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Deltamethrin Increases Neurite Outgrowth in Cortical Neurons through Endogenous BDNF/TrkB Pathways

Daisuke Ihara[1], Mamoru Fukuchi[1,2*], Momoko Katakai[3], Yo Shinoda[3,4], Ritsuko Katoh-Semba[3], Teiichi Furuichi[3], Mitsuru Ishikawa[1,5], Akiko Tabuchi[1], and Masaaki Tsuda[1]

[1]*Department of Biological Chemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama-shi, Toyama 930-0194, Japan,* [2]*Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui-machi, Takasaki-shi, Gunma 370-0033, Japan,* [3]*Laboratory of Molecular Neuroscience, Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278-8510, Japan,* [4]*Department of Environmental Health, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horunouchi, Hachioji-shi, Tokyo 192-0392, Japan,* [5]*Department of Physiology, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan*

ABSTRACT. **Deltamethrin (DM), a type II pyrethroid, robustly increases brain-derived neurotrophic factor (*Bdnf*) expression and has a neurotrophic effect in primary cultures of rat cortical neurons. In this study, we investigated the effect of DM on neurite morphology in cultured rat cortical neurons. DM significantly increased neurite outgrowth, but this increase was abolished when the BDNF scavenger tropomyosin receptor kinase B (TrkB)-Fc was added 10 min before the DM treatment. In contrast, the addition of TrkB-Fc 1 h after the treatment did not affect DM-induced neurite outgrowth. Our previous research has indicated that type II, but not type I, pyrethroids have the ability to induce *Bdnf* mRNA expression, but neither permethrin nor cypermethrin, which are type I and type II pyrethroids, respectively, affected neurite outgrowth in the current study. These results suggest that this effect is not due to increased *Bdnf* expression, and the effect is unique to DM. We previously demonstrated that calcineurin plays a role in the DM-mediated induction of *Bdnf* expression. However, the calcineurin inhibitor FK506 did not significantly affect DM-induced neurite outgrowth. DM-induced neurite outgrowth was abolished by U0126 and rapamycin, indicating the involvement of the mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) pathways. Taken together, these findings suggest that DM activates endogenous BDNF/TrkB-mediated MAPK and mTOR pathways, thereby increasing neurite outgrowth.**

Key words: BDNF, Deltamethrin, MAPK, mTOR, Neurite outgrowth

Introduction

Pyrethroids are a class of synthetic derivatives of pyrethrins, which are insecticidal compounds contained in pyrethrum plants (*Chrysanthemum cinerariifolium*). The main molecular targets of pyrethroids are voltage-gated sodium channels (VGSCs). Pyrethroids prolong the opening of these channels, resulting in a sodium tail current in both vertebrates and invertebrates (Vijverberg *et al.*, 1982; Narahashi, 1985). Insect sodium channels are believed to be much more sensitive to pyrethroids than mammalian channels (Warmke *et al.*, 1997). Because of their high insecticidal activity, low toxicity to mammals, and rapid metabolism (Miyamoto *et al.*, 1995; Ray and Forshaw, 2000), pyrethroid insecticides are widely used.

Pyrethroids have been associated with some toxicity because they target the nervous systems of both mammals and insects. The most common symptom of acute poisoning by type I pyrethroids, which are devoid of an α-cyano moiety, is tremor syndrome (T-syndrome), and that by type

*To whom correspondence should be addressed: Mamoru Fukuchi, Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui-machi, Takasaki-shi, Gunma 370-0033, Japan.
Tel: +81–27–352–1180, Fax: +81–27–352–1118
E-mail: fukuchi@takasaki-u.ac.jp

Abbreviations: BDNF, brain-derived neurotrophic factor; CM, cypermethrin; DM, deltamethrin; ERK, extracellular signal-regulated kinase; GFP, green fluorescent protein; L-VDCC, L-type voltage-dependent calcium channel; MAP2, microtubule-associated protein 2; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; mTOR, mammalian target of rapamycin; PM, permethrin; TrkB, tropomyosin receptor kinase B; VGSC, voltage-gated sodium channel.

II pyrethroids, which have an α-cyano moiety, is choreoathetosis with salivation syndrome (CS-syndrome) (Verschoyle and Barnes, 1972; Barnes and Verschoyle, 1974; Verschoyle and Aldridge, 1980). Recent epidemiological studies have shown a link between environmental and occupational exposure to pyrethroids and abnormalities such as changes in sperm quality and reproductive hormones (Saillenfait *et al.*, 2015). Furthermore, prenatal exposure to pyrethroids appears to be a risk factor for neurodevelopmental delay and autism spectrum disorders (Shelton *et al.*, 2014). Therefore, it is important to elucidate the effects of pyrethroids on the nervous system.

We previously demonstrated that deltamethrin (DM), a type II pyrethroid, triggers a prolonged increase in intracellular calcium concentrations and strongly elevates brain-derived neurotrophic factor (*Bdnf*) expression in primary cultures of rat cortical neurons (Ihara *et al.*, 2012). Furthermore, DM has an antidepressant-like effect in mice (Takasaki *et al.*, 2013). Although DM has a neurotrophic effect and increases neurite outgrowth in cultured neurons (Ihara *et al.*, 2012), it remains unclear how DM affects neurite outgrowth. In the present study, we found that DM increases neurite outgrowth in cultured rat cortical neurons through tropomyosin receptor kinase B (TrkB)-mediated signaling pathways via endogenous BDNF, probably without *de novo Bdnf* transcription. Our present study would provide insight into one of the underlying molecular and cellular mechanisms that DM may be a risk factor for neurodevelopmental disorders, possibly mediated by disturbing neuronal network in the developing brain.

