia, and 3) in relation to control adult rats, old rats treated with reserpine showed a more persistent increase in tongue protrusions than that presented by reserpine-treated adult animals.

The first finding replicates and extends the data of Neisewander et al. (34–36), who showed an increased frequency of tongue protrusions after reserpine treatment in rats. These authors, however, did not quantify the duration of twitching of the facial musculature and the frequency of vacuous chewing movements. In this regard, vacuous chewing movements induced by long-term neuroleptic treatment have been extensively studied (see 47). However, it is still debatable whether neuroleptic-induced purposeless chewing movements are a model of tardive dyskinesia or of acute dystonia. In fact, the latter possibility is supported by the observations that the number of vacuous chewing movements increases just after the first dose of neuroleptic, whereas withdrawal of neuroleptic treatment is followed by a quick decrease to the control level (see 51). Concerning these critical issues for evaluating animal models of tardive dyskinesia, reserpine-induced orofacial dyskinesia (or at least reserpine-induced tongue protrusions) seems to be a better model of tardive dyskinesia. First, reserpine does not cause acute dystonic reactions in humans. Second, acute dystonia frequently develops after the first dose of neuroleptic whereas reserpine produces a decrease in tongue protrusions in animals observed 6 h after the first injection (34,36). The increase in tongue protrusions, however, is not observed until 24 h after the second injection (34). These findings suggest that tongue protrusion is not an acute reserpine-elicited effect, but rather a spontaneous oral dyskinesia that develops as a result of reserpine administration, similar to tardive dyskinesia, which develops as a result of neuroleptic administration. As pointed out by Neisewander et al. (34), two additional observations that support this idea are that reserpine-induced tongue protrusions appear late during the course of administration at lower doses and persist for a long time following termination of administration. The present findings show that the latter observation is also true for the other two behavioral parameters of reserpine-induced oral dyskinesia, i.e., vacuous chewing and twitching of the facial musculature, which lasted 25 and 10 days after reserpine treatment, respectively. In this respect, it should also be noted that whereas Neisewander et al. (36) verified that the increase in tongue protrusions persisted for at least 60 days following termination of 4–6 weeks of treatment with reserpine, we observed that it persisted for 25 days following termination of 3 days of treatment with the drug.

As reviewed by Waddington (47), among the studies which have addressed the effects of age on spontaneous orofacial movements of untreated control animals, those of Rupniak et al. (39), Waddington et al. (50), Gunne et al. (19), Johansson et al. (20), and Waddington et al. (49) have reported that orofacial movements increase with aging. However, those of Gunne & Haggstrom (18) and Mithani et al. (32) did not find such an effect. Differences in recording or categorization of orofacial movements

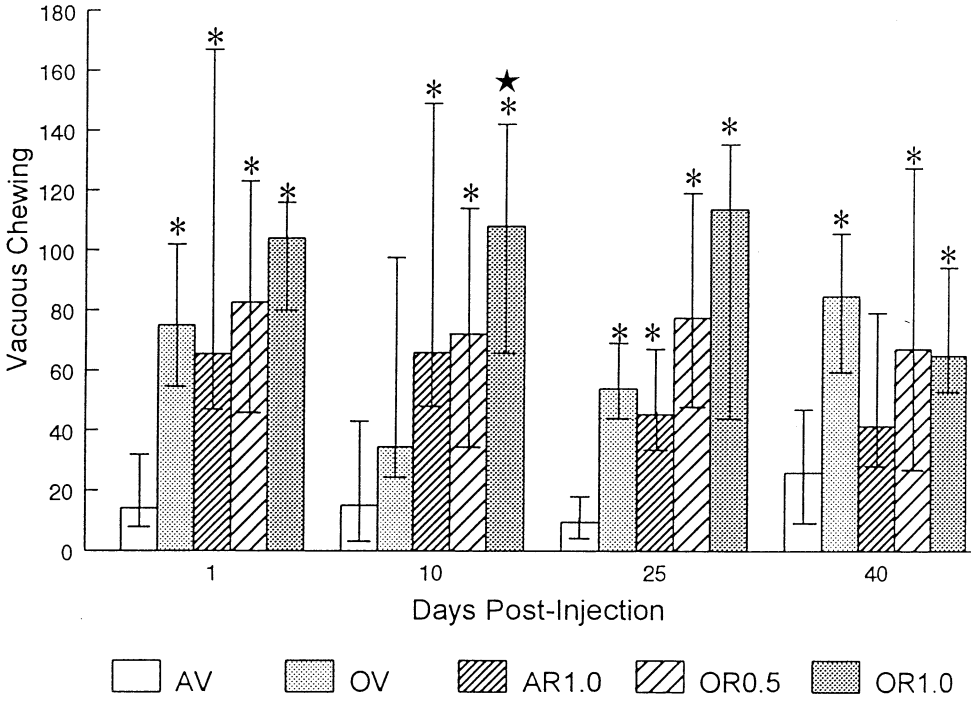

FIG. 2. Median and interquartile range of vacuous chewing frequency exhibited by adult (*A*) or old (*O*) rats treated with vehicle (*V*), 0.5 mg/kg reserpine (*R0.5*) or 1.0 mg/kg reserpine (*R1.0*) and observed 1, 10, 25, and 40 days afterwards (sessions 1, 2, 3, and 4, respectively).
* $p < 0.05$ compared to AV animals; ⋆ $p < 0.05$ compared to OV rats. Kruskal-Wallis analysis of variance and two-tailed Mann-Whitney *U* test.