Materials and Methods

Reagents

Deltamethrin, permethrin and cypermethrin were purchased from Wako Pure Chemicals (Tokyo, Japan). FK506 was obtained from Sigma-Aldrich (St. Louis, MO). TrkB-Fc was purchased from R&D Systems (Minneapolis, MN). U0126 and rapamycin were obtained from Merck Millipore (Darmstadt, Germany).

Cell culture

Primary cultures of cortical neurons were prepared from the cerebral cortices of 17-day-old Sprague-Dawley rat embryos (Japan SLC, Hamamatsu, Japan) as described previously (Imamura *et al.*, 2006; Ihara *et al.*, 2012). Maternal rat and embryos were deeply anesthetized by sodium pentobarbital to obtain rat embryonic brains. Maternal rat was euthanatized by carbon dioxide and rat embryos were done by decapitation under deep anesthesia. All animal care and experiments were approved by the Animal Experiment Committee of the University of Toyama (Approval number: A2011PHA-4) and were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Toyama. Cells were plated at 7.0×10^5 cells/well onto 18-mm coverslips. The coverslips were placed in 12-well plates (AGC ASAHI GLASS, Tokyo, Japan) coated with poly-D-lysine (Sigma-Aldrich).

Analysis of neurite outgrowth

To examine how DM affects neurite outgrowth, a green fluorescent protein (GFP) expression vector was transfected into cells at 4 days *in vitro* using the calcium phosphate co-precipitation method, as described previously (Ishikawa *et al.*, 2010; Ihara *et al.*, 2012). Two days after DNA transfection, the cells were treated with DM and/or other reagents, and immunostaining was performed with anti-GFP (MBL, Nagoya, Japan) and anti-microtubule-associated protein 2 (MAP2) antibodies (Sigma-Aldrich) to identify GFP-positive neurons. It has been previously reported that low molecular weight form of MAP2, MAP2C, is found in both dendrites and axons in immature developing neurons (Meichsner *et al.*, 1993). Probably due to this reason, it was likely hard to distinguish dendrites and axons in this study. Therefore, we focused on GFP and MAP2 positive cells, and evaluated changes in morphology of all neurites. Changes in neurite morphology were evaluated by Sholl analysis (Fig. 1A), as described previously (Sholl, 1953; Ishikawa *et al.*, 2010; Ihara *et al.*, 2012). A series of concentric circles at 20-μm intervals were centered on the cell body and the number of intersections with GFP-positive processes was recorded as an index of neurite outgrowth. Total neurite length was also measured by tracing all of the neurites starting at the cell body and extending within a 400-μm-diameter circle using Image J software. All morphological changes were blindly examined. We analyzed morphologies of about 55 neurons in each experiment. All experiments were independently repeated at least three times (n=3–5).

Statistical analysis

All values are expressed as the mean±SEM. Statistical analyses were performed using repeated measure ANOVA with the Bonferroni/Dunn test (Sholl analysis) or one-way ANOVA with Scheffé's *F*-test (neurite length).

Results

DM increases neurite outgrowth in cultured rat cortical neurons

Treatment of cortical neurons with DM increased neurite outgrowth (Fig. 1B, C), as previously reported (Ihara *et al.*, 2012). We evaluated the effect of DM on neurite outgrowth using Sholl analysis (Sholl, 1953; Ishikawa *et al.*, 2010; Ihara *et al.*, 2012) (Fig. 1A). DMSO, a solvent for DM, did not significantly affect neurite morphology (Fig. 1B). In contrast, neurite outgrowth significantly was enhanced 3 h after DM treatment, and the increases continued 24 h after

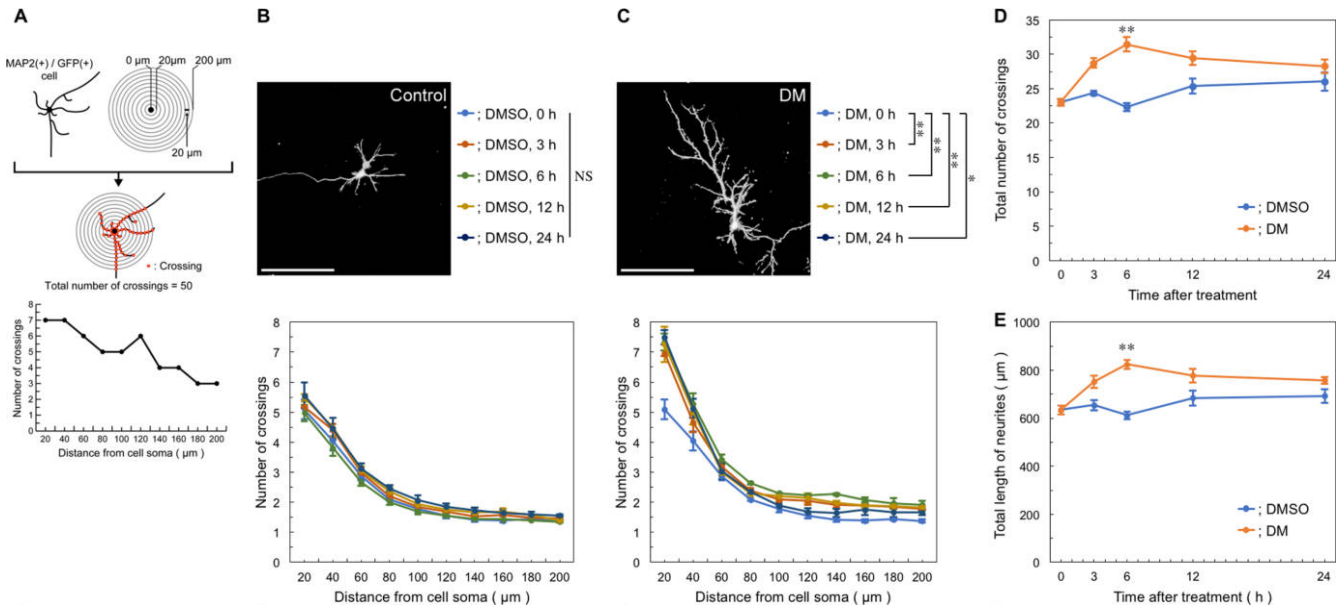

Fig. 1. Effect of DM treatment on neurite outgrowth in rat cortical neurons. (A) Sholl analysis used in this study. To identify GFP-positive neurons, immunostaining was performed with anti-GFP and anti-MAP2 antibodies. Changes in neurite outgrowth were evaluated by Sholl analysis. A series of concentric circles at 20-μm intervals were centered on the cell body and the number of intersections with GFP-positive processes was recorded as an index of neurite outgrowth. (B, C) Representative images of cultured rat cortical neurons 6 h after treatment with DMSO (B) or 1 μM DM (C) (Scale bar=100 μm) and time-course of changes in the number of crossings 0, 3, 6, 12, or 24 h after treatment with DMSO (B) or 1 μM DM (C). *p<0.05 and **p<0.01 versus 0 h, NS: not significant. (D, E) Time-course of changes in the total number of crossings (D) and the total length of neurites (E) in the absence or presence of DM. **p<0.01 versus DM (–) at the same time points. The experiment was repeated independently three times.

treatment (Fig. 1C). However, neurite outgrowth was likely to decline 24 h after the treatment (Fig. 1B–D). We also examined the effect of DM on neurite length of cultured cortical neurons. Similarly, we observed that DM increased neurite length 6 h after the treatment in particular (Fig. 1E). On the other hand, the total number of crossings and neurite length also gradually increased in the absence of DM (Fig. 1D, E), possibly because of physiological neurite outgrowth during culture. Therefore, significant effect of DM on the total number of crossings and neurite length was not observed 12 and 24 h after the treatment (Fig. 1D, E).

We previously reported that DM robustly increased *Bdnf* mRNA expression levels in cultured rat cortical neurons (Ihara *et al.*, 2012). Furthermore, the DM-induced neurite outgrowth was abolished when TrkB-Fc, which blocks BDNF function, was added prior to DM treatment (Ihara *et al.*, 2012). This result indicated that endogenous BDNF is involved in DM-induced neurite outgrowth. Because type II, but not type I, pyrethroids have the ability to induce *Bdnf* mRNA expression in cultured rat cortical neurons (Imamura *et al.*, 2006), we hypothesized that type II, but not type I, pyrethroids would increase neurite outgrowth. Therefore, we tested permethrin (PM), a type I pyrethroid, and cypermethrin (CM), a type II pyrethroid in our current assay. Unexpectedly, in contrast to DM, neither PM nor CM significantly affected neurite outgrowth (Fig. 2). These results suggest that, among the pyrethroids, DM has a unique ability to affect neurite outgrowth in cultured rat cortical neurons.

Effect of TrkB-Fc on DM-induced neurite outgrowth

Consistent with our previous study (Ihara *et al.*, 2012), DM-induced neurite outgrowth was almost fully abolished when TrkB-Fc was added to the neuronal culture 10 min before DM treatment (Fig. 3A). In contrast, DM-induced neurite outgrowth was unaffected when TrkB-Fc was added 1 h after treatment (Fig. 3B). These results suggest that, although endogenous BDNF is necessary for DM-induced increase in neurite outgrowth, DM-induced endogenous BDNF signaling that contributes to DM-induced neurite outgrowth would occur only in the early phase (possibly within 1 h after the DM treatment). In support, transient but slight increases in phosphorylation of TrkB and extracellular signal-regulated kinase (ERK) 1/2 were likely observed 10 min after the DM treatment (Supplementary Fig. 1A). In addition, we found that the cellular levels of BDNF protein did not change 3 h after the DM treatment (Supplementary Fig. 1B). Taken together with the results showing that addition of TrkB-Fc after DM treatment did not affect DM-induced neurite outgrowth, the effect of DM may be mediated by pre-existing BDNF protein in cortical neurons.