may account for these contradictory data. Indeed, the present study shows that, whereas rats of the OV group exhibited a duration of twitching of the facial musculature significantly higher than that of the AV group in all the four observation sessions, the frequency of vacuous chewing movements was significantly higher in three observation sessions and the frequency of tongue protrusion was significantly higher only in one of the test sessions.

The already substantially elevated baseline of age-matched controls made it very difficult to detect any potentiating effect of reserpine on spontaneous oral dyskinesia. Interestingly, in the clinical situation it has also been more difficult to detect any effect of long-term neuroleptic treatment on the increase in involuntary movements in older populations with an already high baseline level of such movements, especially those associated with age-related neurological deficits (see 47). However, because the tongue protrusion baseline of the animals of the OV group was not significantly increased relative to the AV group, we may state that the reserpine-induced enhancement of tongue protrusion frequency in relation to the AV group lasted longer in the old animals. Thus, the present study demonstrates another feature of reserpine-induced oral dyskinesia that is consistent with tardive dyskinesia, i.e., an increased persistence of the condition in the old animals (23,51).

As mentioned before, for many years the theory of the development of tardive dyskinesia has been related to a receptor-mediated supersensitivity to dopamine. In this regard, chronic administration of reserpine increased the density of both D_1 and D_2 dopamine receptors (36). In addition, reserpine-induced oral dyskinesia was blocked in a dose-dependent manner by the D_2-selective antagonist spiroperidol (35). However, some observations suggest that the potentiating effect of age on the persistence of reserpine-induced oral dyskinesia cannot be fully explained by a corresponding facilitatory action of age on dopamine supersensitivity. First, whereas age per se and reserpine treatment produced a phenomenologically similar spontaneous oral dyskinesia, it is well known that the number of dopamine D_2 receptors declines linearly with age (17,22,38,41). Furthermore, as mentioned before, old animals have an impaired ability to develop an up-regulation of striatal ^{3}H-spiroperidol binding sites (37). On the other hand, it is very important to note that the possibility cannot be discarded that an increase in dopamine receptor transduction may be involved in the oral dyskinesia rather than an increase in number of receptors.

As pointed out by Casey (5), a modification of the D_2-receptor supersensitivity hypothesis for tardive dyskinesia suggests that it develops from an imbalance between D_1- and D_2-mediated effects in the basal ganglia. In support of this hypothesis, Marin et al. (28) verified that although withdrawal from treatment with raclopride (a selective D_2 antagonist) or SCH 23390 (a selective D_1 antagonist) enhanced apomorphine-induced stereotypy, co-administration of both drugs at equivalent cataleptogenic doses produced no behavioral supersensitivity. An imbalance between D_1- and D_2-mediated effects could also occur as a result of both aging-induced dopamine receptor down-regulation and reserpine-induced dopamine receptor up-regulation, therefore leading to the emergence of oral dyskinesia. Although this is a speculative hypothesis, it should be noted that whereas the decline of dopamine D_2 receptor with age is a well established phenomenon, a similar decrease of dopamine D_1 receptor has been reported in some experiments (15,16) but not in others (33). Indeed, loss of the D_2 subtype in senescence appears to be a much more consistent phenomenon than loss of D_1 receptors (38). In addition, Neisewander et al. (36)