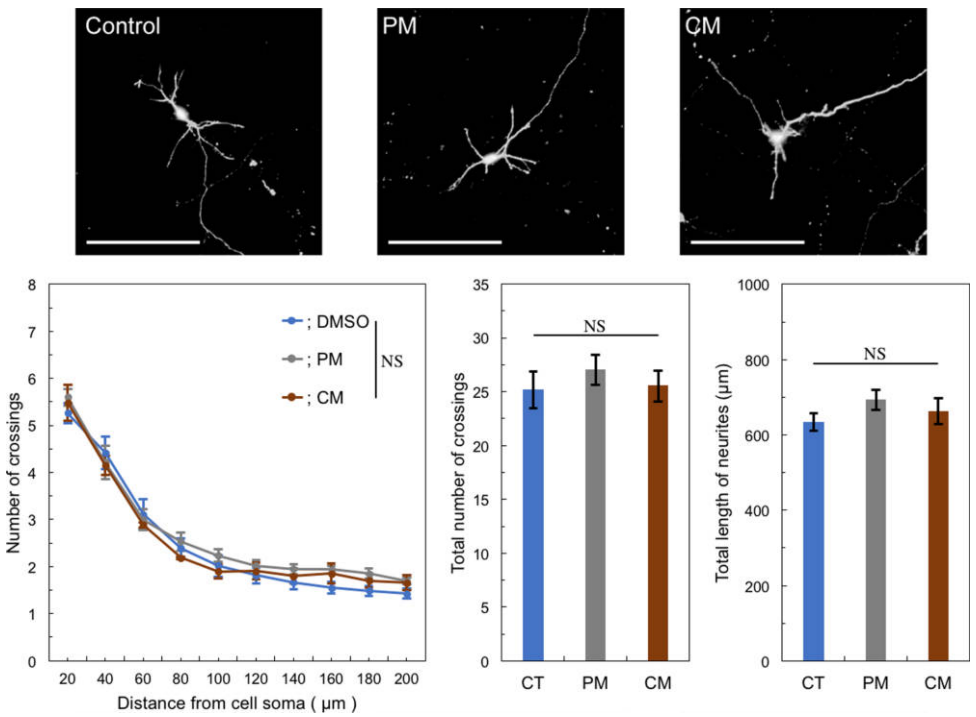

Fig. 2. Effect of PM or CM on neurite outgrowth in rat cortical neurons. Representative images of cortical neurons 6 h after DMSO, PM, or CM treatment (scale bar=100 μm). Changes in the number of crossings, total number of crossings, and the total length of neurites 6 h after each treatment were shown. All pyrethroids were used at a concentration of 1 μM. The experiment was repeated independently four times. NS: not significant.

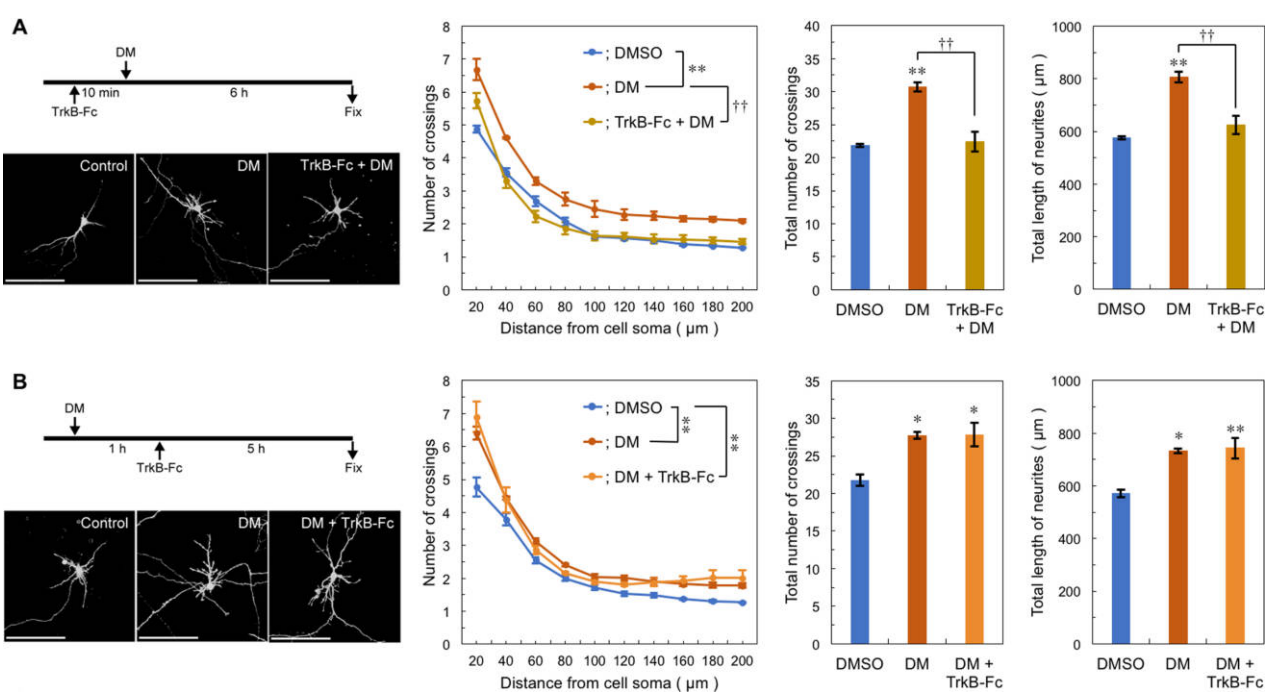

Fig. 3. Effect of TrkB-Fc on DM-induced neurite outgrowth. TrkB-Fc (100 ng/mL) was added (A) 10 min before or (B) 1 h after DM treatment. Cells were treated with 1 μM DM for 6 h (Scale bar=100 μm). The experiment was repeated independently three times. *p<0.05 or **p<0.01 versus DMSO, $^{\dagger\dagger}p$<0.01 versus DM.

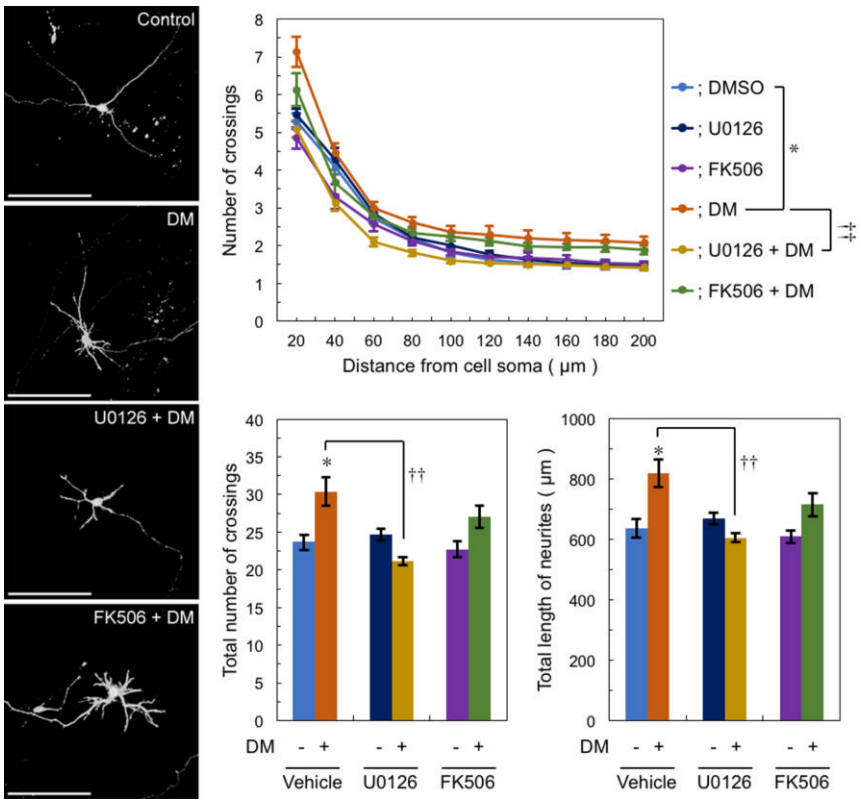

Fig. 4. Effect of U0126 and FK506 on DM-induced neurite outgrowth. U0126 (20 μM) or FK506 (5 μM) was added 10 min before DM treatment. Cells were treated with DM for 6 h (Scale bar=100 μm). The experiment was repeated independently five times. *p<0.05 versus DMSO, ††p<0.01 versus DM. NS: not significant.

On the other hand, we have previously confirmed that sole addition of TrkB-Fc did not affect neurite morphology of cortical neurons (Ihara *et al.*, 2012).

DM-induced neurite outgrowth is dependent on the MAPK and mTOR pathways

We previously found that the DM-induced increase in *Bdnf* mRNA levels was mediated by the ERK/mitogen-activated protein kinase (MAPK) and calcineurin pathways (Ihara *et al.*, 2012). Among these pathways, calcineurin plays a particularly important role in the DM-mediated induction of *Bdnf* mRNA expression (Ihara *et al.*, 2012). However, the calcineurin inhibitor FK506 did not significantly suppress DM-induced neurite outgrowth (Fig. 4). In contrast, U0126, a MAPK/ERK kinase 1/2 inhibitor, significantly suppressed the effect of DM (Fig. 4). These results suggest that the TrkB/MAPK pathway plays a key role in the DM-induced increase in neurite outgrowth. Because FK506 did not significantly affect neurite outgrowth, *de novo Bdnf* expression is unlikely to be necessary for the DM-triggered increase in neurite outgrowth.

Mammalian target of rapamycin (mTOR) has been reported to regulate dendritic morphology (Kumar *et al.*, 2005). Furthermore, BDNF has been shown to control local protein synthesis via mTOR (Takei *et al.*, 2004). Therefore, we examined the effect of rapamycin on DM-induced neurite outgrowth. Although sole addition of rapamycin slightly, but not significantly, increased neurite outgrowth, the DM-induced neurite outgrowth was not observed in the presence of rapamycin (Fig. 5), suggesting that rapamycin blocked the DM-induced neurite outgrowth. These results indicate that DM regulates neurite outgrowth in cortical neurons through the ERK/MAPK and mTOR pathways via endogenous BDNF/TrkB activation.

Discussion

We have demonstrated the involvement of endogenous BDNF in DM-induced neurite outgrowth of cultured rat cortical neurons (Fig. 6). The results obtained using a series of inhibitors and other pyrethroids suggest that the enhancement of neurite outgrowth by DM would be dependent on endogenous BDNF/TrkB-mediated signaling but independent on *de novo Bdnf* expression.