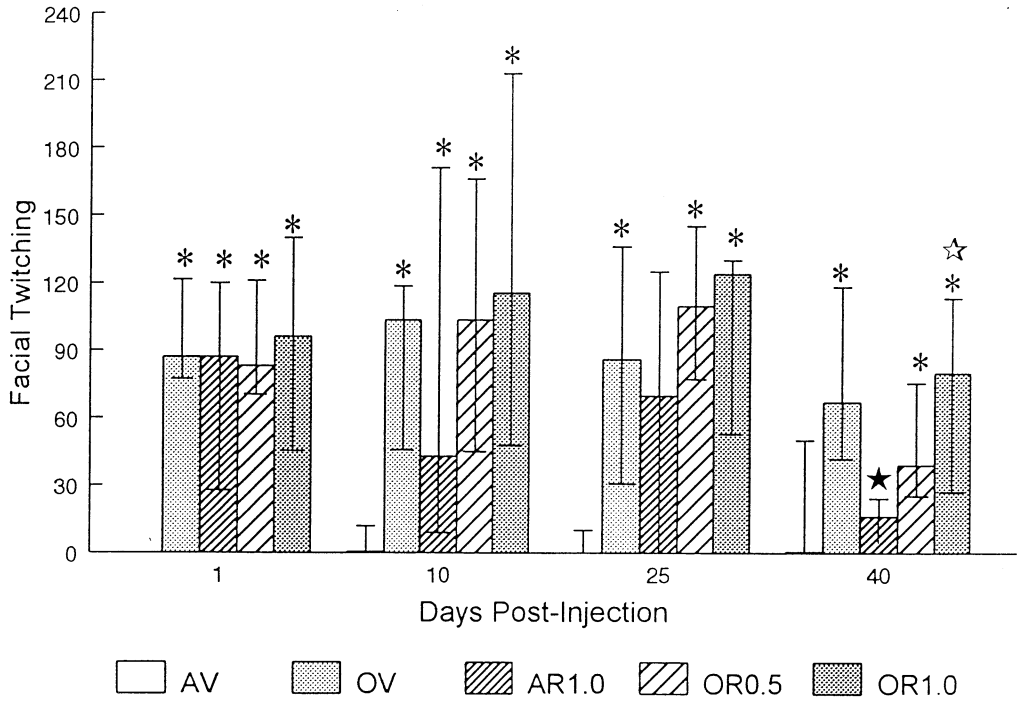

FIG. 3. Median and interquartile range of facial twitching duration (seconds) exhibited by adult (*A*) or old (*O*) rats treated with vehicle (*V*), 0.5 mg/kg reserpine (*R0.5*) or 1.0 mg/kg reserpine (*R1.0*) and observed 1, 10, 25, and 40 days afterwards (sessions 1, 2, 3, and 4, respectively).
* $p < 0.05$ compared to AV animals; ★ $p < 0.05$ compared to OV rats; ☆ $p < 0.05$ compared to AR1.0 animals. Kruskal-Wallis analysis of variance and two-tailed Mann-Whitney *U* test.

verified that reserpine-induced up-regulation of dopamine D_1 and D_2 receptors showed different time courses.

There are several alternative manners in which age could modify the reserpine-induced increase in tongue protrusion frequency. Thus, the effect of age on reserpine-induced oral dyskinesia could be related to changes in the pharmacokinetics of reserpine. This possibility, however, seems unlikely because old rats treated with 0.5 and 1.0 mg/kg reserpine (groups OR 0.5 and OR 1.0) showed very similar increases in tongue protrusion frequency, both of them persisting for a longer time than the increase produced by 1.0 mg/kg reserpine in adult rats (group AR 1.0).

From another standpoint, the similarities of age- and reserpine-induced oral dyskinesia not only support this new animal model of tardive dyskinesia but also suggest the possibility that related mechanisms are involved in each effect. Indeed, as reviewed by Wolfarth & Ossowska (51), the occurrence of involuntary movements, in particular oral dyskinesia in elderly people who had never undergone treatment with neuroleptics before, has been reported frequently. The similarity between this kind of dyskinesia and tardive dyskinesia is so great that it is not possible to differentiate between these disorders on the basis of behavioral symptoms (14). Thus, it has been suggested that long-term neuroleptic treatment does not "cause" the emergence of orofacial movements; rather such prolonged treatment may interact with some substrate of brain aging to result in the premature emergence of an orofacial syndrome that can occur spontaneously in old age (47). Interestingly, whereas increased oxidative stress with cumulative free-radical damage is a well known feature of the aging brain (2,7), the proposal that tardive dyskinesia is due to a neurotoxic effects of free-radical byproducts from catecholamine metabolism has been receiving considerable interest (5,25).

Specifically, neuroleptic drugs, by blocking dopamine receptors, cause a secondary increase in the turnover and metabolism of dopamine, which may lead to increased formation of dopamine quinones as well as of hydrogen peroxide through the activity of monoamine oxidase (25). In support of this hypothesis, some clinical studies have reported beneficial effects of vitamin E (a free-radical scavenger) on tardive dyskinesia (8,9,26), although others did not find such beneficial effects (43). In this context, it seems plausible that reserpine may alter oxidative metabolism even more strongly than neuroleptics because storage of newly synthesized dopamine is prevented and the dopamine is therefore continuously available for metabolism. This possibility would also explain the very fast rate at which reserpine (at high doses) induces oral dyskinesia. Interestingly, and in further support of this hypothesis, we have verified that long-term treatment with GM1 ganglioside or calcium channel blockers inhibits reserpine-induced oral dyskinesia (unpublished data). In this respect, both GM1 and calcium channel blockers have been reported to produce antioxidant/free-radical scavenger effects (27,30).

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