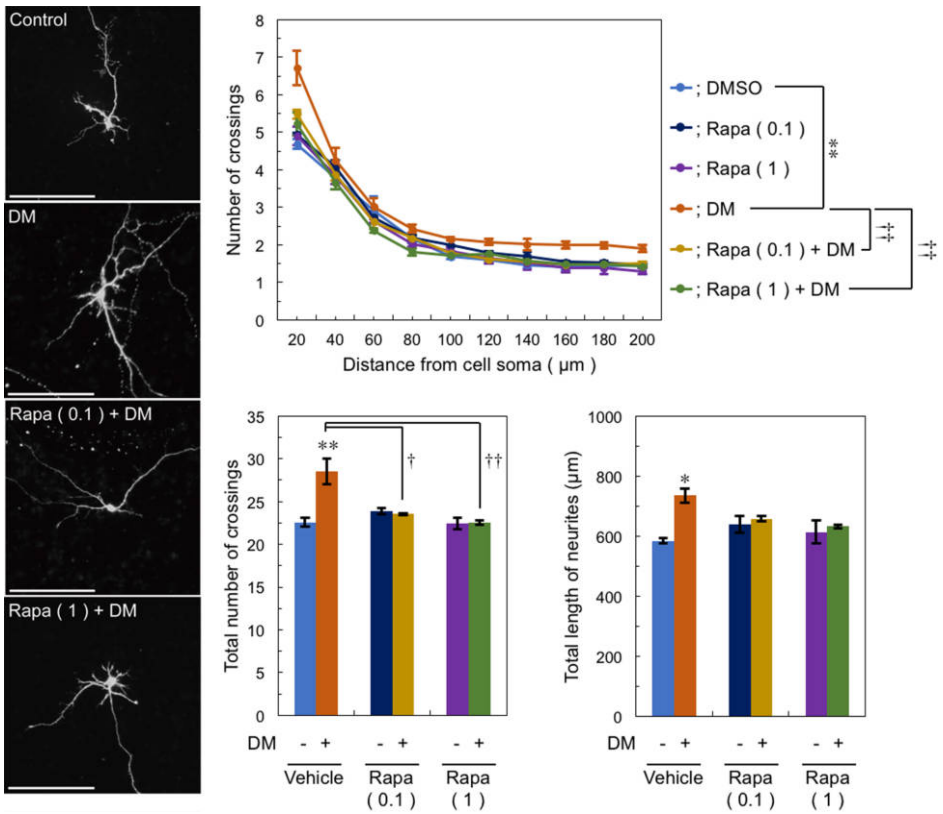

Fig. 5. Effect of rapamycin on DM-induced neurite outgrowth. Cells were treated with 1 μM DM for 6 h. Rapamycin (Rapa; Rapa (0.1)=0.1 μM rapamycin, Rapa (1)=1 μM rapamycin) was added 10 min before DM treatment (Scale bar=100 μm). The experiment was repeated independently three times. *p<0.05 and **p<0.01 versus DMSO, $^{\dagger}p$<0.05 and $^{\dagger\dagger}p$<0.01 versus DM. NS: not significant.

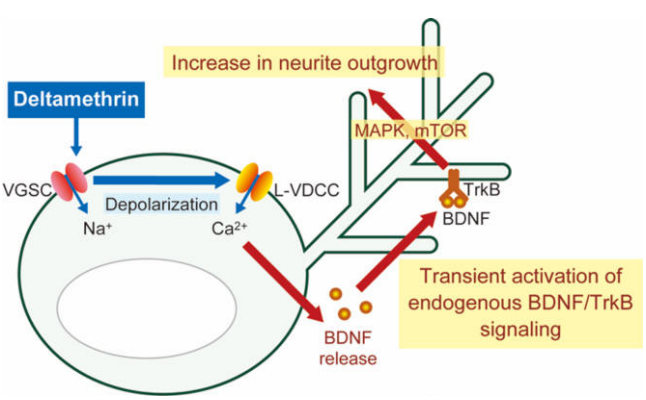

Fig. 6. A schematic of the possible pathway of DM-mediated increased secretion of endogenous BDNF protein, resulting in neurite outgrowth of cortical neurons. DM induces depolarization of rat cortical neurons and triggers calcium influx via L-VDCCs. The increased calcium induces secretion of BDNF protein. Secreted BDNF activates the MAPK and mTOR pathways via TrkB, which promotes neurite outgrowth of cortical neurons. Our results suggest that DM would induce transient and slight activation of endogenous BDNF/TrkB pathway, resulting in neurite outgrowth of cortical neurons.

Although the main molecular targets of pyrethroids are VGSCs, PM and CM did not significantly affect neurite outgrowth. In previous research, DM-induced neurite outgrowth was abolished by nicardipine, indicating the involvement of L-type voltage-dependent calcium channels (L-VDCC) (Ihara *et al.*, 2012). We also found previously that DM produced a prolonged increase in intracellular calcium concentrations (Ihara *et al.*, 2012). CM induces calcium influx into neocortical neurons less potently than DM, while PM has no effect (Cao *et al.*, 2011). These results suggest that the ability to induce calcium influx into neurons, probably via L-VDCC, is necessary for increasing neurite outgrowth.

Because an increase in calcium levels in neurons triggers regulated secretion of BDNF protein (Kuczewski *et al.*, 2009), it is plausible that DM increases intracellular calcium concentration, resulting in increased secretion of BDNF. Although we tried to measure secreted endogenous BDNF protein in the culture medium, the BDNF protein level in the medium was nearly undetectable, possibly due to immature neurons. However, our preliminary data suggested only a slight increase in secreted BDNF protein after DM treatment (Our unpublished observations). We

observed a slight and transient increase in phosphorylation of TrkB and its downstream kinase ERK 1/2 after the DM treatment. Therefore, it is possible that DM slightly increases endogenous BDNF release by inducing calcium influx into the neurons, and secreted BDNF then increases neurite outgrowth via TrkB/MAPK and mTOR pathways. This transient activation of endogenous BDNF/TrkB signaling under the DM treatment might be one of the reason why the effect of DM on neurite outgrowth was optimally observed 6 h after the treatment and the effect was likely to decline 24 h after the treatment. In any case, because of the potent neurotrophic effect of BDNF, a slight increase in BDNF secretion by DM is likely sufficient to enhance neurite outgrowth. However, *de novo* BDNF protein might also contribute to DM-induced neurite outgrowth, and its contribution should be further examined in future.

MAPK and mTOR have been shown to be involved in the regulation of dendritic morphology (Kumar *et al.*, 2005; Jaworski *et al.*, 2005), which is consistent with our present findings that U0126 and rapamycin significantly suppress DM-induced neurite outgrowth. Secreted BDNF regulates local dendritic growth in nearby neurons (Horch and Katz, 2002) and activates local translation in dendrites through mTOR pathways (Takei *et al.*, 2004). These reports, together with our current results, suggest that DM enhances endogenous BDNF signaling, which locally activates translation machinery via MAPK and mTOR pathways, resulting in an increase in neurite outgrowth. Crosstalk between the MAPK and mTOR pathways has been reported to be involved in the regulation of cell survival, differentiation and proliferation in response to biological cues (Mendoza *et al.*, 2011). Furthermore, functional interactions between the ERK/MAPK and mTOR pathways up-regulate the dendritic translation machinery in long-term potentiation (Tsokas *et al.*, 2007). Consistent with these observations, the addition of U0126 or rapamycin almost completely suppressed the effect of DM in the present study, suggesting that the MAPK and mTOR pathways coordinately regulate neurite outgrowth triggered by the insecticide.

Because DM strongly increases *Bdnf* expression in neurons (Imamura *et al.*, 2006; Ihara *et al.*, 2012), DM is anticipated to have trophic effects on neurons. Indeed, we previously reported that DM significantly increases the viability of cultured rat cortical cells (Ihara *et al.*, 2012). Furthermore, DM has an antidepressant-like effect, which is abolished by Trk inhibitor K252a (Takasaki *et al.*, 2013). Therefore, DM may be beneficial for the adult brain. However, DM has been found to negatively affect brain function during development. For example, compared with young adult rats, preweanling and weanling rats exhibit markedly elevated brain DM concentrations after exposure to the insecticide (Kim *et al.*, 2010). Developmental exposure to DM has been reported to be a risk factor for attention-deficit/hyperactivity disorder (Richardson *et al.*, 2015). In addition, childhood exposure to DM at low levels may be related to neurocognitive impairments by 6 years of age (Viel *et al.*, 2015). These neurotoxic effects of DM in the young may be due to immaturity of the blood-brain barriers.

Taken together, we demonstrated that DM has an ability to increase neurite outgrowth of neurons, which is mediated by endogenous BDNF/TrkB-dependent MAPK and mTOR pathways. Our present findings, along with previous observations, suggest that DM disrupts the formation of neural networks, particularly in the developing brain. Despite its possible beneficial effects on the nervous system and in psychiatric disorders such as depression, DM exposure during development may result in an elevated risk of neurological disorders.

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References

Barnes, J.M. and Verschoyle, R.D. 1974. Toxicity of new pyrethroid insecticide. *Nature*, **248**: 711.

Cao, Z., Shafer, T.J., and Murray, T.F. 2011. Mechanisms of pyrethroid insecticide-induced stimulation of calcium influx in neocortical neurons. *J. Pharmacol. Exp. Ther.*, **336**: 197–205.

Horch, H.W. and Katz, L.C. 2002. BDNF release from single cells elicits local dendritic growth in nearby neurons. *Nat. Neurosci.*, **5**: 1177–1184.

Ihara, D., Fukuchi, M., Honma, D., Takasaki, I., Ishikawa, M., Tabuchi, A., and Tsuda, M. 2012. Deltamethrin, a type II pyrethroid insecticide, has neurotrophic effects on neurons with continuous activation of the *Bdnf* promoter. *Neuropharmacology*, **62**: 1091–1098.

Imamura, L., Yasuda, M., Kuramitsu, K., Hara, D., Tabuchi, A., and Tsuda, M. 2006. Deltamethrin, a pyrethroid insecticide, is a potent inducer for the activity-dependent gene expression of brain-derived neurotrophic factor in neurons. *J. Pharmacol. Exp. Ther.*, **316**: 136–143.

Ishikawa, M., Nishijima, N., Shiota, J., Sakagami, H., Tsuchida, K., Mizukoshi, M., Fukuchi, M., Tsuda, M., and Tabuchi, A. 2010. Involvement of the serum response factor coactivator megakaryoblastic leukemia (MKL) in the activin-regulated dendritic complexity of rat cortical neurons. *J. Biol. Chem.*, **285**: 32734–32743.

Jaworski, J., Spangler, S., Seeburg, D.P., Hoogenraad, C.C., and Sheng, M. 2005. Control of dendritic arborization by the phosphoinositide-3'-kinase-Akt-mammalian target of rapamycin pathway. *J. Neurosci.*, **25**: 11300–11312.

Kim, K.B., Anand, S.S., Kim, H.J., White, C.A., Fisher, J.W., Tornero-Velez, R., and Bruckner, J.V. 2010. Age, dose, and time-dependency of plasma and tissue distribution of deltamethrin in immature rats. *Toxicol. Sci.*, **115**: 354–368.

Kuczewski, N., Porcher, C., Lessmann, V., Medina, I., and Gaiarsa, J.L. 2009. Activity-dependent dendritic release of BDNF and biological consequences. *Mol. Neurobiol.*, **39**: 37–49.

Kumar, V., Zhang, M.X., Swank, M.W., Kunz, J., and Wu, G.Y. 2005. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. *J. Neurosci.*, **25**: 11288–11299.

Meichsner, M., Doll, T., Reddy, D., Weisshaar, B., and Matus, A. 1993. The low molecular weight form of microtubule-associated protein 2 is transported into both axons and dendrites. *Neuroscience*, **54**: 873–880.

Mendoza, M.C., Er, E.E., and Blenis, J. 2011. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem. Sci.*, **36**: 320–328.

Miyamoto, J., Kaneko, H., Tsuji, R., and Okuno, Y. 1995. Pyrethroids, nerve poisons: how their risks to human health should be assessed. *Toxicol. Lett.*, **82–83**: 933–940.

Narahashi, T. 1985. Nerve membrane ionic channels as the primary target of pyrethroids. *Neurotoxicology*, **6**: 3–22.

Ray, D.E. and Forshaw, P.J. 2000. Pyrethroid insecticides: poisoning syndromes, synergies and therapy. *J. Toxicol. Clin. Toxicol.*, **38**: 95–101.

Richardson, J.R., Taylor, M.M., Shalat, S.L., Guillot, T.S. 3rd, Caudle, W.M., Hossain, M.M., Mathews, T.A., Jones, S.R., Cory-Slechta, D.A., and Miller, G.W. 2015. Developmental pesticide exposure reproduces features of attention deficit hyperactivity disorder. *FASEB J.*, **29**: 1960–1972.

Saillenfait, A.M., Ndiaye, D., and Sabaté, J.P. 2015. Pyrethroids: exposure and health effects—an update. *Int. J. Hyg. Environ. Health*, **218**: 281–292.

Shelton, J.F., Geraghty, E.M., Tancredi, D.J., Delwiche, L.D., Schmidt, R.J., Ritz, B., Hansen, R.L., and Hertz-Picciotto, I. 2014. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. *Environ. Health Perspect.*, **122**: 1103–1109.

Sholl, D.A. 1953. Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat.*, **87**: 387–406.

Takasaki, I., Oose, K., Otaki, Y., Ihara, D., Fukuchi, M., Tabuchi, A., Tsuneki, H., Tabuchi, Y., Kondo, T., Saitoh, A., Yamada, M., and Tsuda, M. 2013. Type II pyrethroid deltamethrin produces antidepressant-like effects in mice. *Behav. Brain Res.*, **257**: 182–188.

Takei, N., Inamura, N., Kawamura, M., Namba, H., Hara, K., Yonezawa, K., and Nawa, H. 2004. Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. *J. Neurosci.*, **24**: 9760–9769.

Tsokas, P., Ma, T., Iyengar, R., Landau, E.M., and Blitzer, R.D. 2007. Mitogen-activated protein kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. *J. Neurosci.*, **27**: 5885–5894.

Verschoyle, R.D. and Barnes, J.M. 1972. Toxicity of natural and synthetic pyrethrins to rats. *Pestic. Biomech. Physiol.*, **2**: 308–311.

Verschoyle, R.D. and Aldridge, W.N. 1980. Structure-activity relationships of some pyrethroids in rats. *Arch. Toxicol.*, **45**: 325–329.

Viel, J.F., Warembourg, C., Le Maner-Idrissi, G., Lacroix, A., Limon, G., Rouget, F., Monfort, C., Durand, G., Cordier, S., and Chevrier, C. 2015. Pyrethroid insecticide exposure and cognitive developmental disabilities in children: The PELAGIE mother-child cohort. *Environ. Int.*, **82**: 69–75.

Vijverberg, H.P., van der Zalm, J.M., and van der Bercken, J. 1982. Similar mode of action of pyrethroids and DDT on sodium channel gating in myelinated nerves. *Nature*, **295**: 601–603.

Warmke, J.W., Reenan, R.A., Wang, P., Qian, S., Arena, J.P., Wang, J., Wunderler, D., Liu, K., Kaczorowski, G.J., Van der Ploeg, L.H., Ganetzky, B., and Cohen, C.J. 1997. Functional expression of Drosophila para sodium channels. Modulation by the membrane protein TipE and toxin pharmacology. *J. Gen. Physiol.*, **110**: 119–133.

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